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**ІНСТИТУТ БІОЛОГІЇ ТВАРИН**

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# ANTIOXIDATIVE SYSTEM OF BOVINE FOLLICLES REGARDING STAGE OF ESTROUS CYCLE AND FOLLICULOGENESIS

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*Oxidative stress is important for promoting the oocyte maturation and ovulation within the follicle. The aim of the study was to examine activities of the superoxide dismutase (SOD), glutathione peroxidase (GPx) and biological antioxidant potential (BAP) involved in protection against free oxygen radicals and concentration of reactive oxygen metabolites (ROMs) in bovine follicular fluid.*

*Bovine ovaries were obtained from a slaughterhouse. The stage of estrous cycle (follicular or luteal) was identified. Follicular fluids (FF) were collected by puncture from three categories of follicles: small (≤5 mm), medium (6–10 mm) or large (>10 mm) from ovaries in both follicular and luteal stage of estrous cycle.*

*The results indicate a significantly higher activity of SOD in bovine FF from follicles in luteal stage of estrous cycle than in follicular stage (P=0.037). In FF of follicles in both stages of estrous cycle, SOD activity and BAP were significantly higher in FF of small and medium sized follicles than in large ones (P=0.000 both). The ROMs significantly declined from small to large follicles in FF collected during both, luteal and follicular phase of estrous cycle (P=0.005 both). Activity of GPx showed no significant differences regarding neither estrous cycle nor size of follicles.*

*Results indicate similar antioxidative properties of bovine follicles during both luteal/follicular stage of estrous cycle with higher SOD activity in FF of follicles in luteal stage of estrous cycles which undergo atresia.*

**Keywords:** BOVINE FOLLICULAR FLUID, ANTIOXIDATIVE SYSTEM, LUTEAL STAGE OF ESTROUS CYCLE, FOLLICULAR STAGE OF ESTROUS CYCLE

Follicular fluid (FF) accumulates in antral space and surrounds oocyte providing microenvironment for the development of oocytes. FF is a product of plasma filtration and of the secretory activity of granulosa and thecal cells. The molecular composition of follicular fluid includes proteins, nucleic acids and small molecules which show roles in cell nutrition and endocrine signaling and are likely to control the proliferation and development of granulose cells forming the cumulus around the oocyte [10].

Follicular fluid is responsible for providing oocyte protection against oxidation. The ovarian follicles contain macrophages, neutrophils and granulose cells producing reactive oxygen species (ROS) during metabolic processes. ROS must be continuously inactivated by nonenzymatic and enzymatic antioxidants to maintain balance between oxidant and antioxidants to preserve normal cell function [1]. The most important enzymatic molecules are superoxide dismutase (SOD) and glutathione peroxidase (GPx). Superoxide dismutase catalyses the dismutation of superoxide radical to hydrogen peroxide which will be removed by glutathione peroxidase. There were studies dealing with SOD function in oocyte development [2, 4, 12, 13]. The reducing ability of nonenzymatic molecules (vitamin C and E, glutathione and other protein thiols, bilirubin and uric acid) can be measured in biological samples by the ferric-reducing capacity [1].

The aim of the study was to examine activities of the superoxide dismutase, glutathione peroxidase and biological antioxidant potential

(BAP) involved in protection against free oxygen radicals and concentration of reactive oxygen metabolites in bovine FF.

## Materials and methods

***Follicular fluid collection and processing.*** The bovine ovaries (n=76) were collected from naturally cycling animals at a local slaughterhouse and transported to the laboratory within 2 h. Ovaries were transported in 0.9 % NaCl with antibiotics (100 I.U. penicillin/mL and 100 µg streptomycin/mL) and stored at 4–8 °C. In the laboratory, the stage of estrous cycle (follicular or luteal) was identified according to presence or absence of the *corpus luteum* on the ovary as previously described [6]. Follicular diameter was measured and follicles were classified according to diameter: small (≤5 mm), medium (6–10 mm) or large (>10 mm) follicles from ovaries in both follicular and luteal stage of estrous cycle. Ovaries with cystic follicles were excluded from the study. The FF was aspirated from each pair of ovaries using a 2 mL syringe attached to an 18-gauge needle. Follicular fluids from each group from each pair of the ovaries were pooled in one sample for each individual animal. The FFs were centrifuged (1600g for 10 min) for separation of the fluid from the cell fraction. Supernatants were stored at –80 °C until analysis.

***Biochemical analyses.*** Activities of the superoxide dismutase (SOD), glutathione peroxidase (GPx) and biological antioxidant potential (BAP) involved in protection against free oxygen radicals and concentration of reactive oxygen metabolites (ROMs) were analysed in follicular fluids punctured from ovaries in follicular and luteal phase of estrous cycle. Glutathione peroxidase and SOD activities were assayed on an *Olympus AU 400* (*Olympus*, Japan) biochemical analyzer, using the commercial *Ransel* and *Ransod* reagent kits (both *Randox*, Crumlin, UK), respectively. Concentrations of (ROM) and BAP were measured on an *Olympus AU 400* (*Olympus*, Japan) biochemical analyser, using the commercial d-ROM and BAP reagent kits (both *Diacron International*, Italy), respectively.

***Statistical analysis.*** All statistical analysis was performed using *Statistica version 12* (*StatSoft*, Palo Alto, California, USA). Data were expressed as means ± standard deviation (SD). Data were analysed using way repeated measures ANOVA. Significance of follicles size differences within time was tested by Two-way mixed-design ANOVA followed by Tukey's significant difference test in case of normal distribution while a non-normal distribution by Kruskal-Wallis ANOVA and Dunn test for all groups. The level of $P<0.05$ was considered statistically significant.

## Results and discussion

Successful folliculogenesis may depend on balance between ROS and antioxidants. In this study we measured SOD and GPX activities and concentrations of BAP and ROM in bovine follicular fluid regarding to stage of estrous cycle (follicular and luteal) and follicular size.

SOD activity in follicular fluid originates from cells surrounding oocytes. SOD may play role in inhibition of progesterone synthesis [11], inhibition of aromatase activity [7] prevention of damaging effects of ROS and ovulation process [2]. In FF, SOD is responsible for reducing DNA damage and increasing oocyte cytoplasmic maturation [12]. We observed higher SOD activity in FF obtained from follicles during luteal phase ($P=0.037$; fig.). The similar results were obtained by [3]. In FF of follicles in both stages of estrous cycle, SOD activity was significantly higher in FF of small and medium sized follicles than in large ones ($P=0.000$; table). Our results

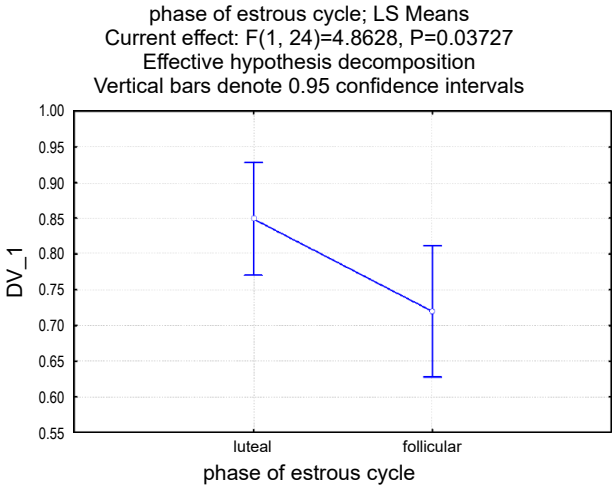


*Fig.* Bovine follicular fluid superoxide dismutase activity from follicles in luteal and follicular phase of estrous cycle

*Table*

**Mean and standard deviation of activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) and concentrations of biological antioxidant potential (BAP) and reactive oxygen metabolites (ROM) in bovine follicular fluid from follicles in follicular and luteal phase of estrous cycle**

| | small (≤5 mm) | | medium (6–10 mm) | | large (>10 mm) | |
|---|---|---|---|---|---|---|
| | follicular | luteal | follicular | luteal | follicular | luteal |
| SOD (U/L) | 944±260 A | 963±229 a | 694±264 AB | 801±260 a | 531±297 C | 590±217 bc |
| GPx (U/L) | 265±107 A | 235±82 a | 265±93 A | 291±69 a | 274±88 A | 296±59 a |
| BAP (mm/L) | 3.63±0.21 A | 3.55±0.26 a | 3.45±0.19 AB | 3.31±0.35 a | 3.06±0.30 C | 3.26±0.20 bc |
| ROM (U CARR) | 106.43±37.41 AB | 117.53±55.47 a | 98.94±43.93 ABC | 85.99±41.98 c | 72.57±16.43 C | 82.37±21.54 bc |

*Note*: Means, within same row, sharing the same superscript are not significantly different from each other (P<0.05). Uppercase and lowercase letters correspond to follicular and luteal phase of estrous cycle, respectively.

are in agreement with [1] in swine and [4] in buffalo FF. For ovulation certain concentration of ROS is needed [8] and maybe high SOD activity in FF prevent ovulation of follicles obtained during luteal phase.

The SOD contributes to the first line of antioxidant protection reducing damage converting superoxide radical to $H_2O_2$. If SOD activity is reduced, catalase activity is also decreased and GPx activity is increased [13]. In our research GPx shows a trend of increased activity during folliculogenesis (P=0.083, table) which may indicate an increase in production of lipid peroxide in follicles during development. [1] reported significant decrease of GPx activity in follicular fluid from medium and large compared with small follicles.

The BAP test measures the concentration of different small nonenzymatic molecules having antioxidant properties such as bilirubin, uric acid vitamin C and E (*Diacron International*, Italy). In our research, BAP concentration was significantly higher in FF of small and medium sized follicles than in large ones regardless stage of estrous cycle (P=0.000). Our results are in agreement with [1] in swine FF. Results indicate that concentration of mentioned molecules decreased during bovine folliculogenesis and antioxidative protection depend on other compounds [5]. Positive correlation between ROS levels in FF and maturation parameters was found in different studies [9]. Among others, superoxide radicals, hydroxyl radical and $H_2O_2$ are the most important. d-ROM test measures hydroperoxides (ROOH which are products of superoxide and hydroxyl radicals peroxidation (*Diacron International*, Italy). In the present study, we showed that concentrations of ROMs during folliculogenesis are reduced in FF collected during both luteal and follicular phase of estrous cycle (P=0.005 both). [1] reported similar results on swine FF with the same test.

## Conclusions

In conclusion, the major finding of the present study is higher SOD activity in FF obtained from follicles during luteal phase and decrease of ROMs level during follicle development. Higher level of SOD indicate lower concentrations of ROS species which are needed for ovulation and one of reasons for anovulation of follicles obtained during luteal phase maybe high SOD activity. Decreased concentration of ROMs suggests that oocyte during folliculigenesis is not subjected to oxidative stress and that balance between oxidants and antioxidants exists.

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# BIOPHYSICAL ANALYSIS OF EMANATED PHEROMONAL ODOR CHANGES IN COWS USING ELECTRONIC NOSE TECHNOLOGY

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*The objective of the study was to investigate the possibility of applying the Electronic Nose (EN) technology to analyze and detect emanated pheromonal odor changes during estrus cycle in cows. In comparison to gas chromatography (GC) headspace samples analysis and blood hormonal (estradiol and progesterone) analysis using Elisa and RIA. Also to establish a protocol to appoint the proper time of artificial insemination (AI) in cows. The study was conducted on 54 Holstein-Friesian cows. Out of sensors of the EN used in this study, sensor#2 showed the highest response to all measured perineal samples, which adhered perfectly to plasma hormone ($E_2$) levels, and GC analysis of the perineal samples which showed progressive change in acetaldehyde as estrus was approached.*

**Keywords:** COWS, ESTRUS, PHEROMONES, ELECTRONIC NOSE (EN), ACETALDEHYDE

Poor estrus detection is a major problem for the AI industry because missed estruses represent lost opportunities for use of semen from genetically superior bulls, since the most fertile semen and the best inseminator in the world can't overcome the problems of inseminating cows at the wrong time [6]. Consequently, a basic understanding of the bovine estrous cycle can increase the effectiveness of reproductive management [9]. It is a tremendous task to detect standing estrus in a cow herd, and nothing can substitute for visually observing the cattle. Several estrus-detection aids are commercially available, but these are just aids. The more time spent with the cattle, the better [11]. Estrus detection aids are heat expectancy charts, pressure-sensitive mount detectors, tail chalk, detector animals, and electronic aids. They may be used to help identify cows that are in estrus but may otherwise go unnoticed [10]. However, none of these techniques has yielded consistent, reproducible data, and estrus detection still relies primarily on the observation of estrous behavior or on the results of a milk progesterone assay, neither of which afford high fertility rates and both of which are labor intensive. The production of a simple, reliable protocol to aid the herdsperson is still not available [11].

In mammals, sexual behaviours of males and females are induced and their hormonal status may be also changed via the stimulation of vomeronasal organ. Vomeronasal organ is primarily responsible for mediating responses to some, but by no means all, pheromone-like signals [4]. Pheromones are chemical substances that are released by animals in order to stimulate modifications in the neuroendocrine system of receiving individuals thus producing a physiological and behavioral response [7]. A pheromonal function has been proposed for the skin glands of the bovine perineum. These glands are specialized sebaceous glands that are located on either side of the vulva, and undergo morphological changes at estrus [1].

The main task in odor recognition is to create a model as similar to the human and animal model as possible. EN are being developed as a system for the automated detection and classification of odors, vapors, and gases. EN is represented as a combination of two components: sensing system and pattern recognition system [8]. Recent advances in artificial olfaction technology have allowed us to monitor perineal odor through estrus [6].

Thus, the objectives of the present study are to: (1) investigate the possibility of applying the EN technology for detecting emanated pheromonal odor changes associated with estrus in cows; (2) assess estrus cycle in cow using the EN technology as compared to conventional methods (i.e., behavioral observations, rectal palpation, and hormonal analysis); (3) establish a protocol to appoint the proper time of artificial insemination in cows.

## Materials and methods

***Animals.*** This study was carried out on 4 healthy Holstein-Friesian dairy cow groups, at the Alexandria Agriculture Farm (Alexandria, Egypt). All cows were housed in a free-stall system and given rations to meet their maintenance and production requirements. Group A cows (n=15) and group C cows (n=9) were observed at their natural midluteal phase of the estrus cycle. While estrus was induced in group B cows (n=15) and group C cows (n=9) by a single intramuscular injection in day 0 with a prostaglandin F2α (PGF2α) — 500 μg cloprostenol in 2 ml of *Estrumate*™ (*Malinkrodt Veterinary Ltd.*, Middlesex, UK), under the supervision of a resident veterinarian. Day of estrus was assessed on basis of progesterone ($P_4$, pg/ml) and estradiol ($E_2$, ng/ml) levels, behavioral observations; Cows were observed 3 times/day throughout the study period and daily rectal palpation for presence of *corpus luteum*.

***Methods.*** *Plasma Hormone Assays.* Blood samples were obtained from the coccygeal vein of each cow in the morning after perineal swaping from the day of PGF2α injection for 9 days (group B) and for 8 days (group D). Each sample was capped, labeled and identified on basis of cow no. and the date of sampling. The samples were centrifuged for ×9 for 10 min and plasma supernatant was decanted and frozen only once at –20 °C. Latter the plasma samples assayed for progesterone ($P_4$) and estradiol ($E_2$) (modified from *DRG diagnostic*, Marburg, Germany).

*Sample collection.* Samples were collected from the perineal region (area around the vulva) [1]. The area was washed with clean tap water and soft brush in order to minimize contaminate fecal odor [6]. The area was dried with soft tissues and left to dry for 10 min. Samples were then taken from a dorsal lateral perineal site using 3 cotton swabs/cow. Samples were collected in pairs of the same tubes: one for the EN and the other for the GC.

*Electronic Nose (EN).* First-tube samples were analyzed using a commercially available portable E-Nose (PEN3, *Airsense Analytics GmbH*, Schwerin, Germany) with an array of 10 different metal-oxide sensors that measure independently and register continuously relative changes in conductance due to a vapor or odor during an experiment. Odors in the headspace (i.e., the space over the cotton swabs) of each sealed tube was carried by the carrier gas (e.g., dry air), and the difference in the sensor output was recorded. The software interacts with the user by displaying the correct time points to connect and disconnect the sample to the E-Nose inlet. All measurements were repeated twice and results files containing sensors patterns for every experiment were saved for subsequent analysis.

*Gas chromatography.* Second tube samples of both studied groups were analyzed using GC (*Auto System XL*, *Perkin Elmer*, USA) at the Pahrmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Alexandria University. The headspace of each sealed tube was aspired and injected immediately into the GC, where oven temperature was kept at 29 °C for 2 min. and then programmed to increase to 56 °C at 5 °C increments. The injector and detector temperatures were set at 180 and 200 °C, respectively. $H_2$ and $N_2$ were set at 45 cm/s flow rates and areas under peaks were calculated using the driving software. Reference standard of acetaldehyde was prepared and measured to calculate its concentration in each sample [12].

*Data Analysis.* All measurements by the E-Nose were analyzed using the Principle Component Analysis (PCA) technique. The greatest variance by any projection of the data comes to lie on the first coordinate, which is called the principal component #1; and the second greatest variance on the second coordinate, which is called the principle component #2. PCA is theoretically the optimum transform for given data in least square terms. Moreover, sensors responses of each individual cows were averaged and compared to investigate

their relative sensitivity for monitoring changes in pheromonal odors in the midluteal phase and in the estrus cycle using ANOVA followed by a Fisher's PLSD *post-hoc* test. Differences were considered to be significant at $P<0.05$.

## Results and discussion

Changes in perineal odor if correctly approached could form the basis of a new method for estrus detection. In the present study perineal odor of cyclic cows was monitored. Moreover estrus was identified using GC for acetaldehyde behavioral observations, and plasma assays for $P_4$ and $E_2$.

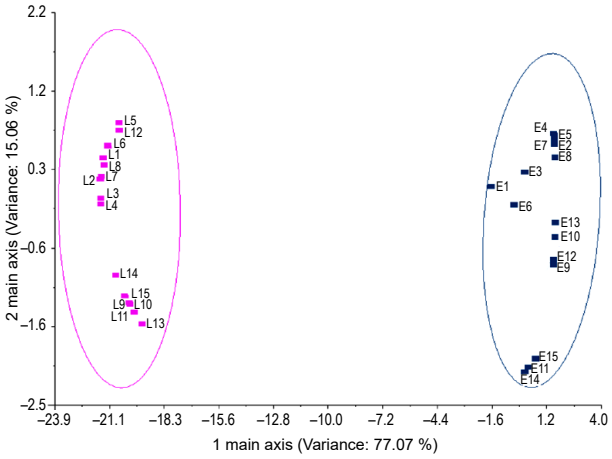


*Fig. 1.* Principal component analysis of perineal odor sample, group A: 15 cows each sampled on the day of estrus (E) and in the midluteal (L). Showing there is difference between the tow clusters of differentiation inspit of there was one of the two needles was congested

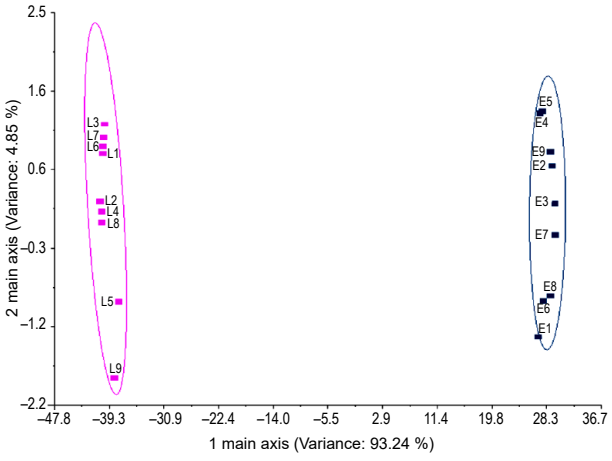


*Fig. 2.* Principal component analysis of perineal odor sample, group C: 9 cows each sampled on the day of estrus (E) and in the midluteal (L). Showing that there is great difference between the tow clusters more than showed in fig. 1

***Experiment I.*** *Group A: 15 non synchronized Holstein Friesian cows* and *group C: 9 non synchronized Holstein Friesian cows.* By PCA, perineal odor data indicated difference between cows (group A) in the midluteal phase (L) and cows in estrus (E) (fig. 1). Only one of the ten sensors was responding (sensor #2) since one of the 2 needles was congested which affected on the chamber flow, thus repeating the experiment with group C was necessary to make sure that the measurements not affected by the needle congestion. In group C by repetition there was three sensors responding (#2, 6 and 8). By PCA the perineal odor data indicated

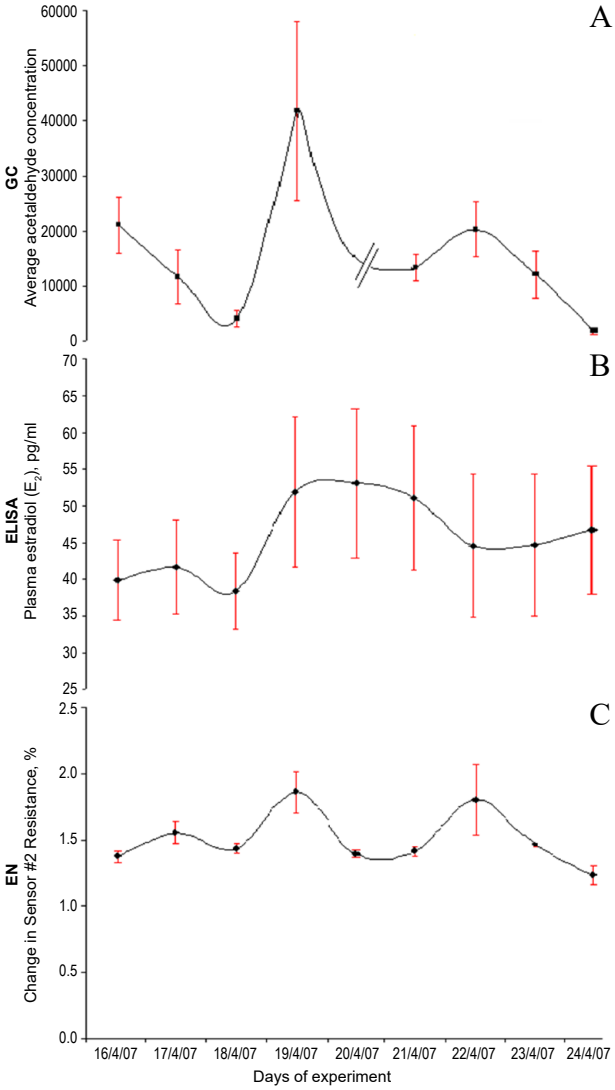


*Fig. 3.* The relationship between A) headspace gas chromatograms (GC) from perineal swabs, B) plasma estradiol concentration through estrus and C) the response of one of 10 sensors (#2) of electronic nose (EN) to perineal odor through estrus. Data are representing as means (±SEM) for 15 cows (group B). Estrus was induced using a single intramuscular injection of PGF2α when midluteal (day 14 or 15).

greater differences than in group A (fig. 2), however in both group A and C didn't show on which day the odor changes occur.

***Experiment II.*** *Group B (15 synchronized Holstein Friesian cows)* and *group D (9 synchronized Holstein Friesian cows).* In group B only 1 sensor showed change in resistance through experiment, this was interpreted to represent a change in perineal odor although there was congestion in one needle which affected chamber flow. It is clear from fig. 3 GC results (except 1st day of GC) coinside with response of sensor #2 and the 1st 4 days of the plasma $E_2$ profile. In group D: with repetition in this group, out of the 10 sensors, three sensors showed change in resistance through experiment (#2, 6 and 8), which was interpreted to represent a change in perineal odor. The SEM bars of plasma estradiol were large due to the individual variation among the cows in spite of using automatic method in $E_2$ kit measurements (ELISA) [2, 3].

These data are in line with observations by [6], how showed strong correlation between concentrations of circulating steroid hormones and signals from bovine perineal swabs that were measured with an EN. The EN sensors responded to changes in volatile substances with changes in resistance. Molecules causing such a response would be likely to be detectable as an odor signal. To date, no group has carried out gas GC of the volatile constituents of the perineum. In light of the studies by [5] acetaldehyde possibly is released from a range of body fluids and may provide a marker for estrus. We used pure acetaldehyde spikes as authentic odor to determine acet-

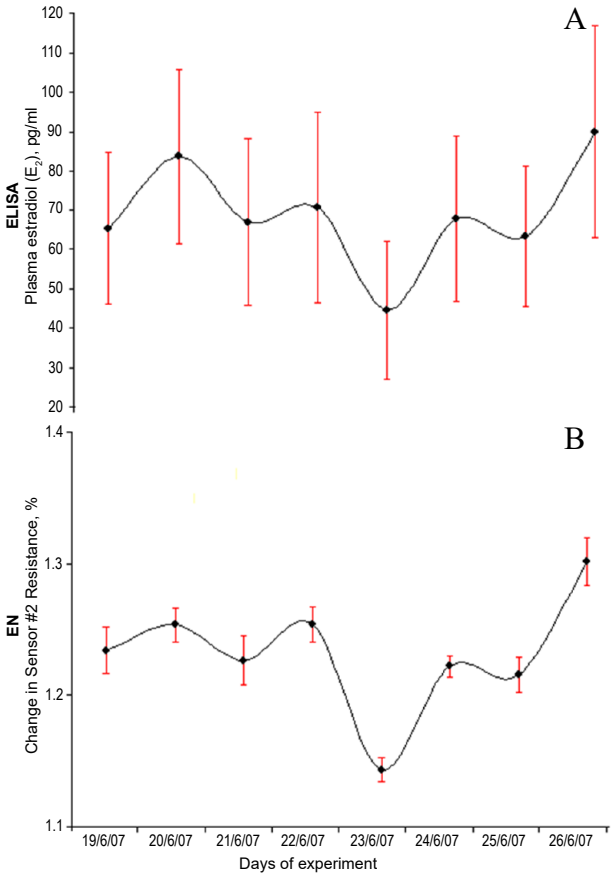


*Fig. 4.* The relationship between A) the response of 1 of 10 sensors (#2) to perineal odor through estrus and B) plasma estradiol concentration through estrus. Data are representing as means (±SEM) of 9 cows (group D). Estrus was induced using a single intramuscular injection of cloprostenol when midluteal (day 14 or 15). EN — Electronic Nose; $E_2$ — estradiol.

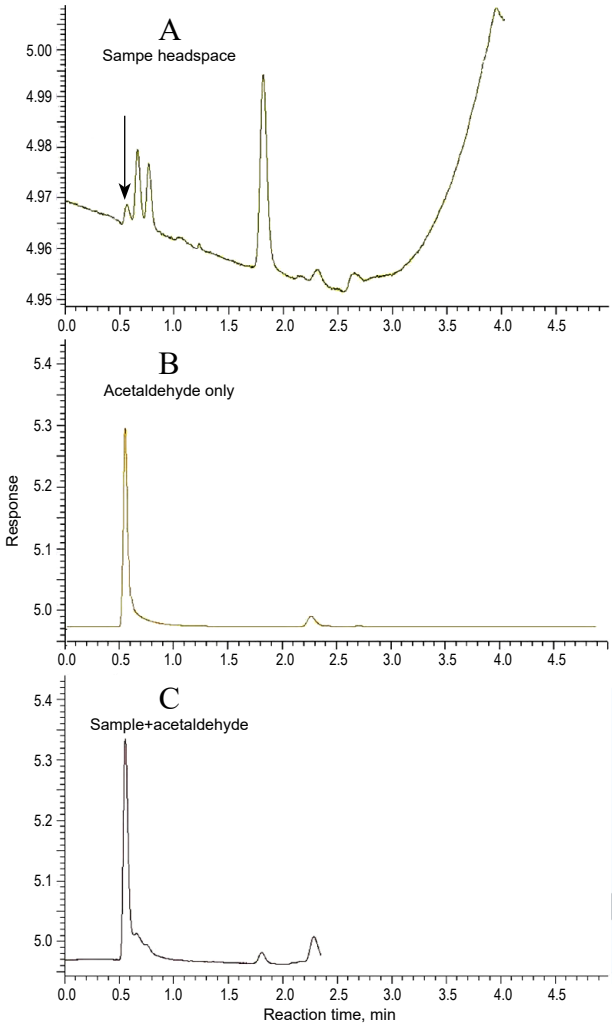


*Fig. 5.* Headspace gas chromatograms from perineal swabs showing coelution of acetaldehyde standard at the same time of retention as the 0.5 min. peak. (A) Sample headspace, (B) headspace from authentic acetalhyde solution, (C) sample was spiked with authentic acetaldehyde.

aldehyde in perineal head space to predict estrus. A peak that eluted at about 0.56 min occurred in all samples across estrus cycle (fig. 5a). This peak had the same retention time as that of authentic acetaldehyde (fig. 5b).

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# EFFECT OF SELENIUM ON OXIDATIVE STRESS AND VIABILITY OF THE RAM SPERMATOZOA DURING THE SPERMATOGENESIS

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*The aim of the experiment was to determine the effect of a single subcutaneous application of Selevit inj. on the volume and density of ejaculates, sperm viability, level of oxidative stress (OS) and apoptosis in semen by using flow cytometry.*

*Ten rams were divided into two groups. The experimental group, (EG; n=6) was injected one time subcutaneously with the Selevit inj. at a dose of 5 ml per animal (11 mg of sodium selenite). The control group, (K; n=4) was subcutaneously administered with physiological solution at a dose of 5 ml per animal. Samples of blood and semen were collected from each ram prior to application of Se and at day one, 14, 26, 38, 50, 62 after selenium injection.*

*Results showed, Se concentration in the blood of EG was significantly higher, but short-term. Se concentration in the semen of the EG was significantly higher during the whole duration of the experiment (62 days). The level of OS was significantly reduced at day one, 14 and 60 after application of Selevit injection. The number of dead spermatozoa was significantly lower in the EG only at day 14 and 26 after Se application. There was only a slight increase in the percentage of sperm in the early phase of apoptosis in EG. There were no significant differences in ejaculate volume and sperm concentration between groups.*

*Single subcutaneous injection of Selevit is sufficient enough to maintain a significant long-term increase of Se concentration in semen and has a positive effect on the level of OS, but there was no substantial influence on the quality of ejaculates.*

**Keywords:** SODIUM SELENITE, SPERMATOZOA, SPERMATOGENESIS, FLOW CYTOMETRY, RAMS

Mammalian spermatozoa have a large content of polyunsaturated fatty acids located in cytoplasmatic membranes, therefore they are highly susceptible to lipid peroxidation (LPO) caused by reactive oxygen species (ROS). The result of the ROS-induced damage is the disruption of sperm function and decreased semen quality [19]. Several defense mechanisms including antioxidants and antioxidant enzymes have been developed to prevent the LPO of sperm and maintain sperm motility and viability [21]. Many reports have suggested that selenium (Se) and vitamin E are important nutrients that act synergistically and can affect many biological processes including spermatogenesis and semen quality [12].

Se is an irreplaceable component of the antioxidant system as an integral part of glutathione peroxidase (GSH-Px). GSH-Px activity has been reported in the semen of several species including the dog, ram, human, rooster, buck and bull. If the Se content in selenoproteins is low, it considerably decreases the possibility of fertilization [6].

Reduced sperm production and poor sperm quality, including impaired motility with flagella defects localized primarily in the midpiece, have been detected in Se-deficient animals [4]. Vitamin E was found to decrease the sperm abnormalities in the head of mice sperm and supplementation with Se and/or vitamin E improved the libido and semen characteristics in rams and bulls [7].

Spermatozoa are produced in the process of spermatogenesis. Spermatogenesis is the process of cell proliferation and differentiation from a spermatogonial stem cell to adult sperm. These complex transformations occur in seminiferous tubules of the mammalian testes and may proceed over an extended period of time, which is species specific [9]. Therefore, it is important to take into account the effect of an applied substance not only shortly after its application, but

also during the course of the one cycle and after its ending what can last several weeks.

Most of the available works describe the positive effects of long-term supplementation with Se, with a preference for the organic form. However, information about the effect of single parenteral administration of the inorganic form of Se on male fertility is inadequate. For this reason, we have decided to test the effect of singel Se administraton on sperm fertility and Se concentration in spermatozoa and blood of rams with respect to the time course of the spermatogenic cycle.

## Materials and methods

Ten adult rams aged 2–3 years and average body weight 63 kg were divided into two groups. The experimental group, (EG; n=6) was injected once subcutaneously with the *Selevit inj.* at a dose of 5 ml per animal (11 mg of sodium selenite — 5 mg of Se and 125 mg of vitamin E per animal). The control group, (C; n=4) was subcutaneously administered only with saline at a dose of 5 ml per animal.

Samples of blood were obtained via jugular puncture. Semen was collected by electroejaculation. Samples were collected before *Selevit inj.* application. and next collections were performed 1, 14, 26, 38, 50, and 62 days post injection with respect to the course of spermatogenesis and the duration of the sperm passage through the epididymis.

***Analysis.*** The concentration of selenium in the blood and the semen was determined using emission spectrometry with inductively coupled plasma on the optical emission spectrometer *Optima 2100DV* at the wavelength of 196.026 nm. The samples were mineralized by wet digestion in microwave laboratory systems *Milestone MLS 1200*.

The semen was evaluated immediately after collection for: volume, concentration, live and dead counts, the level of apoptosis, and the level of oxidative stress (OS). Besides semen's volume other parameters were investigated by flow cytometry on a *BD FACSCanto*™ cytometer (*Becton Dickinson Biosciences*, USA) equipped with blue (488 nm) and red (633 nm) lasers and 6 fluorescence detectors. *BD FACS Diva*™ software was used to analyze the obtained data.

Concentration of sperm was determined by counting beads, 123 count eBeads™ (*eBioscience*, *ThermoFisher Scientific*, USA). The numbers of sperms were expressed as $\log_{10}$ numbers/ml ± standard deviation. Sperm viability was determined by combined staining with propidium iodide (PI) and carboxyl fluorescein diacetate (cFDA). Apoptosis was evaluated with a commercial kit — *FITC Annexin V Apoptosis Detection Kit* (*BD Pharmigen*, USA). This kit contain a PI and Annexin-V labeled with FITC. Oxidative stress (OS) of the sperm cell membrane was assessed using the BODIPY C11 fluorescence dye according to Brouwers and Gadella (2003). In this assay, cells are stained with PI to distinguish living cells from damaged cells.

All results obtained from this experiment were statistically evaluated by two-way ANOVA with the supplementary Mann-Whitney test to compare treatment differences and Tukey's *post-hoc* test to compare time differences.

## Results and discussion

Se concentration in the blood of the experimental group (EG) was significantly higher ($P<0.05$) only 24 hours after *Selevit* injection. In the ejaculates of the EG there were significantly higher values of Se concentration than the control group (C) on day one and 14 (in both collections $P<0.05$) after Se supplementation. Within the time dynamics, a significant increase of the Se concentration in EG was seen between the pre-injection collection and one day post-injection ($P<0.05$). There was no statistical difference between the experimental and control group in the volume and density of the ejaculates.

The higher values of oxidatively stressed sperms were found in the control animals. Significant differences between groups were reported on day 1 ($P<0.05$), 14 ($P<0.05$) and 62 ($P<0.05$). A significant decrease of oxidatively stressed sperm was observed in the EG between the pre-injection collection and 1st day post-injection ($P<0.001$). Then, a gradual increase of OS was recognised in this group until day 50. Another significant decline ($P<0.001$) of the percentage of sperm damaged by oxidative stress was observed between days 50 and 62.

The administration of selenium did not have a significant effect on the number of living

and damaged sperm in the study. Statistically significant decrease of the percentage of dead sperm was observed in the EG on day 26 in comparison to the C ($P<0.05$). The highest number of sperms in the early phase of apoptosis (1–1.2 %) was observed in the experimental group on day 38. However, a significant effect was reported only on day 14. There were higher concentrations of living sperms ($P<0.05$) and significantly lower levels of dead sperms in the EG compared to the C ($P<0.05$).

Many authors have presented the positive effect of selenium on the quality of ejaculate in many animal species [12]. However, some authors reported that the addition of an inorganic form of selenium to the feed did not improve the quality of ejaculates [3].

Our results showed, that the single dose of *Selevit inj.* caused an increase in Se concentrations in ejaculates of experimental rams in all samples. Increased concentrations of the Se in sperm were maintained for at least 62 days. It indicated a long-term supply of sperm by the testes. Therefore, we can conclude that the testes have an efficient mechanism for the use of Se to support spermatogenesis which is in accordance with [16]. According to [20], Se concentrations were higher in the reproductive apparatus (testes, sperm cells) than in other tissues and organs 23 days after intravenous administration of sodium selenite.

The highest values of Se concentrations in semen of the experimental versus control animals were recorded 24 hours after the administration of the *Selevit inj.*, and subsequently at days 50 and 62. [20] demonstrated that in the short period after Se administration, the Se concentration increased in seminal plasma, but no increase in Se in the sperm was observed. Therefore, we can conclude, that the increased Se concentration 24 hours after application was due to an excessive uptake of administered Se by the accessory glands. Additionally, low concentrations in the later collections suggests an inadequate incorporation of Se into sperm transported through the epididymis [15]. Antioxidative protection is ensured through GPX produced by the epithelium of the epididymis during the passage of sperm [14]. Increased concentrations of Se on days 50 and 62 after the Se injection, correspond with the increased incorporation of Se into sperm in the early stages of development, spermatogonia and primary spermatocytes. These conclusions are in accordance with findings of [2]. It has been demostrated that the testes exhibit fairly higher Se concentrations compared to other tissues and organs [1]. [5] also reported that testes of Se-deficient rats maintained Se concentration within normal levels, although its level in other organs declined significantly.

Our results are consistent with the results of [17], who reported no change in the volume and concentrations of ram sperm, even after long-term administration of sodium selenite. In contrast, our results are not consistent with the findings of [10], according to whome an increase of the volume and density of ejaculates and improvement of the sperm motility were observed after Se supplementation.

A significant effect on the viability of sperm in rams supplemented by Se was detected only on day 26, when a significant decrease of dead sperm was observed compared to the control animals. However, there was no increase in the number of living sperm. Similarly, authors [17] and [8] have not reported a significant influence on the number of living and dead sperm after supplementation with both organic and inorganic Se. On the contrary, other authors demonstrated increase of sperm viability after application of Se or combination of Se and vitamin E [7, 12]. The positive effect of Se on sperm viability was also demonstrated *in vitro* [6].

Significant increase of the Se concentrations and a decrease of OS were observed in the EG in most collections compared to day before application of *Selevit inj*. The analyses of first two ejaculate samples indicate the selenium antioxidant protection in seminal plasma and the epididymis secretions. This is consistent with the results of [18], according to which Se is not incorporated by adult sperm. The results of day 62 point to the incorporation of Se in the early stages of sperm development and the subsequent positive influence on the OS level in the samples. However, improvement of sperm viability was not observed. According to [11] application of Se improves the antioxidant properties of seminal plasma by increasing the activity of antioxidant enzymes and reducing the level of lipoperoxidation. [13] found high levels of reactive oxygen species in infertile

patients as well as a greater level of apoptosis compared to healthy subjects. We observed a non-significant increase in sperm in the early phase of apoptosis in EG during the study.

## Conclusion

Based on our results, we can assume that a single subcutaneous injection of *Selevit inj.* is not sufficient to maintain higher levels of blood selenium for longer period. Although there was a long-term increase of selenium concentration in semen and a positive effect on the level of oxidative stress, no significant effect on the quality of ejaculates was observed after a single injection of *Selevit inj*. Based on the number of available studies, as well as our results, it can be concluded that the final concentration of selenium in the testes and its effect on the quality of the ejaculate, may depend on the form of the administered selenium, the method of administration and the duration of selenium supplementation.

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# THE IMPACT OF TWINNING AND STILLBIRTH ON REPRODUCTIVE AND ECONOMIC PERFORMANCE IN LARGE HUNGARIAN DAIRY HERDS

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*The aim of our study was to quantify the effect of twinning and stillbirth on the main reproductive parameters, and to estimate the resulting economic loss on large-scale commercial dairy farms.*

*The data of 3.660 calvings that occurred on five large Hungarian Holstein-Friesian farms in 2016 and 2017 were analysed retrospectively. Information about twin calvings, stillbirths and uterine treatments were gathered. The main reproductive indices (i.e. calving to conception interval — CCI, services per conception — SPC, and first service conception risk — CR1) were calculated based on cow-level data. Statistical analyses were performed by using linear and logistic regression, and Dunnett-test. The economic losses related to twinning and stillbirth were estimated by partial budget analysis (1 EUR = 320 HUF).*

*Overall, twinning and stillbirth occurred in 4.1 % and 6.9 % of the calving events. Twinning was more likely, whereas stillbirth was less likely in multiparous cows (odds ratio [OR]: 4.18 and 0.64, P<0.0001 and P=0.0015, respectively). Following twin calving, CCI and SPC were increased by 12.8 days and by 2.8, respectively, whereas CR1 was reduced by 7.1 percentage points. Twinning caused an estimated loss of 50.4 EUR/case. The analysed reproductive parameters were not impaired after stillbirth. Although, the reduction of calf number and the excess semen usage led to 112.5 EUR estimated loss per stillbirth case, on average.*

*Twinning and stillbirths are significant sources of economic loss on dairy farms, therefore, their prevalence should be reduced.*

**Keywords:** DAIRY CATTLE, REPRODUCTION, TWINNING, STILLBIRTH, ECONOMICS

The events of the peripartal period require special attention in dairy cattle, since the majority of those animal health problems that may potentially reduce productive and economic performance occur during this period [7]. The prevalence of twin calving is approximately 10 %, on average, although it varies widely among dairy farms [6, 12]. Twin pregnancies increase the risk of pregnancy loss, dystocia, stillbirth, calf mortality, and culling of the dam, moreover, gestation length and birth weight of the calves are reduced. Freemartinism occurs in 92–98 % of those twin pregnancies, when calves from both sexes are born [6, 8, 12].

Stillbirth is defined as the death of a calf just prior to, during, or within 24 to 48 h of parturition, following a normal gestation length [3, 10]. The prevalence of stillbirth ranges from 0.1 to 19.2 %, but on most farms it is between 6.5 and 9.3 % [1, 9, 10]. Stillbirth reduces milk production mainly during early lactation, and increases the risk of culling of the cow, as well [2, 5].

The aim of our study was to survey the occurrence of twinning and stillbirths, and to quantify their effects on the reproductive and economic performance on Hungarian dairy farms.

## Materials and methods

The study was conducted in five large commercial Hungarian dairy herds. In each herd, the number of cows exceeded 390, treatments were regularly recorded in the farm management software (RISKA, *Systo Kft*., Budapest, Hungary), and shared the same reproductive advisor. Herd size, milk production and culling data of the studied herds are shown in table 1.

The protocol of reproductive examinations and treatments was identical on every studied

*Table 1*

**Herd size, production and culling data of the surveyed herds (average of 2016 and 2017)**

| Herd | Number of cows | 305-day milk yield, kg | Number of milkings per day | Annual culling rate, % |
|---|---|---|---|---|
| A | 420 | 8,776 | 2 | 30.5 |
| B | 400 | 8,691 | 3 | 35.8 |
| C | 547 | 10,349 | 3 | 31.4 |
| D | 502 | 8,618 | 3 | 30.0 |
| E | 396 | 8,720 | 2 | 33.7 |

farm. Data were collected from the farm management software about the calvings that occurred in 2016–2017, as well as about the postpartum treatments and reproductive parameters. Those cows were considered affected with retained placenta, in which the fetal membranes were still present on the next day following calving. Uterine inflammation was diagnosed based on the evaluation of the discharge. The prevalence of twinning and stillbirth was quantified, furthermore, the main reproductive indices (calving to conception interval — CCI, first service conception risk — CR1, services per conception — SPC) were calculated, as well. The relationship of twinning and stillbirth with retained placenta, uterine inflammation, CCI, and CR1 were analysed. Statistical analyses were performed by using linear and logistic regression, and Dunnett-test. Data were analysed in *R version 3.4.2* (*R Core Team*, 2017).

In the partial budget model for the quantification of the economic losses caused by twinning and stillbirth, calf revenue, cost of open days and insemination cost were taken into account [11]. When calculating calf revenue, we assumed that calves are sold. According to Northern American studies, single and twin calvings yield 0.93 and 1.70 live calves, respectively, and twin calves weigh 15 % less compared to single calves, on average [4]. Stillbirth reduces calf revenue, and the insemination cost of producing the stillborn calf is also incurred. Average prices and costs of the studied farms were used for the calculations; each extra open day was assumed to cost 2.5 EUR (1 EUR = 320 HUF). The economic analysis was performed in *Microsoft Excel 2016*.

## Results and discussion

Altogether 3,660 calving events occurred in the studied period, of which 4.1 % was twin calving and 6.9 % was stillbirth (table 2). Twinning was more likely, whereas stillbirth was less likely in multiparous compared to primiparous cows.

The average CCI, CR1 and SPC were 139.8 days, 16.3 % and 5.74, respectively. The major reproductive parameters of the cows with twins or stillbirth by parity are shown in table 3. Following twin calving, a remarkable, although, nonsignificant decline was observed in the reproductive parameters. Following stillbirth, no marked decline in the reproductive indices was found.

The reproductive performance following twinning and stillbirth was compared to those cows, which gave birth to a single, live calf, and were free from postpartum uterine diseases ("healthy" cows, table 4). Despite the remarkably poorer reproductive performance in cows after twin calving, these differences remained not significant (P>0.05). Fertility parameters declined after stillbirth, however, their differences compared to those of the "healthy" cows did not prove to be significant (P>0.05).

The decline of the reproductive performance following twinning is probably attributable to the disorders of the uterine involution. However, we could not observe the negative effect of twin calving on the performance of the primiparous cows, which is most probably related to the low prevalence of twinning at first calving. The reproductive parameters did not significantly decline following stillbirth. This may be explained by these cows being subject to uterine treatments, which

*Table 2*

**The occurrence of twinning and stillbirth by parity (n=3,660)**

| | n | Prevalence, % | Parity | n | Prevalence by parity, % | OR[a] | 95 % CI[b] | P |
|---|---|---|---|---|---|---|---|---|
| Twinning | 149 | 4.1 | 1 | 16 | 1.3 | Reference | | <0.0001 |
| | | | ≥2 | 133 | 5.5 | 4.18 | 2.5–7.45 | |
| Stillbirth | 251 | 6.9 | 1 | 113 | 9.0 | Reference | | 0.0015 |
| | | | ≥2 | 138 | 5.7 | 0.64 | 0.48–0.84 | |

*Note*: [a] — odds ratio, [b] — 95 % confidence interval.

*Table 3*

**The major reproductive parameters in case of twinning and stillbirth (n=3,660)**

| Parity | Twinning/Stillbirth | N | CCI[a], days | Difference | SPC[b] | Difference | CR1[c], % | Difference |
|---|---|---|---|---|---|---|---|---|
| Primiparous | Single calf | 1,233 | 141.6 | Reference | 5.3 | Reference | 18.6 | Reference |
| | Twins | 16 | 124.8 | –16.7 | 4.2 | –1.1 | 9.1 | –9.5 |
| Multiparous | Single calf | 2,278 | 138.0 | Reference | 5.9 | Reference | 15.4 | Reference |
| | Twins | 133 | 156.9 | 18.9 | 9.2 | 3.3 | 9.6 | –5.9 |
| *Altogether* | *Single calf* | *3,511* | *139.4* | *Reference* | *5.7* | *Reference* | *16.6* | *Reference* |
| | *Twins* | *149* | *152.2* | *12.8* | *8.5* | *2.8* | *9.5* | *–7.1* |
| Primiparous | Live calf | 1,136 | 141.9 | Reference | 5.1 | Reference | 19.1 | Reference |
| | Stillbirth | 113 | 134.8 | –7.1 | 6.9 | 1.8 | 11.8 | –7.2 |
| Multiparous | Live calf | 2,273 | 139.4 | Reference | 6.1 | Reference | 14.9 | Reference |
| | Stillbirth | 138 | 124.9 | –14.5 | 5.1 | –1.0 | 18.7 | 3.8 |
| *Altogether* | *Live calf* | *3,409* | *140.4* | *Reference* | *5.7* | *Reference* | *16.4* | *Reference* |
| | *Stillbirth* | *251* | *129.7* | *–10.7* | *6.0* | *0.3* | *15.4* | *–1.0* |

*Note*: in this and the next table [a] — calving to conception interval, [b] — services per conception, [c] — first service conception risk.

*Table 4*

**The major reproductive parameters of cows with twins and stillbirth compared to "healthy" cows (i.e. cows that gave birth to one live calf and were free from postpartum uterine diseases) (n=3,660)**

| | n | CCI[a], days | Difference | SPC[b] | Difference | CR1[c], % | Difference |
|---|---|---|---|---|---|---|---|
| "Healthy" | 2,008 | 130.4 | Reference | 4.9 | Reference | 18.7 | Reference |
| Twinning | 149 | 152.2 | +21.8 | 8.5 | +3.6 | 9.5 | –9.2 |
| Stillbirth | 251 | 129.7 | –0.7 | 6.0 | +1.1 | 15.4 | –3.3 |

was often not performed in cows that gave birth to live calves, although fertility could also be negatively affected in these animals if a subclinical uterine disease was present.

The prevalence of retained placenta and uterine inflammation was 13.3 and 29.4 %, respectively. The risk of retained placenta was increased by twinning (OR=2.22, P<0.0001) and stillbirth (OR=1.23, P<0.0001), as well. The risk of uterine inflammation was not related to stillbirth (OR=1.05, P=0.1364), although it was reduced after twinning (OR=0.76, P<0.0001).

The results of the economic analysis are shown in table 5. The number of extra calves could not compensate for the extra cost of open days and inseminations following twin calving.

*Table 5*

**Economic analysis of twinning and stillbirth, EUR**

| Cost factor | Twin calving | Stillbirth |
|---|---|---|
| Calf revenue | +25.3 | –64.2 |
| Open days | –32.0 | +26.8 |
| Insemination | –43.8 | –75.0 |
| *Altogether* | *–50.4* | *–112.5* |

*Note:* Positive numbers indicate economic gains, negative numbers indicate economic losses.

The economic loss due to stillbirth exceeded the cost of twinning.

## Conclusions

Based on our results, twinning was more common, whereas stillbirth was less common in multiparous compared to primiparous cows. Stillbirth caused larger losses than twinning, mainly due to the foregone calf revenue, and the cost of semen used to produce the stillborn calf. The risk of retained placenta was increased by twinning and stillbirth, as well. Both twinning and stillbirth should be considered significant sources of economic loss in large dairy herds.

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# RELATIONSHIP BETWEEN SOMATIC CELL COUNT AND OCCURRENCE OF INTRAMAMMARY PATHOGENS IN DAIRY COWS

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*The goal of this observation was to evaluate relationship between somatic cell count and intramammary pathogens occurrence in milk of dairy cows.*

*Somatic cell counting was performed by new on-farm commercial device Deleval Cell Counter (DCC) and laboratory Fossomatic cell counting (FSCC). 100 sensoric unchanged mixed milk samples collected during milking time on dairy farm were analysed in this study for detection of somatic cell count by both methods. Quarter milk samples (n=389) of all selected cows were cultured.*

*Increased somatic cell count was detected in 46 mixed milk samples by DCC and in 58 by FSCC. Of total quarter milk samples bacteria were determined in 76 (19.5 %). The most prevalent bacteria were Enterococcus spp. (26.3 %), followed by E. coli (25 %), A. viridans (15.7 %), coagulase-negative staphylococci (11.8 %), Proteus spp. (9.2 %), Streptococcus spp. (6.6 %) and S. intermedius (2.6 %). Contagious isolates (S. aureus) were detected in 3 quarter milk samples (4 %). Agreement between DCC and microbiological culture was found in 90 %, and between FSCC and bacteriological incidence in 84 %.*

*Higher SCC was detected in milk samples contaminated by bacteria than in healthy milk ($P<0.001$). Presence of individual species of intramammary pathogens was not related to different levels of SCC.*

*The presented data illustrated that bacteria are predominant causes of subclinical mastitis. However, in some milk samples with increased SCC no bacteria were detected. This means that it could have been caused by numerous other agents or factors for mastitis in dairy industry.*

**Keywords:** BACTERIA, COWS, SOMATIC CELL COUNT

In spite of strong scientific effort in the prevalence of mastitis no important elimination of this disease in dairy industry all over the world has been reached. Severe intramammary inflammation remains major problem causing high losses of dairy farms. According to recent sources prevalence of mastitis has been extremely different in separate countries and regions. The incidence of mastitis in dairy herds results from a complex interaction between the infectious agents, poor management practices, genetic and environmental factors stressing the defence of udder. The most common potential risk factors for mastitis in dairy farms are classified as quarter, cow and environmental risk factors [5]. Numerous authors have pointed out risk factors for CM associated with farm management, hygiene management, the breeding environment, milking technology, feeding, the calving season and preventive health management [1]. In an individual herd, cow factors are responsible for the differences among cows in contracting CM. A great number of individual cow-specific risk factors for CM have been identified, including breed, parity, period of lactation, udder and teat morphology, age at first calving, milk leakage, udder oedema, milk production, somatic cells count, and reproductive disorders [7, 8, 12].

For rapid analysis of SCC many assays and devices were invented, whose principle is performed on the base of chemical reaction between DNA of white blood cells and testing reagent. The most commonly used on-farm rapid test is California mastitis test or its modifications. However this test has served only for a semi-quantitative analysis, and counting of accurate SCC cannot be performed. It yields only approximate value of SCC and subsequently degree of subclinical mastitis. Second on-farm test is Eimu Cell Check Test that has been more sensitive and determines lower SCC compared to requirements of farmers originated from West European countries, who prefer mean SCC below 100 000 SC/ml. However, ECC test is also a semi-quantitative without the accurate SCC in milk.

In commercial environment new devices are used that ensure accurate reliable counting of

somatic cells in milk samples. Delaval Cell counter is one of them and therefore it was tested in the field conditions, whereby benefits and liabilities were evaluated. Agreement between SCC analysed by DCC in the field and SCC analysed in the laboratories of The Breeding Services of the Slovak Republic was accomplished. Moreover, relationship among SCC detected by these methods with occurrence of bacteria and SCC in milk samples contaminated by individual species of intramammary pathogens was evaluated.

## Materials and methods

Experimental dairy herd consisted of 335 lactating cows with average milk yield of 7,100 kg. All the animals were housed in a free stall system with the deep straw bedding. Of complete herd SCC was analysed in mixed milk samples and bacterial culture was performed in quarter milk samples of 100 dairy cows.

***Somatic cell counting.*** Sensorically unaltered mixed milk samples were tested by Delaval Cell Counter (DCC, *DeLaval International AB*, Tumba, Sweden; Obr. 2) within 1 h after milking. DeLaval cell counter (DCC) is a portable device designed for on-farm somatic cell count (SCC) analysis in bovine milk within 1 min. Laboratory counting of SC was carried out in milk samples preserved with 0.1 % $K_2Cr_2O_7$ by fluoro-opto-electronic method in accredited laboratory of The Breeding Services of the Slovak Republic the following day (*Fossomatic™ FC*, Denmark). For the evaluation of SCC in milk samples we used a scale based on SCC. Milk samples with SCC up to 400 000 SC/ml were considered as a threshold or negative and samples above the 400 000/ml were considered positive.

***Bacteriological analyses.*** Bacteriological examinations were performed to detect bacterial genus *Staphylococcus* spp., S*treptococcus* spp., *Enterococcus* spp., *Proteus* spp. and were evaluated according to commonly accepted rules. Milk samples (10 µl) were cultured at the respective veterinary practice according to their routine procedures, usually employing Columbia Blood Agar Base with 5 % of defibrinated blood, Staphylococcal medium N°110, Baird-Parker agar, Edwards Medium, Mac Conkey Agar (*Oxoid*, *OXOID Ltd.*, Basingstoke, Hants, UK) incubated at 37 °C for 18–24 h. Besides evaluation of bacterial growth characteristics another assays were used to bacterial species determination: pigment and coagulase production, catalase activity, haemolysis, Gram staining and other virulence factors. Commercial kits *STAPHYtest 24*; *STREPTOtest 24*, resp. *ENTEROtest 24* (*Erba Lachema*, Brno, Czech Republic) were used for the identification of bacteria. All kits were evaluated by the programme *TNW Pro-Auto 7.0®* (*Erba Lachema Ltd.*, Brno, Czech Republic) according to the manufacturer's instructions (*Erba Lachema*, Brno, Czech Republic). *S. aureus* was identified by means of typical colony morphology, and α- and β-hemolysis and positive coagulase reaction. Coagulase-negative staphylococci (CNS) were identified by typical colony morphology and negative coagulase reaction.

***Statistical analyses.*** Agreement of increased SCC and occurrence of bacteria in milk was assessed by percentage. Moreover statistical analyses was tested between SCC in contaminated milk by intramammary pathogens by Student's *t*-test and relationship between SCC and bacterial species by ANOVA test.

## Results and discussion

Increased somatic cell count was detected in 46 mixed milk samples by DCC and in 58 by FSCC. Of total quarter milk samples bacteria were determined in 76 samples (19.5 %) in 47 dairy cows. The most prevalent bacteria were *Enterococcus* spp. (26.3 %), followed by *E. coli* (25.0 %), *A. viridans* (15.8 %), coagulase-negative staphylococci (11.8 %), *Proteus* spp. (9.2 %), *Streptococcus* spp. (5.3 %) and *S. intermedius* (2.6 %). Contagious isolates (*S. aureus*) were detected in 3 quarter milk samples (3.9 %). Presence of individual species of intramammary pathogens was not related to different levels of SCC (table 1).

Agreement between DCC and microbiological culture was found in 90 %, and between FSCC and bacteriological incidence in 84 %. Higher mean SCC was detected in milk samples contaminated by bacteria than in healthy milk samples ($P<0.001$), whereby mean SSC of contaminated milk analysed by FSCC was 3514.8 and by DCC was 1549.8. Opposite mean SCC

of uncontaminated milk was 253 counted by FSCC and 187.4 by DCC (table 2).

Somatic cells are indicators of both resistance and susceptibility of cows to mastitis and can be used to monitor the level or occurrence of subclinical mastitis in herds or individual cows. SCC is a useful predictor of intramammary infection (IMI). An increased SCC in milk has a negative effect on the quality of raw milk [10]. Relationship between SCC and prevalence of subclinical mastitis and occurrence of intramammary pathogens has been observed in many studies. Based on obtained results closed relationship between increased SCC and IMI was noticed in our study, whereby SCC was higher (P<0.001) in infected milk. A study to determine the relationship between SCC and mastitis etiological agents was carried out by [4]. They reported that milk samples with SCC lower than 200,000 cells/ml were mostly (59.6 %) culture negative. Coagulase-negative staphylococci (CNS), *S. aureus* and *Streptococcus spp.* were mostly detected in samples with 200,000 to 2,000,000 of SCC/ml. However, in our study in milk infected by *S. aureus* mean SCC was lower than 200,000. Coagulase-negative staphylococci and *Streptococcus* spp. were detected in milk samples ranging from 1,219,000 to 2,376,667 SC/ml. In Poland observed samples having more than 2 million/ml of SCC were infected mainly with CAMP-negative and CAMP-positive streptococci and Gram negative bacilli. The highest SCC (≥10 million/ml) in foremilk samples was associated with intramammary infections caused by *Arcanobacterium pyogenes* (95.5 %), *Streptococcus agalactiae* (57.6 %) and Gram-negative microorganisms (46.5 %). Very high SCC (≥5 million/ml) was connected with infections caused by *Prototheca* sp. (64.5 %), yeast-like fungi (60.2 %) and *Streptococcus* sp. (55.1 %). *S. aureus* (76.2 %), CNS (84.2 %), Gram-positive bacilli (72.4 %) and *Corynebacterium* sp. (83.2 %) caused an increase in SCC that was smaller than 5 million/ml. Review by [2] makes it obvious that milk SCC (×1 000/ml) ranged from 27 to 600 in culture negative quarters, from 191 to 9 433 in quarters infected with *S. aureus*, from 561 to 4 758 in quarters infected with *Streptococcus agalactiae*, from 809 to 1 944 in quarters infected with *Streptococcus dysgalactiae*, from 851 to 1 085 in quarters infected with *Streptococcus uberis*, from 590 to 9 009 in quarters infected with coliforms, from 90 to 3 040 in quarters infected with CNS and from 128 to 1 352 in quarters infected with *C. bovis*. In South African study [9] of the eight pathogens that were quantified according to SCC levels, *S. dysgalactiae* had overally the highest SCC followed by *S. aureus* (STH and STA) and *S. agalactiae*. Although only small numbers of *T. pyogenes* were isolated in this data set, more than 84 % of these bacteria had SCC exceeding 200 000 cells/mL. However, some bacterial species were isolated at an SCC of below 200 000 cells/mL: *S. dysgalactiae* (4.2 %), *S. aureus* (STH) (5.9%) and *S. agalactiae* (9.0%). A study conducted by [6] showed that the mean SCC for samples identified with the CNS strains *S. chromogenes* and *S. hyicus* was 168 000 cells/mL and 193 000 cells/mL compared with 39 000 cells/mL from uninfected quarters. According to Brasilian study the geometric means of the bacteriological examination results were in milk samples with no

*Table 1*

**Distribution of intramammary pathogens and SCC in dairy cows**

| Bacteria | Number of positive quarter milk samples | DCC (x±sd) | FSCC (x±sd) |
|---|---|---|---|
| Contagious pathogens | 3 (3.9 %) | 146.0 | 145.0 |
| *Staphylococcus aureus* | 3 | | |
| Environmental pathogens | 73 (96.1 %) | | |
| *Enterococcus* spp. | 20 | 1833.1 | 4123.8 |
| *E. coli* | 19 | 1591.7 | 4349.8 |
| *Aerococcus viridans* | 12 | 1678.4 | 3363.9 |
| CNS | 9 | 1219.0 | 2377.8 |
| *S. epidermidis* | 5 | | |
| *S. chromogenes* | 3 | | |
| *S. warneri* | 1 | | |
| *Proteus* spp. | 7 | 1710.3 | 3078.0 |
| *Streptococcus* spp. | 4 | 2736.7 | 5854.8 |
| *S. intermedius* | 2 | 231.0 | 335.5 |
| Totally | 76 | 1549.8 | 3461.8 |

*Table 2*

**Milk SCC analysed by two methods**

| | DCC (x±sd) | FSCC (x±sd) |
|---|---|---|
| Milk with bacterial contamination | $1549.8^{***}$ | $3461.8^{***}$ |
| Healthy milk | $187.4^{***}$ | $253^{***}$ |

*Note:* x — mean, sd — standard deviation.

growth 52,000, coagulase-negative staphylococci 85,000, *S. aureus* 587,000; other streptococci 432,000 and *S. agalactiae* (1,572,000; 333,000 [3]. In another study average SCC of the *A. viridans* infected cows was significantly higher ($1000.0\times10^3$ cells/mL) ($P<0.01$) compared to healthy cows ($72.4\times10^3$ cells/mL) [11].

## Conclusion

The presented data illustrated that bacteria are predominant causes of subclinical mastitis. However in some milk samples with increased SCC no bacteria were detected. This suggests that it could have been caused by numerous other agents or factors for mastitis in dairy industry.

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# STANDARDIZING OUTPUT-BASED SURVEILLANCE TO CONTROL NON-REGULATED DISEASES OF CATTLE IN THE EU (SOUND-control, COST action-CA17110)

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*The European Union (EU) overviews the control, eradication and surveillance of many cattle diseases especially those that are detrimental to animal or human health, cause huge economic losses or are subjected to trade restrictions. Other cattle diseases are left to be controlled by each Member State (MS). However, these diseases can also cause economic losses, which are usually hidden at first glance, as they often have subclinical or chronic courses. Trade of live cattle can introduce those diseases into countries that have already eradicated or have never even had them. Therefore, there is a gap in knowledge of how different EU countries control each disease and what their current disease status is.*

*A team of 76 veterinarians, epidemiologists, statisticians, economists, sociologists, stakeholders etc. from 30 different countries have come together to participate in an action called Standardizing output-based surveillance to control non-regulated diseases of cattle in the EU (SOUND control) CA17110 funded by COST (European Cooperation in Science and Technology). The aim of the project is to gather information about the different control programmes and produce an output-based framework. Five working groups have been formed. Work group 1 (WG1) aims to gather information on existing control programmes for non-EU regulated cattle diseases in participating countries and produce a list of relevant stakeholders. The focus is on diseases not listed under categories A or B in the new European Animal Health Law. WG2 aims to identify which data are required to serve as input for an output-based framework, and to assess the data availability and quality for each country. WG3 aims to find methods that enable output-based comparison of different control programmes, identify gaps and also evaluate cost-effectiveness, social acceptability and generalizability of those methods. WG4 is entrusted with the development of a joint research agenda about possibilities to combine heterogeneous inputs into an output-based framework that is applicable to a large number of non-regulated cattle diseases. Finally, WG5 will be responsible for the dissemination of knowledge achieved during the SOUND-control action to a wider audience and to encourage the incorporation of an output-base framework both on national and European level. The action has started in 2018 and will end in October 2022.*

*The objective of the SOUND control action is to explore and implement a widely adaptable output-based framework to substantiate the confidence of freedom and costs-effectiveness in current surveillance, control or eradication programmes for non-regulated cattle diseases in Europe. The results of this project will facilitate safe trade and support the improvement of disease control measures, which is of great importance for the EU agriculture since the cattle sector contributes one third to the total gross production value.*

**Keywords:** BOVINE, CONTROL PROGRAMMES, EUROPE, INFECTIOUS DISEASES, DISEASE MODELLING

The European Union (EU) overviews the control, eradication and surveillance of many cattle diseases especially those that are detrimental to animal or human health, cause huge economic losses or are subjected to trade restrictions. Other cattle infectious diseases (like bovine viral diarrhoea, infectious bovine rhinotracheitis infectious pustular vulvovaginitis, paratuberculosis, etc.) are left to be controlled by each Member State (MS) [7]. However, these diseases can also cause economic losses, which are usually hidden at first glance, as they often have subclinical or chronic courses [4]. They also reduce the welfare of affected animals [3]. Trade of live cattle can introduce those diseases

into countries/farms that have already eradicated or have never even had them [7].

For notifiable cattle diseases, EU regulations (Regulation (EU) 2016/429, Council Directive 82/894/EEC) [6] are in place to help harmonise requirements for the free status of the animal, herd and/or country. The study design, sampling scheme and type of tests are generally prescribed by EU regulations, so-called "input-based standards". In contrast, "output-based standards" do not prescribe what needs to be done, but rather what must be achieved. Enabling standardised comparison between outputs of Control programmes (CPs) is important in light of intracommunity trade of cattle with potential substantial economic consequences. Some EU countries are implementing regional or national CPs for non-regulated cattle diseases for which, no generally accepted rules or guidelines are currently in place. Consequently, countries are generally developing their own CPs, often with considerable country-level variation in respect to both programme design and implementation [5].

COST (European Cooperation in Science and Technology) is a Horizon 2020 EU funded organization that funds research and innovation networks. COST actions help connect research initiatives across Europe and beyond and enable researchers and innovators to grow their ideas in any science and technology field by sharing them with their peers. COST Actions are bottom-up networks with duration of four years that boost research, innovation and careers [1].

## Materials and methods

SOUND control is currently a team of 76 veterinarians, epidemiologists, statisticians, economists, sociologists, stakeholders etc. from 30 different countries, but other people with relevant expertise and experience (Veterinary science: Veterinary medicine (miscellaneous); Veterinary science: Databases, data mining, data curation, computational modelling; Animal and dairy science: Applied mathematics, statistics, non-computational modelling) may join. SOUND control stands for Standardizing OUtput-based Surveillance to control Non-regulated Diseases of cattle in the EU and is funded by COST. SOUND control aims to use innovative methods for standardizing output-based surveillance to control non-regulated diseases in the EU. Regardless of the heterogeneities in the data, a joint understanding about the requirements and characteristics needed for proof of freedom will be developed. Cost-effectiveness of different control programmes will be compared. SOUND-control will assist with initiatives to explore and implement a widely adaptable, output-based framework to substantiate confidence of freedom from infectious diseases and assess epidemiological and economic equivalence of control efforts. With the new Animal Health Law, it is anticipated that disease control will progressively change towards output-based approaches. SOUND-control will provide requirements and demands for a single general regulatory framework, adaptable to multiple diseases, which aims to enhance the safety of trade [5].

Five working groups have been formed.

- Work group 1 (WG1) aims to identify and reach an agreement on the requirements (both scientific and practical) that should be met by a framework that aims at an objective comparison of the output of CPs for non-regulated cattle diseases in the EU; and to identify the non-regulated cattle diseases for which control, eradication and/or surveillance are currently being conducted in the EU. The framework should be applicable to a large number of diseases, but will initially be designed for a few example diseases with considerable variation between member states (MS). The focus is on diseases not listed under categories A or B in the new European Animal Health Law.
- Work group 2 (WG2) aims to evaluate the availability and quality of the heterogeneous data that is needed as an input for an output-based framework. This will be evaluated at each level of aggregation and for each of the countries involved in the SOUND-control Action.
- Work group 3 (WG3) aims to evaluate existing methods enabling output-based comparison of CPs and identify gaps.
- Work group 4 (WG4) will encourage research initiatives that aim to take the next steps into development of innovative methodologies that tackle the gaps identified in WG3, including mathematic, epidemiologic, economic and social science methods, and facilitate the possibilities for

short term scientific missions (STSMs) to combine expertise from different research areas.

● Dissemination of knowledge that is achieved during the SOUND-control Action and encouraging the incorporation of an output-based framework both on national and European level will be the obligation of all WG but specifically of Work group 5 (WG5).

The action has started in late 2018 and will end in October 2022 [5].

## Results and discussion

Since the beginning of the action three meetings have been held. The kick off meeting was in Brussels, Belgium (29.10.2018), the second meeting was a WG1 meeting in Porto, Portugal (21.1.2019) and the third meeting in Utrecht, Netherlands was a WG1 workshop as well as WG meetings and a management committee meeting (25–26.3.2019). Two STSMs have already taken place. The main deliverables are going to be an overview of non-EU regulated diseases with control programmes, contact list of relevant stakeholders, handbook describing the different control programmes, review paper comparing methodologies for evaluation of control programmes, SOUND-control website and hopefully an output-based framework for the control of non-regulated cattle disease in the EU [5].

## Conclusions

Surveillance, control or eradication of non-listed cattle diseases in the EU is important because it leads to a reduction in disease burden, improvement in the overall health and welfare of livestock, reduces medicine use and improves sustainability of livestock production. However, for countries that have achieved, or are implementing, control and eradication of these diseases, trade between different regions or countries currently poses a very tangible risk of reintroduction of infections and diseases into susceptible populations [7].

In SOUND-control, the available methods will be evaluated and required improvements and initiatives will be identified to refine existing and developing innovative methods for field application of an output-based framework that is ultimately widely applicable to a large range of cattle diseases in the EU. Transfer of new knowledge will enable countries to learn from each other and increase the probability of success with improved country-level efforts towards surveillance, control or eradication [5].

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# RELATIONSHIP BETWEEN HERD SIZE, MILKING TECHNOLOGY AND MILK PRODUCTION PARAMETERS ON LARGE-SCALE HUNGARIAN DAIRY FARMS

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*The aim of the study was to survey the milking technology on the large-scale (≥50 cows) Hungarian dairy farms and to compare the different types of milking parlour with herd size, average daily milk yield, average daily milk production per each cow and average somatic cell count (SCC).*

*The milking technology was surveyed by using a questionnaire on 380 large-scale commercial Hungarian dairy farms in 2017, and it was compared with the official farm milk production data of March 2017. Three herd size categories (Group 1: 50–300 cows, Group 2: 301–600 cows and Group 3: >600 cows) were set up and the relationship between milking technology, herd-size and milk production parameters were analysed by using two-way ANOVA and Tukey-test.*

*In Group 1, 2 and 3 (1) the number of farms was 142, 142 and 96; (2) the average herd size was 165, 437 and 958 cows; (3) the average daily milk yield was 24.73, 29.42 and 32.25 kg; (4) the average daily milk production per each cow was 21.07, 25.83 and 28.49 kg; and (5) the weighted average SCC was 432.5, 412.7 and 341.9×10³/ml. As the herd size increases, so does the average daily milk yield and the average daily milk production per each cow, however, the average SCC decreases significantly (P<0.001). The type of milking parlour had a significant effect (P=0.027) on the average SCC, and dairy units having herringbone milking system produced the lowest quality of milk with an average of 430.0×10³ SCC/ml.*

*The type of milking parlour has an impact on milk quality, however further research is required in this regard.*

**Keywords:** DAIRY FARM, MILKING TECHNOLOGY, HERD SIZE, MILK YIELD, SCC

Milking technology greatly influences the organization of animal movements, the selection of milking routines, the influence of the quality of human labour, the hygiene of technology and the technical efficiency of milking equipment during the time of milking. Technological diversity has an impact on milk production, particularly on milk quality. The most common milking system on large dairy farms is herringbone. In addition parallel, tandem and carousel milking technology is used, but in recent years the spread of robot milking systems has also started [3]. [5] compared three types of milking technology (pipeline, parallel milking parlour and robot). In the case of pipeline milking, the germ count (18,000 CFU/cm³) was one and a half times higher than that of the parallel milking parlour (11,500 CFU/cm³) and robot (6,200 CFU/cm³). Somatic cell count (SCC) was 279,000/cm³ in pipeline milking, 281,600/cm³ in parallel and 195,600/cm³ in robot milking system. The lowest milk fat% (3.75 %) was also measured in pipeline milking system, while the milk fat% was 3.83 % in parallel and 3.88 % in robot milking system. Further research has also confirmed that in case of robot milking, the daily milk production is higher and the SCC is lower compared to the traditional milking parlour (herringbone) [1; 2].

The aim of the study was to survey the milking technology on the large-scale (≥50 cows) Hungarian dairy farms and to compare the different types of milking parlour [herringbone (H), parallel (P), carousel (C) and other (O)] with herd size, average daily milk yield, average daily milk production per each cow and average somatic cell count (SCC).

## Materials and methods

The milking technology was surveyed by using a questionnaire on 380 large-scale commercial Hungarian dairy farms in 2017, and it was

compared with the official farm milk production data of March 2017. On the surveyed farms milk performance test is conducted monthly that is based on individual milk samples from all milking cows. The herd size of the Hungarian dairy farms varies largely, therefore three herd size categories (Group 1: 50–300 cows, Group 2: 301–600 cows and Group 3: >601 cows) were set up. Data were processed in *MS Excel 2013* software (*Microsoft Corporation*, Redmond, WA, USA). The relationship between milking technology, herd-size and milk production parameters were analysed. Statistical analysis was performed by using two-way ANOVA and Tukey-test in *R version 3.5.1.* [4].

## Results and discussion

The most common type of parlour is the herringbone, but with the increase number of cows, the number of parallel and carousel milking parlour also increases (fig.).

The differences by the type of parlour of herd size, average daily milk yield, average daily milk production per cow and SCC are shown in table 1.

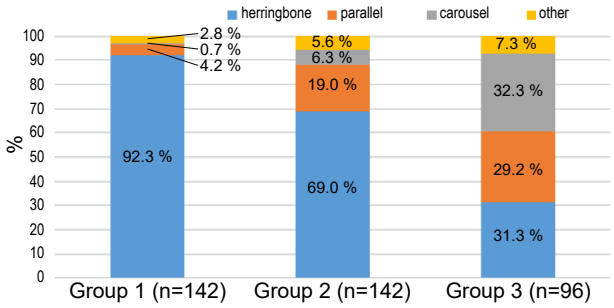


*Fig.* The distribution of farms using different type of milking parlour per herd size groups (n=380)

As the herd size increases, so does the average daily milk yield and the average daily milk production per each cow, however, the average SCC decreases significantly (P<0.001) (table 2).

The type of milking parlour had a significant effect (P=0.027) on the average SCC, and dairy units having herringbone milking system produced the lowest quality of milk with an average of 430.0×10³ SCC/ml (table 3).

The lower milk production in smaller dairy farms can be explained with lower standards of housing, feeding, milking technology and genetics. The type of milking parlour has an impact on milk quality. Herringbone parlours mostly used in older

*Table 1*

**Milk production parameters in different herd size groups and milking parlour (n=380)**

| | Parlour | Average number of cows | Average daily milk yield, kg/day | Average daily milk production per cow, kg/day | Average SCC, ×10³ cell/ml |
|---|---|---|---|---|---|
| Group 1 | herringbone | 164 | 24.69 | 21.03 | 441.50 |
| | parallel | 167 | 24.59 | 21.12 | 364.14 |
| | carousel | 152 | 19.95 | 17.19 | 368.00 |
| | other | 180 | 26.72 | 22.71 | 258.75 |
| Group 2 | herringbone | 420 | 29.03 | 25.48 | 432.42 |
| | parallel | 486 | 31.32 | 27.78 | 347.74 |
| | carousel | 477 | 29.32 | 25.06 | 356.00 |
| | other | 437 | 27.89 | 24.31 | 453.75 |
| Group 3 | herringbone | 787 | 30.28 | 26.51 | 376.93 |
| | parallel | 937 | 33.44 | 29.41 | 342.21 |
| | carousel | 1055 | 32.35 | 28.80 | 317.45 |
| | other | 1227 | 34.29 | 30.76 | 316.67 |

*Table 2*

**Milk production parameters by herd size groups (n=380)**

| | Number of farms | Average number of cows | Average daily milk yield, kg/day | Average daily milk production per cow, kg/day | Average SCC, ×10³ cell/ml |
|---|---|---|---|---|---|
| Group 1 | 142 | 165 | 24.73$^{a}$ | 21.07$^{a}$ | 432.5$^{a}$ |
| Group 2 | 142 | 437 | 29.42$^{b}$ | 25.83$^{b}$ | 412.7$^{a}$ |
| Group 3 | 96 | 958 | 32.25$^{c}$ | 28.49$^{c}$ | 341.9$^{a}$ |

*Note*: $^{a, b, c}$ groups with different superscripts differ significantly (P<0.05).

*Table 3*

**Milk production parameters by the type of milking parlour (n=380)**

| Parlour | Number of farms | Average daily milk yield, kg/day | Average daily milk production per cow, kg/day | Average SCC, $\times 10^3$ cell/ml |
|---|---|---|---|---|
| herringbone | 259 | 27.03[a] | 23.40[a] | 430.00[a] |
| parallel | 61 | 31.71[b] | 27.94[b] | 346.75[a] |
| carousel | 41 | 31.38[ab] | 27.70[ab] | 327.15[a] |
| other | 19 | 30.06[ab] | 26.41[ab] | 363.79[a] |

*Note*: [a, b] Milking parlour types with different superscripts differ significantly ($P<0.05$).

milking systems. Higher cow number and milk production enable the use of newer technologies, which could have an impact on production.

The average SCC in Hungary is high in all groups, which is not only the most commonly used indicator of milk quality but could influence the quantity of milk production, as well. It makes necessary to explore the weak points in the dairy production. Regarding to this study milking technology and herd size have impact on the milk production parameters, as well. Further researches are needed to explain the influences of each segment of the used technologies in Hungary.

## Conclusions

The lower milk production in smaller dairy farms can be explained with lower standards of housing, feeding, milking technology and genetics. The type of milking parlour and the number of cows has an impact on milk production, however further research is required in this regard.

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# SURGICAL MANAGEMENT OF PERONEAL NERVE PARALYSIS IN CALF

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*This article describes a case of peroneal nerve paralysis of left hind limb in 2 month old Holstein calf, weight 60 kg. The final diagnosis of the case was done based on clinical investigation. The severity of the paralysis was based on clinical findings as well as peroneal nerve motor and sensory function. Protective bandage and medical treatment was applied for three weeks. Due to poor prognosis after three week tendon transposition was performed.*

*The operation was performed in general anaesthesia. Musculus vastus lateralis was dissected at the insertion and musculus extensor digitalis longus and musculus fibularis tertius were dissected at the origin. Ends of tendons were sutured by using the Bunnel suture with 4 simple interrupted sutures around. A bandage was applied to the extremity for three weeks.*

*Postoperatively, no complications such as suture dehiscence or failure of the tendon anastomosis was seen. After the period of three weeks the bandage was removed and calf was allowed to move freely and put weight on leg correctly.*

*Results of the case confirm that peroneal paralysis can be successfully treated by a tendon transposition technique.*

**Keywords:** PARALYSIS, PERONEAL NERVE, CALF, MEDICAMENT THERAPY, MUSCLE TRANSPOSITION

Traumatic injury of peripheral nerves is a worldwide problem and can result in significant disability [10]. Partial nerve injuries can have variable therapeutical response depend on complete nerve laceration. The generation of endogenous neurotrophic factors such as brain-derived neurotrophic factor and glial cell-derived neurotrophic factor are known to play a critical role in supporting axon regeneration during peripheral nerve repair [12]. Hind limb peripheral nerve abnormalities in adult cattle are most commonly associated with calving trauma most often to the sciatic (peroneal branch) and obturator nerves. In calves, femoral nerve injury may be associated with forced extraction, particularly in posterior presentation [4]. The sciatic nerve branches into the peroneal and tibial nerve dorsal to the stifle. In case of high nerve damage (gluteal area) for example due to abscess or irritating injections, hip, stifle, and hock appear dropped and the fetlock may be knuckled. The limb can support weight but may drag along the ground in advanced stadium. Lack of sensitivity may occur distal to the stifle if the nerve is severely damaged [7]. The common peroneal nerve originates as a terminal branch of the sciatic in the mid-thigh region. The nerve crosses the lateral aspect of the stifle joint and then divides into superficial and deep branches. The peroneal nerve supplies motor innervations to the muscles that flex the hock (cranial tibial, *peroneus tertius*) and extend the digits (digital extensors). It also supplies sensory innervations along the cranial aspect of the tarsus and metatarsus [3]. The peroneal nerve is the cranial division of the sciatic nerve. It passes superficially over the lateral femoral condyle and the head of the fibula, which makes it vulnerable to external trauma or pressure from recumbency. An affected animal stands with the digit knuckled over onto the dorsal surface of the pastern and fetlock. The hock may appear to be overextended. Testing of reflexes may demonstrate that hock flexion is absent, but stifle and hip flexion are normal [1]. The prognosis in peroneal paralysis is considered to be guarded, although it depends on the cause, its direct relationship to the nerve and the severity of the lesion [2].

## Materials and methods

Holstein calf weighting 60 kg was admitted to the Clinic with knuckling left hind limb. It was diagnosed as peroneal paralysis based on clinical observation and examination results including leg posture, walking, needle pricks and

responses of the animal to passive forcing (extensor pushing and flexor pulling reflexes). Examination revealed moderate muscle atrophy below to knee joint of the left hind limb with sensory analgesia (superficial and deep) on the lateral and dorsal surfaces, and proprioceptive deficit. All cranial nerve reflexes were normal. At first medical treatment was administered with vitamins B two times in week, anti-inflammatory drugs (NSAIDS), and protective bandage for 28 days. The medical treatment was not successful and therefore we decided to perform tendon transposition. Animal was prepared with 12 hours starvation and last 8 hours without water. Patient was sedated with diazepam (0.5 mg/kg) intramuscularly (IM). Surgery was performed in general anaesthesia with xylazine (0.25 mg/kg) and ketamine (2 mg/kg) IM. Calf in general anaesthesia was placed in right lateral recumbency. Surgical site was clipped and scrubbed at the proposed incision site. The incision was made parapatelar about 20 cm long. After preparation of muscles we identified *musculus vastus lateralis* and the *musculus extensor digitorum longus*. The *musculus vastus lateralis* was dissected at the transition of the muscle into the tendon and *musculus extensor digitorum longus* was released at the origin. Both of free ends of the *musculus vastus lateralis* and the origin of *musculus extensor digitorum longus* with *musculus fibularis tertius*, were brought together and connected with the Bunel suture technique with non-absorbable suture material (*Tervalon EP 6*). In addition four simple interrupted sutures were placed around the muscle anastomosis with absorbable material (*Chirlac EP 4*). Surgery site was flushed with sterile solution and subcutaneous suture was performed with absorbable suture material (*Chirlac EP 4*). After suture of subcutaneous we continued with suture of skin with U-suture and we used non- absorbable material (*Tervalon EP 6*). Operation wound was controlled every second day. Skin suture was removed after 10 days. The leg was kept in bandage for 21 days. Medicament care after operation involved antibiotic ceftiofur (*Cevaxel-RTU*, 50 mg/ml) in doses 3 mg/kg subcutaneously during 10 days and nonsteroidal anti-inflammatory drugs flunixin meglumine (*Flunixin a.u.v.*, 50 mg/ml) in doses 2.2 mg/kg intramuscularly during 4 days after surgery.

## Results and discussion

Three weeks after surgical intervention we removed the bandage and calf was allowed to walk free showing mild limping. The calf put weight on the leg correctly with complete surface of sole. Two months after surgery calf was walking uneventfully. Post operatively no complications with wound like suture dehiscence or failure of tendon anastomosis were seen but clinical investigation of surgery area on day 5 showed swelling in the place of anastomosis of muscles. The swelling area size was about 5 cm and was located under skin. Sonography confirmed muscle tissue. For the anastomosis of muscles we used non-absorbable material (*Tervalon EP 6*). The most probable reason of non-absorbable sutures eliciting intense inflammatory response may be attributed to being a foreign body reaction in muscle tissue based on the finding that non-absorbable material produces more reactivity to internal tissue than external [9]. The swelling was non-palpable on day 21 after surgery. Aetiology of peripheral nerve dysfunction is commonly based on trauma. Trauma to a nerve may be associated with direct injury or it may arise secondary to pressure from oedema, neoplasia, or fractures. Aetiology also influences prognosis [3]. According to authors [8] and [11], medicament therapy should be performed in case of peroneal paralysis. Treatment of sciatic nerve dysfunction includes bandaging to prevent abrasions of the fetlock and encouragement of weight bearing [4]. We performed medicament therapy with vitamins B, anti-inflammatory drugs, and applied the protective bandage on the leg for 28 days to prevent complications. Result of medicament therapy was negative therefore we decided for surgical intervention. There was positive result after 21 days after surgery. Similar result with a surgical treatment of peroneal paralysis caused by intramuscular injection was reported by [5] in sheep. Recently, the same treatment was used with positive outcome in calves [6].

## Conclusion

The aim of our study was to present possibility of surgical management of peroneal nerve paralysis. Surgery tendon transplantation proce-

dures appear to be superior to conservative treatments in the management of patient with peroneal nerve paralysis. This method helps to avoid complications associated with injury of affected limb.

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# LONGEVITY OF DAIRY COWS — ENERGY PROFILE

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*In the study we compared parameters of energy profile in relation to number of lactation of dairy cows. We analysed blod serum to glucose, non-esterified fatty acids, β-hydroxybutyrate, total cholesterol, triacylglycerols and total lipids of Slovak Pied dairy cows with number of lactation (I L — 6 cows, II L — 5 cows, III L — 6 cows, IV L — 5 cows). Blood samples were collected 20 days a.p. and 20, 40, 60, 80 days p.p.*

*There were observed lower concentrations of glucose and BHB in groups of cows I L and II L compared with cows of groups of III L and IV L. The highest concentrations of glucose was recorded in cows IV L 20 days a.p. (4.32±0.09; P<0.01). NEFA and TL were increased with a.p. period. TCH values in groups III L and IV L, were lower than in groups of cows I L and II L (P<0.01; P<0.05). In the assessment of concentration of TG was found the highest concentrations in group of cows I L during ante-partum (0.22±0,03; P<0.05). Cows during a.p. had significantly higher TG concentrations compared to cows in postpartal period (P<0.05). These results showed dynamic changes in the energy profile during a.p. and p.p. which reflect the physiological response of the organism to the variation of metabolic functions occuring from gestational to a lactating state in dairy cows. Our results indicate that older cows have higher levels of blood GL, BHB and NEFA levels, which proves that dairy cows with higher number of lactations have a better adaptation to the metabolic challenge, for example to milk production, in terms of maintenance of glycemia.*

**Keywords:** ENERGY PROFILE, DAIRY COWS, NUMBER OF LACTATION, LONGEVITY

Transition period and the early lactation phase, are characterized by sudden episodes of metabolic and hormonal changes, such as the parturition and the onset of lactation, which include alterations in the energy balance that lead to increased lipomobilization with consequent elevation of plasma concentration of nonesterified fatty acids. This period is considered the most critical period in the lactation cycle because 50 % of transition cows may be affected by disease.

## Materials and methods

Glucose (GL), β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), total cholesterol (TCH), triacylglycerols (TG) and total lipids (TL) were evaluated in dairy cows of Slovak Pied Cattle. The dairy cows (n=22) were classified into 4 groups based on the number of lactation: dairy cows at 1st lactation (I L) (n=6), dairy cows at 2nd lactation (II L) (n=5), dairy cows at 3rd lactation (III L) (n=6), dairy cows at 4th lactation (IV L) (n=5). The blood samples were collected by direct puncture of *v. jugularis* during *ante-partum* (20 days *ante-partum*) and *post-partum* (20, 40, 60 and 80 days *post-partum*).

Mean production age was 3.45 lactation. Total mixed rations (TMR) with different levels of ME were offered to dairy cows twice daily, nutrient composition of the TMR varied with the stage of pregnancy and lactation. Meals for the cows were based on meadow hay, lucerne silage, haylage, green fodder and concentrate. Chemical components of meals meet the needs of cows in dry period and different period of lactation. Diet was suited to the energy requirements of late pregnancy, early and mid-lactation cows. The dairy cows had free access to drinking water. Before sample collection, the animals were clinically examined by standard clinical examination procedures. No treatments were administered before the start of the experiment. The concentrations of glucose, TCH, TG, and BHB were determined using commercial diagnostic kits (*Randox*) on automatic biochemical analyser ALIZE (*Lisabio*, France). Total lipids were analyzed using commercial diagnostic kits (*Ecomed*) by spectrophotometric method. The concentrations of NEFA

were by spectrophotometric method. Evaluation of the obtained results was performed by the assessment of mean values (x) and standard errors (SE) in each monitored group of dairy cows. Significance of differences in the mean values in relation to the several monitored periods was evaluated by one way analysis of variance (ANOVA). Significance of differences in the mean values between groups was evaluated by Tukey's multiple comparisons test. Statistical analyses were done with the *GraphPad Prism 3.0*.

## Results and disscusion

In our study, prepartum serum NEFA concentrations for cows with 1st lactation exceeded the threshold of 0.4 mmol/l proposed by [9] as indicating prepartum negatively altered metabolic status and the serum concentration (0.7 mmol/l) — in cows with 4th lactation 40 days p.p. proposed to indicate postpartum negatively altered metabolic status. NEFA concentrations increased at calving, reached peak concentrations on day 20 p.p. for primiparous and multiparous cows and started to decrease thereafter (fig. 1). This increase is a result of a decrease in dry matter intake prior to parturition and of hormonal changes before and at parturition that stimulate mobilization of NEFA from adipose tissue to provide energy for parturition and lactogenesis. The high postpartum levels of NEFA would indicate that primiparous cows were mobilizing more long chain fatty acids from adipose tissue than multiparous cows [12], which would agree with the results of [4].

[3] found higher concentrations of β-hydroxybutyrate in the postpartum period compared to prepartum period. Our results comply with these findings. BHB concentrations were low at calving, rose sharply up to 20 days *post-partum* and slowly decreased thereafter (fig. 2), but they remained higher than the *ante-partum* levels, reflecting the negative energy balance and the consequently mobilization of body reserves [5] associated with the onset of lactation. High serum concentrations of BHB have been associated with reduced immune system functionality [6], disease risk (displaced abomasum, retained placenta etc.), and less milk production [7], and many other transition and early lactation cow disorders [9].

The blood glucose level is regarded as one of the indicators of energy status in the cow. Glucose was significantly lower in I L, II L and IV L after calving, but in III L were observed significantly lower values than before calving. Overall glucose was higher in older cows (IV L) than in younger cows (I L) (fig. 3.). Similar result was found by other researcher, demonstrating that older cows had higher overall glucose than younger cows [12]. Our results comply with these finding, as after parturition we found decreasing mean values of glucose compared with cows in the prepartum

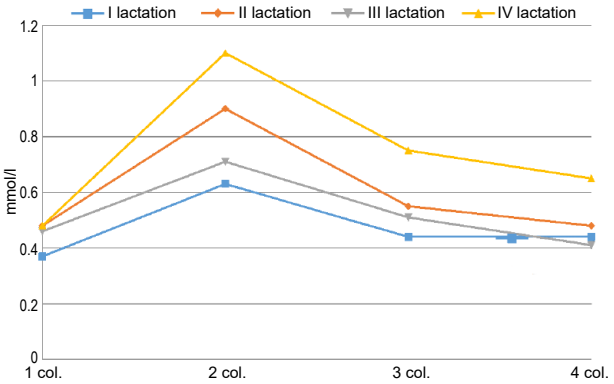


*Fig. 1.* Non-esterified fatty acids concentration

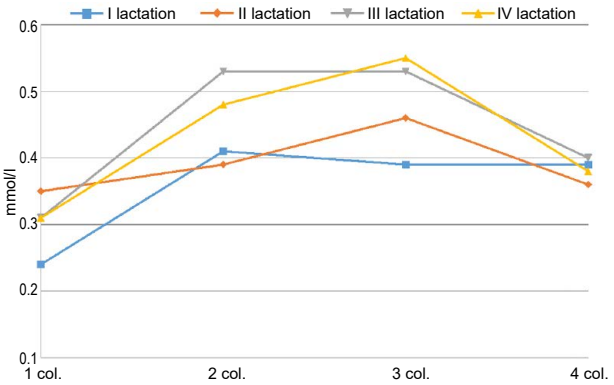


*Fig. 2.* β-hydroxybutyrate concentration

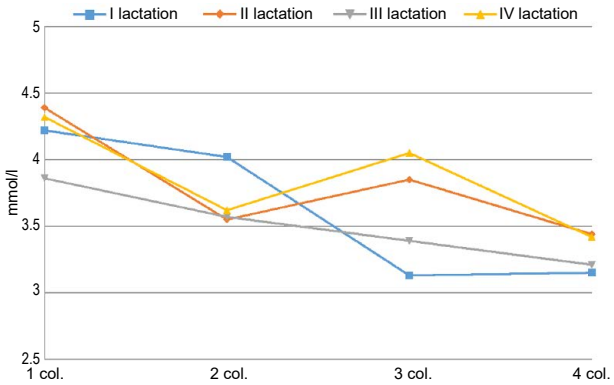


*Fig. 3.* Glucose concentration

period. [1] observed cows from 4 days before until 36 days after calving, and recorded lower glucose concentrations in the early postparturient period, with a significant increase on the 27th day *post-partum*. The blood glucose level was higher in lactating cows this may be due to high energy diet feeding during lactation period and also for taking the extra amount of feed than the requirement of animal for milk production and maintenances.

Cholesterol concentration increased during postpartum period in cows with I, II, III and IV lactation, although multiparous cows had higher cholesterol around day 60 p.p. than primiparous, reflecting an increased lipid uptake by the liver (fig. 4). Our results comply with findings by [2]. According to [8], hypercholesterolaemia can be considered physiological during lactation, either as a result of lipid mobilization caused by glucagon or to an increase in the synthesis of plasmatic lipoproteins. However, the increase in cholesterol concentrations may be due to a greater energy demand than that supplied by the offered diet. Rise in cholesterol is associated with an improvement in energy balance.

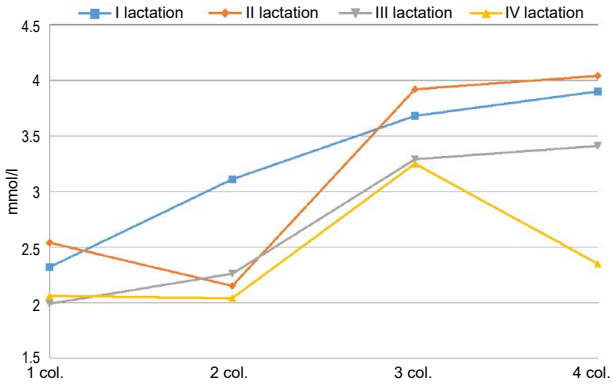


*Fig. 4.* Total cholesterol concentration

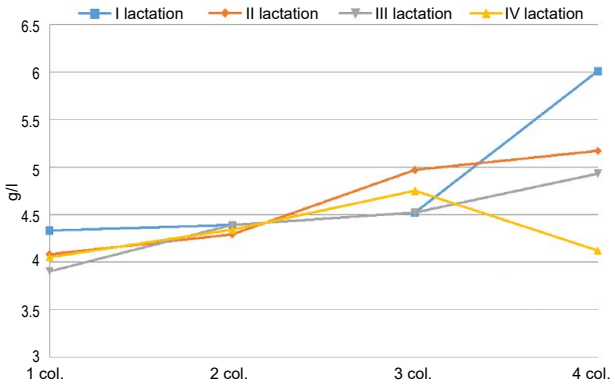


*Fig. 5.* Total lipids concentration

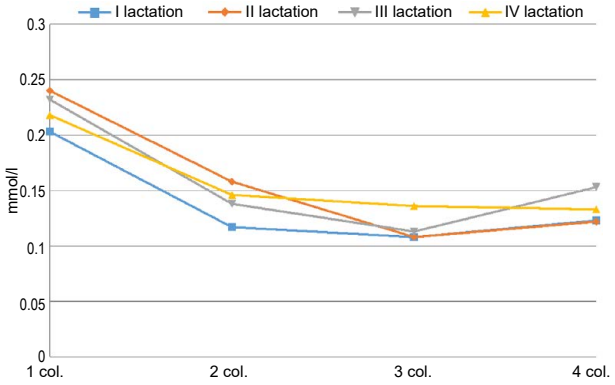


*Fig. 6.* Triacylglycerols concentration

[10] reported that total lipids level increased significantly during the mild and late gestation, postpartum and early lactation compared to dioestrus, early gestation. Our results also show significantly higher values of TL before parturition than in cows after calving (fig. 5).

The same authors found that total cholesterol and triacylglycerols resulted significantly affected by the physiological status, in fact, both showed substantial increases during the mild lactation (fig. 6).

Probably because, during the puerperal period, there is an increase in the demands for regulatory mechanism, responsible for all the processes involved with milking. At this purpose, characteristic changes in lipid metabolism were found during pregnancy and lactation in most mammals. Endocrine profiles change and lipolysis and lipogenesis are regulated to increase lipid reserve during pregnancy, and, subsequently, these reserves are utilized following parturition and the initiation of lactation [11]. Similar results, however, were found by other researchers, demonstrating that concentrations of total lipids and triacylglycerols increased at parturition, despite the kind of fed administered.

## Conclusion

During the study we found lower concentrations of glucose and BHB in groups of cows I L and II L compared with cows of groups of III L and IV L .The highest concentrations of glucose was recorded in cows IV L 20 days a.p. (4.32±0.09; P<0.01). NEFA and TL were increased with a.p. period. TCH values in groups III L and IV L, were lower than in groups of cows I L and II L (P<0.01;

P<0.05). In the assessment of concentration of TG was found the highest concentrations in group of cows I L during *ante-partum* (0.22±0,03; P<0.05). Cows during a.p. had significantly higher TG concentrations compared to cows in postpartal period (P<0.05).These results showed dynamic changes in the energy profile during a.p. and p.p. which reflect the physiological response of the organism to the variation of metabolic functions occuring from gestational to a lactating statein dairy cows. Our results indicate that older cows have higher GL, BHB and NEFA levels, which proves that dairy cows with higher number of lactations have a better adaptation to the metabolic challenge, for example to milk production, in terms of maintenance of glycemia.

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# SURVEY ON THE COURSE OF PUERPERIUM AND ON FERTILITY AFTER IMPLEMENTATION OF THE *IVET*® BIRTH MONITORING SYSTEM IN HEIFERS

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*The aim of this study was to investigate the influence of the birth monitoring system iVET® on the puerperium, milk yield and fertility of the dam in the subsequent lactation.*

*On a large dairy farm in Saxony-Anhalt, the visual birth monitoring of the heifers was complemented by the automated iVET® birth monitoring system. The iVET® consists of two components: a transmitter, which is inserted into the vagina of the animals to be monitored and a receiver which must be installed above the calving pen. During the birth process, the transmitter is forced out of the vagina and sends a signal to the receiver which then triggers an SMS or phone call to the person in charge. In the control group (n=192), birth monitoring was performed by farm personnel in the same way as before the start of the study. In the iVET® group (n=167), a distinction was made between the animals in which the transmitter remained longer (24h+group, n=88) or shorter than 24 hours (24h-group, n=79). The experimental phase started with the recording of the calving process and ended at day 200 p.p. To assess the heifer's fertility, the onset of ovarian activity was determined by ultrasound examination of the ovaries. The following fertility measures were calculated: first service conception rate (FCR), overall pregnancy rate (PR), mean pregnancy index (PI), conception rate (CR), mean interval from calving to first insemination (CFI), mean days open (DO), mean interval from first insemination to conception (FIC), mean calving interval (CI).*

*In the iVET® group, significantly fewer animals calved without assistance and there were significantly more calvings with extreme difficulty especially in the 24h+group. The iVET® group had a significantly higher number of injuries, the injuries were more severe, the healing progressed more slowly and these animals developed an endometritis significantly more frequently than the control group. In the control group, significantly more animals had active ovaries when they were first examined on Day 10 p.p. than in the iVET® group. The iVET® group had a signifianty longer CFI, but a significantly shorter FIC than the control group. Concerning the other fertility measures, there were no significant differences. In the 24h+group, the milk yield of the first 100 days p.p. was significantly lower than in the 24h-group, but there was no significant difference between the 100-d-yield of the iVET® group and the control group. The number of animals which had to be culled before Day 200 p.p. was significantly higher in the iVET® group than in the control group.*

*Birth monitoring by means of the iVET® system impaired the course of labour and in consequence the puerperium. The evaluation of calving ease and the examinations during puerperium showed that a retention time of transmitters in heifers of more than 24 hours cannot be recommended. This limitation makes the use of the birth monitoring system problematic; under real-life conditions it is hardly possible to predict the beginning of birth with sufficient accuracy with an acceptable amount of effort.*

**Keywords**: CATTLE, HEIFERS, BIRTH, MONITORING, PUERPERIUM, FERTILITY, MILK YIELD

The objective of successful management of the cow at calving time is to ensure delivery of a viable calf and smooth transition of the cow from the dry to the milking string without complications. The two major problems encountered at calving time are dystocia and perinatal mortality [6]. The adverse effects of poor calving management are numerous and well documented. Dams with dys-

tocia often show retained fetal membranes and metritis and in consequence poor fertility measures and impaired milk yield in the following lactation [1–4]. A close calving monitoring, particulary in heifers, therefore is an integral part of successful calving management. The objective of this study was to investigate the influence of the birth monitoring system *iVET*® on the puerperium as well as on the milk yield and on the fertility of the dam in the subsequent lactation.

## Materials and methods

The study was conducted from July 2013 to July 2014 on a large dairy farm in Saxony-Anhalt in Germany (877 lactating and 123 dry German Holstein cows, 941 replacement heifers and 227 calves <6month of age).The visual birth monitoring of the heifers was complemented by the automated *iVET*® birth monitoring system. The *iVET*® birth monitoring system consists of two components: of a transmitter (fig. 1), which is inserted into the vagina of the animals to be monitored and of a receiver (fig. 2) that must be installed above the calving pen.

During the birth process, the transmitter is forced out of the vagina and sends a signal to the receiver which then triggers an SMS or phone call to the person in charge. Pregnant heifers were examined clinically 3 to 2 weeks before the calculated calving date, and only clinically healthy heifers were used. Those heifers were housed in a free stall barn with straw bedding and fed a total mixed ration. The median age of the heifers at calving during the study period was 782 d. A total of 359 heifers were allocated randomly to two groups. 192 heifers were assigned to the control group in which birth monitoring was performed by farm personnel in the same way as before the start of the study. 167 heifers were assigned to the *iVET*® group (study group) in which the *iVET*® system was used. In the *iVET*® group a distinction was made after calving between the animals in which the transmitter remained longer or shorter than 24 h. The experimental phase started with the recording of the calving process and ended at day 200 p.p. Labor was assessed in terms of calving ease. Animals were clinically examined 4 times *post-partum* (day 1, day 10, day 21, day 42).

*Fig. 1.* Transmitter of the *iVET*® birth monitoring system


*Fig. 2.* Receiver unit of the *iVET*® birth monitoring system

Injuries of the vulva and the vagina due to calving and the healing process were scored. To assess the heifer's fertility, the onset of ovarian activity was determined by ultrasound examination of the ovaries. The following fertility measures were calculated: first service conception rate (FCR), overall pregnancy rate (PR), mean pregnancy index (PI), conception rate (CR), mean interval from calving to first insemination (CFI), mean days open (DO), mean interval from first insemination to conception (FIC), mean calving interval (CI). Additionally, body condition score (BCS), occurrence of peripartal diseases, culling data and the 100d-milk-yield we reevaluated.

## Results and discussion

The interval from insertion of the *iVET*®-transmitterto first birth alarm averaged 74.6±89.2 h. In the *iVET*® group, significantly fewer animals

calved without assistance (P≤0.05). Also, there were significantly more calvings with extreme difficulty (table 1).

The heifers of the *iVET*® group suffered from a higher number of injuries, the injuries were more severe (table 2), and the healing progressed more slowly. Furthermore, these animals developed an endometritis significantly more frequently (47.4 % vs. 33.1 %, P≤0.05), and it lasted significantly more often until the end of puerperium on day 42 p.p. (31.5 % vs. 19.6 %, P≤0.05). The experimental arrangement did not reveal any influence on the frequency of retained placenta, on the occurrence of metritis, and on the trend of BCS. Within the *iVET*® group, animals in which the transmitter remained longer than 24 h significantly more often had extreme calving difficulties, and they significantly less often calved without as-

*Table 1*

**Calving ease in study groups (n = number of animals)**

| % (n) | control | *iVET*® | <24 h° | ≥24 h |
|---|---|---|---|---|
| Score 1* | 44.8a (86) | 27.0b (45) | 44.3c (35) | 11.4d (10) |
| Score 2 | 12.0 (23) | 17.4 (29) | 22.8 (18) | 12.5 (11) |
| Score 3 | 15.6 (30) | 17.4 (29) | 15.2 (12) | 19.3 (17) |
| Score 4 | 23.4 (45) | 28.1 (47) | 16.5c (13) | 38.6d (34) |
| Score 5 | 4.2a (8) | 10.2b (17) | 1.3c (1) | 18.2d (16) |
| Total | 100 (192) | 100 (167) | 100 (79) | 100 (88) |

*Note*: within a row: a vs. b; c vs. d — P≤0.05. º <24 h — *iVET*® remained shorter than 24 h; ≥24 h — *iVET*® remained 24 h or longer. *Score 1 — spontaneous calving, no assistance needed; Score 2 — very easy extraction, 1 person, max. 1 min; Score 3 — easy extraction: 1–2 persons, extraction force without effort, quickly (within 5 min); Score 4 — moderately severe extraction: 2 persons, moderate extraction force, duration 5–15 min, stretching of soft birth canal necessary; Score 5 — severe extraction: 2 persons, maximal extraction force, duration 15–25 min, stretching of soft birth canal necessary, only very slow progress (<1 cm per expulsive strain).

*Table 2*

**Injuries of the *vestibulum vaginae* and the vagina 1 p.p. (n = number of animals)**

| % (n) | | Control | *iVET*® | <24 hº | ≥24 h |
|---|---|---|---|---|---|
| Injuries of the *vestibulum vaginae*# | degree 1 | 34.6a (66) | 19.8b (32) | 22.8 (18) | 16.9 (14) |
| | degree 2 | 59.2 (113) | 69.1 (112) | 74.7 (59) | 63.9 (53) |
| | degree 3 | 6.3 (12) | 11.1 (18) | 2.5c (2) | 19.3d (16) |
| Injuries of the vagina* | no injury | 54.7a (41) | 37.7b (61) | 55.7c (44) | 20.5d (17) |
| | degree 1 | 28.0a (21) | 16.7b (27) | 16.5 (13) | 16.9 (14) |
| | degree 2 | 17.3a (13) | 45.7b (74) | 27.9c (22) | 62.7d (52) |

*Note*: within a row: a vs. b; c vs. d — P≤0.05. # Injuries of the *vestibulum vaginae*: degree 1 — noor mild injuries; degree 2 — marked injuries <2 cm deep; degree 3 — severe injuries ≥2 cm deep. * Injuries of the vagina: degree 1 — lesion <2 cm deep and up to 10 cm long; degree 2 — lesion ≥2 cm deep and/or ≥10 cm long. º <24 h — *iVET*® remained shorter than 24 h; ≥24 h — *iVET*® remained 24 h or longer.

*Table 3*

**Fertility measures (n = number of animals)**

| | | Control | *iVET*® | <24 hº | ≥24 h |
|---|---|---|---|---|---|
| CFI | d, mean ± standard deviation (n) | 87.7a±21.1 (138) | 100.7b±25.4 (108) | 98.1±23.9 (64) | 104.5±27.4 (44) |
| DO | | 112.9±38.5 (94) | 111.9±35.8 (70) | 108.2±35.6 (42) | 117.5±35.9 (28) |
| FIC | | 27.2a±33.1 (94) | 16.8b±25.5 (70) | 14.9±23.7 (42) | 19.7±28.2 (28) |
| CI | | 392.9±38.5 (94) | 391.9±35.8 (70) | 388.2±35.6 (42) | 397.5±35.9 (28) |
| PI | | 1.8±0.9 (94) | 1.5±0.7 (70) | 1.5±0.8 (42) | 1.5±0.6 (28) |
| PR | % (n) | 68.1 (94) | 64.8 (70) | 65.6 (42) | 63.6 (28) |
| FCR | | 31.9 (44) | 38.0 (41) | 40.6 (26) | 34.1 (15) |
| CR | | 31.3 (94) | 35.4 (70) | 35.3 (42) | 35.4 (28) |

*Note*: within a row: a vs. b — P<0.05. º <24 h — *iVET*® remained shorter than 24 h; ≥24 h — *iVET*® remained 24 h or longer. FCR — first service conception rate; PR — overall pregnancy rate; PI — mean pregnancy index; CR — conception rate; CFI — mean interval from calving to first insemination; DO — mean days open; FIC — mean interval from first insemination to conception; CI — mean calving interval.

sistance than the animals in which the transmitter remained for less than 24 h (table 1).

Additionally, they showed a poorer performance in some examinations during the puerperium. In the control group, significantly more animals had active ovaries when they were examined on day 10 p.p. (81.9 % vs. 63.7 %, P≤0.05) and on day 42 p.p. (97.9 % vs. 91.2 %, P≤0.05). The fertility measures are presented in table 3. Animals of the *iVET*® group took a longer time to first insemination than animals of the control group, but became pregnant more quickly (P≤0.05). Thus, DO and the expected calving interval were almost equal in both groups. Concerning the other measures of fertility, there were no significant differences. Comparisons of some fertility analyses showed that the animals of the *iVET*® group with a longer retention time of the transmitter did not perform as well as those with a shorter retention time.

However, these differences were not significant. In the group of heifers with the longer retention time, the milk yield of the first 100 days p.p. was significantly lower than in the group with the shorter retention time (2910.4±454.2 kg vs. 2723.8±483.6 kg, P≤0.05). The yield of the control group (2770.9±526.4 kg) was between the yields of the other two groups. In the *iVET*® group significantly more heifers were culled than in the control group (22.2 % vs. 10.9 %, P≤0.05). The number of animals that had to be culled was three times higher in the group with the longer retention time than in the group with the shorter retention time (33.0 % vs. 10.1 %, P≤0.05). In contrast to [5], who tested the birth monitoring device C6, the *iVET*® system was not well tolerated by all heifers and caused irritation and discomfort to the heifer which may lead to neuro-hormonal alterations of the birth process. This could be one reason for the high percentage of dystocia and injuries. An obvious problem was that the *iVET*® seemed too large for heifers. As a result of the current investigation, the *iVET*® birth monitoring system has already been modified and a smaller version for heifers or smaller cows has been developed.

## Conclusions

Birth monitoring by means of the *iVET*® system did not improve the course of the puerperium or the fertility of the heifers. The evaluation of calving ease and the examinations during puerperium showed that a retention time of transmitters in heifers of more than 24 hours cannot be recommended. This limitation makes the use of the birth monitoring system problematic; under real-life conditions it is hardly possible to predict the beginning of birth with sufficient accuracy with an acceptable amount of effort. Therefore, this device was lacking in several aspects and should be improved and evaluated further before its use in primiparous cattle can be recommended. Further controlled experiments were needed to eliminate major drawbacks.

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# EFFECTS OF A-TOCOPHEROL AND SELENIUM INJECTION ON SERUM CORTISOL IN DAIRY COWS UNDERGOING ABDOMINAL SURGERY

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*The present trial was aimed to study the effects of vitamin E and selenium treatment on blood cortisol concentrations in dairy cows stressed by omentopexy.*

*Twenty dairy cows with left abomasal displacement were used in this study. The cows were randomly divided into two groups. Ten hours before surgery 6 g of DL-α-tocopheryl acetate (6 mg/kg) and 67 mg of sodium selenite (0.1 mg/kg) in volume of 40 ml (Vitaselen®) were administered subcutaneously to 10 cows; the control animals (n=10) received an equivalent volume of injectable water (40 ml).*

*The serum vitamin E increased several times ten hours after vitamin E and Se injection and rose continuously to the highest average concentration 21.6 mg/l at hour 24 after the surgery. The highest selenium concentration was seen ten hours after selenium administration with holding the increased concentrations in comparison to initial ones during the whole study. The highest cortisol concentrations were reached at one hour after surgery in the experimental and control group (56.7±28.8 and 65.3±26.1 μg/l, respectively). The ANOVA revealed a significant effect of vitamin E and selenium injection on plasma cortisol (P<0.05).*

*The decrease of blood cortisol in our study may suggest that vitamin E and selenium supplementation may be an effective method to minimize stress response in dairy cows.*

**Keywords:** SURGICAL STRESS, VITAMIN E, SELENIUM, CORTISOL

It has been demonstrated that a number of manipulations including transport [6], feed deprivation [7], therapeutic manipulation [14], and surgery [2], increased secretion of cortisol from the adrenal cortex in cattle. Several studies demonstrated that the stress reaction has enhancing effects on free radical production, thus contributing to an increased lipid peroxidation in animals [13]. Although stress reactions are organised to protect the homeostatic state of animals, they contain elements that may either enhance or diminish susceptibility to the disease process; in many instances, however, stress reactions themselves may induce pathologic change [3]. Elevated lipid peroxidation can play an important role in the pathogenesis of many health disorders in animals [16]. The lipid peroxidation can be monitored by analysing of oxidative status by assessment of pro-oxidants, oxidative damaged molecules (i.e. lipid peroxidation products), and antioxidative status of organism [1]. Biological systems contain powerful enzymatic (glutathione peroxidase, catalase, and superoxide dismutase) and nonenzymatic (tocopherol, b-carotene, ascorbic acid, and glutathione) antioxidant systems protecting organic compounds against the harmful effects of free radicals.

The present trial was aimed to study the effects of vitamin E and selenium treatment on blood cortisol in dairy cows stressed by omentopexy.

## Materials and methods

Twenty Holstein-Frisian lactating dairy cows, mean age 4.41±1.34 years (x±sd), admitted for treatment of left abomasal displacement, were used in the study. All of them were within first six weeks after calving. Their mean body weight was 586±65 kg (x±sd). They were randomly divided into two equal groups (n=10) according to the order of admission to the clinic. The surgery followed on the subsequent day to correct the abomasal displacement. Ten hours before surgery 6 g of DL-α-tocopheryl acetate (6 mg/kg) and 67 mg of sodium selenite (0.1 mg/kg) in volume of 40 ml (*Vitaselen®*) were administered subcutaneously to 10 cows; the control animals (n=10) received an equivalent volume of water for injections (40 ml). Abdominal surgery (omentopexy) was performed in a standing position 16–24 hours after admission. The mean duration of preparation for surgery lasted approximately 30–40 minutes, and the surgery approximately 40 minutes. Pro-

casel (2 % procaine-hydrochloride) was used for local anaesthesia. All experimental animals were housed in pens with straw bedding and were fed concentrates and hay. All cows recovered and left the Clinic on day 4 or 5 after the omentopexy.

Blood samples were drawn from the jugular vein before vitamin E/Se injection, just prior to surgery, immediately after surgery, then 15, 30, 60 minutes, and 2, 5, 10, and 24 hours after surgery. The blood samples were stored at 4 °C maximally for two hours before centrifugation. The plasma and serum were obtained and then stored frozen at –80 °C until analyses. The α-tocopherol concentrations in serum were determined in saponified samples by high performance liquid chromatography (HPLC) using a fluorescent detector. The concentrations of selenium in serum were measured using the fluorimetric method. The serum cortisol concentrations were determined by chemiluminescent enzyme immunoassay (*Immulate®/ Immulate® 1000 Cortisol immunoassay*, DPC L.A., USA). Statistical analysis was carried out by a two-factorial analysis of variance (one repeated factor: time, one grouping factor: treatment) with the *post-hoc* Bonferroni test (*IBM* SPSS Statistics 23, 2015). Significance was declared at P<0.05.

## Results and discussion

The subcutaneous injection of vitamin E and selenium resulted in a rapid increase (P<0.05) in blood α-tocopherol and selenium concentrations (table).

The serum vitamin E increased to six-fold value ten hours after administration of *Vitaselen®* and rose continuously to the highest average concentration 21.6 mg/l 24 hours after the surgery. There were no changes in serum vitamin E concentration in the control group during the study. The serum selenium concentrations of the experimental group showed a similar dynamic like α-tocopherol. The highest selenium concentration was seen ten hours after selenium administration with holding the increased levels in comparison to initial ones during the whole study.

*Table*

**Concentrations of blood α-tocopherol, selenium, and cortisol in operated dairy cows after vitamin E/Se or placebo treatment (mean±SD)**

| Sampling time | Group | α-tocopherol, mg/l | Selenium, μmol/l | Cortisol, μg/l |
|---|---|---|---|---|
| Before injection | E | 2.38±1.71 | 0.75±0.19 | 8.13±4.60 |
| | C | 2.27±1.28 | 0.91±0.22 | 7.87±4.14 |
| Before surgery | E | 13.4±5.16* | 1.25±0.20* | 10.9±7.10 |
| | C | 2.33±1.23 | 0.91±0.23 | 13.7±11.6 |
| Immediately AS | E | 15.9±3.85* | 1.12±0.20* | 48.0±24.8 |
| | C | 2.02±0.85 | 0.88±0.21 | 58.9±41.0 |
| 15 min AS | E | 15.2±4.15* | 1.10±0.20 | 43.8±20.7 |
| | C | 2.01±0.90 | 0.89±0.19 | 59.7±30.6 |
| 30 min AS | E | 16.5±3.60* | 1.18±0.22* | 44.5±19.4 |
| | C | 2.05±0.79 | 0.86±0.18 | 61.1±28.7 |
| 60 min AS | E | 16.7±3.81* | 1.16±0.22* | 56.7±28.8 |
| | C | 2.18±1.00 | 0.84±0.17 | 65.3±26.1 |
| 2 hours AS | E | 17.6±3.18* | 1.09±0.26* | 26.6±16.5* |
| | C | 2.13±0.84 | 0.90±0.21 | 49.9±21.7 |
| 5 hours AS | E | 19.0±3.08* | 1.08±0.19* | 15.8±7.97 |
| | C | 2.11±0.91 | 0.88±0.21 | 21.8±11.1 |
| 10 hours AS | E | 20.6±2.53* | 1.06±0.16* | 9.04±5.19 |
| | C | 2.66±2.53 | 0.95±0.25 | 12.6±8.02 |
| 24 hours AS | E | 21.6±2.60* | 1.10±0.15 | 9.41±9.70 |
| | C | 2.51±1.57 | 0.98±0.25 | 10.7±5.43 |
| Group effect | | P<0.05 | P<0.05 | P<0.05 |
| Time effect | | P<0.05 | P<0.05 | P<0.05 |

*Note:* E — experimental group (Vit E/Se), C — control group (placebo), AS — after surgery, NS — not, * means within sampling times differ (P<0.05) (Bonferroni test).

Serum cortisol concentrations increased in both groups after surgery (table). The highest values were reached at one hour after surgery in both groups. A return to concentrations similar to the initial ones was observed 24 hours after the surgery. The ANOVA revealed significant effect of vitamin E and selenium injection on plasma cortisol concentrations ($P<0.05$).

The concentrations of vitamin E measured in our study were similar to those found by [15] in dairy cows around calvings. The serum concentrations of α-tocopherol found ten hours after vitamin E and selenium injection were approximately as high as those in cattle reported by [10] who used DL-α-tocopheryl acetate for intramuscular injection in a similar dosage (4500 IU per 250–300 kg body weight). Thus, it can be assumed that concentrations of vitamin E, reached in the experimental animals within the surgical procedure, were high enough to be effective on a lipid peroxidation or other physiological reactions associated with stress response in animals. Similarly, the subcutaneous administration of selenium elevated blood selenium in experimental dairy cows within the whole experimental period (24 h) what could create a different metabolic condition in animal tissues affecting multiple biochemical processes and reactions. This dynamics are similar as previously reported by [5] after subcutaneous Se injections of 0.13 mg/kg in feedlot heifers. In dairy cows receiving Se injections in the present study, mean concentrations of serum Se observed prior to the injection in the experimental and control group (0.75 and 0.91 μmol/l, respectively) were in the range of reference intervals for dairy cows [9].

[11] concluded that 69.4 % of 307 baseline cortisol samples had concentrations below 3 μg/l, whereas 13.7 % of the samples contained 6 μg/l cortisol or more. The relatively higher mean cortisol values prior to surgery in the present study (higher than 7.5 μg/l) are suggested to be results of both sickness and transport stress of the animals. The effect of surgery on plasma cortisol was significant; however, cortisol concentrations fell near to pre-surgery values by the end of the trial. Thus, the pattern of cortisol response to surgery was similar to the pattern seen in 5–6 month-old cattle after amputation dehorning [17]. Comparable to some other studies, a significant effect of vitamin E and selenium administration on cortisol levels could be observed in the present study. The reduction effect of vitamin E on the production of cortisol was shown in cattle [8]. In addition, maternal Se supplementation of dams resulted in lower circulating cortisol concentrations in lamb offspring compared with lambs born from non-supplemented ewes [4]. In an experiment with transportation stress in sheep a depressive effect of trace element supplementation, including selenium, could be seen on cortisol levels in ewe lambs [12]. It is speculated that Se can act directly on blood cortisol by affecting the free radical-antioxidant capacity balance.

## Conclusion

In conclusion, we have demonstrated that the single injection of α-tocopheryl acetate and sodium selenite significantly increases the serum vitamin E and Se within 10 hours. In addition, a significant reduction of blood cortisol was found in treated cows. These decreases in blood cortisol may suggest that vitamin E and selenium supplementation may be an effective method to minimize stress response in dairy cows.

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# SELECTED ACUTE PHASE PROTEINS IN DAIRY COWS WITH CHRONIC DIARRHEA CAUSED BY *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*

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*Paratuberculosis, or Johne's disease, is one of the most important intestinal chronic progressive granulomatous infections of ruminants with major economic impacts. The objective of this study was to evaluate the alterations in the concentrations of selected acute phase proteins in dairy cows with chronic diarrhea and seropositive to Mycobacterium avium subsp. paratuberculosis (MAP).*

*Blood samples from 44 dairy cows affected by chronic diarrhea were included into the study. The animals were seropositive for MAP antibodies, and showed obvious clinical signs of the disease (diarrhea, loss of body weight, general wasting). Nineteen clinically healthy and MAP negative cows were taken as a control group. Immunoenzymatic ruminant MAP-Ab test was used for specific detection of anti-Mycobacterium avium subsp. paratuberculosis antibodies in blood serum (Prionics Lelystad, The Netherlands). The concentrations of selected acute phase proteins — serum amyloid A (SAA), haptoglobin (Hp) and C-reactive protein (CRP) were measured in blood serum. SAA was assessed by sandwich enzyme linked immunosorbent assay (ELISA) using commercial multispecies kits and haptoglobin was determined according to itsbiochemical activity to bind haemoglobin using commercial colorimetric kits (Tridelta Development, Ireland) in microplates. CRP was measured by solid-phase ELISA assay using commercially available tests (Life Diagnostics, Inc., USA).*

*The evaluation of the concentrations of SAA and Hp showed a trend of higher values in cows with diarrhea, however, the differences were statistically not significant. On the other hand, the concentrations of CRP were significantly higher in healthy animals compared with diarrheic cows ($P<0.001$). These differences may be related to differences in the reactivity among several acute phase proteins, since Hp is characterized by more prolonged response. Thus, haptoglobin may be preferable in the field to evaluate disease processes, especially the course of chronic diseases. One of the reasons for lower concentrations of CRP in cows with chronic diarrhea may be its excessive loss through the gastrointestinal mucosa.*

*Because the pathogenesis of Mycobacterium avium subsp. paratuberculosis infection in cows and clinical manifestation of chronic diarrhea with subsequent protein-losing enteropathy has so far lacked knowledge of their impact on the changes of acute phase protein values, the results of the presented study represent significant widening of the knowledge in this area of research. They suggest a significant effect of chronic diarrhea in MAP seropisotive cows on the changes in the concentrations of some acute phase proteins.*

**Keywords:** COWS, DIARRHEA, PARATUBERCULOSIS, INFLAMMATION, PROTEINS

Chronic diarrhea associated with malabsorption is less frequently documented in older or adult cattle, but represents a great problem on the affected farms causing high economic losses due to decreased milk production, higher risk of early culling, as well as decreased slaughter value [12, 17]. There are several pathogens that may cause chronic diarrhea in adult cattle. Paratuberculosis is one of the most important intestinal chronic progressive granulomatous infections of ruminants with major economic impacts [10]. The disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) through oral infection from forage, water, milk or environment contaminated with faeces containing bacteria from the infected animals [15]. The infection develops into granulomatous lymphadenitis, thickening and oedema of the intestinal mucosa resulting in intermittent treatment-resistant diarrhea [2, 14]. The intestinal absorption of nutrients decreases consequently resulting in malabsorption associated with protein losing enteropathy, and in the advanced stages of the disease in decreased concentrations of blood proteins [16]. On the other site, the infection with MAP may be accompanied by immune and inflammatory reactions of the body, manifested also by the activation of macrophages, release of tumor necrosis factor alpha (TNF-α) and other cytokines, as well as by increased production of acute phase proteins [1, 3]. However, the mechanism of these reactions is not yet completely understood. Similarly, little is known about the effect of chronic

diarrhea on the production of acute phase proteins in cows seropositive for MAP-antibodies (MAP-Ab). Therefore, this study was aimed to evaluate the concentrations of some acute phase proteins in chronic diarrhea associated with paratuberculosis in dairy cows seropositive for MAP-antibodies.

## Materials and methods

Blood samples from 44 dairy cows affected by chronic diarrhea manifesting for more than two weeks were included into this study. The sampled animals were of a low land black spotted breed, Slovak spotted breed and their crossbreeds at the age of 3.5 to 8 years, and were from four conventional dairy farms with similar feeding and management regimes, and occurence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infections in the herd. The evaluated cows were positive for MAP-antibodies. All of these animals showed clinical signs of the disease, characterised by persistent diarrhea, reduced milk yield, loss of body weight, and general muscle wasting. 19 clinically healthy MAP-negative cows without any signs of diseases and in good general condition were selected as control animals. Blood samples were taken from these animals from *v. jugularis* into serum gel separator tubes without any additives or anticoagulants (*Meus*, Piove di Sacco, Italy). Blood samples were centrifuged at 3000 g for 20 min. The harvested serum was dispensed into plastic tubes, and stored at –20 °C until it was analysed.

Immunoenzymatic ruminant MAP-Ab test was used for specific detection of anti-*Mycobacterium avium* ssp. *paratuberculosis* antibodies in blood serum (*Prionics Lelystad*, The Netherlands). To evaluate the changes in selected acute phase proteins the serum concentrations of serum amyloid A (SAA, μg/ml), haptoglobin (Hp, mg/ml) and C-reactive protein (CRP, μg/ml) were evaluated. SAA was assessed by sandwich enzyme linked immunosorbent assay (ELISA) using commercial multispecies kits (*Tridelta Developmet*, Ireland). Haptoglobin was determined according to its biochemical activity to bind haemoglobin using commercial colorimetric kits (*Tridelta Development*, Ireland) in microplates. CRP was measured by solid-phase ELISA assay using commercially available tests (*Life Diagnostics, Inc.*, USA). The absorbancies were read on automatic microplate reader Opsys MR and the results were calculated using the computer software *Revelation QuickLink version 4.25* (*Dynex Technologies*, USA).

The statistical analyses were done with the programme *GraphPad Prism V5.02* (*GraphPad Software Inc.*, California, USA). Descriptive statistical procedures were used to calculate arithmetic means (x) and standard deviations (SD) for each evaluated variable and group of animals. The significance of differences in values between cows with chronic diarrhea and healthy animals, as well as between highly and weaker MAP-positive cows was examined by unpaired *t*-test.

## Results and discussions

The data obtained in healthy and sick cows are presented in table and fig. The evaluation of the concentrations of SAA and Hp showed a trend of higher values in cows with diarrhea, however, the differences were statistically not significant. On the other hand, the concentrations of CRP were significantly higher in healthy animals compared with diarrheic cows (P<0.001). While the concentrations of SAA were approximately 1.7-fold higher in diarrhoic cows compared with healthy ones, the values of Hp were about 2.9-fold higher in sick animals.

*Table*

**Differences in the concentrations of SAA, Hp and CRP between cows with chronic diarrhea and clinically healthy cows (mean±SD)**

| Variables | Groups of cows | | P value |
|---|---|---|---|
| | Chronic diarrhea (n=44) | Healthy (n=19) | |
| SAA, μg/ml | 27.44±45.51 | 15.69±11.85 | n.s. |
| Hp, mg/ml | 0.238±0.374 | 0.080±0.020 | n.s. |
| CRP, μg/ml | 45.45±61.65 | 150.10±72.80 | <0.001 |

*Note*: P value — significance of the unpaired *t*-test, n.s. — not significant.

The differences in the rate of increase of SAA and Hp may be related to differences in the reactivity among several acute phase proteins, since Hp is characterized by more prolonged response. Thus, haptoglobin may be preferable in the field to evaluate disease processes, especially the course of chronic diseases. The behavior of CRP in cattle affected by chronic diarrhea associated with protein-losing was not yet described. One of the reasons

for lower concentrations of CRP in cows with chronic diarrhea may be its excessive loss through the gastrointestinal mucosa.

[7] found an increase of the concentrations of haptoglobin (by 26 %) and serum amyloid A (by 37 %) in cattle infected with *Mycobacterium bovis*. Furthermore, they determined a good prognosis for animals with Hp values between 0.1 and 1.0 g/l. The increase of SAA and Hp might be related to the tissue damage caused by the bacteria. Increased Hp and SAA concentrations were observed also in our study in cows seropositive for MAP-antibodies clinically manifested by chronic diarrhea due to severe inflammatory changes in the intestinal wall. While the concentrations of SAA were approximately 1.7-fold higher in diarrhoic cows compared with healthy ones, the values of Hp were about 2.9-fold higher in sick animals. These differences in the rate of increase may be related to differences in the reactivity among several acute phase proteins, since Hp is characterized by more prolonged response. Thus, haptoglobin may be preferable in the field to evaluate disease processes, especially the course of chronic diseases [9]. The wider range of measured concentrations and higher values of standard deviations suggest that there are great differences in the reactivity of animals to respond to various inflammatory stimuli. Different disease severity might be another reason for wider range of values, i.e. more severe disease processes are accompanied by higher concentrations of acute phase proteins [6]. Elevated concentrations of SAA were found also in patients with inflammatory bowel disease associated with active gut inflammation and in mice exposed to *Mycobacterium avium* subsp. *paratuberculosis* manifested with colitis and weight loss [4, 11]. In ruminants, CRP it is a constitutively synthesised protein, with only a minor increase during disease processes [5]. In the present study, the concentrations of CRP were lower in cows with chronic diarrhea compared with clinically healthy animals. The behavior of CRP in cattle affected by chronic diarrhea associated with protein-losing was not yet described. One of the reasons for lower concentrations of CRP in cows with chronic diarrhea may be its excessive loss through the gastrointestinal mucosa, similarly to typically elevated fecal loss of $\alpha_1$-antitrypsin in human patients, as well as in dogs suffering from protein-losing enteropathy [8, 13]. However, further studies are needed to evaluate the usefulness of CRP in the differential diagnosis of chronic diarrhea in cattle associated with various degree of hypoproteinemia.

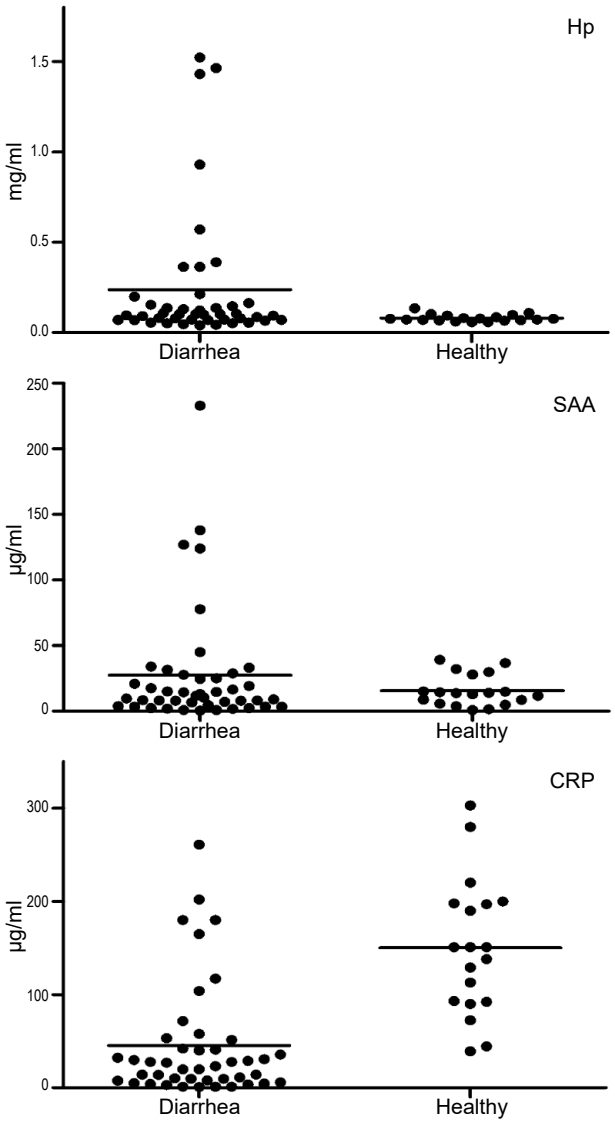


*Fig.* Distribution of individual values of analyzed acute phase proteins in cows with diarrhea and clinically healthy cows

## Conclusions

Because the pathogenesis of *Mycobacterium avium* subsp. *paratuberculosis* infection in cows and clinical manifestation of chronic diarrhea with subsequent protein-losing enteropathy has so far lacked knowledge of their impact on the changes of acute phase protein values, the results of the presented study represent significant widening of the knowledge in this area of research. They suggest an effect of chronic diarrhea in cows seropositive for

paratuberculosis on changes in the concentrations of the evaluated acute phase proteins. The differences in the rate of increase of SAA and Hp may be related to differences in their reactivity and haptoglobin may be preferable in the field to evaluate disease processes, especially the course of chronic diseases. The behavior of CRP in cattle affected by chronic diarrhea associated with protein-losing was not yet described. One of the reasons for lower concentrations of CRP in cows with chronic diarrhea may be its excessive loss through the gastrointestinal mucosa. However, further investigations are needed to establish the diagnostic accuracy of serum protein protein electrophoresis in the differential diagnosis of chronic diarrhea in cattle.

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# EVALUATION OF REPRODUCTIVE PERFORMANCE ON LARGE-SCALE HUNGARIAN DAIRY FARMS

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*The aim of our study was to survey the reproductive performance of replacement heifers and cows in large commercial dairy herds by collecting the most commonly used reproductive indices and to introduce novel parameters to evaluate fertility in dairy units.*

*The authors surveyed the major reproductive indices on 34 large commercial dairy farms from all the statistical regions in Hungary between May and November 2015. Individual data were gathered for 50,396 heifers first inseminated between 1 January 2011 and 31 December 2014, and for 25,672 cows that calved between 1 January 2014 and 31 December 2014 in the surveyed herds. The number of cows covered 14.6 % of the total Hungarian milk recorded Holstein-Friesian cow population.*

*The average (±standard deviation) age at first service, age at first calving and average first service conception rate (CR1) were 15.53±1.59 months, 25.61±2.22 months and 47.10 %, respectively. 8.6 % of the inseminated heifers were culled prior to first calving, at 23.94±3.95 months of age, on average. For cows, calving interval (CI) was 435 days (392–490), CR1 was 26.52 % (11.26–51.40 %), and services per conception (SPC) was 4.04 (2.56–6.16), respectively. The breeding interval (IBI) was 31.38 days (22.00–56.03), and the proportion of reproductive culling was 31.68 % out of all premature disposals (7.57–69.70 %), on average.*

*The use of some relevant parameters (PR, CR1, CCI) is enough for the daily routine, but in-depth analysis is required when the reproductive performance is diminishing.*

**Keywords:** DAIRY CATTLE, REPRODUCTION, REPRODUCTIVE PARAMETERS, PREGNANCY RATE, CALVING INTERVAL

Milk is the primary source of income on commercial dairy farms. In turn, milk production is fundamentally influenced by reproductive performance [13]. In order to evaluate reproduction effectively, adequate reproductive parameters are required [10]. However, evaluation of reproductive performance may vary from farm to farm, because of the lack of consistency in the usage of the reproductive indices [6]. Therefore, the aim of our study was to survey the reproductive performance of replacement heifers and cows in large commercial dairy herds by collecting the most commonly used reproductive indices and to introduce novel parameters to better standardize the evaluation of fertility in dairy units.

## Materials and methods

The major reproductive indices were surveyed on 34 large commercial dairy farms from all the statistical regions in Hungary between May and November 2015. The average herd size was 755 dairy cows (291–2,502) and the average 305-day milk yield was 10,014 kg (8,330–12,541). Individual data were gathered for 50,396 heifers first inseminated between 1 January 2011 and 31 December 2014, and for 25,672 cows that calved between 1 January 2014 and 31 December 2014 from the farm management software RISKA (*Systo Ltd.*, Hungary) in the surveyed herds. The number of cows covered 14.6 % of the total Hungarian milk recorded Holstein-Friesian cow population. Data were managed in *Microsoft Excel 2013* (*Microsoft Corporation*, Redmond, WA, USA). Statistical analyses were performed in *R version 3.3.2* [15].

## Results and discussions

In heifers, age at first service (AFS), age at first calving (AFC), and first service conception rate (CR1) were the most commonly used parameters. However, the parameters of the culled heifers were rarely considered. The major reproductive indices of heifers are shown in table 1. The average CR1 of heifers was 47.10 %. Altogether,

*Table 1*

**The major reproductive parameters of replacement heifers in the studied herds**

| Parameter | Mean | Standard deviation |
|---|---|---|
| Age at first service, months | 15.53 | 1.59 |
| Age at first calving, months | 25.61 | 2.22 |
| Days from first service to culling | 246.25 | 107.10 |
| Age at culling, months | 23.94 | 3.95 |

8.6 % of the inseminated heifers were culled prior to first calving, at nearly two years of age.

In our study, age at first calving was much higher than 24 months, which is generally considered the optimal AFC from an economic point of view. However, the average AFC has decreased by more than two months in the last two decades in Hungary (fig. 1).

The decrease of AFC is probably attributable to the improvement of heifer management practices, e.g. the implementation of oestrus detection aids [4, 5, 15]. However, since the parameters of the culled heifers were rarely taken into consideration, farm managers were not aware of the economic losses that originated from keeping those heifers, which were culled prior to first calving.

For cows, many conventional reproductive indices were widely used, such as productivity, calving interval (CI), calving-to-conception interval (CCI), services per conception (SPC), CR1, breeding interval (IBI), and the percentage of pregnant cows (PP). The major reproductive parameters of cows are summarized in table 2. Large differences were found among the reproductive performance of cows in different herds, since CI, SPC and IBI ranged from 392 to 490 days, 2.56 to 6.16, and 22.00 to 56.03 days, respectively. The average CR1 was 26.52 % (range: 11.26–51.40 %). Culling rate of the herds (mean ± standard deviation) was 29.5±8.2 %. The proportion of reproductive culling was 31.68 % out of all premature disposals (7.57–69.70 %), on average.

*Table 2*

**The major reproductive parameters of cows in the studied herds**

| Parameter | Mean | Standard deviation |
|---|---|---|
| Calving interval, days | 435.2 | 23.7 |
| First service conception rate, % | 26.52 | 9.41 |
| Services per conception | 4.04 | 0.72 |
| Breeding interval, days | 31.38 | 9.83 |

Reproductive indices were much poorer than the most frequently used reference values even in the best herds [6]. Calving interval was more than one month longer than 400 days that is often cited as the realistic goal for large dairy herds [1]. In Hungary, CI increased substantially since the 1970s, however, the increasing trend stopped some years ago and now a decrease in CI can be observed (fig. 2). The improvement of the reproductive performance in dairy cows may be related to the widespread implementation of intensive management practices, e.g. transrectal ultrasonography [3, 7, 8].

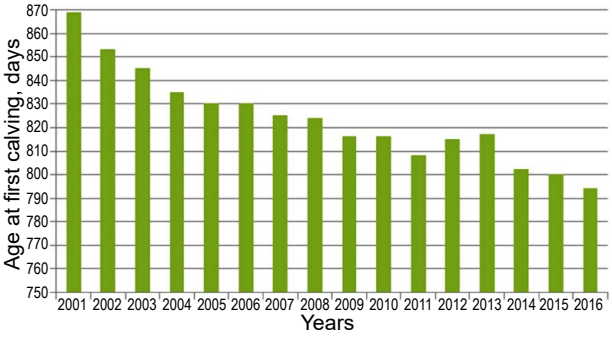


*Fig. 1.* The average age at first calving in Hungarian dairy herds (2001–2016). Source: *National Food Chain Safety Office — Livestock Performance Testing Ltd.*, 2017 [11]

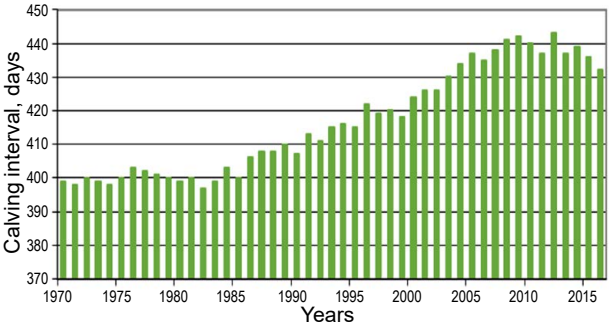


*Fig. 2.* The average calving interval in Hungarian dairy herds (1970–2016). Source: *National Food Chain Safety Office — Livestock Performance Testing Ltd.*, 2017 [11]

However, calving interval should only be used with caution, because this parameter does not take primiparous cows (that make 30–40 % of the herds) into account. Productivity was also a widely used parameter, although, the method of its calculation is heterogenous among farms. Productivity is originally the number of pregnant cows and those cows that calved in the previous 90 days altogether, divided by the number of cows

in the herd, expressed as a percentage [10]. Although SPC is an important parameter of fertility, its role should not be overemphasized, because other parameters, e.g. CCI, are more relevant from an economic point of view. Moreover, if the work of inseminators is evaluated based on SPC, they might inseminate only those cows that will be the most likely to conceive, but in turn, CCI will largely increase, causing serious economic losses.

In North America, heat detection rate (HDR), conception rate (CR) and pregnancy rate (PR) are the most commonly used reproductive parameters in dairy herds [2, 12]. HDR is the number of cows that were inseminated as a proportion of the total number of cows that were eligible for insemination in a 21-day-long period (that is equal to the length of the estrus cycle; [9]. CR is in fact the reciprocal of SPC, and indicates the proportion of successful inseminations (i.e. those that resulted in a pregnancy) within the total number of inseminations. PR is the number of cows that became pregnant as a proportion of those cows that were eligible for insemination during a 21-day-long period. The relationship among HDR, CR and PR can be described by a simple mathematical equation: HDR × CR = PR. Corrected pregnancy rate (cPR), as a novel parameter, was introduced to overcome inaccuracies stemming from the differences of the Hungarian and Northern American culling policies [10]. cPR is the PR calculated for all cows within 200 days in milk. PR and cPR strongly correlate with the traditional measures of fertility, however, carry more relevant and up-to-date information about the performance of the herd [10]. Therefore, besides the widely used traditional reproductive parameters, the use of PR and/or cPR would be very beneficial. Reproductive performance must be evaluated taking several indices into account.

## Conclusions

The reproductive performance of replacement heifers and dairy cows is suboptimal on the Hungarian Holstein-Friesian farms, however, significant improvements can be observed in the recent decades. In order to evaluate the changes in performance effectively, reproductive parameters should be tracked on a regular basis. The use of some relevant parameters (PR, cPR, CR1, CCI) is enough for the daily routine, but in-depth analysis is required when the reproductive performance is diminishing.

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# IMPACT OF *AD LIBITUM* MILK FEEDING REGARDING WEIGHT GAIN AND BEHAVIOUR OF SIMMENTAL CALVES

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*The aim of this study was to find out how ad libitum milk intake affects weight gain, drinking behavior and cross-sucking in simmental calves. Right after birth 97 simmental calves were assigned either to the control group (RES: restricted group, fed only twice a day) or to the test group (ADL: milk was ad libitum available). All calves were housed in single boxes with straw bedding for the first two weeks of life and in group housing after this. Calves were fed colostrum in the beginning and acified milk afterwards. The individual milk intake and bodyweight was recorded, as well as the cross-sucking via video recording.*

*Until weaning the weight gain of the ADL calves was higher than that of the restricted group. This became also evident in the higher weight at the end of the trial. Although the calves of both groups took in the same amount of starter feed the ADL animals had a higher weight. The ADL fed calves drank more often but smaller portions throughout the day. There was no difference in drinking speed between the groups. Regarding the results of this study the ADL drinker increases the cross-sucking of calves kept in groups. The ad libitum drinker represents an animal-friendly feeding method for calves because the animals can perform their drinking behavior longer and more frequently during the day, no hunger periods occur and thus this feeding method better meets the natural behavior. By the higher frequency of milk intake during the day and the therefore higher suckling motivation the ad libitum drinker encouraged the cross-sucking of the calves in groups.*

**Keywords:** CALVES, *AD LIBITUM* DRINKING, CROSS SUCKING, DRINKING BEHAVIOUR

Successful rearing of calves is influenced by different factors such as housing, feeding, hygiene and drinking supply. An optimal drinking supply from the first day of life to at least the 5th week of life has a positive effect on the life performance of cattle [10]. [2, 3, 6] found out that an unrestricted intake of milk in the first 3 or 6 weeks respectively influences the weight gain, the number of sick days and the vitality of the calves in a positive way. Dam calves drink 6–10 times daily with an average milk intake of 8–12 l [1]. Restrictive feeding with buckets 2–3 times daily results in a significantly lower milk intake. Thereby, an early intake of roughage shall be achieved. By short milk intake the natural sucking need of the calves is not satisfied which can result in behavioral disorders like cross-sucking [4, 7]. The new findings concerning the *ad libitum* drinker and the metabolic programming could lead to a revision of the current recommendation for the rearing of calves.

## Materials and methods

The trial was divided into two groups. The first was fed milk restrictedly (RES, control group) and the second was fed *ad libitum* (ADL, trial group). In total, there were 8 groups with 12 calves each, 4 fed restrictedly and 4 fed *ad libitum*. During the first two weeks of life the calves were kept in single boxes interspersed with straw and rehoused afterwards into sections with deep bedding.

From the first day of life the calves were provided with hay, water and concentrated feed. The first 5 days the calves were fed colostrum and afterwards whole milk. During single housing the calves of the control group were fed acified milk in buckets twice daily (1st week of life 2.5 l each time, 2nd week 3 l). The trial group was provided round the clock with buckets of acified milk, each containing 13 l. From the 3rd drinking the drinker was acified with 2.0 ml acid per liter milk to a pH-value

of 5.5. During group housing the individual supply with milk and concentrated feed was controlled by a computerised automat. The drinking plan shows the offered amounts of milk (table 1).

*Table 1*

**Schedule for calves milk intake in the two feeding groups**

| | Restricted feeding | | | *Ad libitum* feeding | |
|---|---|---|---|---|---|
| Day | Beginning [l] | End [l] | Day | Beginning [l] | End [l] |
| 1–7 | 5.0 | 5.0 | 1–28 | *ad libitum* | *ad libitum* |
| 8–14 | 6.0 | 6.0 | 29–42 | 25.0 | 8.0 |
| 15–21 | 6.0 | 8.0 | 43–70 | 8.0 | 0.0 |
| 22–42 | 8.0 | 8.0 | | | |
| 43–70 | 8.0 | 0.0 | | | |

The drinking behavior was examined with 22 calves (11 ADL, 11 RES) from 3rd–14th day of life. The daily milk intake in l, the number of meals per day, the duration of meals in minutes, the total duration of drinking per day in minutes and the drinking speed in l per minute was recorded. To measure the drinking behavior, a special weighing system was developed. Video recordings were taken of 6 groups over 4 weeks each to measure cross-sucking. One sucking-action was defined when the muzzle of one animal had contact to ear, abdomen, elbow or genital area of another animal and simultaneously performed a head butt and/or overstretched its neck area. The contact had to last a minimum of 5 sec. The sucking act ended when there occurred no more sucking for a minimum of 10 seconds.

## Results and discussion

***Milk intake and weight development.*** During the first 4 weeks the ADL animals took in 2.1 l of milk more than the calves of the RES group. Between the 28th and 42nd day the weaning of the ADL animals started and from day 43 on both groups were weaned beginning with 8 l (fig. 1). During the first 4 weeks, the medium daily weight gain of the ADL animals was 300 g higher than that of the RES group. Therefore, not only the weight gain but also the end weight of the ADL animals was higher (fig. 2).

[4] showed that calves are able to take in a great amount of milk in one meal.

The capacity of the abomasum in their study was 6.8 l. In the present study this quantity was confirmed. Some calves could take in even more, e.g. one calf drank 7.2 l on the 14th day of life.

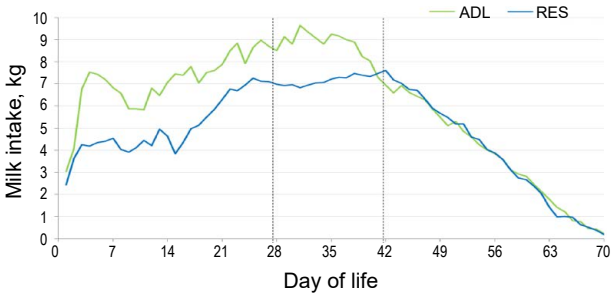


*Fig. 1.* Milk intake of *ad libitum* and restricted fed calves during the first 10 weeks of life

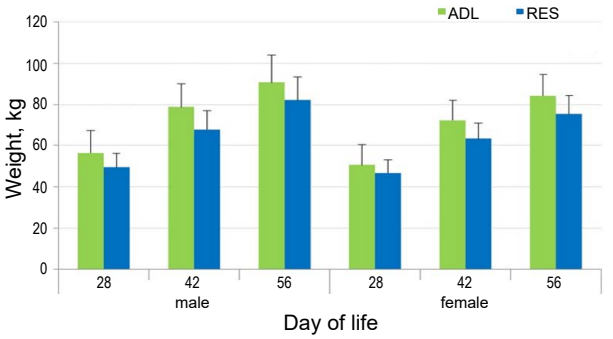


*Fig. 2.* Development of live weight of *ad libitum* (ADL) and restrictive (RES) fed calves in dependence upon gender

In week 5 and 6 the effect of weaning was noticeable in the ADL group. The weight gain sank and adjusted to that of the control group.

***Drinking behavior.*** Calves with unrestricted milk supply took in nearly twice the amount of milk compared to the control group during the first 2 weeks of life (ADL: 7 l; RES: 4 l) and drank twice as often during the day (ADL: 6.6; RES: 3.6). This does not comply with the number of meals of 9 to 10 times that a calf shows in the presence of the dam [11] but it becomes clear that calves take in more than 2 meals when given the possibility.

The duration of an average ADL meal was significantly shorter than that of a RES calf (ADL: 4.6 min.; RES: 6.7 min.). Concerning the total duration of drinking during the day, the ADL calves spent significantly more time for their meals than the RES animals (ADL: 26.2 min.; RES: 16.4 min.). The standard deviation was strikingly high, 21.1 min. during the first week with the ADL animals. These points to the great individual differences in the drinking behavior between the calves. No considerable differences in drinking intensity (drinking speed) could be detected between the groups in the first or second week, unlike [9], who found that in the 3rd week the restrictively fed

*Table 2*

**Drinking behavior (frequency and duration of milk intake, total drinking duration, intensity of drinking) of *ad libitum* (ADL) and restrictive (RES) fed calves in the first and second week of life**

| | Week 1 | | Week 2 | |
|---|---|---|---|---|
| | ADL | RES | ADL | RES |
| Milk intake, l/day | $7.0^{a}$±2.8 | $4.2^{b}$±1.8 | $7.2^{a}$±2.9 | $4.1^{b}$±1.9 |
| Frequency of drinking, n/day | $6.5^{a}$±3.9 | $2.1^{b}$±0.8 | $6.7^{a}$±3.3 | $3.3^{b}$±1.9 |
| Duration of meal, min | $4.8^{b}$±2.6 | $8.2^{a}$±3.5 | $4.3^{b}$±2.5 | $5.5^{a}$±2.6 |
| Total drinking duration per day | $28.0^{a}$±21.1 | $16.2^{b}$±7.3 | $24.3^{a}$±12.6 | $16.5^{a}$±10.1 |
| Intensity of drinking, l/min | $0.31^{a}$±0.13 | $0.29^{a}$±0.15 | $0.34^{a}$±0.17 | $0.29^{a}$±0.16 |

calves had a higher drinking speed of 0.41 l/min than the *ad libitum* calves with 0.35 l/min.

As the restrictive drinkers were supplied only twice a day the motivation for milk intake at the meals seems to have been so high that a higher drinking speed occurred in their study. The more frequent visits at the drinking automat and the higher drinking speed of the restrictively fed calves are behavior patterns that point to the unfulfilled need of milk intake.

***Cross-sucking.*** Cross-sucking occurred from introducing the calves into group housing on day 15. Almost exclusively the genital region was sucked. There was no difference between the genders. Most of the sucking occurred immediately after milk drinking. The intensity of sucking behavior was individually different (0–7 times/day and calf). Comparing the groups, the calves of the ADL group sucked their box companions more than twice as often as the calves of the RES group (ADL: Ø 2.46/day; RES: Ø 1.04/day).

Cross-sucking is a common problem of the motherless rearing of calves that on the one hand leads to health problems and on the other hand to sucking at lactating cows at a later age of the heifer [8]. In contrast, cross-sucking does hardly ever occur in a mother-bound rearing [5].

## Conclusions

The *ad libitum* drinker is animal friendlier because the calves can perform their drinking behavior longer and more often throughout the day, no hunger times occur and therefore this housing form better meets the natural behavior. It remains to be clarified which housing measures can be taken to fulfill the sucking motivation following the milk meals of the *ad libitum* drinker.

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# HERD HEALTH MANAGEMENT IN THE TRANSITION PERIOD

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*Transition cow diseases are a multifactorial complex. Veterinaries need reliable indicators to identify risk animals, take treatment decisions or monitor the metabolic state of the herd. The identification and development of prognostic markers, accompanied by sound metaphylactic treatment protocols are needed.*

*For the trial 80 German Holstein dairy cows (≥2nd lactation, clinically healthy and pregnant) were selected from the herd. The study included an intense analysis of each animal from 14 days ante-partum until 49 days post-partum: daily milk yield, monthly milk content analysis, clinical state throughout the trial, ultrasonography of the liver and back-fat tissue measurement, liver biopsies, blood and urine sampling, rumination and locomotion behaviour. To evaluate a metaphylactic treatment protocol with Butaphosphan and Cyanocobalamin two groups received a treatment with Catosal® at either a low or high dosage (5 ml and 10 ml/100 kg body weight, 10 % Butaphosphan and 0,005 % Cyanocobalamin) and two placebo-groups were formed (5 ml and 10 mL NaCl 0,9 %/100 kg body weight).*

*We identified "high risk" animals based on their metabolite profiles and that these metabolic alterations were already present prepartum. The cows in the spring-calving group exhibited higher clinical scores (e.g. concerning the genital tract, the gastro-intestinal tract and treatment frequency), fat accumulation in the liver and higher serum fatty acid concentrations, indicative for a more pronounced energy deficit in this group. By the analysis of each group separately at the separate time points the effect of the treatment with Butaphosphan and Cyanocobalamin emerged. In the "high-risk" group a long-lasting effect (day 28 postpartum, 3 weeks after treatment) was observed.*

*Further analysis is needed to identify the metabolites involved in the alterations observed across the transition period, as well as describing "high-risk" animals and treatment effect with Butaphosphan and Cyanocobalamin and bringing the observed metabolic alterations on a production level.*

**Keywords:** COWS, TRANSITION PERIOD, PREGNANCY, LACTATION, DRY PERIOD

Dairy cow's metabolism undergoes, as in every female mammal, dramatic metabolic changes throughout the period of gestation, giving birth and the onset of lactation (= the transition period) [23, 24, 31].

During the dry period a ration comparably low in energy and protein content is fed and in the common free-stall housing system the daily demand for exercise is relatively low, as well as the contact with humans [18]. At the happening of parturition this situation changes dramatically. The birth itself is comparable to an extreme exertive physical effort, such as running a marathon. However, further accompanied by massive tissue damage due to the expulsion of the foetus [2, 8, 11]. The event of calving does not only implicate physical stress but also affects the animal on various psychological levels. In most housing/management systems calving implicates the separation of the animal from its cohort into a new one (calving pen) and therefore a new environment, various handling procedures by humans (e.g. pushing, calving assistance and milking) plus the pain and unexpected physical happenings during delivery may induce fear and stress [5, 8]. The onset of milk production further causes massive alterations in the mammary tissue. The sudden increase in nutrient and energy demand for milk production causes the re-routing and excessive strain of various metabolic pathways [24].

A physiologically normal negative energy balance is observed during this time, since the animal is not able to adequately increase its feed intake to meet the energy demand caused by the ongoing tissue recovery and milk production [25]. This negative energy balance causes lipomobilisation from the fat depots generated in the previous lactation and dry period [27]. In this transition period the liver plays a key role, responsible for metabolizing the non-esterified fatty acids (NEFA)

originated from mobilized triacylglycerols (TAG), in the beta-oxidation cycle to acetyl-CoA, which is either entering the Krebs-Cycle or being metabolized to ketone bodies [9, 13, 22]. The liver metabolism is therefore accelerated within a few days from a very low-demanding state to the highest demand in metabolic capacity throughout the lactation cycle [5]. Further on, the onset of lactation causes a shift in the mineral household. If not adequately prepared for this situation during the dry period, the risk for imbalances like hypocalcaemia increases [12].

But as mentioned above, the cow is not only confronted with these massive metabolic alterations, but also needs to adapt to a new social structure and housing system in the fresh cow pen, as well as to the new daily routines (e.g. milking, feeding times) [6, 7]. If this area is not adequately designed and managed within the farm's daily routines the cows daily feed intake and resting behaviour will be insufficient [17, 20].

This component is even more accentuated when health problems are already present, such as lameness, preventing the animal from a normal activity and therefore further decreasing the dry matter intake (DMI) [10].

This short summary of factors and aspects illustrates that transition cow diseases are a multifactorial complex. In our modern total confinement free-stall housing systems four main influencing factors are identified: nutritional imbalance, lameness and deficiencies in housing and management (e.g. stocking density, time budgeting, feed supply, professionalism in milking, animal monitoring and general husbandry) [29].

The named factors lead to nutritional imbalances with decreased DMI causing a negative energy balance in a unphysiological magnitude, leading to an insufficient energy supply to support tissue healing and immune defence against infectious diseases [19, 30].

The result is an array of different production diseases which are all somehow interrelated and often with a synergistic action [27]:

— the genital tract is unable to re-shape and heal properly leading to retained placenta, lochiometra, metritis, endometritis [16];

— at the mammary gland severe udder oedema, mastitis and udder eczema may be observed;

— in the gastrointestinal system ruminal microbial fermentation, and peristaltic and absorption processes at various locations (forestomach, abomasum and lower intestines) are disturbed causing malabsorption of nutrients, absorption of toxins, abnormal gas production leading to different clinical signs such as diarrhoea and a displaced abomasum [3];

— a dysregulated mineral household, exhaustion and endotoxemia cause muscle weakness and circulatory problems leading to the inability to arise [12, 21];

— extreme lipomobilisation may lead to ketosis and an overload of the liver with fatty acids (fatty liver syndrome) [15, 24];

— the decrease in the sole fat cushion due to excessive lipomobilisation increases the risk for sole ulcers and an endotoxemia (due to a metritis, mastitis or ruminitis) may induce laminitis [1, 4];

— but also other organ systems such as the lung are at a higher risk for infectious diseases due to the general immune-suppression [19].

As described above, the liver holds a key function in this aspect by metabolizing the fat reserves and thereby supplying the body with energy in this critical period [5, 24]. Observations from farmers and veterinarians, also confirmed by different studies, show that certain cows seem to be more metabolically robust than others [14, 26]. However, underlying pathomechanisms and the reasons for this individual susceptibility are not clear.

To solidly consult their costumers, veterinaries in the field need reliable indicators to identify risk animals, take treatment decisions or monitor the metabolic state of the herd — ideally implemented in cow-site tests. Therefore, the identification and development of prognostic markers, accompanied by sound metaphylactic treatment protocols are needed.

The aim of the study presented therefore is to:

— investigate pathomechanisms in the transition dairy cow disease complex with a special focus on the liver fat metabolism;

— identify possible prognostic markers;

— develop non-invasive methods to determine the liver fat content by ultrasound;

— test a metaphylactic treatment protocol with *Butaphosphan* and *Cyanocobalamin* (*Catosal*®, Bayer).

## Materials and methods

An on-farm randomized, prospective, three-fold blinded study was performed on a 660-cow dairy in Saxony (Germany), between November 2015 and November 2016. The cows were housed in TMR-based free-stall system with deep bedding boxes during lactation and deep bedded straw pack during the dry period. During the spring and summer period the dry cows were allocated on pasture. The herd was characterized by an average milk production per lactation of 10,744 kg and a fat and protein content of 3.74 % and 3.33 % during the 12 months of the trial duration.

For the trial 80 German Holstein dairy cows were selected from the herd. Inclusion criteria were: ≥2nd lactation, clinically healthy and pregnant. The average lactation number of the selected animals was 3.9±1.8 (mean ± SD) at the calving in the trial and the 305d milk production in previous lactation was 10,944±2,013 kg. The study included an intense analysis of each animal from 14 days *ante-partum* until 49 days *post-partum*.

To evaluate a metaphylactic treatment protocol with *Butaphosphan* and *Cyanocobalamin* following treatment groups were established: two groups receiving a treatment with *Catosal*® at either a low or high dosage (5 ml and 10 ml/100 kg body weight (BW), 10 % *Butaphosphan* and 0,005 % *Cyanocobalamin*) and two placebo-groups (5 ml and 10 mL NaCl 0,9 %/100 kg BW). The animals were treated at six time points: –7/–6/–5 prepartum and 1/2/3 days postpartum.

To gain a sound and encompassing data set to describe the metabolic and production state of the animals throughout the trial following aspects/variables were documented, sampled and analyzed:

— exact documentation of the production state through daily milk yield measurements and monthly milk content (fat%, protein%, urea and somatic cell count) analysis;

— daily exact documentation of the clinical state throughout the trial using standardized clinical examination and scoring protocols;

— ultrasonography of the liver and back-fat tissue measurement (7x throughout the trial);

— liver biopsies (4x, -14 d, 7, 28 and 32 days peripartum) for fatty acid fraction analysis, histopathology;

— blood sampling (8x) for fatty acid pattern, clinical chemistry, haptoglobine concentrations;

— urine sampling (15x) for clinical chemistry;

— rumination and locomotion behaviour of the animals.

## Results and discussion

Especially cows in the group that have calved into the study in spring are conspicuous since their metabolic state seems to be altered to a much lower degree across the calving, compared to animals entering the study in winter 2015 and summer/autumn 2016. Analysis of the ration documentation revealed the feeding of different grass silage silos in the identified time periods, hinting towards an influence of the silo quality on the energy and nutrient supply of the animals.

When analysing the identified groups in regard to their clinical scores, clinical chemistry and histopathological data a clear differentiation according to health status was observed. The cows in the spring-calving group exhibited higher clinical scores (e.g. concerning the genital tract (metritis, endometritis), the gastro-intestinal tract (abnormal feces, abomasal displacement) and treatment frequency), fat accumulation in the liver and higher serum fatty acid concentrations, indicative for a more pronounced energy deficit in this group. It was therefore concluded that these animals may be classified as "high-risk" cohort due to their exposure to an inadequate feed quality. These differences in metabolic state were already present prepartum in the "high-risk" group.

By the analysis of each group separately at the separate time points the effect of the treatment with *Butaphosphan* and *Cyanocobalamin* emerged. In the "high-risk" group a long-lasting effect (day 28 *post-partum*, 3 weeks after treatment) was observed.

## Conclusion

The first preliminary results showed that we were able to identify "high risk" animals based on their metabolite profiles and that these metabolic alterations were already present prepartum. Further statistical analysis of the dataset is needed to identify the metabolites involved in the alter-

ations observed across the transition period, as well as describing "high-risk" animals and treatment effect with *Butaphosphan* and *Cyanocobalamin* and bringing the observed metabolic alterations on a production level.

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# A CASE REPORT: SHEEP ENDOPARASITISM DYNAMICS UNDER SEMI-DRY CONTINENTAL CLIMATE OF KARCAG, HUNGARY

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*The main aim of the study was to have a preliminary assessment of the endoparasites which are infecting sheep under semi-dry continental climate in Karcag, Hungary.*

*Two groups of Hungarian Merino sheep were assigned as treated (N=40) and untreated (N=20) after selecting the animals randomly. Only the Treated group was drenched two times with commercially available deworming drugs. Faecal samples were collected individually from both the groups to perform faecal egg count; body condition score and FAMACHA scores were also taken to assess body and anaemia conditions respectively. The temperature and humidity conditions were also obtained to check the optimum environmental condition that could influence the worm burden. The case study was done at the University of Debrecen Experimental Animal Farm, Karcag.*

*Parasitic nematodes, namely, trichostrongylid/trichostrongyles nematodes, Protostrongylus sp. and Strongylus sp. were found to be more predominant species affecting the sheep and up to some degree with the tapeworm Moniezia sp. and the coccidian, Eimeria sp. During the study period, there was no clinically significant anaemic condition and the animals were found to have fairly good body condition. Yet, the infection intensity, mainly of the trichostrongyles, was significant even after the second drenching. This may be due to the optimum environmental condition coinciding with the grazing period which increased the parasitic loads in the fields. Another possibility is the presence of resistant worm population as there is no proper assessment of the effectiveness and/or resistance of the drenching drugs commonly used in Hungary.*

*The study is only a preliminary report of endoparasitism of sheep under a particular Hungarian climatic condition. It is obvious that parasites, mainly of the nematodes, do occur and may be an increasing concern with time. Keeping this in mind, there is a need for a wider prevalence study and if possible, anthelmentic resistance studies of the commonly used drugs to check their efficacies.*

**Keywords:** SHEEP, ENDOPARASITE, NEMATODES, SEMI-DRY, ENVIRONMENT

Small ruminats such as sheep and goats are extremely susceptible to endoparasites. The endoparasites that are commonly found globally in sheep and goats are: *Dictyocaulus* sp. or *Muellerius capillaris* (lung worms); and the important *Trichostronglidae* family like *Haemonchus contortus*, *Ostertagia ostertagii*, and *Trichostrongylus axei*; *Fasciola hepatica* (liver flukes); and coccidia such as *Eimeria* or *Isospora* [6]. The cost of control of this problem in sheep industry was estimated to be tens of billions of dollars globally [5]. The health condition of sheep is also affected by the climate change, which further can give rise to appearance of parasite species that might not have been significant before [1]. Climate also influences infective larval availability and subsequently the rates of infection, through direct effects on the development and survival and translation of larvae onto pasture. Parasitic infection is inevitable in grazing sheep and the control of disease is generally done commonly by the use of anthelmintic drugs, known as drenching, to suppress egg output and consequently reduce the infection pressure. The intensive farming system with shared grazing pastures also increase the parasitic load of the grazing grounds. The overuse of anthelmentics and in inappropriate dose may also give rise to resistance. The spread and increasing prevalence of anthelmintic resistance threatens the feasibility of this approach and the sustainability of parasitism control in sheep [3].

Hungary generally has a semi-dry continental climate [2] and the knowledge of animal parasitism in this conditions is quite less. As of the moment

in Hungary, there is only a few published information on endoparasitism. Only [4] reported the status of haemonchosis and the subsequent benzimidazole resistance in the south-western part of Hungary. Moreover, there are no standard treatment protocols against parasitic infections with most of the farmers are treating randomly and not according to the real infecting parasite species. Thus, there is a need to have a proper prevalence study of parasites infecting sheep industry in Hungary. The present study is a preliminary report keeping in view of this.

## Materials and methods

The presented study was performed from December 2017 till October 2018 at the University of Debrecen Experimental Animal Farm, Karcag. Two groups of Hungarian Merino sheep were assigned as treated (N=40) and untreated (N=20) after selecting the animals randomly. The treated group was drenched twice, that is, in December 2017 and May 2018 using commercially available ivermectin and levamisole as per the manufacturer's instruction. Faecal samples were collected individually from both the groups along with Russell body condition score (BS) and FAMACHA (FM) during December 2017, March, May, July and October 2018. The collected faecal samples were used to perform faecal egg count (FEC) by modified McMaster technique. The BS and FM score were taken to assess body and anaemia conditions respectively. The temperature and precipitation levels were also obtained from the meteorology department. From the FEC data (not shown), we determined the infection percentage (infected animals/total×100) and the infection level (weighted infection/infected individuals). Correlation analysis between BS and FM; variance analysis for the infection level of the different parasite species, FS and BS between the two groups were performed with *MS Excel* and *SPSS 13.1*.

## Results and discussion

The main infecting parasites was found to be of trichostrongylids, *Strongyloides* sp., *Protostrongylus* sp., up to some extent with *Moniezia* sp., and *Eimeria* sp. (table). The infection level varied as per the season and interpreted as light, moderate and heavy.

*Table*

**Infection percentage and infection level between the groups**

| | | Untreated | | Treated | |
|---|---|---|---|---|---|
| | | Infection, % | Infection level | Infection, % | Infection level |
| 2017/12 | *Protostrongylus* | 27 | light | 14 | light |
| | *Trichostrongylid* | 70 | moderate | 10 | light |
| | *Strongyloides* | 20 | moderate | – | – |
| | *Moniezia* | 17 | light | – | – |
| | *Eimeria* | 7 | light | – | – |
| 2018/03 | *Protostrongylus* | 19 | light | 30 | light |
| | *Trichostrongylid* | 23 | light | – | – |
| | *Strongyloides* | 23 | light | – | – |
| | *Moniezia* | – | – | – | – |
| | *Eimeria* | 12 | light | 15 | light |
| 2018/05 | *Protostrongylus* | 19 | light | – | – |
| | *Trichostrongylid* | 77 | moderate | 10 | light |
| | *Strongyloides* | 15 | light | 10 | light |
| | *Moniezia* | – | – | – | – |
| | *Eimeria* | – | – | – | – |
| 2018/07 | *Protostrongylus* | – | – | – | – |
| | *Trichostrongylid* | 100 | moderate | 40 | light |
| | *Strongyloides* | 20 | light | – | – |
| | *Moniezia* | – | – | – | – |
| | *Eimeria* | 50 | light | 25 | light |
| 2018/10 | *Protostrongylus* | – | – | – | – |
| | *Trichostrongylid* | 62 | light | 36 | light |
| | *Strongyloides* | 23 | light | 18 | light |
| | *Moniezia* | – | – | – | – |
| | *Eimeria* | 27 | light | 9 | light |

The trichostrongylid parasites were found to occur in the highest proportion throughout the whole study period for the untreated group and even reached a 100 % infection rate in July 2018, which means all the test animals had this worm burden. Yet the infection level was moderate and did not result in any mortality. In the same group, the *Protostrongylus* sp. infection was variable and was not at all detected after the second drenching. *Strongyloides* sp. remained consistently below 25 %. In case of the treated group, the infection seemed fairly under control except for the trichostrongylids, which saw a jump in the infection rate after the second drenching just as in the other group. This rise could be due to the exposure in the pasture which might have resistant worms (fig. 1). In both the groups, *Monieszia* sp. and *Eimeria* sp. were found to be variable and of little importance as in both the groups, their infection rates are fairly low all throughout except in July 2018 for which *Eimeria* sp. recorded up to 50 % in the untreated group.

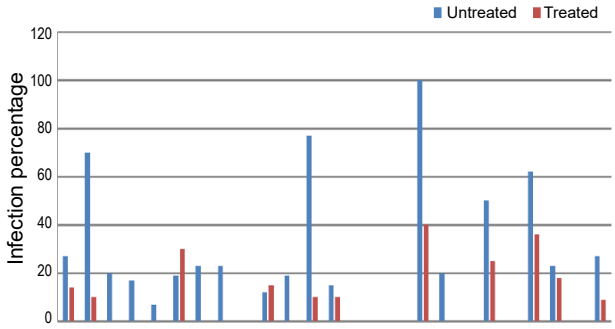


*Fig. 1.* Infection information of the parasites and drenching time

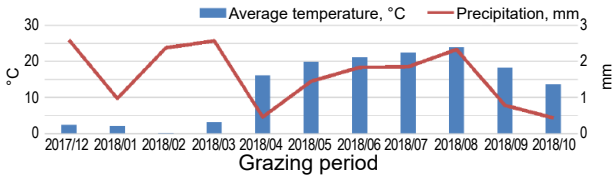


*Fig. 2.* Average temperature and precipitation conditions of during the study period

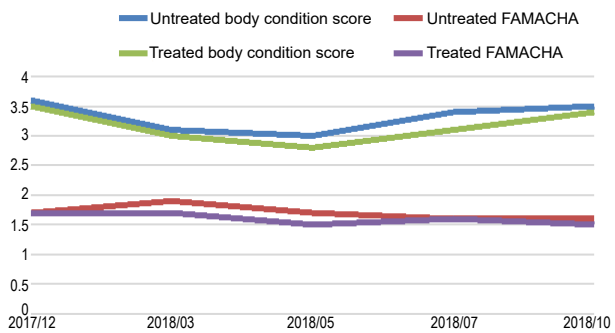


*Fig. 3.* Average Russell body conditional score and FAMACHA score

Between the two groups, there is a significant difference (P=5 %) for the trichostrongylid worms in December 2017, May and July 2018. Yet, it can also be seen that infection levels of the parasites was still high even after the second drenching. This may be attributed to the rising temperature and precipitation (fig. 2). This gave an optimum environment for the infective larva from the already contaminated pasture and infect the grazing animals.

There is no correlation between the BS and FM scores as well as no significant difference (P=5 %) between the two groups for both the scores as seen from fig. 3. These gave an idea that the animals were healthy and no severe anaemia was detected. Haemonchosis may be ruled out in the study as no severely anaemic animal was recorded as well as any clinical signs suspicious of *Haemonchus contortus* were never found during the study period. Nevertheless, a differential diagnosis for the trichostrongylids is necessary.

## Conclusions

From this preliminary study, it can be seen that parasitic nematodes are fairly common, the main concern being the trichostrongylid nematodes. Even though the animals presented a good body conditions, the study indicated that the animals harbour these parasites and keep on contaminating the grazing grounds. This affirms that the semi-dry conditions of the study area can still support the parasites albeit in lesser degree. In addition to this, anthelmintic are given without a proper examination of the animals whether drenching is required or not, as usually done by the sheep farmers Thus, this may contribute to anthelmintic resistance in the near future.

**Prospects of future research.** The prospective future researches which can be done from here are:

I. a proper prevalence study of endoparasites of sheep in Hungary climate as so far there is no such published data;

II. accurate and economical differential diagnosis of the trichostrongyles is also needed for proper treatment as this group of parasite is most important;

III. resistance study commonly used drugs;

IV. pasture management and other alternative controls.

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# THE EFFECT OF THE QUANTITY AND QUALITY OF MILK REPLACER INTAKE ON STARTER FEED INTAKE IN HOLSTEIN CALVES

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*The aim of this study was to evaluate the effects of increasing amounts and quality of liquid feed of calves on starter feed intake in real farm situations.*

*Three calf rearing system were compared. We offered different type and amount of solids content of liquid feed by using whole milk and two different milk replacer (MR). The dry matter intake in group A, B and C were accordingly 898; 1033; 1050 g/calf/day. In group A whole milk and MR1 were used, instead of group B and C where MR1 or MR2 were used exclusively. The MRs differed in CP level (21 % vs. 27 %), in proportion of palm/coconut oil (80/20; 60/40) and in technology of production (spray drying vs. spray cooling).*

*The statistical analyses shown strong connection between the amount and quality of milk replacer intake and starter feed intake ($P<0.001$) before, under and after the weaning period. Between week 2 and 7 the calves in group C ate three times more starter than members of group A and B (129 g/day; 135 g/day; 464 g/day). This difference remained significant later on too. The data of group B differs significantly ($P<0.05$) only the 9$^{th}$ week of life from group A. The feeding systems also effect changes in BW ($P<0.01$) and ADG ($P<0.001$).*

**Keywords**: CALVES, FEED INTAKE, MILK REPLACER QUALITY, FEED INTENSITY, DRY MATTER INTAKE

Creating the conditions necessary for a successful early weaning is an especially important criterion in calf nutrition. To meet this objective, the development of the rumen must be supported by the use of appropriate technologies. Before weaning, the calf must be able to successfully prepare for the intake of adequate amounts of dry matter and should have a sufficiently mature rumen to allow the efficient utilisation of feed. If the calf is weaned before it meets the above conditions, this inevitably results in lower performance and slower body weight gain [3]. According to many research and farm practices, the conventional feeding programs cause a higher starter feed intake in dairy calves. [1, 2, 5, 7] report about the disadvantages of providing more milk or milk replacer include reduced solid feed intake during the milk-feeding period. After research of [4], the increase in calves' weight gain has come to the fore. If we focus on the development of rumen only in the week of choice, we increase the incidence of health problems caused by choice. A smooth transition from liquid feed (milk or milk replacer) to solid feed (grains or forage) is important in minimizing weight loss and distress at weaning [6].

According to the challenge of the age, we need to find solutions for calf rearing that provide high growth strength and high starter feed intake at the same time. We want to contribute to this problem solving with the data measured by us in working farm situations.

## Materials and methods

***Experimental animals and housing.*** We compared data measured under different liquid feeding system in calf rearing on a HF dairy farm (1800 cows). 30, 45 and 20 HF heifers were used in the three different groups (A, B, C). The calves received 3.5 litres of colostrum via an oesophageal tube within 2 hours of birth. 12 to 24 hours after birth, the calves were transferred from the calving barn to the calf rearing unit, where they were placed in individual straw bedding calf hutches (*Calf-Tel Pro II*, *Hampel Corporation*, Germantown, Wisconsin, USA) in the order of their birth.

***Feeding.*** The calves received liquid feed twice a day from bucket at 12 hours intervals. All of the groups we used the same feeding and weaning method: increased the amount of liquid in the first weeks (A and B for 3 weeks, C only for 1 week),

*Table 1*

**The liquid feeding systems in groups**

| | A | B | C |
|---|---|---|---|
| Base of the liquid feed | 1/3 whole milk + 2/3 MR1 | MR1 | MR2 |
| CP/Fat content in MR | 21/17 | 21/17 | 27/17 |
| Dilution ratio | 12,5 % | 14,5 % | 14 % |
| Average solid feed intake from liquid, g/calf/day | 898 | 1033 | 1050 |
| Palm oil / coconut oil ratio, % | 80/20 | 80/20 | 60/40 |
| Technology of production | spray drying | spray drying | spray cooling |

then we decreased the amount of liquid on the 8th week of life and we finished the liquid nutrition at the start of week 9. In group A from day 1 to day 21 only MR1 were used and after 21 day we fed with a mix of 2/3 MR1 and 1/3 whole pasteurized milk. In group B and C we used only MR in whole experimental period and we used a higher dilution ratio, to compare the effects of the dry matter intake from liquid feeding. The main components of both milk replacers were whey powder and 15 % skimmed milk powder but they differ in CP level, in proportion of palm/coconut oil. They also differs in technology of production, which can modify the digestibility of the ingredients. The MR1 (*Sprayfow Yellow*, *Trouw Nutrition*, Sloten, The Netherlands) were made with spray drying system, where the added fat forms a uniform coating around the protein. The MR2 (*Nukamel Performer*, Weert, The Netherlands) were made with spray cooling system, which means the added fat forms a cross-linked structure around the protein, making it easier to digest. The differences between the liquid feeding systems in the study are shown in table 1.

Drinking water was available from the first day, while calf starter from day 7 *ad libitum*. The nutrient content of starter diets (*UBM Feed Ltd.*, Hungary) is shown in table 2.

***Samplings and measured parameters.*** Individual starter feed intake was recorded for all animals every day. For ease of comparison, the feed intake was averaged weekly for each group. All calves were measured after the birth. Since the data of the 3 groups here are from 3 different experiments, the times of weight measurements

*Table 2*

**Nutrient content of starter diets, % (as specified by the manufacturer)**

| Nutrient content, % | |
|---|---|
| Moisture | 11.19 |
| Dry matter | 88.81 |
| Crude protein | 20.19 |
| Crude oils and fats | 4.49 |
| Crude fibre | 10.40 |
| Crude ash | 6.44 |
| Total sugar | 8.01 |
| Starch content | 14.22 |
| NDF | 32.41 |
| ADF | 14.26 |
| N-free extract | 45.50 |
| NEm, MJ/kg | 7.43 |
| NEg, MJ/kg | 4.84 |

are not the same in the groups. Group A was measured after one week of the weaning, while calves from group C when we finished the liquid feed. The time in days of the last weight measured is seen in table 3 in the results section.

***Statistical analyses.*** Statistical analysis was done using the *R Commander 3.4.1* program type (*Free Software Foundation Inc*., 1991). One-Way ANOVA and Kruskal-Wallis procedures of the programme were use for analysing and compare the variances. Differences were considered as significant if $P<0.05$.

## Results and discussion

***Starter feed intake.*** The fig. 1 shows the feed intake of the groups. The statistical analyses shown strong connection between the amount and quality of liquid feed and starter feed intake

*Table 3*

**Body weight and average daily gain in groups**

| | Groups | | | Statistical analyses | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | P | SEM | CV | A-B | B-C | A-C |
| Weight at birth, kg | 39±3 | 38±4 | 39±4 | | 0.37 | 0.09 | | | |
| Weight at end, kg | 74±7 | 77±7 | 81±7 | <0.01 | 0.76 | 0.09 | | 0.07 | <0.01 |
| ADG | 550±93 | 624±106 | 769±99 | <0.001 | 13.36 | 0.20 | <0.01 | <0.001 | <0.001 |
| Age of measure, day | 64 | 60 | 56 | | | | | | |

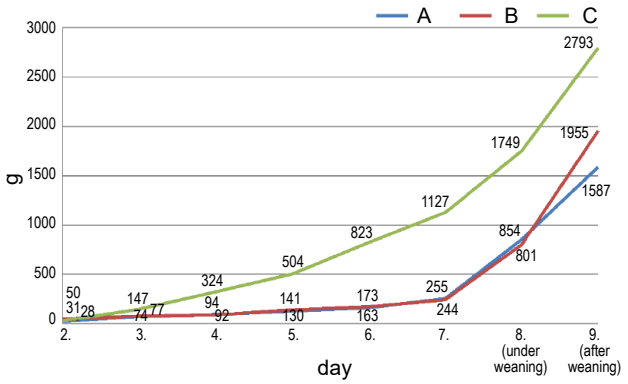


*Fig.* Feed intake of the groups

(P<0.001) before, under and after the weaning period. Except the 2nd weeks of life, the starter feed intake of group C was significant higher (P<0.001) than other groups. The data of group B differs significantly (P<0.05) only the 9th week of life from group A.

To compare the line of A with B, it can be stated that higher intake of dry matter may increase the starter feed intake after stopping liquid feeding, but has no effect on the consumption during the drinking time. This can be explained by the increased body weight caused by more intense nutrient supply and thus by the greater need for it. To compare the line of B with C, it can be seen that, with the same dry matter intake, the milk replacer that has a better digestibility due to its production technology increases the uptake of the starter feed even at higher protein intake. If we think about the rumen development as one of the most determinative thing in the calves rearing, the consistent starter feed intake like in group C is more favorable. In group A and B only one week is available for the rumen to prepare for that amount of starter feed which can be supply the life and growth needs without the nutrients which came from the liquid feeding.

***Body weight, daily weight gain.*** Regardless of the measurement at different times, the difference between the groups is clearly visible in table 3. Nowadays, the basic goal of calf rearing is to double the birth weight by the time of the weaning. Due to the higher protein level and better digestibility the group C reach higher ADG and BW in less time than group B, altough they got the same solid feed intake from MR.

## Conclusions

According to our data, the digestibility and composition of the milk replacer have an effect on preweaning starter feed intake. The increased daily gain due to the higher protein and dry matter intake from milk replacer, can effect on starter feed intake under and after the weaning period. Creating the conditions necessary for a successful early weaning is an especially important criterion in calf nutrition. To meet this objective, the development of the rumen must be supported by the use of appropriate technologies.

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# QUANTITATIVE INSULIN SENSITIVITY CHECK INDEXES IN EARLY POSTPARTUM COWS AND CALVES KEPT IN A BEEF SUCKLER SYSTEM

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*The aim of our study was to determine indexes of insulin resistance (IR) in early postpartum period in cows and their calves kept in a beef suckler system. The study was conducted on 13 Simmental cows and their calves. We calculated following indexes values: RQUICKI (Revised Quantitative Insulin Sensitivity Check Index) and its modified version $RQUICKI_{BHB}$, HOMA (Homeostatic Model Assessment) and QUICKI (Quantitative Insulin Sensitivity Check Index) based on serum concentrations of glucose, insulin, NEFA (non esterified fatty acids) and BHB (β-hydroxybutyrate), sampled 6, 12 and 48h and 7 and 14 d pp. Cows were grouped according to lactation number ($2^{nd}$ to $4^{th}$ lactation, $1^{st}$ group; and $5^{th}$ to $8^{th}$ lactation, $2^{nd}$ group) and calves according to gender.*

*Results showed that RQUICKI was higher in $1^{st}$ group of cows ($P>0.05$) and showed progressive increase during pp period in both groups. In $1^{st}$ group of cows RQUICKI was lower 6 and 12 h pp than 14 d p.p. ($P=0.01$). HOMA was higher in $2^{nd}$ group of cows ($P>0.05$); HOMA was higher 12 h pp than 48 h, 7 and 14 d p.p. ($P=0.01$) as well as 6 h pp than 7 and 14 d p.p. ($P=0.01$). QUICKI showed the same trend in both groups, progressive decrease from 6h to 14 d p.p. ($P=0.001$, $1^{st}$ group; $P=0.001$, $2^{nd}$ group). In calves, HOMA was higher in females ($P>0.05$). In females HOMA was lower 6 h pp than 7 and 14 d p.p. ($P=0.03$). $RQUICKI_{BHB}$ was higher in females than in males ($P=0.03$). $RQUICKI_{BHB}$ showed progressive decline from 6 h p.p. to 14 d p.p. in females ($P=0.004$). Changes in trends of IR indexes may help in analysis of decreased sensitivity or responsiveness to the metabolic actions of insulin as well as in determination of metabolic status of animals.*

**Keywords**: COWS, CALVES, EARLY POSTPARTUM PERIOD, INSULIN RESISTANCE, BEEF SUCKLER SYSTEM

Insulin is a key regulator of glucose homeostasis. Insulin resistance (decreased sensitivity or responsiveness to the metabolic actions of insulin) is determined by both genetic and environmental factors. Insulin resistance is defined as a condition when higher than normal insulin concentrations are needed to achieve normal metabolic responses [9].

In high-yielding postpartum (p.p.) dairy cows insulin resistance (IR) develops to help directing nutrients from insulin sensitive tissues such as skeletal muscle and adipose tissue to the lactating mammary gland and for the growing fetus, as an important homeorhetic adaptation mechanism of mammals [1]. In dairy cows genetically selected for high-milk production, these homeorhetic adaptation mechanisms are driven to extremes. Insulin resistance in the transition period has been associated with several pathological conditions like ketosis, cystic ovarian disease, fatty liver and the fat cow syndrome is a well-known problem [5, 10, 12]. The etiology of IR in veal calves may be complex and multifactorial [7]. It has been shown that a prolonged intake of high levels of milk replacement (large amounts of lactose and fat) may induce problems with glucose homeostasis and insulin sensitivity in heavy veal calves (>4 month old), as characterized by high incidences of hyperglycemia and hyperinsulinemia [7, 8]. These problems may ultimately result in (pro)inflammatory stress and metabolic diseases.

In humans, surrogate indices for insulin sensitivity have been proposed based on the analysis of glucose, insulin, NEFA (non esterified fatty acids), and BHB (β-hydroxybutyrate) in a single blood sample after an overnight fast. The surrogate indices most frequently used are the homeostasis model of IR (HOMA-IR), the quantitative insulin sensitivity check index (QUICKI), and the revised quantitative insulin sensitivity check index (RQUICKI) [11, 13].

In order to prevent the development of IR, it is of importance to understand the patho-physiological mechanisms of IR and to identify early biomarkers of decreased insulin sensitivity.

## Materials and methods

The animals were kept in a beefsuckler system. Most of the year, cows were kept on pasture but during the late autumn/winter period, when all calving took place (November 2012 to March 2013) they were housed in a barn. During the indoor period, whole corn silage, meadow hay (mixed herbs) and a concentrate, based on ground corn, ground soybean and sunflower pellets, were fed to cows twice a day. The calves were kept in separate wooden boxes next to their mothers and were allowed to nurse *ad libitum* 4 times per day until 14 d of age. Age of cows ranged from 3.5 to 10.5 y (6.58±2.93; mean ± SD); lactation number ranged from 2nd to 8th. Average milk yield during lactation was 5 660 kg. All animals were clinically healthy.

Blood samples were collected from October to March 2013 from each cow and her calf and were taken at 6, 12 and 48 h and 7 and 14 d after calving. Samples were taken by jugular vein puncture (v. jugularis externa) into test tubes containing gel (*BD Vacutainer*® tubes, *BD Diagnostics*, Plymouth, UK). After coagulation and centrifugation (2000×g for 20 min, at +4 °C) serum samples were frozen and stored –20 °C until analysis. The concentrations of glucose and NEFA (non-esterified fatty acids) were determined by spectrophotometry using a biochemistry analyzer *SABA 18* (AMS, Rome, Italy) and reagents (*Herbos Diagnostics*, Sisak, Croatia). The concentrations of insulin was determined using an immunoenzymometric (IEMA) assay in accordance with the instructions provided by the manufacturers (*Insulin Bovine Elisa*, *DRG Instruments GmbH*, Marburg, Germany). Based on serum concentrations of glucose, insulin, NEFA and BHB we calculated following indexes values: RQUICKI (Revised Quantitative Insulin Sensitivity Check Index) and its modified version $RQUICKI_{BHB}$, HOMA (Homeostatic Model Assessment) and QUICKI (Quantitative Insulin Sensitivity Check Index).

Cows were grouped according to lactation number (2nd to 4th lactation, 1st group; and 5th to 8th lactation, 2nd group) and calves according to gender. All data were statistically analysed using *Statistica version 10* (*StatSoft Inc.*, Tulsa, USA). The results are presented in tables as means and standard deviation (mean ± SD). The significance of differences within the group between the sampling periods using time of sampling as factor was assessed by analysis of variance of repeated measurements and Tukey's test in case of normal distribution while a non-normal distribution was checked by Kruskal-Wallis ANOVA. The level of significance was set at $P \leq 0.05$.

## Results and discussion

Insulin resistance is a phenomenon that occurs in early lactation in cows and neonatal calves if fed by large amounts of lactose and fat, and is characterised by lower insulin production and lower tissue response to insulin. Results showed that RQUICKI was higher in 1st group of cows ($P>0.05$) and showed progressive increase during p.p. period in both groups, which was in accordance with results [6]. Value of RQUICKI index is dependant on the insulin value in dry cows, but on NEFA value in early lactation cows [3]. In 1st group of cows RQUICKI was lower 6 and 12 h p.p. than 14 d p.p. ($P=0.01$). Decreased RQUICKI values are in accordance with higher insulin resistance [3, 4]. RQUICKI index, compared to HOMA and QUICKI indexes, is the better predictor of metabolic status [3]. There are scarce reports on the relationship between HOMA values and insulin resistance in cows. HOMA was higher in 2nd group cows ($P>0.05$). In 1st group of cows, HOMA was higher 12 h p.p. than 48 h, 7 and 14 d p.p. ($P=0.01$). Similarly, in 2nd group of cows HOMA was higher 6 h p.p. than 7 and 14 d p.p. ($P=0.01$). [5] and [3] found

*Table 1*

**Values of RQUICKI, RQUICKI$_{BHB}$, HOMA and QUICKI in cows in 2$^{nd}$–4$^{th}$ and in 5$^{th}$–8$^{th}$ lactation during different sampling times**

| Parameter | N | Sampling time | | | | | P-value |
|---|---|---|---|---|---|---|---|
| | | 6 h* | 12 h* | 48 h* | 7 d* | 14 d* | |
| *2$^{nd}$–4$^{th}$ lactation* | | | | | | | |
| RQUICKI | 4 | 0.52±0.07$^{a}$ | 0.50±0.05$^{a}$ | 0.76±0.10$^{ab}$ | 0.74±0.08$^{ab}$ | 1.17±0.49$^{b}$ | 0.01 |
| RQUICKI$_{BHB}$ | 4 | 0.64±0.14$^{a}$ | 0.61±0.06$^{a}$ | 1.13±0.16$^{a}$ | 1.11±0.25$^{a}$ | 7.26±6.70$^{a}$ | >0.05 |
| HOMA | 4 | 3293±1747$^{ab}$ | 4401±2901$^{a}$ | 900±460$^{b}$ | 880±397$^{b}$ | 808±974$^{b}$ | 0.01 |
| QUICKI | 4 | 2.21±0.24$^{a}$ | 0.44±0.06$^{b}$ | 0.61±0.08$^{b}$ | 0.60±0.06$^{b}$ | 0.75±0.23$^{b}$ | <0.01 |
| *5$^{th}$–8$^{th}$ lactation* | | | | | | | |
| RQUICKI | 5 | 0.53±0.15$^{a}$ | 0.58±0.17$^{a}$ | 0.59±0.07$^{a}$ | 0.68±0.17$^{a}$ | 0.83±0.13$^{a}$ | >0.05 |
| RQUICKI$_{BHB}$ | 5 | 0.73±0.27$^{a}$ | 0.77±0.31$^{a}$ | 0.76±0.18$^{a}$ | 0.96±0.47$^{a}$ | 1.01±0.19$^{a}$ | >0.05 |
| HOMA | 5 | 4932±4932$^{ac}$ | 3962±3849$^{c}$ | 2289±1727$^{bc}$ | 888±315$^{bc}$ | 730±99$^{b}$ | 0.01 |
| QUICKI | 5 | 2.20±0.53$^{a}$ | 0.47±0.08$^{a}$ | 0.51±0.07$^{b}$ | 0.61±0.08$^{b}$ | 0.62±0.02$^{b}$ | <0.01 |

*Note:* $^{a, b, c}$ — different superscripts in the same row shows significant difference within the group (P<0.05); ***** — hours/days postpartum; results presented as mean ±SE; NS — non significant.

*Table 2*

**Values of RQUICKI, RHUICKI$_{BHB}$, HOMA and QUICKI in female and male calves during different sampling times**

| Parameter | N | Sampling time | | | | | P-value |
|---|---|---|---|---|---|---|---|
| | | 6 h* | 12 h* | 48 h* | 7 d* | 14 d* | |
| *Female calves* | | | | | | | |
| RQUICKI | 4 | 0.52±0.11$^{a}$ | 0.50±0.08$^{a}$ | 0.50±0.09$^{a}$ | 0.54±0.11$^{a}$ | 0.43±0.10$^{a}$ | >0.05 |
| RQUICKI$_{BHB}$ | 4 | 3.94±4.34$^{aA}$ | 1.72±1.08$^{b}$ | 1.05±0.56$^{b}$ | 1.70±1.19$^{b}$ | 1.07±0.15$^{b}$ | <0.01 |
| HOMA | 4 | 2474±1805$^{a}$ | 1950±1241$^{a}$ | 4800±5106$^{a}$ | 5317±4913$^{b}$ | 5696±4009$^{b}$ | 0.03 |
| QUICKI | 4 | 0.51±0.12$^{a}$ | 0.52±0.09$^{a}$ | 0.48±0.11$^{a}$ | 0.46±0.12$^{a}$ | 0.43±0.08$^{a}$ | >0.05 |
| *Male calves* | | | | | | | |
| RQUICKI | 5 | 0.50±0.08$^{a}$ | 0.43±0.05$^{a}$ | 0.56±0.08$^{a}$ | 0.58±0.10$^{a}$ | 0.51±0.09$^{a}$ | >0.05 |
| RQUICKI$_{BHB}$ | 5 | 1.42±0.84$^{aB}$ | 0.89±0.16$^{b}$ | 5.22±3.59$^{b}$ | 0.74±1.80$^{b}$ | 1.00±0.29$^{b}$ | <0.01 |
| HOMA | 5 | 2413±2250$^{a}$ | 5891±2921$^{a}$ | 2763±1422$^{a}$ | 2846±2483$^{a}$ | 5760±4104$^{a}$ | >0.05 |
| QUICKI | 5 | 0.53±0.11$^{a}$ | 0.41±0.04$^{a}$ | 0.48±0.06$^{a}$ | 0.48±0.06$^{a}$ | 0.43±0.08$^{a}$ | >0.05 |

*Note:* $^{a, b, c}$ different superscripts in the same row shows significant difference within the group (P<0.05); $^{AB}$ — uppercase superscript shows significant difference (P<0.05) between groups; ***** — hours/days postpartum; results presented as mean ±SE; NS — non significant.

lower HOMA values than were established in our study. Insulin resistant individuals will have increased HOMA values [4]. Countrary, values in our study showed decresing trend. QUICKI showed the same trend in both groups, progressive decrease from 6 h to 14 d p.p. (P=0.001, 1$^{st}$ group; P=0.001, 2$^{nd}$ group). Results by [4] and [3] showed lower QUICKI values than were found in our study. There is insufficient data on QUICKI values in dairy cows. RQUICKI$_{BHB}$ showed no significant differences in our study.

In calves, results for insulin sensitive check indexes are scarce. RQUICKI showed no significant differences. HOMA was higher in females (P>0.05) than in male calves. In females HOMA was lower 6 h p.p. than 7 and 14 d p.p. (P=0.03). QUICKI showed no significant differences, but values in our study were in accordance with the results obtained by [2]. RQUICKI$_{BHB}$ was higher in females than in males 6 h p.p. (P=0.03). RQUICKI$_{BHB}$ showed progressive decline from 6 h p.p. to 14 d p.p. in females (P=0.004).

## Conclusions

Accoridng to decreased values of QUICKI in both cows and calves, higher insulin resistance can be proposed. Results in cows also showed increased RQUICKI and decreased HOMA what can be found in insulin non-resistant individuals.

Results in female calves showed RQUICKI decrease while both calves groups showed incresed HOMA values, what can be linked to higher insulin resistance. Reduced insulin sensitivity was shown in the examined population, meaning that insulin resistance is present in the population.

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Ukrainian Association for Buiatrics

# XIX Middle-European Buiatrics Congress

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Abstracts of Reports

# THE ECBHM: AN OPPORTUNITY FOR OUR BUIATRITIANS?

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The European College of Bovine Herd Health Medicine (ECBHM) began as a small working group on specialization in Buiatrics in Europe as a response to significant changes in cattle production with increasing unit sizes, more intensive production systems, coupled with increasing consumer demand for safe food enhanced animal welfare and environmental protection. These changes had resulted in a demand for change in a predominant professional role of cattle vets reacting to problems ("fire-brigade service") and treating individuals or small groups of animals to individuals proactive in the management and healthcare of the herd. A provisional organizing committee (POC) was subsequently established on 24th November 1998 in Paris. The POC (Drs. Baumgartner, Lekeux, Navetat, Klee, Noordhuizen and Schelcher) worked towards establishing a European College and seeking accreditation by the EBVS. This came finally on October 20th 2003. Professor Wolfgang Klee received then a letter from the President of EBVS informing that the ECBHM had been successful in achieving provisional recognition as the ECBHM. Since then, a huge work has been done, keeping the original objectives, and adjusting tis activity to the EBVS rules. The primary objective of the College shall be to advance health oriented bovine production management in the herd context in Europe and increase the competency of those who practice in this field by establishing guidelines and standards of training for postgraduate education and experience prerequisite to become a specialist in the specialty of bovine health management; examining and authenticating veterinarians as specialists in bovine herd health management to serve the veterinary patient, its owner, the consumer of products originating from the bovine and the public in general, by providing expert care for cattle; encouraging research and other contributions to the science and practice of bovine herd health management including; animal husbandry, internal medicine, surgery, obstetrics and reproductive management, as they relate to the epidemiology, pathogenesis, diagnosis, therapy, prevention, and control of diseases directly or indirectly affecting *bovidae* and the maintenance of healthy productive herds.

The ECBHM-Diploma is a professional opportunity, as the only one internationally recognized Buiatritians Specialization Title in Europe; it is a way to be part of an international network of bovine specialists; it is a fix source of continuing Education Courses with the most up to date knowledge. In the Middle European Countries there is a very important bovine producing sector, and we find many bovine specialists working in it: practitioners, professors and researchers. Goals and work, but also advantages of being EBCHM-Diplomate are the same for our MEB colleagues. However, the implication of these outstanding professionals has been tight up to now in our College. Despite of this, we are convinced that the ECBHM needs the feedback and work from these MEB-bovine veterinarians, and on the other side, that a closer connection between MEB Buiatritians and ECBHM will enrich both, enormously.

**Keywords:** BOVINE MEDICINE, HERD MEDICINE, SPECIALIZATION, EASTERN COUNTRIES

# EXAMPLES OF UTERINE CONTRACTILITY PATTERNS IN EARLY POSTPARTUM COWS WITH RETAINED FETAL MEMBRANES AS RELATED TO VARIOUS BLOOD $CA^{2+}$ CONCENTRATIONS: A PRELIMINARY STUDY

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Recent knowledge about the characteristics of mechanical activity of the early postpartum uterus in hypocalcaemic animals with retained placenta is controversial. The aim of this preliminary study was to illustrate early postpartum uterine contractility patterns in cows with normo- and various degrees of hypocalcaemia.

Intrauterine pressure (IUP) was measured with a Labview based, digital, open tip catheter system, to quantify contractility of the early postpartum uterus in dairy cows with retained fetal membranes at a large-scale Hungarian dairy farm. Fourteen to 17 hours after calving a 4-hour continuous recording took place, followed two times by further 1-hour recordings in 12-hour intervals collecting pressure signals from the previously gravid uterine horn. Contractions frequency, amplitude, duration, mean and total areas under the pressure curves were calculated. Coccygeal blood was withdrawn at the beginning of the first and at the end of all recordings and $Ca^{2+}$ was measured on site within 30 minutes of sampling. Cows were considered hypocalcaemic with initial blood $Ca^{2+}$ values less than 1.06 mmol/l (group 1, n=6). In a cow, milk fever had spontaneously developed and will be discussed individually. Normocalcaemic cows were involved as controls (group 2, n=5). Statistical analyses included two-sample *t*-tests, repeated measures ANOVA and correlation analysis.

Significant time-related decline occurred in all uterine contractility parameters among the 12-hour intervals recordings ($P<0.001–0.05$) without showing significant group differences, except that of the 36$^{th}$ hour recording, when contraction frequency was significantly higher in group 1. Initial blood $Ca^{2+}$ concentrations in group 1 ranged between 0.79–1.04 mmol/l, representing a mild hypocalcaemia, where the lowest value in one case was 0.67 mmol/l at the end of the 4-hour long IUP recording session. However, the initial blood $Ca^{2+}$ concentration in the clinically diseased hypocalcaemic cow before any treatment was as low as 0.48 mmol/l. This cow showed typical signs of milk fever with recumbency and had a toneless uterus at that stage.

Blood $Ca^{2+}$ concentrations remained significantly lower in group 1 at all time points ($P<0.01–0.05$), as compared with group 2 but a time-dependent change could not be observed. Within the 4 consecutive hours of the first IUP sessions no consequent IUP changes were found. Blood $Ca^{2+}$ level did not show significant correlations with any of the IUP parameters.

Mild hypocalcaemia does not seem to affect early postpartum uterine contractility pattern in cows with retained fetal membranes, however, severe hypocalcaemia with clinical symptoms was accompanied with the loss of uterine contractility.

**Keywords:** DAIRY COW, INTRAUTERINE PRESSURE, RETAINED FETAL MEMBRANES, HYPOCALCAEMIA

# EVALUATION OF PASSIVE TRANSFER WITH BRIX REFRACTOMETER AND COMPARISON WITH OTHER SEMIQUANTITATIVE TESTS IN GOAT KIDS

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The aim of this study was to evaluate a Brix refractometer in determining the level of passive transfer (PT) in newborn goat kids and to determine the PT status by semiquantitative tests (total protein — TP, glutaraldehyde coagulation test — GCT and gammaglutamyl transferase — GGT).

The study consisted of 75 newborn Saanen goat kids. On the 1st, 2nd and 3rd days after birth, blood samples were collected from the kids. IgG (Goat IgG-ELISA), Brix%, TP, GCT and GGT levels were measured in serum samples.

On the 1st and 2nd days, serum Brix% in the kids was measured as 9.33±0.17 % and 9.17±0.14 %, respectively. In the first- and second-day serum samples of the kids, IgG was 817.76±37.34 mg/dl and 1173.29±47.81 mg/dl, respectively, GCT was 15.24±2.84 min and 11.98±2.41 min, respectively, GGT was 1298.07±133.29 U/L and 692.26±79.86 U/L, respectively. Brix% and IgG were positively correlated on day 1 ($r=0.43$, $P<0.001$) and day 2 ($r=0.25$, $P<0.05$). IgG was similarly correlated with TP and, GCT on 1st and 2nd days, and with GGT on the 1st day after birth. The highest sensitivity and negative predictive ratio of Brix% were detected on day 2; specificity, positive predictive value and accuracy were found to be highest on the 1st day after birth.

Brix refractometer was found to be more sensitive for detection of PT status in kids on the 1st and 2nd days after birth such as TP and GCT, whereas GGT as an early indicator of PT, was useful only on the first after birth. As a result, we conclude that Brix refractometer could be used to determine the passive transfer status in goat kids.

**Keywords:** GOAT KID, COLOSTRUM, PASSIVE TRANSFER, BRIX REFRACTOMETER, SEMIQUANTITATIVE TESTS

# *BURKHOLDERIA CEPACIA* COMPLEX PNEUMONIA IN CALVES: A CASE REPORT

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*Burkholderia cepacia* complex (*B. cepacia*), is group of Gram-negative aerobic closely related species of bacteria. Organisms from this complex are considered ubiquitous microorganism and opportunistic human pathogens. *B. cepacia* complex were described as a reason of cystic fibrosis, lung transplantation, and chronic granulomatous disease in humans. We present cases of pneumonia in 2 beef calf herds. *Burkholderia cepacia* was identified in lung and nasal swaps cultures.

Animals were diagnosed with use anatomopathological methods. Samples of lung, liver, spleen, and kidney tissue from the calf were collected for bacteriological culture. The samples were diagnosed with use RT-PCR and ELISA test.

A 7 young death calf were submitted for examination to the diagnostic laboratory. The calf came from two beef calf herds of 42 and 36 animals in which a high calf mobility and mortality rate had occurred. Postmortem examination of the calves revealed a lobular fibrio-necrotic pneumonia mainly involving the lung cranial lobes. Fibrinous pleurisy was also evident in pneumonic areas. The cut surface of the lung was red and airless with multiple necrotic foci ranging from about 2 to 5 mm in diameter. No pathological changes in other organs were observed.

Cultures of calf lung yielded several colonies of *Burkholderia cepacia* complex. *B. cepacia* complex were also isolated from the nasal swabs taken from calf with signs of pneumonia. Animals from infected farms were tested for BVDV. In both herds antibody — ELISA and real-time RT-PCR results were negative.

To our best knowledge this is first report of *B. cepacia* complex pneumonia in cattle. The disease was associated with severe pneumonia with 80–90 % mobility and up to 45 % mortality, not responding to standard therapy. Moreover, results of our study indicate, that *B. cepacia* complex can have infectious character in calves, with out influence of BVD virus as the most common immunosupresive factor in both described outbreaks.

**Keywords:** CALVES, *BURKHOLDERIA CEPACIA*, PNEUMONIA

# STUDY OF CEFAPIRIN RESIDUES IN MILK FROM COWS AFFECTED BY VARIOUS FORMS OF ENDOMETRITIS AFTER THEIR TREATMENT BY THE MEDICINAL PRODUCT *CEFMETRIN*

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The purpose of the research was to establish the withdrawal period of the residues of the medicinal product *Cefmetrin* with milk after treatment of cows affected by various forms of endometritis.

Cows affected by acute and subclinical endometritis (5 animals in each group) were injected into the uterus with the medicinal product *Cefmetrin* at a dose of 19 g (the contents of one syringe dispenser) using a catheter. A single dose contained 500 mg of cefapirin administered in the form of 640 mg of cefapirin benzathine. Milk was collected from cows of two groups within six milkings in a row (for 3 days). The first selection of milk was carried out in 6 hours after the injection. A study of the content (availability) of β-lactam antibiotics in the selected milk was carried out using the *4 Sensor Ultra test kit* for immunoreceptor determination of β-lactams, tetracyclines, streptomycins and chloramphenicol in mixed milk samples. 0.2 ml of milk was added to a well with the prepared reagent, mixed, kept for 3 minutes and then a test strip was inserted into the well and kept for about 7 minutes until color reactions appeared on it. The sensitivity of the *4 Sensor Ultra test kit* for determining cefapirin was 10–20 μg/l of milk, it is 3–6 times less than MRL of cefapirin in milk for human consumption adopted in the European Union.

It was found that all milk samples that were taken within six milkings in a row for 3 days after intrauterine administration of *Cefmetrin* did not contain residues of β-lactam antibiotics and other antibiotics, including residues of cefapirin with its possible metabolites.

Milk from all cows affected by various forms of endometritis, which were injected into the uterus by *Cefmetrin* once at a dose of 19 g (the contents of one syringe dispenser) containing 500 mg of cefapirin administered in the form of 640 mg of cefapirin benzathine according to the requirements of the package-leaflet, did not contain residues of β-lactam antibiotics within all six milkings for 3 days, including cefapirin with its possible metabolites. On the basis of the conducted research, it was concluded that it is not advisable to establish a withdrawal period for the milk from cows affected by various forms of endometritis, which are injected *Cefmetrin* in the recommended therapeutic dose.

**Keywords:** COWS, RESIDUES, CEFAPIRIN, ENDOMETRITIS

# THE ACID-BASE BALANCE IN NEWBORN KIDS BEFORE AND AFTER COLOSTRUM INTAKE

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The purpose of our study was to obtain physiological reference values in neonatal kids depending on the colostrum intake. The research was focused on the changes of acid-base balance and basic biochemical parameters in neonatal kids before and 2 hours after colostrum intake.

Total of 66 blood samples were taken from 33 neonatal kids. The samples were collected immediately after birth and 2 hours after first colostrum intake. Blood was collected from jugular vein and sample was analyzed immediately by the automatic acid-base analyzer. Blood pH, partial pressure of carbon dioxide ($pCO_2$), partial pressure of oxygen ($pO_2$), bicarbonate concentration ($cHCO_3^-$), base excess (BE), oxygen saturation ($cSO_2$), total carbon dioxide ($TCO_2$), sodium ($Na^+$), potassium ($K^+$), calcium ($Ca^{2+}$), chloride ($Cl^-$), glucose (Glu), lactate (Lac) and creatinine (Crea) were measured. The results obtained were tested for the homogenity of variances (Hartley-Cochran-Bartlett test) and the normality of distribution (Shapiro-Wilk test). The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Fisher LSD *post-hoc* test.

There were no statistically significant differences in acid base parameters such as $pO_2$, $cHCO_3^-$, $TCO_2$, $cSO_2$ and biochemical parameters such as $Na^+$, $K^+$, $Ca^{2+}$ between two groups — before colostrum intake (BF) and after colostrum intake (AF). There were statistically significant differences in acid base parameters such as pH, BE, $pCO_2$ between these groups. Acid-base values of pH, BE and $pCO_2$ and biochemical values of chloride and glucose were statistically significant on the $P<0.001$ level. Values of lactate were statistically significant on the $P<0.01$ level and values of creatinine were statistically significant on the $P<0.05$ level.

The results presented in our study are important for veterinary practice and can improve the neonatal care especially for impaired kids. Furthermore, we would like to emphasize that there is a need for next research focusing on neonatal kids. As the goat farming is increasing there are still not sufficient information in this field compare to other domestic species.

**Keywords:** ACID-BASE BALANCE, BLOOD, KIDS, GOATS, COLOSTRUM

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# CASE REPORT: TRACE MINERAL DEFICIENCY WITH CONCURRENT DETECTION OF *TRYPANOSOMA THEILERI* IN A SUCKLER COW HERD IN GERMANY

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Grazing cattle have different trace mineral requirements than dairy cattle and deficiencies leading to weakness and low production are described for many regions of the world. Three recumbent suckler cows from one farm were presented within two weeks. All of them were kachectic, unable to rise and showed variable mineral and trace mineral deficiencies. Cows were treated with an intensive downer cow-care-protocol and parenteral fluid and electrolyte therapy, mineral substitution, NSAIDs and antibiotics. In one cow *Trypanosoma theileri* was detected in blood smears. Two cases were poorly responsive to treatment and were euthanised after 8 and 22 days of treatment. One case was in a detrimental condition on arrival and died within one day. Postmortem examination of each case resulted in the common diagnoses of muscular dystrophy in the hindlimbs, kachexia and scleral edema. Liver copper content, measured in one animal, was extremely low. A diagnostic follow up herd visit was performed. The farmer fed no concentrate until just before the first farm visit. A mineral mixture was offered *ad libitum* on pasture with variable acceptance by the animals. The cows were very uneven in body condition, had a rough hair coat. In a selection of animals, serum selenium and copper values were analysed and were below reference. All other tested minerals and electrolytes were in the reference range. *Trypanosoma theileri* was detected using PCR in 10 out of 27 cases. In a second farm visit liver biopsies of 9 cows were taken and copper content in dry matter was below reference. Ration analysis was performed and farmer was adviced to adjust feeding regime. This case reports documents the importance of surveillance of the mineral status in suckler cow herds, especially when forage quality is low. The significance of the detection of *Trypanosoma* sp. is so far not known, as there are no data available for the occurrence and distribution of this parasite in suckler cow herds in Germany.

**Keywords:** COWS, *TRYPANOSOMA THEILERI*, TRACE MINERAL DEFICIENCY

# *LISTERIA MONOCYTOGENES* — MICROBIOLOGICAL CRITERIA INDICATE THE ACCEPTABILITY OF SAFETY MEAT RAWS

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The aim of research was development indices of improved horizontal method of *Listeria monocytogenes* detection in meat raws (beef, pork, mutton and meat of goat).

The basis of the horizontal method for the *Listeria monocytogenes* detection in meat raws (beef, pork, mutton and meat of goat) is developing the strategies for improving the horizontal method of *Listeria monocytogenes* determination in meat by using the research suspension.

The meat raws are irreplaceable in human diet and their consumption affects the health on the population. In the countries of the European Community, considerable attention is paid to the improvement of the legislative framework for controlling the traceability of meat raws material throughout the entire food chain — from field to table.

The developing the for improving the horizontal method of *Listeria monocytogenes* detection in meat with the help of research suspension, prepared in the ratio of 1:5 (samples of meat in the amount of 10−11 g and 50−55 $cm^3$ of initial selective enriched medium (half of Fraser broth), and further incubation of the suspension for 21−23 hours at temperature of 31±1 °C and secondary enrichment. After the first initial enrichment the received culture in the amount of 0.05−0.06 $cm^3$ is transferred in to the test tube that contains 5−6 $cm^3$ of second time enriched medium (Fraser broth). Then the environment with crops is incubated for 46−48 hours at temperature of 37 °C. After that the primary (5−6 $cm^3$) and the secondary (2.5−3.0 $cm^3$) enriched culture in terms of selective environment PALKAM-agaris in oculatedandis carried out to get clearly separated colonies of *Listeria monocytogenes* for 24±2 hours at temperature of 37±1 °C and for 46±2 hours at temperature of 37±1 °C.

The results of our research showed that *Listeria monocytogenes* colonies were found in for 24±2 hours at temperature of 37±1 °C. They were of small size about 1.5−2.0 mm in diameter of grey-green or olive-green color, sometimes with a black halo. In 46±2 hours at temperature of 37±1 °C they were of green color with deeply sunk centre and black halo in the following samples of in meat raw: 2 samples of beef and 3 samples of pork in at production in processing enterprises; 3 samples of pork, and 2 samples of mutton and 1 samples in meat of goat on agro-food markets.

The improved horizontal method of *Listeria monocytogenes* detection in meat raw have a reliability of 99.8 %. A method we propose is a qualitative technique of improving the horizontal method of *Listeria monocytogenes* detection in meat raw (beef, pork, mutton and meat of goat) along with other methods of determining meat raw safety.

**Keywords:** MEAT RAWS, *LISTERIA MONOCYTOGENES*, SAFETY

# AUTOGENIC VACCINES ARE AN EFFECTIVE FOR CONTROL OF EPIZOOTIC PROCESS FOR MASTITIS IN COWS

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The main etiologic agent of cows mastitis is considered conditionally pathogenic microflora (streptococci, staphylococci, mycoplasmas) and their associations, which are usually activated under the influence of adverse animal retention factors. Prevention and treatment of mastitis is complicated by the prohibition of the use of a large number of antibacterial preparations. In recent years, in the scientific literature more and more messages appear on the successful use of autogenic vaccines to prevent mastitis. To study the effectiveness of the use of an experimental vaccine produced from autogenous strains in one of the dairy farms of the Lviv region.

Dairy farm has a herd of 600 dairy cows. For the bacteriological research samples of milk and the secretion of udder from cows with clinical and subclinical forms of mastitis the content of the uterus from cows with postpartum endometritis and specimens of faeces from newborn calves suffering from diarrhea were selected. From the 12 biomaterials, 20 isolates were isolated and identified, including *E. coli* — 9, *Str. pneumoniae* — 4, *Str. dysagalactiae* — 3, *St. aureus* and *St. intermedius* — for 2 isolates.

The analysis of the dynamics of titer of agglutinins in serum of blood of cows vaccinated with vaccine from autogenous strains, shows that the highest antigenic activity possessed by the *E. coli* immunogen (1: 2048 — mean titers in cows before calving and 1: 448 — in 2 months after the calving), lower — immunogens *Str. pneumoniae* i *Str. Dysagalactiae* (1:448, 1:256 — middle titer before calving and 1:96, 1:64 2 months after calving), and the lowest — immunogens *S. aureus i S. Intermedius* (1:112, 1:64 — middle titer before calving and 1:32, 1:28 in 2 months after calving). This indicates a high immune response of vaccinated animals, which had expressed projective effect as indicated by the data of the analysis of the zootechnical and economic parameters of the farm before and after applying the vaccine. Thus, the morbidity of cows for subclinical mastitis decreased 5 times, clinical — 6 times, on endometritis — 6 times, and the incidence of newborn calves by gastrointestinal diseases — 8 times.

Thus, the use of autogenic vaccines is effective, and therefore, a perspective direction for the prevention of diseases of cows mastitis is associated with this pathology.

**Keywords**: MASTITIS, VACCINE, UDDER, MILK, IMMUNOGEN

# INDICATORS OF QUALITY AND SAFETY OF RABBIT MEAT DURING STORAGE

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For the investigation 52 rabbits which entered the State Laboratory of Veterinary and Sanitary Expertise of the Central Market in the city of Bila Tserkva, Kyiv region were used.

Organoleptic methods (GOST 20235.0-74) were used: determination of the appearance and color of the carcass surface, the position of the muscles in the cut, the consistency, the smell, transparency and aroma of the broth; physical and chemical (GOST 29235.1-74): determination of the hydrogen index, ammonia and ammonium salts and products of primary decomposition of proteins in the broth; bacteriological research (GOST 20235.2-74): microscopic analysis, determination of the bacteria of the coliform sticks in meat, bacteria of the genus *Salmonella*.

We investigated 52 carcasses of rabbits. Changes were found in 14 carcasses (26 %). The organoleptic study revealed changes in the meat that are of a sanitary value. During the organoleptic studies on the third day in three carcasses, changes in the indicators of freshness of meat were found, in particular: the surface of carcasses — slightly sticky, darkened, sometimes moisturized, the color of the internal fatty tissue — with a reddish tinge; the muscles in the dark red section, leave a damp spot on the filter paper. The fovea during the touch of a finger equalizes for one minute; the carcasses became smelly; during the test, the broth is muddy, with an unpleasant odor. In 8 carcases (15 %), changes were detected during meat intake, while in 6 carcases (11 %), spoilage was found during storage and sale on the agro-industrial market (dark-red meat, friable consistency, with a faint smell). We have also detected such defects of meat as tanning, mildew and rotting. During the physical and chemical analysis of rabbit meat revealed pH changes as a measure of deterioration of meat. During the mildew of the meat there was a shift of pH to the sour side (pH=5.8), and for decay — in alkaline (pH=7.6 and above). During the bacterioscopy studies, traces of muscle breakdown and the presence of gram-negative sticks in the smear were found in the number of 53±3 microbial cells in the field of vision, which is 5 times the permissible norm. Non-compliance with commodity neighborhoods was identified during implementation, in particular: teaching rabbits with carbohydrates, which led to the identification of bacteria of the genus Salmonella in rabbit meat. In 3 carcasses (5 %) found bacteria of the coli group in an amount that exceeds the permissible standards; in one carcass (2 %) the meat was found to be fermented with bacteria of the genus *Salmonella*. Since the presence of bacteria of the genus *Salmonella* in rabbit meat is unacceptable, the affected carcass was disposed of. The remaining 5 carcasses were sold during the 4$^{th}$ day of sale on the agro-industrial market.

On the basis of the comprehensive research, factors that reduce the health and hygiene quality of the meat of the studied carcasses of rabbits were revealed, namely: violation of slaughter technology (bad bleeding), cooling of rabbits after slaughter ("tanning" of meat), violation of the temperature regime storage during sales of rabbit meat; absence of disinfection of counters and refrigerating chambers (insemination of carcasses with microflora); non-compliance with the principles of commodity neighborhood during implementation (implementation of waterfowl carcases along with rabbits).

**Keywords:** RABBIT MEAT, AGRO-INDUSTRIAL MARKET, QUALITY INDICES, SAFETY INDICATORS, NORMATIVE DOCUMENTS

# RELATIONSHIPS BETWEEN MILK YIELD AND SUSCEPTIBILITY TO DISEASE IN DAIRY COWS

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Breeding for high milk yield is often blamed for increasing the susceptibility to disease and shortening productive life. However, the results of previous studies are contradictory. If, in fact, such a correlation really does exist it would have considerable consequences, according to German and European law, up to and including a ban of the practice of breeding to increase milk production.

The aim of these investigations was to examine the extent to which the level of milk yield and other factors (husbandry conditions, feeding, management) affect animal health, including immunocompetence, as well as the productive life. The investigations were carried out in 16 randomly selected German Holstein herds, 8 of them with high milk yield (10,421±1,111 kg/year, herd size 83–264 cows) and 8 herds with lower milk yield (8.298±701 kg/year, herd size 71–208 cows). In addition to clinical and laboratory diagnostic tests, the cows were vaccinated with a live vaccine against BVD (*Bovela®*, *Boehringer Ingelheim Vetmedica GmbH*, Ingelheim am Rhein, Germany) for the determination of immunocompetence.

Despite significantly different milk production, there were no differences in the prevalence of subclinical ketoses or other diseases between the two groups. The same applied to the cortisol concentration in feces and the formation of neutralizing BVD antibodies after vaccination. Age of leaving the herd averaged 64.2 months in the high-performance group (medium performance group: 66.0 months), the average culling rate in this group was 23.1 % (lower performance group: 28.0 %) and the average productive lifetime 35.8 months (lower performance group: 37.4 months). There was no significant correlation between the level of milk yield and the parameters mentioned above. The same was true for the cell content in milk. However, there were significant group differences in milk yield per day of life (high performance group: 15.7±2.5 kg, low performance group: 13.0±1.4 kg) and lifetime production (31.047±8.247 vs. 26.093±4.185 kg).

The relationships between high production, reduced fertility and susceptibility to diseases are much more complex than are considered in current discussions and must therefore be viewed in a more differentiated manner. The health of dairy herds is not primarily dependent upon the milk yield, but is rather related to management including feeding, housing conditions and disease prevention. If these underlying conditions are all right it is possible to achieve very high milk production without negative effects on animal health.

**Keywords:** COWS, HERMAN HOLSTEIN HERD, MILK YIELD, CATTLE DISEASES

# THE EFFECT OF *MYCOPLASMA BOVIS* INFECTION ON PERIPHERAL BLOOD LEUKOCYTE ACTIVITY IN THE CALVES

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*Mycoplasma bovis* is known as etiologic agent of pneumonia, arthritis and mastitis in cattle. It was previously confirmed that *M. bovis* possesses both immunostimulating and immunosuppressive properties. The aim of the study was to evaluate the effect of *M. bovis* on bovine peripheral leukocyte activity *in vivo* conditions.

The study was carried out on clinically healthy calves divided into two groups: experimental and control. The experimental calves were infected with the field *M. bovis* strain three times at 48 hour intervals. Instead the control animals were administered with phosphate buffered saline. Blood samples were collected each day up to day 9 following the first *M. bovis* infecting dose and then weekly until day 30 when the calves were euthanized to obtain the lung samples. In the blood samples phagocytic activity (*Phagotest*™) and oxygen metabolism (*Phagoburst*™) of peripheral blood leukocytes were evaluated using flow cytometry according to the manufacturer's instruction (*Glycotope Biotechnology GmbH*, Berlin, Germany). The *M. bovis* antigen was detected in the lung samples by immunohistochemistry using mouse anti-*Mycoplasma bovis* monoclonal antibody (*Millipore*).

Positive immunolabelling for *M. bovis* in the bronchiolar epithelial cells in the lungs of the experimental calves confirmed the infection efficacy. The percent of phagocytic granulocytes in the blood of experimental calves did not significantly differ from the control. However, the mean fluorescence intensity (MFI) for granulocytes visibly increased on day 9 post the first infecting dose and it was significantly higher than the control on day 16. Following the calf infection the percent of phagocytic monocytes was increased throughout the study when compared to the control, with the exception of days 9 and 16. The MFI for monocytes in the experimental calves was in general slightly higher than the control. For the oxygen metabolism the percent of activated leukocytes was significantly increased on day one post the first infecting dose of *M. bovis* however after that it suddenly decreased and had similar or lower values than the control up to day 30. However the MFI was generally increased in the experimental calves throughout the study when compared to the control.

The general stimulation of phagocytic activity and oxygen metabolism of peripheral blood granulocytes and monocytes following the calf infection with *M. bovis* can show the activation of host defence mechanisms for the pathogen elimination.

**Keywords:** *MYCOPLASMA BOVIS*, CATTLE, IMMUNE RESPONSE

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# CLINICAL EFFICACY OF IVERMECTIN AGAINST CERTAIN GASTROINTESTINAL NEMATODES OF CAMELIDS IN THE STARI GRAD ZOO AT ĐURĐEVAC, CROATIA

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The objective of this study was to determine the clinical effectiveness of ivermectin (*Biomectin* 1 %) against certain gastrointestinal nematodes of camelids in the Stari Grad Zoo at Đurđevac, Croatia.

One castrated dromedary camel, three bactrian camels (two females and one male) and a pair of llamas were used in this clinical study. All animals were aged 1–3 years and were kept in a fenced area with a sandy base for exercise, with access to stables during the night. Animals had free access to good quality meadow hay, adequate concentrate for camelids and drinking water. The female camels and both llamas occasionally consumed less food, had messy hair and occasionally had short-term diarrhoea. Faecal samples were collected twice prior to treatment (in April and May) and three times every two months (July, September and November) after SC administration of 1 % ivermectin (*Biomectin*) at a SC dose of 0.3 mg/kg. Faecal examination was performed by the flotation method using $ZnSO_4$ (371 g zinc sulfate in 1000 ml water). From each animal, 3 g faeces was mixed with 10 ml prepared $ZnSO_4$ solution, and the sample was centrifuged at 1200 rpm for 5 minutes. Every sample was checked by the McMaster's test (MMT) to determine the number of eggs per gram (EPG) of faeces for each type of GI parasite.

A variety of gastrointestinal nematodes were identified prior treatment, including undifferentiated strongyles, *Nematodirus* spp., and *Strongyloides* sp. (in llamas only). Prior to treatment, the average EPG in all camelids was 28.42±9.72 (*Nematodirus* sp.), 78.08±37.06 (strongyles) and 56.05±12.00 (*Strongyloides* sp., in llamas only). After treatment, EPG was reduced to 5.05±3.19 (*Nematodirus* sp.) and 3.17±3.12 (strongyles). In July, 66.67 % of samples were negative for undifferentiated strongyles and only 16.67 % for *Nematodirus* sp. eggs. All three MMT tests after treatment were negative for *Strongyloides* sp. in llamas.

After ivermectin treatment, animals had a better appetite, shiny hair and solid faeces. Ivermectin (*Biomectin* 1 %) at a SC dose of 0.3 mg/kg, caused a reduction in egg production of *Nematodirus*, *Strongyloides*, and undifferentiated strongyle species, as determined by faecal egg counts in camelids at the Stari Grad Zoo at Đurđevac, Croatia.

**Keywords:** CAMELIDS, GASTROINTESTINAL PARASITES, IVERMECTIN

# INFLUENCE OF PARITY ON BLOOD SERUM CONCENTRATIONS OF MACROMINERALS IN DAIRY GOATS DURING EARLY LACTATION

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The objective of this study was to determine the influence of parity on blood serum macromineral concentrations in Saanen dairy goats during early lactation before the weaning of goat kids.

A total of 18 Saanen dairy goats (7 primiparous and 11 multiparous) between 1 and 4 years of age were used in this research. Goats were kept in individual boxes. According to standard farming practice, animals were fed twice daily and had free access to drinking water. They were fed with good-quality meadow hay (2.2 kg per doe daily) at the same time every day. Every day, each animal received 0.98 kg of concentrate of known chemical composition (1.54 % calcium, 0.60 % phosphorous with a Ca:P ratio of 2.57:1). All does were categorized on a scale from 1 to 5 into medium does with the body condition score (BCS) ≥2.75<3.50. Blood samples were taken every five days, starting on the 5$^{th}$ day until the 40$^{th}$ day of lactation. Calcium, phosphorous, potassium, magnesium, sodium and chloride serum concentrations were determined.

In primiparous dairy goats, the average serum macromineral levels were: calcium 2.28±0.27, phosphorous 2.05±0.46, sodium 148.12±6.12, potassium 4.87±0.53, chloride 107.28±4.25, and magnesium 1.21±0.41 mmol/L. The average levels of macrominerals in multiparous goats were: calcium 2.36±0.19, phosphorous 2.38±0.62, sodium 147.73±6.37, potassium 4.79±0.49, chloride 108.64±3.77, and magnesium 1.23±0.11 mmol/L. The average parity was 3.2 in multiparous does with an average litter size 1.55±0.59, while in primiparous does, this was 1.29±0.41. Phosphorus values were higher at the beginning of lactation than in mid lactation in multiparous does. Multiparous does had calcium levels below the normal range (2.3–2.9 mmol/L) until the 20$^{th}$ day of lactation (from the first sampling 2.07±0.28 to 2.23±0.27 mmol/L), as a clinical sign of moderate hypocalcaemia.

In this study, all goats had average macromineral levels within the physiological range for the species. Only multiparous does had calcium levels below normal range until the 20$^{th}$ day of lactation, and higher phosphorus values. Analyses of macromineral serum levels in dairy goats during lactation can be helpful for early detection of certain metabolic disorders.

**Keywords:** DAIRY GOATS, MACROMINERALS, PARITY

# POSSIBILITY OF MODULATION OF THE BOVINE UTERINE PERFUSION WITH THE USE OF SILDENAFIL CITRATE IN DAIRY COWS DURING LUTEAL PHASE OF THE OVARIAN CYCLE

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A proper perfusion of the organs guarantee its proper functioning. Modulation of perfusion can be used to compensate for deficits as well as to create overperfusion which could have a curing effect for the tissue. The aim of the study was to evaluate the influence of the Sildenafil citrate after different rout of administration on the blood flow in the uterus of cows during dioestrus.

Uterine blood flow was examined in healthy, adult cows at the 6–8 day of the ovarian cycle. Experiment was divided on two parts depending on rout of sildenafil administration. In 1st experiment six cows received 200 mg of sildenafil diluted in 10 ml of warm saline into the body of the uterus and in second experiment another five cows received Sildenafil intravaginaly in the form of vaginal suppositories containing 200 mg per animal. In both cases a placebo infusion and suppository was also given to the cows. Analysis the maximum velocity in m/s (V max) in the aorta was performed and selected parameters of the blood flow (pulsatile index, PI; resistance index, RI; systolic peak velocity SPV; end diastolic velocity, EDV; flow velocity integral, FVI; systolic peak velocity: end-diastolic velocity ratio, SV/DV) were measured in the uterine artery (*arteria uterina*) before and after sildenafil infusion. In addition, Color Doppler examination of the uterine wall perfusion was evaluated and obtained results were analyzed with the *Pixel Flux* software (*Chameleon*, Germany). Animals were examined before and five times after drug application (two times at 15 min intervals, and three times at 2 h intervals). Statistical analysis was based on program *Statistica version 7.1* (2.3.1–2.3.2) (*StatSoft*, USA). The comparison of values of the evaluated parameters before and after sildenafil treatment was performed by Wilcoxon test, and $P<0.05$ was defined as representing a significant difference.

A significant decrease of values of PI and SV/DV ratio as well as an increase of end diastolic velocity and time averaged maximum velocity was noted. With the use of color coded sonography, the increased intensity of the blood flow in the uterine wall was observed in both method of sildenafil administration. After administration of sildenafil, significant differences in values of all parameters except SPV occurred ($P>0.05$).

It was concluded that intrauterine as well as intravaginal administration of sildenafil during dioestrus can increase uterine tissue perfusion. Further studies are indicated if this phenomenon could be useful for the uterine disorders treatment as a main or additional method of treatment.

**Keywords:** COWS, SILDENAFIL CITRATE, UTERINE PERFUSION

# IMPACT OF FARM INDIVIDUAL ACTION PLANS ON LAMENESS PREVALENCE, PRODUCTIVITY AND WELFARE OF DAIRY CATTLE

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Orthopedic disorders causing lameness belong to the most common and economically most relevant production diseases of dairy cattle worldwide. Lameness has severe economic implications while causing a serious impact on animal welfare. Reasons for orthopedic disorders are multifactorial and can be divided in cow-, housing- and management-related risk factors. The aim of the study was to assess the impact of farm individual action plans on lameness prevalence, productivity and welfare of dairy cattle.

Five dairy farms in eastern Germany with high prevalence of lameness were visited between January 2017 and February 2019. The average number of milking cows per herd was 675, ranging from 257 to 1137 cows. All farms housed German Holstein cows as the predominant breed in freestall barns with cubicles and fed total mixed ration and used the herd management system *Herde®* (*DSP Agrosoft GmbH*, Ketzin, Germany). Regular hoof trimming was conducted at a minimum of twice per year and new cases of lame cows were treated at least once per week. The same veterinary hoof care professionals always visited farms. Information regarding animals, performance, housing, diet, management practices, biosecurity and claw health management were collected using direct observation of the cows and their environment, interview with the herd manager during the visit and analysing of herd data. Cows were evaluated for lameness using a 6-point locomotion scoring (LS) system (modified according to Starke et al., 2007), where 1 = regular locomotion, without lameness, 2 = imperfect locomotion, 3 = slight lame, 4 = moderately lame, 5 = severely lame and 6 = highly severely lame. Clinical case of lameness was indicated by a LS≥3. Furthermore body condition score (Edmonson et al., 1989), integument alterations (Lombard et al., 2010) and cleanliness (Reneau et al., 2005) of cows were assessed. Regarding the aims of the farm and the collected data, we developed an individual action plan together with the farms management, the herd manager and the farms external consultants and accompanied the implementation. Frequency, interval and topic of the following farm visits were adapted to the action plan.

Farms were visited between 2 and 24 times. At the first farm visit the farms were characterized by: average annual milk yield per cow and lactation of 9,779 kg (range from 8,387 kg to 11,542 kg), average life span production of 31,635 kg (17,631 kg to 54,908 kg), 31 % (20 % to 46 %) average culling rate and an average of 3 (2.4 to 4.6) lactations in herd until culling. The median lameness prevalence was 54 % (35 % to 80 %). The following conditions were considered when developing the farms individual action plans: efficiency, feasibility, sustainability and profitability. Optimizing herd health documentation, raising the knowledge level about claw health and intensifying the hoof trimming and treatment were the most common objectives. Possible effects of the action plan were steadily monitored. Resulting conclusions led to adjustments to the action plan. About six month after the first farm visit, the mean lameness prevalence decreased from 54 % to 32 % (15 % to 50 %). Annual milk yield per cow and lactation increased to 9825 kg (8,424 kg to 11,747 kg) and life span production increased to 32,616 kg (21,419 kg to 53,042 kg).

Using an in-depth analysis to assess and eliminate the farm-related risk factors for orthopedic disorders helps to develop an effective farm individual action plan. With consistent implementation, decreasing of lameness prevalence is possible. Hence, productivity and welfare of dairy cattle increase.

**Keywords:** DAIRY CATTLE, LAMENESS, GERMAN HOLSTEIN BREED, DAIRY FARMS

# THE EFFECTS OF DIFFERENT STARCH LEVEL STARTERS WITH OR WITHOUT AMYLASE ON PERFORMANCE AND HEALTH PARAMETERS IN CALVES

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The purpose of this study is to evaluate effects of calf starter feeds with different starch levels on feed intake, body weight gain, feed conversion, blood glucose levels, rumen pH, wither height, chest widht, body length, clinic and respiratory score in 0–56 day calves.

The research was carried out in calf unit in İtimat Agriculture and Animal Husbandry. Therefore, 6 groups were formed and 90 Holstein-Friesian female calves were used totally as 15 calves in each group. Starter feed containing 23 %, 28 % and 33 % starch in 88 % dry matter and same feeds with amylase enzyme (*RumiStar*, *DSM Animal Nutrition & Health*, Turkey) added at a dose of 1 kg/ton were given to groups respectively. The groups were named as 23E–, 23E+, 28E–, 28E+, 33E– and 33E+ according to the starch content of the calf starter feeds and whether or not the enzyme is contained. Body weights of the calves measured at 0, 28, and 56 days; calf starter consumption and feed conversion were calculated weekly. On 56$^{th}$ day, approximately 10 ml of blood sample was taken, glucose values were measured. In addition, on 56$^{th}$ day, wither height, chest widht, body length was measured and clinic and respiratory score was recorded. Ph measurement was made in rumen fluid taken on 56$^{th}$ day. Statistical analysis of datas was performed by one-way ANOVA method and pearson chi-square method for clinic and respiratory score records was applied with SPSS package program.

The average starter feed consumption until 56$^{th}$ day was 12.2 kg and there was no difference between groups. No difference was found between groups for daily body weight gain, feed conversion, wither height, chest widht, body length, clinic and respiratory score. However, the pH value of 23E+ feds was higher than 33E+ feds ($P<0.05$). Then, 23E– feds had the lowest blood glucose levels (72.9 mg/dL) and were found different from 23E+, 28E– and 33E+ feds.

It was concluded that calves fed 23E+ diet have higher rumen pH, which may contribute to health and performance.

**Keywords:** CALVES, STARTER, RUMEN pH, HEALTH

# THE INFLUENCE OF GENETIC AND PARATYPIC FACTORS ON THE DURATION AND THE EFFECTIVENESS OF LIFETIME USE OF DAIRY CATTLE

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The influence of genetic and paratyphoid factors and manifestation of phenotypic characteristics on indicators of productive longevity of dairy cattle have been studied.

Studies on cows of Holstein (n=2902), Ukrainian Black- (n=14876) and Red-and-White (n=2176) dairy breeds. The variability of productive longevity of dairy cattle is significant influenced by housing conditions, a little less — by the year and the season of birth and the first calving. Born in the autumn-winter period cows had the highest rates of duration and effectiveness. The season of the first calving didn't show any consistent pattern, but birth and the first calving year had almost the same impact on productive longevity indicators. Animals that have not reached the breed standard in live weight in a certain age period, in the future, had lower indicators of lifetime use and productivity. In order to prolong the productive longevity of cow their first calving should be planned at the age of 25–29 months. Among the animals of Holstein and Ukrainian Red-and-White the cows with 121–150 days of the first service period had the longest use in herds and had the highest milk yield, and among Ukrainian Black-and-White with the duration of the indicated period 151–180 days. There was observed the significant impact of milk yield during first and best lactation regardless of breed of mothers, mothers of mothers and mothers of fathers on the milk yields of descendants of the same lactations, but not on their productive longevity. High productivity of cows and their female ancestors in most cases led to a reduction of the duration of productive use and lactation period of daughters and grandchildren, reducing their lifelong productivity and premature dropping out of the herd.

The indicators of the duration and effectiveness of life-long use of the daughters of long-lived Holstein breed were lower not only than their mothers, but also lower than the average of the herd. Descendants of Ukrainian Black-and-White, Red-and-White breeds had a little higher indicators of productive longevity, than the average per herd: lifetime yield was higher by 10.4 and 28.9 % respectively, and the number of lactations for life – by 6.4 and 22.7 %.

Such Holstein breeders as Rock 373840409, V. M. Dan 5510544, V. Teksel Kin 393522 (Canadian breeding) E. Samb 3035115974 (Hungarian selection), Lord 661288 (German breeding), Valentin 373840175, Matador 373840109 (Russian breeding) improved productive longevity of daughters by some separate features, and also the breeders of Ukrainian Black-and-White, Red-and-White breeds Abrykos 5806 and Khlor 2052.

The nonadditive type of inheritance cows for the first lactation characterized by a longer duration of productive use and higher lifelong yields than the ones with additive inheritance. During selection cows with "over-domination" and "domination of the mother" and "domination of the father" the forms of yield inheritance should be preferred since these animals were characterized in most cases by the highest rates of duration of productive use and lifetime productivity.

One-factor dispersion analysis has established that the most significant impact on the productive longevity of dairy breeds were made by genetic factors, namely, the origin of the father, conditional pedigree by Holstein breed and linear affinity. Factor "Herd" had the greatest impact on the productive longevity of cows among the paratypes factors and much smaller is birth and the first calving year and birth and first calving season.

**Keywords:** BREED, COWS, DURATION OF PRODUCTIVE USE, LIFETIME PRODUCTIVITY, GENETIC FACTORS, PHENOTYPIC FEATURES, ENVIRONMENTAL FACTORS, CORRELATION COEFFICIENTS, POWER OF INFLUENCE

# MACRO- AND MICROELEMENTS OF BLOOD AND ITS ANTIOXIDANT ACTIVITY IN LACTATING COWS UNDER THE ACTION OF IODINE CITRATE IN DIFFERENT DOSES

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The earlier studies indicate the important role of iodine in the life of the organism. The determinative influence of this element on the reproductive ability of females and their productivity has been proved. However, the use of mineral salts of iodine in animal rations is limited by their high chemical reactivity. Currently, the nanotechnology for the production of carboxylates of macro- and microelements has been developed in Ukraine, including iodine citrate, which makes it possible to use organic compounds of iodine as an environmentally safe feed additive. The purpose of the research was to find out the effects of various doses of iodine citrate on the content of macro- and trace elements in the blood and antioxidant protection of the body of cows in the first period of lactopoesis.

The research was carried out on 15 full-year-old cows of Ukrainian black-and-white milk breed, formed in the winter-stool period, having been anchored and divided into three groups, 5 animals in each, the analogs by age (3–5 lactation), body weight (580–620 kg), the period of lactation (1st month after calving). Cows of group I (control) received the basic diet, which was normalized according to the physiological state, productivity and body weight. The animals of the II (experimental) group from 18–23 to 78–83 days of lactation received daily iodine citrate in the feed of the basic diet at a rate of 0.6 mg I/kg of dry matter of the diet, and animals of the III (experimental) group received the basic diet and the iodine citrate at the rate of 0.06 mg I/kg of dry matter of the diet. For biochemical studies, selected samples of venous blood were used in the preparatory (prior to feeding I citrate) and experimental (60 days of supplementation of the iodine supplement) periods. The processes of mineral metabolism were evaluated by the concentration of Ca, P, Fe, Zn, Cu in the blood, and the antioxidant defense of the organism by the content of lipid hydroperoxides, TBK-active products and its catalase, glutathione peroxidase and superoxide dismutase activity.

The probable influence of iodine citrate on mineral metabolism and the antioxidant protection of cows in the beginning of lactation has been established. In particular, in the blood of cows of II and III groups, the content of Ca ($P<0.001$), P ($P<0.05$), Fe, and Zn was increased only by 0.6 mg of iodine. These data indicate a more pronounced effect of higher applied dose of iodine citrate for the exchange of Zn, and Ca, P and Fe — for both concentrations of iodine. The applied doses of iodine citrate resulted in activation of the enzyme level of antioxidant protection of the organism, as evidenced by an increase in the catalase activity ($P<0.05$), glutathione peroxidase ($P<0.05$) and superoxide dismutase ($P<0.05$) blood counts in both experimental groups. However, the content of lipids hydroperoxy decreased significantly only in the blood of cows under the action of lower (0.06 mg I) dose.

Consequently, the use of iodine citrate, obtained by the method of nanotechnology, in doses of 0.6 and 0.06 mg I/kg of dry matter of the diet in the first 3 months of lactation causes an increase in Ca, P and Fe content, and Zn — only under the action of 0.6 mg of iodine citrate in the blood during the 1st period of lactopoesis and enhances the enzyme level of antioxidant protection of their organism. The obtained results of the research can be used to substantiate the approbation of iodine citrate application to their diet on the sufficient lactation cows in the range of experimentally determined doses (0.06–0.6 mg I/kg of dry matter of the diet).

**Keywords:** MINERAL METABOLISM, ANTIOXIDANT ENZYMES, LACTATION

# ASSESSMENT OF DIFFERENT PAIN MANAGEMENT METHODS FOR THE TREATMENT OF CLAW LESIONS IN MEAT MERINO EWES

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Pain management during veterinary procedures is a significant component of animal welfare and has legal as well as ethical implications. Even though intravenous regional anaesthesia (RIVA) is an accepted method for painful procedures involving the distal digits of sheep, this anaesthetic technique is rarely used in the field. The primary goal was to investigate the feasibility, safety and efficacy of the RIVA in sheep. A secondary goal was to examine whether the anaesthetic procedure can be improved by combining the RIVA with sedation and whether these methods have a positive effect on postoperative wellbeing.

36 Meat Merino sheep with contagious interdigital dermatitis and 12 healthy control sheep were used. Behaviour was observed during treatment of the lame sheep using various pain management protocols and during routine claw trimming of the healthy sheep, and all the sheep were observed after the procedures. The observed behaviours were assessed using scores and the scores compared among the animals of the 4 study groups (control, RIVA, sedation with Xylazine hydrochloride + RIVA, placebo).

The RIVA was successfully conducted in sheep. Local reactions at the application sight and in the tourniquet area in two animals resolved completely. A significant reduction in defensive movements during the painful procedure confirmed the efficacy of the RIVA. Stress-associated behaviours such as head shaking and idle chewing occurred with similar frequency in RIVA- and placebo-animals, leading to the conclusion, that stress levels due to the handling in dorsal recumbency were comparable between the two groups. Sedation reduced the frequency of pain- and stress-associated behaviours such as guarding, favouring limbs, vocalisation, idle chewing and bruxism. Xylazine hydrochloride-RIVA-animals showed better weight-bearing in the affected limb, better food uptake and ruminated more postoperatively than sheep from the other treatment groups.

Concluding, the RIVA in sheep is straightforward, safe and effective. Additional sedation reduces the stress- and pain-response. This pain and stress management has a positive effect on postoperative wellbeing of sheep. However it is clear, that the investigated pain management methods are not sufficient to treat post-operative pain and need to be extended by further components.

**Keywords**: EWES, MERINO BREED, CLAW LESIONS, XYLAZINE HYDROHLORIDE

# DETERMINATION OF SERUM ALBUMIN IN LARGE ANIMALS

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The serum albumin is most commonly photometrically determined by reaction with bromcresol green (BCG) or bromocresol purpure (BCP) in biochemical laboratories. Both colors are known to react differently to albumin of vary animal species, and BCP shows more significant differences in this regard. Our aim was to examine the reactions of these dyes with serum albumin from large animals and compare these results with electrophoresis as a reference method. On this basis, decide on the suitability of these dyes for the determination of serum albumin in cows, calves, goats and horses.

Commercial diagnostic kits were used for the analyzes and were followed as recommended by the manufacturers. Measurement was performed on the *Cobas Mira Plus* automatic analyzer. Electrophoretic separation was performed on an agarose gel with a *Sebia* diagnostic kit. Blood was collected from the animals in a conventional manner and processed immediately or frozen (–18 °C) until analysis was performed.

In the determination of albumin in cows, higher results were obtained by the BCG method ($P<0.05$). However, this increase in the results averaged about 1 g/l. On the other hand, using BCP, the differences were statistically significant ($P<0.001$) and were up to 15 g/l lower. In calves, the BCG method provided an average of about 2.0 g/l higher ($P<0.01$), but the BCP method was again more than 10 g/l lower ($P<0.0001$). In cattle, statistically significant differences were not measured by the BCG method, again in the BCP method, statistical significance ($P<0.01$) and about 8 g/l lower results. Regarding to horses, both methods were not statistically significant, and the BCP method had very good agreement with the results obtained by electrophoresis (mean difference of approximately 0.3 g/l).

Determination of serum albumin belongs to routine biochemical examinations with simple analysis. Despite the simplicity of performance, however, the analysis of albumin is connected with some difficulties. One of this difficulties is calibration of the method. The certified reference material ERM DA 470k/IFCC has been used less efficiently for the metrological continuity of work calibrators. In addition, there is not enough consistency among manufacturers using the same methods, and methodological differences can cause deviations of up to 17 %. In spite of all the differences numerous papers in human medicine show that, the BCP method has better results and it is recommended to use this method. However, our results show that BCP method completely fails in veterinary medicine, and although the BCG method gives slightly better results than electrophoresis (about 1–2 g/l higher) and these results can be used in clinical practice. We don't recommend to use the BCP method in veterinary laboratories. Also field practitioners who have samples analyzed in human laboratories should be informed that the laboratory uses the BCG method.

**Keywords**: SERUM ALBUMIN, BROMKRESOL PURPLE, BROMKRESOL GREEN, ELECTROPHORESIS

# ASSOCIATIONS OF REPRODUCTIVE MANAGEMENT AND PERFORMANCE IN PRIMI- AND MULTIPAROUS COWS ON LARGE DAIRY FARMS

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The aim of this study was to analyse the associations among management practices and reproductive performance in primiparous and multiparous dairy cows on large commercial dairy farms.

Personal interviews were performed between 22 May and 6 November 2015 in order to survey the reproductive management practices on 34 Holstein-Friesian large commercial dairy herds in Hungary. Individual data of 23,781 cows that calved between 1 January 2014 and 31 December 2014 were also gathered from the farms participating in the survey. The associations of the management practices and reproductive performance by parity were analysed retrospectively by mixed effects models. Data were managed in *Microsoft Excel 2013* (*Microsoft Corporation*, Redmond, WA, USA). Statistical analyses were performed in *R version 3.4.0*.

Mean±SD size of the studied herds was 755±470 cows (range: 291–2,502), whereas the 305-day milk yield of the herds amounted to 10,014±965 kg (range: 8,330–12,541). Primiparous cows had shorter breeding interval (42.2 vs. 43.2 days, $P<0.001$), shorter calving to conception interval (152.3 vs. 161.8 days, $P<0.001$), higher first-service conception risk (24.8 vs. 17.3 %, $P<0.001$) and higher probability of pregnancy at 200 days in milk (65.2 vs. 55.4 %, $P<0.001$) compared to multiparous cows, however, no differences between parities were found regarding days to first service (75.7 vs. 75.6 days, $P>0.05$). The use of voluntary waiting period was linked to larger increase in calving to conception interval ($P<0.05$) and greater decline in the probability of pregnancy at 200 days in milk ($P<0.001$) in multiparous cows. Primiparous cows experienced larger improvement in days to first service ($P<0.001$), breeding interval ($P<0.05$), calving to conception interval ($P<0.01$) and probability of pregnancy at 200 days in milk ($P<0.001$) than their multiparous counterparts when estrus synchronization was used (vs. not used). Early pregnancy diagnosis and pregnancy recheck improved breeding interval ($P<0.01$ for both practices), calving to conception interval ($P<0.01$ and $P<0.001$, respectively) and the probability of pregnancy at 200 days in milk ($P<0.001$ for both practices) to a larger extent in primiparous cows.

Primiparous cows generally experienced larger improvement in reproductive parameters when estrus synchronization, early pregnancy diagnosis and pregnancy recheck were applied compared to their multiparous herdmates. Therefore, our study has shown that the associations of reproductive management practices and parameters are different in primi- and multiparous cows.

**Keywords:** DAIRY CATTLE, REPRODUCTION, PARITY, MANAGEMENT, PREGNANCY DIAGNOSIS

# ZINC DEFIENCY IN BREEDS OF DAIRY COW IN THE CZECH REPUBLIC

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The aim of this study was to evaluate serum zinc levels and determine deficiency of this microelement in the Czech Republic.

Serum zinc concentrations were measured in 583 Holstein high-yield dairy cattle. Samples were collected as a part of monitoring herd's health status during performing the metabolic profile test. Those tests has been performed on 28 selected farms during year 2018, either for preventive or diagnostic reasons. There is one to five groups containing 5–10 animals is chosen for a metabolic profile test (e.g. group after calivng, 1/2 lactation, end of lactation and dry period). Blood samples were collected from the the coccygeal vein into serum separation tubes. Serum levels of zinc were measured by atomic absorption spectrometry (AAS) at wavelength 213.9. The measured values of samples were then compared with a reference physiological range of 12.0–15.0 μmol/l. The data were evaluated by using a standard deaviation.

According to comparation with physiological range 5 groups were created: <8.0 μmol/l — 34 pcs, 6 % (σ 1.24); 8.01–10.0 μmol/l — 98 pcs, 17 % (σ 0.55); 10.01–11.9 μmol/l — 148 pcs, 25 % (σ 0.56); 12.0–15.0 μmol/l — 225 pcs, 39 % (σ 0.83); 15.01 μmol/l and more — 78 pcs, 13 % (σ 2.43). Standard deviation helped us to determine that highest variability of values were in groups with highest and lowest zinc levels, which might be due to wide range of results in those groups. In other groups variability of zinc status was low.

Serum zinc levels in high-yield cows are very variable in the Czech Republic. In the 39 % of samples physiological level of zinc was measured. As deficiency we consider a value lower to 12 μmol/l, in our study it was 48 % of the samples.

**Keywords:** ZINC DEFICIENCY, METABOLIC PROFILE TEST, DAIRY CATTLE

# RISK ANALYSIS CONCERNING LUMPY SKIN DISEASE INTRODUCTION TO UKRAINIAN TERRITORY. ASPECTS OF LSD PREVENTION

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The lumpy skin disease (LSD) is viral vector-borne disease, caused by the Capriposvirus. The disease incidence could reach up to 90 %, with the mortality rate about 45 %, and significant economic losses consist of rubbish and death of the diseased livestock. The decreasing of productivity, quality of milk and leather raw materials, abortions, stillbirth, and infertility could be detected. The consequences of the LSD are devastating not only for agricultural regions, but also at the national level. According to the O.I.E. classification, the LSD is classified as particularly dangerous and subjects to mandatory notification. The disease prevention is based in mass alive vaccines application. Since last couple of years LSD is potentially hazardous for our country.

The epidemic situation concerning LSD was studied in the affected countries, most closely located to Ukraine, using the O.I.E. data and personal communications. The risks for disease introduction were calculated by the ball rate factors-assessment matrix. The populations of potential LSDV vectors were tested for virus presence using in house PCR protocol for Capripoxvirus. The comparative review of vaccines for LSD prevention.

The confirmed high level risks (12 balls) for LSDV introduction in cattle herds from Russia, Central Europe and Turkey. Situation regarding LSDV introduction to Ukraine is likely to be non-optimistic. Russia, Caucasian countries, and Bulgaria high LSD-associated risks put our territory on high range of risk regarding LSDV introduction. Disease introduction probabilities could be estimated as extremely high and high from the side of Russia. The first way for possible introduction could be potentially associated with warm and wet summer-spring period, sufficient for growing of the population of different insects, potentially could be LSDV transmission factors in the wildlife and farming animals, especially backyards kept on free pastures.

Polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), as very fast tools for agent's identification are widely used and recommended by OIE. In house PCR test has been development in NSC IECVM and SSRILDVSE based on FAO protocol. The PCR-based screening of the midges, and biting-flies demonstrated absence of viral DNA in samples of insects collected in Sumy and Kharkiv regions.

As far as LSD vaccines are concerned, only live attenuated vaccines against LSD are currently commercially available. RM-65 attenuated sheep pox vaccine at the recommended dose for sheep has limited effectiveness in protecting animals from LSD. The Neethling attenuated lumpy skin disease virus vaccine is highly effective in the prevention of morbidity, thus confirming the need to use homologous vaccines for the control of Capripoxvirus infections. Nevertheless, some safety issues have been reported that are linked to generalize clinical reactions due to vaccination with LSD strains that can be observed.

The high level risks (12 balls) for LSDV introduction are existing for Ukraine. Effective prevention could be realized by the application of regular surveillance of disease, including monitoring of the vectors populations. Vaccines reserve could be also created for the specific disease prophylaxes.

**Keywords:** LUMPY SKIN DISEASE (LSD), CAPRIPOSVIRUS, UKRAINE

# TREATMENT OF NANO PREPARATION IN LIPOSOMAL FORM OF CATTLE ENDOMETRITIS

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Endometritis remain one of the main bovine postpartum diseases that cause of the infertility, survival and welfare of dairy cows. Endometritis is an inflammatory disease caused by pathogenic bacteria and associated with delayed uterine involution and poor reproductive performance. Antibiotics are generally used in the treatment of endometritis; however, frequent usage of them is limited via the emergence of antibiotics multidrug resistant.

The main goal of research was the development and improvement of effective new nano-preparation for treatment of cattle endometritis. Thus we elaborated a new complex liposomal preparation with silver nanoparticles, vitamins and hormones.

For study of the therapeutic effectiveness of new liposomal nano-product we used cows with clinic symptoms of endometritis. The first group of cows received commercial preparation with AgNPs (control) at the dose of 20 ml/day during 6 days. Second group of animals was intrauterus treated of liposomal nano-preparation "Argoton" at the same dose. The animals condition was monitored by ultrasound and blood sampling was done for determine of hematological and biochemical parameters.

Using to the preparation "Argoton" for the cows with endometritis of the first group led to a decrease the concentrations of aspartate-aminotransferase and alkaline phosphatase in comparison with the control group, which was been administered the preparation "Sumer silver". The increase in the concentration of urea on the 4$^{th}$ and 14$^{th}$ day after the introduction of drugs with silver nanoparticles by 1.5 and 2.5 times was detected in the blood serum of both experimental groups, especially in the second experimental group with "Argoton". While the lower content of uric acid on the 4$^{th}$ day after the introduction of preparations with silver nanoparticles in both groups was observed. In the experimental group with "Argoton", its level was reduced from 239±27 to 53.6±6.5 μmol/L, and remains at approximately this level throughout the study period. The significantly change the content of cholesterol, total protein, albumin, magnesium, phosphorus, calcium, estradiol and progesterone in the blood serum cows after treatment were not observed.

The results showed that new nano-preparation is effective substance for treatment of cattle endometritis without antibiotics (effectiveness more 98 %). It was confirmed by biochemical analysis of blood samples obtained before and after drug administration.

We explored the possibility of use the nanoparticle in liposomal form as new alternative drugs to fight against uterine infections in dairy cattle. The present study showed that the new liposomal preparation was effective on treatment of cattle endometritis without antibiotics.

**Keywords:** COWS, LIPOSOMAL PREPARATION WITH SILVER NANOPARTICLES, ENDOMETRITIS, BLOOD

# AMINO ACID COMPOSITION OF GRASS SILAGES CONTAINING DIFFERENT LEVELS OF TRUE PROTEIN IN TOTAL CRUDE PROTEIN

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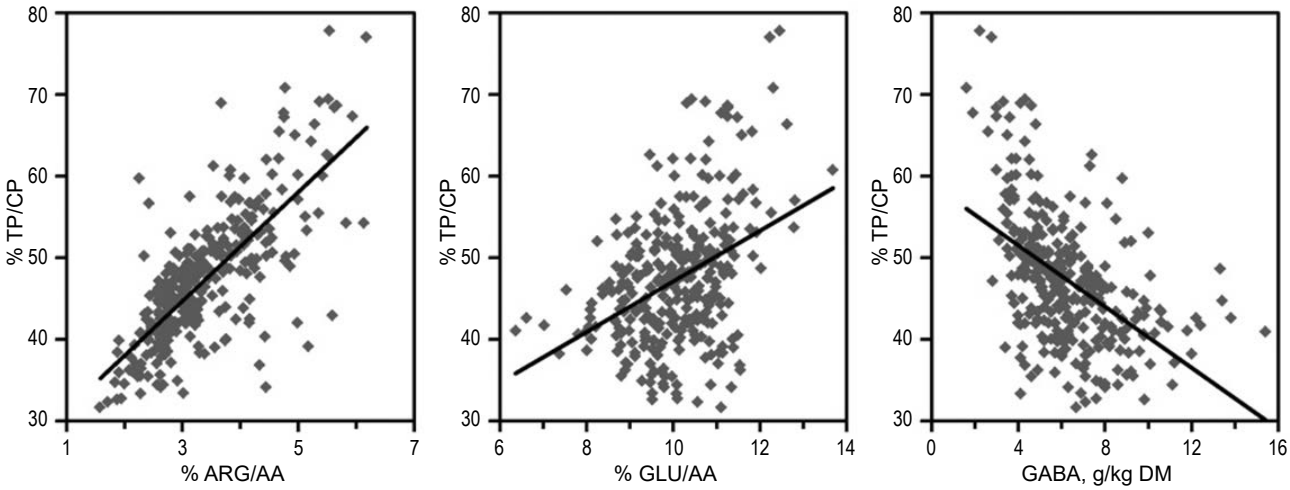
Grass silage is an often used compound in the diet of dairy cattle. However, its quality and composition may be influenced by numerous factors. It is well known that after harvesting grass for silage production, plant enzymes like proteases degrade the true protein (TP). As long as the grass is not wilted yet, enzymes in the plant cells can still function, hence the percentage of TP in total crude protein (CP) decreases. Simultaneously, the amino acid (AA) composition of the grass changes. 311 grass silages were analyzed for their contents of AA, TP and CP. The aim of the present study was to investigate whether the AA profile changes with different percentages of TP in CP.

The AA composition of all 311 grass silages was analyzed via VDLUFA III, method 4.11.1 by *Evonik Nutrition & Care GmbH*, Hanau, Germany. Assayed AA were: MET, CYS, LYS, THR, ARG, ILE, LEU, VAL, HIS, PHE, GLY, SER, PRO, ALA, ASP, GLU as well as the biogenic amine GABA (γ-aminobutyric acid). The Institute for Animal Nutrition, University of Veterinary Medicine Hannover Foundation, analyzed TP and total CP contents. TP contents were determined using the Barnstein method, corresponding to VDLUFA III, method 4.4.1. The amount of total CP was analyzed via KJELDAHL according to VDLUFA III, method 4.1.1. The concentration of every single assessed AA was converted into a percentage of the sum of all measured AA. Statistics were evaluated via Spearman rank correlation (src).

Statistics revealed a highly significant correlation ($P<0.001$) between the contents of arginine (src = 0.73), glutamate (src = 0.36), GABA (src = –0.52) and the respective percentages of TP in CP in the grass silages, as can be seen in fig. 1–3. The other AA were not or had only low correlations with TP and CP.




Correlation between arginine (*fig. 1*, left), glutamate (*fig. 2*, center) and GABA (*fig. 3*, right) and the percentage of TP in total CP

As opposed to the literature, a change in AA composition was only noticeable for arginine, glutamate and GABA. Deficiencies of arginine or glutamate in the forages or higher concentrations of ornithine and/or biogenic amines [e.g. GABA (from GLU), putrescine, spermidine, spermine and thermospermine (from ARG)] could be a result of plant protein degradation. This information may aid in finding an answer to the question: How are sensorially ordinary grass silages with low TP in CP a cause of dairy herd diseases?

**Keywords:** COWS, GRASS SILAGES, AMINO ACID PROFILES, HERD DISEASES

# THE RELATIONSHIP BETWEEN THE NUMBER OF SOMATIC CELLS (SCC) IN THE MILK AND THE CONCENTRATION OF VITAMIN A, E AND β-CAROTENE IN THE BLOOD SERUM OF DAIRY COWS

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The aim of this study was to compare SCC in the milk and the concentration of vitamin A, E, and β-carotene in the blood serum of dairy cows during the first month of lactation.

Samples of milk from 50 selected dairy cows were examined from 10th to 30th day of lactation in the Holstein dairy cows with average yield of 10,500 kg of milk for standard lactation, The number of somatic cells (SCC) and serum concentrations of vitamin A, E and β-carotene were determined in the milk. Selected dairy cows were categorized into 5 groups of 10 pieces according to number of somatic cells. Group no. 1 consisted of 10 dairy cows with SCC to 100,000 in 1 ml, group no. 2 consisted of 10 dairy cows with SCC 100,000–200,000, group no. 3 consisted of 10 dairy cows with SCC 200,000–400,000, group no. 4 consisted of 10 dairy cows with SCC 400,000–800,000, and group no. 5 consisted of 10 dairy cows with the number of somatic cells above 800,000. Somatic cells were determined on Fossomatic instrument, the vitamins concentrations ware determined by the HPCL method. The statistical evaluation of the results was carried out using the ANOVA method.

Concentrations of vitamin A, E and beta-carotene were significantly different among the groups of dairy cows. In the group no. 1, the vitamin A concentration was 1.13 μmol/l, vitamin E concentration was 6.31 μmol/l and β-carotene 4.81 μmol/l. With the increasing number of SCC in milk, concentration of these micronutrients decreased, and in the group no. 5 concentrations were very low. Level of vitamin A was 0.69 μmol/l, vitamin E 4.22 μmol/l and β-carotene 1.59 μmol/l. The differences between group no. 1 and no. 5 were statistically significant. In the vitamin A, group no. 1 vs. no. 5 $P<0.001$, group no. 1 vs. no. 4 $P<0.001$, group no. 2 vs. no. 5 $P<0.001$. In vitamin E, group no. 1 vs. no. 5 $P<0.01$, group no. 2 vs. no. 5 $P<0.001$. And in β-carotene, group no. 1 vs. no. 5 $P<0.05$, and group no. 1 vs. no. 4 $P<0.05$.

On the 1st month of lactation in dairy cows, a significant difference in vitamin A, E and β-carotene was find out according to SCC in the milk. In the group with SCC up to 100,000 in 1 mil of milk were the statistically higher concentrations of vitamin A, E and β-carotene than in the group of cows with number of somatic cells greater than 800,000.

**Keywords:** DAIRY COW, SCC, VITAMIN A, VITAMIN E, β-CAROTENE

# BIOCHEMICAL CHANGES IN FOLLICULAR FLUID AND VENOUS BLOOD DURING ACUTE RUMINAL ACIDOSIS IN HEIFERS

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The aim of this study was to evaluate biochemical changes in composition ofollicular fluid and blood during acute ruminal and metabolic acidosis in dairy heifers.

Ten Holstein heifers were synchronized by cloprostenol (500 µg i.m. per cow, *Oestrophan*®, *Bioveta a.s.*, Ivanovice na Hane, Czech Republic). Seven days later dominant follicles were ablated to start the new follicular wave. Two days later (day 0, D0), stimulation using FSH was initiated. A total dose of 345 µg FSH (*Pluset*®, Calier SA, Spain) was administered intramuscularly in eight doses at 12 h intervals (D0–D3) in order to induce production of follicular fluid for the whole experimental period. The first sampling (venous blood, follicular fluid) was performed on D3 (time 0, T0). Than metabolic acidosis was induced by oral administration of sucrose at a dose of 9 g/kg of bodyweight dissolved in 10 L of warm tap water givenas a ruminal drench. After this treatment, the heifers were not fed until the last sample was collected on D5. Subsequent samplings were collected after 8, 12, 16, 24, 32, 40 and 48 hours (T8–T48) of each cow. Samples of follicular fluid obtained by transvaginal follicular aspiration (TVFA) and peripheral blood obtained by indwelling jugular catheters were examined for biochemical parameters: urea, glucose (Glu), non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), sodium (Na), phosphorus (P) and magnesium (Mg).

During the experiment, changes in acid-base balance variables in blood were determinated to monitor acute metabolic acidosis development. Values of pH reached the minimum 16 h after sucrose treatment (ST) — 7.30. The lowest values of $HCO_3^-$ were observed 24 hours after ST (18.75 mmol/l) as well as the lowest values of BE (–6.61 mmol/l).

Statistically significant decrease (T0 vs. time after ST) were recorded in ureaconcentration (5.09 vs. 2.33 mmol/l), NEFA (0.90 vs. 0.17 mmol/l), BHB (0.3 vs.0.08 mmol/l), Mg (1.00 vs. 0.78 mmol/l) and statistically significant increase in P concentration (2.20 vs. 3.18 mmol/l) in blood. Statistically significant decrease were recorded in ureaconcentration (4.57 vs. 1.99 mmol/l), BHB (0.40 vs. 0.07 mmol/l) and statistically significant increase in glucose (4.19 vs. 6.64 mmol/l), Na (141.5 vs. 165.0 mmol/l), P (2.74 vs. 3.45 mmol/l) in FF.

Ruminal and subsequent metabolic acidosis significantly influenced evaluated blood parameters. The composition of follicular fluid reflected changes of blood composition. We supposed that the affection of follicular fluid by metabolic acidosis can impair fertility in dairy cows.

**Keywords:** FOLLICULAR FLUID, RUMINAL ACIDOSIS, HEIFER, TRANSVAGINAL ASPIRATION

# DEVELOPMENT OF SERUM VITAMIN E, A AND β-CAROTENE LEVELS IN HEIFER CALVES DURING THE FIRST 8 WEEKS OF LIFE

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The aim of the study was to determine the development of vitamin E, A and β-carotene levels in the serum of Holstein heifer calves during the pre-weaning phase (first 8 weeks of life). Another objective was to evaluate the correlations between serum vitamin levels and live weight in the heifer calves.

Serum vitamin E, A and β-carotene levels were measured in 11 Holstein heifer calves. The calves were included in the study in their first week of life. All the calves received 2 litres of colostrum within 2 hours from birth. Another 2L of colostrum were fed within 4–6 hours from the first dose of colostrum. Then the calves received milk replacer. Blood sampling and determination of serum vitamin E, A and β-carotene levels were performed weekly, from the week (wk) 1 to wk 8 of the calves' life. Blood was collected from the jugular vein into serum separation tubes. Serum levels of vitamins E, A and β-carotene were measured by HPLC. The calves were weighed after every blood sampling. The data were processed by one-way ANOVA.

Serum levels of vitamin A did not change significantly during the first 8 weeks of life ($P>0.05$). However, significant differences in serum vitamin E levels occurred between the week 1 and wks 3, 7, 8 ($P<0.05$), and between the wk 2 and wk 3 ($P<0.001$), wk 4 ($P<0.01$), wk 5 ($P<0.01$), wk 6 ($P<0.01$), wk 7 ($P<0.001$), and wk 8 ($P<0.001$). Vitamin E serum levels were increasing during the first 8 weeks of life. There were significant differences in serum β-carotene between the wk 2 and wks 5, 6, 7 ($P<0.05$). Significant correlations between serum vitamin A and E levels and live weight were found during the first 8 weeks of life ($P<0.01$). No significant correlations were observed between serum β-carotene levels and live weight ($P>0.05$).

The serum vitamin A levels were suboptimal throughout the pre-weaning phase, whereas the vitamin E levels were within the reference range. This implies that the milk replacer was insufficient in vitamin A, but had an optimal vitamin E content. Positive correlations were found between live weight and serum vitamins A and E during the the first 8 weeks of life. The serum level of β-carotene had no effect on the calves' growth.

**Keywords:** VITAMIN E, VITAMIN A, β-CAROTENE, HEIFER CALVES

**Acknowledgements.** This study was supported by the institutional research fund of the Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno.

# A TWO-STAGE APPROACH FOR THE REDUCTION OF THE MAP PREVALENCE IN CATTLE HERDS AS PART OF REGIONAL CONTROL PROGRAMS — EXAMPLES AND EXPERIENCES

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Paratuberculosis (Johnes disease) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and leads to substantial economic losses in infected herds. Examples of regional control programs, consisting of the identification of MAP-positive herds followed by voluntary control measures, are presented.

In course of the program for the reduction of the MAP prevalence in Lower Saxony, Germany, dairy farms are obliged to test bulk milk samples for MAP antibodies, followed by testing of individual animals in seropositive farms. Subsequently, farmers can decide to join the accompanying MAP control program. Within the first 11 months of the program 6,035 bulk tank samples were tested, 13 % were MAP-positive and 670 farms joined the MAP control program.

Within the voluntary certification program in Hesse, Germany, the MAP herd status is evaluated using boot swab sampling (PCR and culture). In positive farms, animals are tested by milk or blood ELISA-serology. In case of double positive results, a fecal examination can be performedadditionally. 100 farms participated in the voluntary program until the end of 2018. Of these farms, 60 were MAP-negative and 33 positive, respectively (no status assigned in 7 farms). In participating farms, the mean intra herd prevalence decreased from 7.56 % to 4.06 %.

The program for the abatement of MAP infections in cattle herds in Thuringia, Germany, is based on a yearly fecal examination of all adult cattle within a herd. In 2017, fecal samples from 28,941 animals were tested of which 1.8 % were MAP-positive. Of the 136 participating farms, 64 are MAP-negative and 72 positive, with 39 of the positive farms in the last step of the program before achieving a MAP-unsuspected status.

The biennial survey of the MAP herd status by boot swabs (PCR and culture) is the base of the MAP program in Tyrol, Austria. Positive farms may have their animals tested by fecal sampling and join the MAP control program. In 2016/17 boot swab samples from 4,206 farms were tested with 0.97 % positive farms. In these farms 2,151 fecal samples were collected of which 2.3 % were MAP-positive. Altogether, 131 farms joined the voluntary MAP control program until summer 2018.

The programs presented show, that a two-stage approach consisting of the evaluation of the MAP herd level, followed by the testing of single animals, is generally well accepted by the stakeholders and therefore seems a promising way for the surveillance and control of MAP infections in cattle herds.

**Keywords:** CATTLE HERDS, JOHNES DISEASE, *MYCOBACTERIUM AVIUM* SPP. *PARATUBERCULOSIS*, GERMANY, AUSTRIA

# THE EFFECT OF LAMENESS ON MILK YIELD AND FERTILITY IN AUSTRIAN DAIRY COWS — RESULTS FROM THE NATIONAL EFFICIENT COW PROJECT

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Lameness is one of the three major factors influencing the profitability and economic stability in modern dairy farming. It is associated with pain and has a clear negative effect on welfare. The objective of this study was to analyze the effect of lameness on milk yield and fertility in Austrian dairy cows within one 305-day lactation period.

Within the scope of the big national "Efficient cow" project data on locomotion scores and lameness episodes, milk production and fertility parameters were- collected from 2013 to 2015 in 5392 Brown-Swiss, Simmental and Holstein cows from 166 dairy herds from all over Austria. All thecows were scored every 60 days during one 305-day lactation period for locomotion (Sprecher method), and were grouped regarding their observed locomotion score and number of lameness observations during the lactation period into five groups (LOC-G 1: never lame; LOC-G 2: only two observations with locomotion (LOC) score 2; LOC-G 3: more than two observations with LOC-score 2 and one LOC-score 3 observation; LOC-G 4: two and more observations of LOC-score 3; LSC-group 5: one or more observations of LOC-score 4 and 5). The impact of lameness on milk yield and selected fertility parameters was calculated by various statistical tests and a mixed ANCOVA-model using various covariates and fixed effects.

The statistical model calculation for all breeds showed significant differences in milk yield and milk protein yield between non-lame and lame cows. Regarding milk yield per 305-day lactation differences between cows of LOC-G 1 and LOC-G 4 (–234 kg) became evident. The milk protein yield per 305-day lactation resulted in significant differences in cows of LOC-G 1and 2 compared to LOC-G 4 (–13 kg) for all breeds and for Holstein cows (–23 kg) respectively.

In regard of the fertility parameters evaluated (calving to conception interval, time from the first to the successful insemination, calving interval) between never lame cows (LOC-G 1) of all the 3 breeds and the other lameness groups were particularly statistically significant for each breed.

The mean calving interval for cows of LOC-G 3, LOC-G 4 and LOC-G 5 of all breeds was significantly longer compared to never lame cows. The mean calving interval for never lame Holstein cows was 392.5 days compared to 425.3 and 429.0 days for LOC-G 5 and LOC-G 4 respectively. The mean calving to conception interval, the mean calving interval were significantly lower in never lame cows within the first 100 DIM compared to cows with lameness during the first 100 DIM. First service conception rate was assessed to be the highest for never lame cows (50 %), the poorest results with 35.4 % were observed for cows of LOC-G 4 ($P<0.05$).

This was the first study of the impact of lameness in dairy cows on milk yield and selected fertility parameters in Austria. The results indicated that the milk yield and even fertility parameters were significantly negatively influenced in cows being moderately and severely lame on repeated observation dates compared to never lame cows.

**Keywords:** LAMENESS, CLAW LESIONS, MILK PRODUCTION, FERTILITY PARAMETERS, COW

# LIMB FRACTURES IN 98 CATTLE — TREATMENT AND OUTCOME

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Food animals are excellent orthopedic patients due to their quiet temperament, their long lying periods, their excellent bone healing potentialand their good acceptance of external coaptation devices on their limbs. The objective of this study was to evaluate the records of 98 bovine patients treated due to limb fractures during a 17-years period.

The records of 98 bovine patients of our clinic suffering from limb fractures were analyzed retrospectively regarding the species, breed, and age of the animals, age, localization and type of fracture, the applied treatment method, and the final outcome.

Of a total of 98 cattle of different breeds, 33 were male and 65 were female. They had a mean age of 281.4 days (±403.9), and 33.7 % of them were only some hours to up to 26 days old. 58.9 % of fractures were located at the rear and 41.9 % at the front limbs. Fractures of the metatarsus (23.4 %) and metacarpus (29.2 %) were most common, with lower fracture incidence of the tibia (15.9%) and femur (14%). 59 patients with fracture were treated by external coaptation (cast, PVC-splints), 9 surgically by internal fixation, and in 29 cattle no treatment at all was performed due to poor prognosis.

A success rate of 83.6 % could be revealed by conservative and of 66.7 % by surgical treatment respectively. The conservative treatment in one calf failed, and therefore subsequently a surgical treatment was applied with good final outcome. In total, 38 cattle experienced complications, which were mild in cattle treated conservatively and all of them healed successfully. However, 8 of 9 surgically treated patients developed complications, resulting in euthanasia of 3 of them. The other 5 patients had a satisfactory final outcome. Statistical analysis revealed no significant correlation between the age of fracture and success rate. However, a logistic regression analysis showed that each day of treatment delay in a fracture patient led to a negative drift of 3.8 % for the success rate.

This retrospective study showed that conservative treatment of long bone fractures in cattle was associated with a significantly higher success rate of 83.6 % then surgery with 66.7 %. Similar success rates for fracture treatment in bovines were reported by others. This favorable success ratefor treatment of limb fractures in young and adult cattle should encourage to apply conservative treatment in particular in metacarpal, metatarsal, phalangeal fractureseven in practice. In contrast, for proximal bovine limb fractures, internal fixation is the applied method of choice. In any case, an adequate and professional emergency management of limb fractures, a correct decision to apply conservative or surgical treatment, and an adequate treatment at an early stage of fracture occurrence improve the likelihood for a successful final outcome.

**Keywords:** LONG BONE FRACTURE, TREATMENT, EXTERNAL COAPTATION, FRACTURE SURGERY, CATTLE

# BACTERIOLOGICAL STUDIES FOR CHARACTERIZING INFECTIOUS SITUATIONS AT THE CLAWS OF DAIRY CATTLE

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Polybacterial skin infections of the bovine foot are the most common causes of lameness on dairy farms worldwide. Primary and complicated secondary infections at the distal limb like *Dermatitis digitalis*, interdigital phlegmons, septic arthritis, and claw horn disruption lesions are the important lameness causing foot lesions. Their etiology is multifactorial, but infectious processes are implicated in disease pathogenesis. It may be that mostly they are opportunistic pathogens infecting pre-existing lesions and are not solely responsible for lesion initiation. The cultivation methods are imperfect and show often random results only, similar to an ice peak phenomenon.

But at least, a correct recognition is an important requirement for a treatment decision including antimicrobial resistance testing. In addition to the Spirochetes associated with Digital dermatitis, *Fusobacterium necrophorum* and several other bacteria such as *Bacteroides* spp., *Dichelobacter nodosus*, *Porphyromonas levii* and *Trueperella pyogenes* have been suggested to play a role in the pathogenesis. Nevertheless, most of that research was done long ago and, for example, the taxonomical changes since then make interpretation of the results challenging.

We have carried out microbiological studies on the pathogen involvement in sole ulcers as well as phlegmonous inflammations as a model case for other local infections. Especially the use of MALDI-TOF MS facilitates the differentiation considerably, but also shows the wide variety of the microbiome, especially in superficial infections. In comparison to earlier studies, species such as *Helcococcus kunzii*, which is thought to have pathogenic potential, could also be identified.

For the future, comparative investigations between bacteriological investigation and molecular biological diagnostics are to be considered.

**Keywords:** DAIRY CATTLE, CLAWS, *DERMATITIS DIGITALIS*

# HAPTOGLOBIN AND SERUM AMYLOID IN SERUM OF DAIRY COWS WITH CLINICAL AND SUBCLINICAL MASTITIS

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The aim of the study was to compare serum levels of acute phase proteins (haptoglobin and serum amyloid A) between healthy dairy cows and those with subclinical or clinical mastitis at 3 weeks *post-partum* under field conditions. Also the relationship between milk somatic cell count (SCC) and hematological parameters was evaluated.

The study included 107 Holstein-cross, primi- and multiparous dairy cows from a 600 head herd. The cows with clinical metritis and lamenes were excluded. The cows were evaluated for clinical signs of mastitis at the milking parlour. Milk SCC was measured at 7 and 21 days postpartum. Blood samples were collected at 1 week prepartum (T1) and 21 days postpartum (T2) from the coccygeal vein into serum separation tubes. Serum was frozen at –20 °C. Serum haptoglobin was analyzed by colorimetry (*Konelab 20XT*), SAA by *Sandwich ELISA* (*BIOTEK Instruments Inc.*, USA). Hematology was performed with the analyser *BC-2800 Vet* (*Mindray*, China). Based on milk SCC values at 21 days postpartum, the cows were divided into 4 groups: group 1 — healthy cows, 0–100 thous. cells/mL (n=70); group 2 — SCC 101–200 thous. cells/mL (n=9); group 3 — SCC 201–800 thous. cells/mL (n=10); group 4 — high SCC and clinical cases, SCC >801 thous. cells/mL (n=18). Differences between the groups in serum SAA, haptoglobin levels, hematological parameters (white blood cells, lymphocytes, monocytes, granulocytes, etc.) and leucogram (band cells) were evaluated. The data were processed by one-way non-parametric ANOVA.

At T1 serum levels of SAA and haptoglobin were generally low. An increase in serum SAA and haptoglobin between T1 and T2 was statistically significant only for the clinical mastitis group 4 ($P<0.001$), averaging 0.483 mg/L and 0.998 mg/mL at T2, respectively. For the healthy group 1, no increase between T1 and T2 was noted. At T2, serum levels of SAA and haptoglobin were significantly higher in group 4 than in the other groups ($P<0.01$), exceeding markedly the reference haptoglobin values reported for healthy cows. Also the haptoglobin level was significantly higher in group 3 than in group 1 at T2. The healthy group 1 had average SAA and haptoglobin levels at T2 of 0.097 and 0.117 mg/mL, respectively. SAA and haptoglobin were highly correlated in groups 4 and 3 at T2 ($r=0.61$ and 0.79, resp., $P<0.001$). No significant differences in hematological parameters and band cell percentages were found between the groups.

Clinical mastitis/high SCC significantly increased serum haptoglobin and SAA as compared both with the healthy udder cows (SCC <100 thous.) and the cows with elevated SCC (200–800 thous.). We did not find any differences in serum SAA between the healthy udder cows (SCC <100 thous.) and the cows with elevated SCC (200–800 thous.), whereas serum haptoglobin was significantly increased with elevated SCC. Hematological parameters and leucogram were not significantly influenced by increasing milk somatic cell counts, not even by clinical mastitis.

**Keywords:** COWS, MASTITIS, MILK SOMATIC CELLS, ACUTE PHASE PROTEINS

# DOT BLOTTING ANALYSIS AS A WAY OF CELLULAR PRION TOTAL LEVEL DETERMINING

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Transmissible spongiform encephalopathies are fatal diseases caused by pathogens of protein nature — prions. Scrapie is diagnosed in sheep and goats and bovine spongiform encephalopathy is diagnosed in cattle. These diseases have a long period of incubation which is required for the replication and accumulation of the pathogen protein. The cellular prion ($PrP^C$) is a membrane protein of normal cells and it is involved in important processes. But under conditions of neurodegeneration $PrP^C$ becomes a substrate for the formation of abnormal pathological prion. The study of cellular prion level in different tissues is an important for understanding the mechanism of neurodegeneration. The aim of study was the determination of the $PrP^C$ total level in spleen, jejunum, liver, kidneys, muscle and brain of laboratory rats.

Research was carried out using the six months males of laboratory rats *Rattus norvegicus var. alba*, *Wistar* line. The animals were decapitated and the tissues were selected. The dot blotting analysis of tissues was carried out. For that, the tissue was homogenized and lysed in a special buffer with the addition of 0.001 % mixture of proteinase inhibitors (*Sigma*, Germany) as well as centrifuged. The protein level was measured by Lowry method.

The samples with the same concentration of the protein were deposited on polyvinyl diftorid (PVDF) membrane (*Millipor*, USA). The monoclonal primary antibodies (Antibody mAB6H4; *Prionics*, Switzerland) and secondary polyclonal goat anti-mouse antibodies, which are conjugated with alkaline phosphatase (*Sigma*, Germany), were used too. Detection of the immune complexes was carried out using a substrate for alkaline phosphatase CDP-Star (*Tropix*, UK). Visualization was performed using X-ray film *Retina XBM* (*Lizoform Medical*, Ukraine) and film development kit for films (*Kodak*, Japan) (Vlizlo V. V., 2012). The cellular prion total level was 100 % in jejunum, 97.41 % in medulla oblongata, 91.72 % in spleen, 52.63 % in cerebellum, 40.71 % in liver, 29.84 % in kidneys and 17.14 % in femoral muscle.

Prion pathologies arise mainly as a result of oral infection, while eating affected meat products or feed, as evidenced in experiments on monkeys (Verbitsky P. I., 2005). So the highest $PrP^C$ level in jejunum cells contributes to prionopathy. In experimental mice pathological prion was found in the spleen and lymph nodes on 5–13$^{th}$ week after injection, in the spinal cord on 13–17$^{th}$ week, and in the cerebrum on 17–19$^{th}$ week. Pathological changes in the brain appeared on the 25$^{th}$ week, and the clinical symptoms of encephalopathy appeared from 34$^{th}$ week. Pathological changes were observed only in the brain (Yuan J. et al., 2010).

Obviously, due to the cellular prion expressing spleen is the organ of prion replication. Neurons and glial cells express high level of $PrP^C$ too so they are very sensitive to abnormal prion lesions. This may explain a considerable degradation of neurons during prionopathies. Total $PrP^C$ level in other investigated tissues is lower but the infection spared depends on the $PrP^C$ production in this tissues.

The cellular prion is revealed in spleen, jejunum, liver, kidneys, muscle and brain of laboratory rats by dot blotting analysis. This confirms that $PrP^C$ is playing an important physiological function and investigated tissues are potentially dangerous because express cellular prion which is a precursor of pathological prion. The infection spared depends on the production of $PrP^C$ in the tissues.

**Keywords:** RATS, DOT BLOTTING ANALYSIS, CELLULAR PRION, TRANSMISSIBLE SPONGIFORM ENCEPHALOPATIES

# FORMATION OF MILK PRODUCTION OF BLACK-AND-WHITE CATTLE IN THE WESTERN REGION OF UKRAINE

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The main aim was to investigate the formation of milk productivity of black-and-white cattle.

Studies conducted on black-and-white cattle in the "Milk River" farm in the Sokal and Brody offices breeding reproducers "Breeder" Lviv region and plant breeding "Yamnytsya" Ivano-Frankivsk region. Milk productivity was studied using zootechnical materials accounting. The force of influence on performance metrics was calculated by Single-factor disperse analysis method. The results of research were treated by variation statistics.

Black-and-white cattle in the western region of Ukraine are characterized by high milk productivity throughout all studied lactation. In the firstborn, depending on the farm, milk productivity was from 4592 to 6032 kg, the fat content in milk — from 3.73 to 3.86 %. The milk productivity of the cows increased to the 3rd–4th lactation, and then gradually decreases. In experimental farms there were 2.3 to 14.7 % of cows with milk productivity during better lactation of 8000 kg and more. The coefficients of the variability were 13.3–27.4; the fat content in milk was 3.1–6.4, milk fat — 13.8–26.9 %, the coefficient of repeatability of milk productivity — 0.404–0.753, the fat content in milk — 0.242–0.781, the relative variability and the content of fat in milk — 0.282–0.254.

The formation of milk productivity of the cows was influenced by the intensity of their weight and linear growth during the period of growth, as well as the live weight after the 1st, 2nd and 3rd calving and the size of the body of the cows after first calving. The correlative variability of the live weight of animals during the period of growth and feeding was 0.018–0.604, the body measurements during the period of cultivation and fertilization — 0.170–0.458, live weight after the 1st, 2nd and 3rd palates, and infusion — 0.413–0.551, the body measurements of the foetuses and infusion — 0.297–0.478. The most significant impact on the future dairy productivity of the cows was made by their live weight at the age of 18 months and after the first calving, high-altitude measures, the circumference of the chest on the shoulder blades, and the skid length of the trunk, and the smallest — the live weight at birth and the circumference of the heel.

The influence of the lines on yield of milk, depending of the farm and lactation, was 9.6–39.0, the fat content of milk — 2.9–32.2 and the yield of milk fat — 9.7–38.8 %, the strength father's influence — 6.9–49.3; 7.4–68.4 and 6.8–51.0 % respectively. The coefficients of inheritance on the path along the "mother-daughter", depending of the farm and lactation, were within 0.034–0.618, fatty milk — within 0.032–0.762.

A black-and-white cattle in the western region of Ukraine is characterized by high milk productivity. The formation of milk productivity of the cows was influenced by the intensity of their weight and linear growth during the period of growth, as well as the live weight after the first, second and third calving and the size of the body of the cows after first calving. Significant influence on the milk productivity of cows was caused by their linear affiliation and parentage.

**Keywords:** BLACK-WHITE CATTLE, MILK PRODUCTIVITY, CORRELATION COEFFICIENTS, INHERITANCE AND REPEATABILITY, POWER OF INFLUENCE

# SUPEROXIDE DISMUTASE ISOFORMS IN TISSUES OF REPRODUCTIVE ORGANS IN BULLS

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The content of AFO is optimally supported by the antioxidant system. In it superoxide dismutase (SOD) plays the key role. In the reproductive organs of males the enzyme exists in three genetically predisposed forms — mitochondrial, cytosolic and extracellular. Therefore, it is important not only to state the changes in the activity of SOD, but also the redistribution of enzyme isoforms, when studying spermiogenesis and after ejaculation.

The aim is to investigate the content of SOD isoforms in the tissues of the reproductive system of the bulls.

Tissues of the testicles and epididymis, which were taken after the slaughter of the bulls (n=5) were used. Epididymium spermatozoa were washed with 0.9 % NaCl solution. Tissues were homogenized at 4 °C in 0.25M sucrose at 6000 rpm within 2 minutes. Homogenate was centrifuged for 15 min at 8000 rpm, supernatant was taken for study of enzyme isoforms. SOD isoforms were detected after electrophoresis in 10 % polyacrylamide gel by staining gel plates using Beauchamp and Fridovich method in our modification. Content of isozymes was calculated using *TotalLab TL120* program.

Five isoforms of SOD were detected in testicle tissues, epididymis and in spermatozoa. In testicle tissue isoform distribution was: 2.4–2.8 % S1 and S2 isoforms, 23.6–24.6 % S3 and S5, and 46.6±0.9 % S4 isoforms. In epididymis head 10.4±0.4 and 58.3±1.7 %, correspondingly, S1 and S4 isoforms, on 3.3 % and 12.6 % ($P < 0.001$) lower in body and 5.6±0.6 and 43.2±0.6 % in tail. Content S3 and S5 isoforms in epididymis head, respectively, 12.4±3.5 and 6.2±1.3 %, in body — 1.2 and 15.1 % ($P < 0.001$) higher and in tail — 17.0±0.2 and 22.2±2.6 %. The content of S2 isoform in epididymis head was 12.6±0.3% and remains at same level in tissues of body and tail.

Content of SOD isoforms in epididymal sperm depends on localization in morphological parts of the epididymis. S1 isoform content in spermatozoa of epididymis head was 18.4±1.5 %, increased to 29.1±3.0 % in spermatozoa from body and tail. S2 isoform content in spermatozoa with change in morphological part: head → body → tail of epididymis increases from 19.6±1.6% to 6.7 and 14.7 % ($P < 0.05$), respectively. S3 isoform content was high (14.7±1.6 %) in body of epididymis, lower by 5.1 % in tail and the lowest (4.5±0.6 %) in head. S4 isoform content in spermatozoa is reduced with a change in part of epididymis: head → body → tail with 52.3±5.6 %, 29.5 % ($P<0.001$) and 30.5 % ($P<0.01$), respectively. S5 isoform content was low (5.2–5.9%) in sperm from head and tail and on 1.9 % higher in epididymis body.

There are 5 isoforms of SOD in tissues of testicles and epididymis and in spermatozoa. In tissues of bull testicles, activity of SOD is mainly realized by S3, S4 and S5 isoforms, in epididysis by S2, S3, S4 and S5, and in sperm from epididymis: heads — S1, S2 and S5, bodies — S1, S2, S3 and S4 and in tails S1, S2 and S4.

**Keywords:** SUPEROXIDE DISMUTASE, ISOFORMS, REPRODUCTIVE ORGANS, SPERMATOZOA, BULLS, ELECTROPHORESIS

# OCCURRENCE OF SELECTED CALF DIARRHEA AGENTS IN AUSTRIA

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Calf diarrhea is one the most important diseases affecting calves worldwide. The high morbidity and mortality of the disease leads to tremendous production losses. Aim of this study was to determine the occurrence of selected viral, bacterial and parasitic causes of calf diarrhea on Austrian farms and to validate commercially available rapid tests for the detection of calf diarrhea agents.

Farm animal veterinarians and farmers from all over Austria were contacted directly (e-mail, telephone, congresses) and asked to participate. Calves less than six months of age with diarrhea were included in the survey which was carried out from November 2017 to June 2018. At the farm a personal interview was conducted, fecal samples were collected *per rectum* and clinical examination was performed on all included calves. Four different immunochromatographic rapid tests (A-D) for the detection of *Giardia intestinalis*, *Cryptosporidium parvum*, *Clostridium perfringens*, *E. coli* (F5), Rotavirus and Coronavirus were performed on-site on individual samples. At the university parasitological examination for *Giardia* spp. (immunofluorescence microscopy), *Cryptosporidium* spp. (phase-contrast microscopy) and *Eimeria* spp. (light microscopy, Mc-Master technique) was performed by one of the authors (KL). For virological and bacteriological examination samples were sent to the appropriate laboratories at the university where the fecal samples were screened for bovine Coronavirus, bovine Rotavirus A, *E. Coli* (F5, F41), *Salmonella* spp., *Campylobacter jejuni* and *C. perfringes* (a, Pi, $p_2$).

In total 177 samples from calves with diarrhea originating from 70 farms were collected and completely analyzed. Bacteriological examination of the 177 (100 %) samples yielded positive results for *C. jejuni* (8.5 %), *E. coli* (98.3 %), *C. perfringens* (29.9 %) and *Salmonella* spp. (1.1 %). *Eimeria* spp., *Giardia* spp. and *Cryptosporidium* spp. were found in 15.3 %, 27.1 % and 55.4 %, respectively. Virological examination showed 33.9 % and 23.7 % of the analyzed samples positive for bovine Coronavirus and bovine Rotavirus A, respectively. Rapid test A was positive for Rotavirus (25.4 %), Coronavirus (3.4 %), *E. coli* (1.7 %) and *C. parvum* (36.7 %). Rapid test B, C and D were positive for *Giardia intestinalis* (9.0 %), *C. parvum* (46.9 %) and *C. perfringens* (31.1 %).

Results confirm the widespread occurrence of the selected calf diarrhea agents on Austrian farms and that there are great differences in sensitivity and specificity of rapid tests for the detection of calf diarrhea agents.

**Keywords:** ENTEROPATHOGENS, PREVALENCE, DIAGNOSTICS, RAPID TESTS

# DISTRIBUTION AND CLINICAL AND BIOCHEMICAL STATUS OF D-HYPOVITAMINOSIS IN CALVES OF BLACK-AND-WHITE BREED IN WINTER-STALL PERIOD

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Many scientific papers, which comprehensively covered violations of D-vitamin and phosphorus-calcium metabolism devoted to pathology of metabolism in young cattle. A characteristic feature of most of these diseases is that they are mostly hidden the stage of flow at which the developing pathobiochemical disorders, back even to the stage of pathognomonic symptoms. Among the latter, D-hypovitaminosis is particularly common. It is characterized by a violation of the formation of bone tissue and its calcification with the following functional changes in the nervous, cardiovascular, digestive and respiratory systems. In addition to the lack of vitamin D, other hypovitaminosis — A, $B_1$, C, as well as zinc deficiency, manganese, copper and cobalt contribute to the development of this pathology. Also, one of the important reasons is a deficiency or violation of the optimal ratio of Calcium and Phosphorus. It was found that it should be in the diet of ruminants 1.5–2:1. The diagnosis of the disease at this stage can be made only by special, including laboratory methods of investigation.

When performing the work, general clinical and laboratory methods were used. The state of mineral metabolism in calves was determined by the serum content of total calcium (arsenazo-III reagent), inorganic phosphorus (by the method of UV detection of phosphomolibdate complex), and the activity of alkaline phosphatase (by the method of Wagner, Putilin and Kharabuga).

The distribution of D hypovitaminosis among calves 1-3 months of age was examined using clinical and laboratory methods, in OOO "Mojari" (Mogari village, Ovruch district, Zhytomyr region). The course of pathology in calves had two forms: subclinical and clinically expressed, which was much less common. During the clinical study, changes typical for D-hypovitaminosis were noted. Clinical symptoms of the disease were diagnosed in 53 calves (35.6 %), and subclinical course was noted in 97 animals (64.6 %).

The analysis of the content, feeding and the results of blood studies suggest that the main etiological factors of D-hypovitaminosis in calves is insufficient insolation with non-motorized content, low availability of their main nutrients and biologically active substances, namely feeding animals with insufficient intake of vitamin $D_2$ with feed (hay, haylage, silage, straw), excess Calcium and deficiency or excess Phosphorus. The imbalance of phosphorus-calcium nutrition is complicated by a pronounced deficiency of vitamin $D_2$ (65.4–89.5 %), deficiency of trace elements — Cobalt, Cuprum, Zinc, the provision of which was, respectively, 65.7, 71.3, and 81.4 % of the need. This imbalance of minerals in the objects of the environment is the cause of specific diseases in animals, including D-hypovitaminosis. Subclinical course of D-hypovitaminosis in calves was not expressed. Under these conditions, the most characteristic symptoms of the clinical course of the disease were characterized by softening and partial resorption of the last rib, pain of the backbone, thickening of the joints, curvature of the limbs (X-shaped formulation of the forelimbs), and resorption of the last tail vertebrae within 10 cm of the distal parts of the tail. The imbalance of minerals in the feed included in the diet of calves had a significant impact on the blood counts of calves. Significant violations of the clinical status in young cattle for subclinical and clinically expressed course of D-hypovitaminosis are confirmed by the results of biochemical blood tests: hypocalcemia, respectively, in 80 % of the diseased young animals — 2.05±0.05 mmol/l, hypophosphatemia — 20 % 1.65±0.04 mmol/l, is likely to increase the activity of alkaline phosphatase ($P<0.001$). The activity of total alkaline phosphatase for D-hypovitaminosis increases by 1.6 times, indicating a violation of the mineralization of bone tissue.

D-hypovitaminosis was registered in winter-spring period. The main causes of D-hypovitaminosis in calves 1–3 months of age are: low supply of vitamins $D_2$ and $D_3$, Cobalt, Copper and Zinc, excessive Calcium, high calcium-phosphorus ratio (2.6–4.3:1 against 1.5–2.0:1). Characteristic symptoms of D-hypovitaminosis in 1–3-month-old calves is softening and partial resorption of the last rib, pain of the backbone, thickening of the joints, curvature of the limbs, resorption of the last tail vertebrae.

**Keywords:** CALVES, TOTAL CALCIUM, INORGANIC PHOSPHORUS, D-HYPOVITAMINOSIS

# DIGITAL AMPUTATION FOLLOWED BY SCREW FIXATION OF THE SUBSEQUENT PROXIMAL PHALANX LUXATION IN AN ALPACA STALLION

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Digital amputation is the most commonly applied treatment for severe deep digital sepsis involving the distal and proximal phalanges in cattle. To our knowledge there are no previous publications reporting digital amputation and its sequelae in new world camelids. This case report describes the digital amputation through the proximal phalanx (P1) for treatment of septic arthritis of the proximal interphalangeal joint (PIJ) in an alpaca stallion, and the treatment of subsequent luxation of P1 using internal fixation.

A 4-year-old alpaca stallion was presented with severe lameness (3/5 in walk) and an infected, 10×5 cm sized wound on the abaxial aspect of the medial digit of the left front limb reaching from the PIJ to the distal interphalangeal joint. During cleaning maggots, necrotic tissue and fibrin were flushed out. At first, wound debridement, curettage, administration of antibiotics, NSAIDs, and bandage changes every other day was applied. However, after initial improvement and after termination of administration of antibiotics and NSAIDs, lameness re-appeared and a fistula developed. Radiographs showed an axial subluxation of P2 and gas inclusions in the region of PIJ, leading to the diagnosis of septic arthritis of the PIJ in combination with subluxation. Under general anaesthesia, the wound was diligently debrided and the joint flushed. The leg was stabilized with a half limb cast. The fistula healed and the alpaca was discharged showing a mild lameness in walk (1/5) only.

After four weeks of repeated bandage changes and wound management the fistula recurred and instability of the joint was palpated. Radiographs revealed a complete axial luxation of P2 and diffuse areas of radiolucency indicating osteomyelitis of P2 and the distal part of P1. Under general anaesthesia, an amputation through the distal aspect of P1 was performed and the amputation wound was closed with sutures. The wound healed by first intention and the alpaca was free of lameness in walk, pace and gallop for 5 years.

In 2018, the stallion was presented with severe, sudden onset lameness (4/5) of the left front limb in walk. A painful swelling was identified at the lateral aspect of the fetlock joint. Radiographs showed abaxial luxation of P1. Under general anaesthesia, the luxation was treated by internal fixation of the displaced lateral P1 to the remnant of the medial P1 using a cancellous bone screw inserted at their proximal aspects, and by a Robert Jones bandage. After four weeks of box rest the stallion was discharged from the hospital, and after eight additional weeks of box rest with gradual increase of controlled exercise and bandaging, the stallion showed no lameness in walk and pace.

After searching in all available databases (MED-LINE/PubMed, Google etc.), it seems that this is the first report describing digital amputation and its sequelae in an alpaca. Thus initial screw fixation of the remaining P1 at the time of digital amputation is an interesting possibility for future investigations. Despite the worry that alpacas as tylopoda might have problems when walking on one digit, the final, successful outcome is encouraging.

**Keywords:** ALPACA STALLION, DIGITAL AMPUTATION, LAMENESS

# CRITERIA FOR ASSESSING THE QUALITY AND SAFETY OF BEEF IN THE AGRO-INDUSTRIAL MARKET

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Beef samples of *NOR* and *PSE* obtained from cattle carcasses from different enterprises of the Kyiv region on the agro-industrial markets of Bila Tserkva (no. 1, no. 2 and Indoor market), improvement and development of express methods for the determination of beef of NOR quality and PSE.

Organoleptic, biochemical, physico-chemical, microscopic, microbiological, morphological, biometric (GOST 7269–79 and GOST 23392–2016).

Beef produced from quality beef *NOR* was registered at 85.2 %, quality of *PSE* — 11.4 %, *DFD* — 19.6 %. Sufficiently often (31.0 %) is found beef, which requires special treatment to improve its quality. The pH of beef quality *of PSE, DFD* in one hour after the slaughter of cattle was 5.11±0.14 and 6.21±0.17, respectively, in comparison with the NOR quality (6.02±0.12). PSE and DFD had worse organoleptic characteristics, lower biological value compared to NOR beef. The relative biological value of beef PSE was, on average, 67.3 %, and beef DFD — 62.4 %. The water content of PSE beef was 1.07 times higher than that of NOR beef and 1.2 times less in DFD beef. The content of dry matter was the lowest in beef quality of PSE — 20.14 %, which is 21.2 % lower, compared to beef NOR values. The content of ash in beef of all categories of quality ranged from 1.03 to 1.19 %. The protein content was the highest in DFD quality beef, which is 1.5 times more than NOR quality beef. The fat content was the lowest in PSE quality beef, which is 0.7 times less than that of NOR quality beef. The beekeeping capacity of the beef was the lowest quality PSE — 52.27±2.31 %, which is 1.2 times less, and in the beef quality DFD — 0.7 times more than in the quality of NOR. The content of glycogen was lowest in beef-grade DFD — 127.65 mg%, which is 54.9 % less, and in beef quality PSE — lower by 7.13 %, compared to NOR indicators. The content of lactic acid was the largest in beef PSE quality — 1.23 times, and in beef quality DFD — 3.4 times less, compared with NOR indicators. The content of glucose was higher in beef-quality PSE — 1.3 times, and in DFD — 1.6 times less, compared to NOR indicators of quality. The content of tryptophan in the beef quality of PSE and DFD was less than 1.08–1.05 times compared to NOR beef quality. The content of oxyproline in beef PSE and DFD was greater by 1.05–1.1 times compared to NOR beef. The protein-quality index was lower in beef quality PSE. An express photometric method of improving the determination of the total content of pigments in the beef of NOR, PSE, DFD quality, which was 98.3 % probable compared to pH. The established optical density indicators for the total content of pigments in beef of NOR quality range: 8.43–10.17 B, PSE 1.68–2.41 B and DFD 16.22–18.89 B.

For the determination of NOR, PSE, DFD quality beef, in addition to the existing complex of organoleptic, biochemical studies, it is necessary to use morphological and photometric methods to determine the total content of pigments and color intensity applied for under no. 03329, u 2007 03330 on the issuance of Ukraine's Declarative Patents for invention.

**Keywords:** BEEF, QUALITY NOR, PSE, DFD, RESEARCH COMPLEX, PIGMENT CONTENT AND COLOR INTENSITY

# GLUTATHIONE REDOX STATE, GPX ACTIVITY AND SE CONCENTRATION IN DAIRY COWS DURING NEGATIVE ENERGY BALANCE

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The aim of research was to evaluate glutathione redox state, GPx activity, Se concentration and also NEFA and BHB concentrations in 15 Holstein dairy cows during negative energy balance.

Blood samples were collected 4 times during this period — 7 days a.p., calving day, 7 days p.p. and 14 days p.p. All of the cows had gone through two or more lactations. The BCS was recorded at every single blood collection of the cows. Serum NEFA and BHB concentrations and whole blood GPx activity were measured using standardized kits supplied by *Randox Laboratories*. Reduced and oxidized glutathione concentrations were measured with a BIOXYTECH GSH/GSSG-412 kit (*Oxis-Research*, USA) using a colorimetric enzymatic method. The selenium concentration in whole blood was analyzed using atomic absorption spectrometry. The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Fisher LSD *post-hoc* test. The relationship between parameters was evaluated by the correlation coefficient and the significance of correlation using linear regression analysis.

A significantly increased NEFA concentration was recorded on calving day ($P<0.05$) and 7 days p.p. ($P<0.01$) compared to 7 days a.p. An increase in BHB concentration was also observed after parturition in our study, but was not, however, significant ($P>0.05$). The GSH concentration was significantly decreased on calving day and 7 days p.p. ($P<0.05$) as compared to 7 days a.p. The mean GSSG concentration was significantly higher 7 days p.p. as compared to calving day ($P<0.01$) and 14 days p.p. ($P<0.05$). The differences in GSH/GSSG ratio were not, however, significant ($P>0.05$). The significant decrease in GPx activity was found 14 days p.p. as compared to 7 days p.p. ($P<0.05$). No significant differences ($P>0.05$) in Se concentration between individual groups were found. Between the GSSG concentration and the GSH/GSSG ratio a significantly negative ($r = –0.84$; $P<0.001$) correlation was found. A significantly positive correlation was found between the BCS value and the GSSG concentration ($r = 0.44$; $P<0.05$). The BCS value was also negatively correlated to GSH/GSSG ratio ($r = –0.30$) but it was not, however, significant ($P>0.05$).

The results of our study indicate significant changes of antioxidant markers during negative energy balance and also confirm that during the periparturient period oxidative stress occurs in dairy cows. It seems that BCS value correlates to antioxidant markers and could influence the level of oxidant processes in cows during the periparturient period.

**Keywords:** REDUCED GLUTATHIONE, OXIDIZED GLUTATHIONE, OXIDATIVE STRESS, NEGATIVE ENERGY BALANCE, DAIRY COWS

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# EFFECTS OF TETRACYCLINE INJECTION ON BLOOD CALCIUM AND RUMINAL ACTIVITY IN SHEEP

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Tetracycline is widely used in the treatment of the foot rot in ruminants. They chelate with $Ca^{2+}$ ions causing a depression of levels of ionised calcium. The objective of the study was to assess effects of tetracycline administration on serum calcium concentrations and the frequency of ruminal contractions.

Rumen contractions were monitored by auscultation in 23 sheep prior to administration of oxytetracycline and recorded every 12 hours for 84 hours after intramuscular injection of the antibiotic. Blood for calcium analyses was collected by venepuncture of the jugular vein before and 24, 48, 72, and 96 hours after administration of oxytetracycline. The serum calcium concentrations were determined by atomic absorption spectrophotometry. Analysis of variance (ANOVA) was used to analyse the time effect of tetracycline treatment on the rumen contractions and serum calcium concentrations.

There was a significant decrease ($P<0.01$) in ruminal contractions following application of oxytetracycline, with a maximum decrease at 24 hours following oxytetracycline application and a return to the initial rumen contraction frequency by 60–72 hours following oxytetracycline application. The oxytetracycline administration resulted in serum calcium decrease from 2.42 mmol/l to 2.26 mmol/l 24 hours after the administration ($P<0.01$).

In conclusion, the administration of tetracycline in sheep can be associated with a decline in ruminal motility potentially causing production losses, particularly in lactating ewes. Despite the resulting transient production decreases, oxytetracycline remains the antibiotic drug of choice for the treatment of bacterial infections in small ruminants, foot rot especially.

**Keywords:** SHEEP, TETRACYCLINE, RUMINAL ACTIVITY

# HAPTOGLOBIN CONCENTRATIONS IN DAIRY COWS WITH INFLAMMATORY DISEASES

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Haptoglobin is an acute phase protein produced primarily in the liver in response to pro-inflammatory cytokines. The objective of this field study was to determine if common inflammatory diseases like mastitis and claw inflammatory disorders are associated with increased serum haptoglobin. Moreover, the sensitivity and specificity of haptoglobin levels were tested.

237 Holstein dairy cows were included in the study; farm feeding management was based on TMR and the dairy cows were housed in free stalls with cubicles. Health condition of mammary gland and claws was examined in the crush. The subclinical mastitis was diagnosed by using California mastitis test. Blood samples were obtained from the jugular vein at the time of the clinical examination and treatment. Serum haptoglobin concentration was measured by colorimetric assay (*Tridelta Development*, Ireland). For statistical analyses the dairy cows were divided into two groups: INFLA (cows with inflammation) and control (no inflammation found). Differences in serum haptoglobin levels were tested by *t*-test. The threshold level of haptoglobin for calculation of sensitivity and specificity was 0.05 g/L.

Clinical mastitis, subclinical mastitis, and inflammatory claw disorders, including digital dermatitis, interdigital dermatitis, pododermatitis, interdigital hyperplasia, and subclinical laminitis were found in 204 dairy cows (INFLA). 33 dairy cows were found to be free of inflammatory changes (control). Cows with inflammation had higher serum haptoglobin than controls (INFLA: 0.21 g/L; control: 0.06 g/L; $P<0.01$). The sensitivity detecting dairy cows with inflammatory disorders by serum haptoglobin levels was 84 %, whereas the specificity in the control group of 33 clinically unsuspicious cows was 68 %.

Results of the study show that the inflammatory disorders in dairy cows are associated with increased concentrations of the serum haptoglobin. However, sensitivity and specificity of the serum haptoglobin are rather low for detection of inflammatory processes in dairy cows. Therefore, a use of serum haptoglobin for monitoring of inflammatory diseases on the dairy farm level can be recommended only with limitation.

**Keywords:** DAIRY COWS, HAPTOGLOBIN, INFLAMMATION

# LINK BETWEEN SOLE ULCER AND SUBCLINICAL LAMINITIS

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Subclinical laminitis is a multifactorial syndrome with complex pathophysiology and significant economic impact on dairy industry. One of the effects it has on the welfare of cattle is that it predisposes to the development of other lesions on the foot, predominantly sole ulcers and white line disease. The aim of the study was to investigate if the subclinical laminitis actually predisposes dairy cows to the development of sole ulcer.

The data used in this study were obtained on 220 Holstein Friesian dairy cows during 2 sessions of routine orthopaedic and claw trimming visits (autumn 2016 and spring 2017) as well as within a period between them when lame cows were treated. All of the cows were kept on manure solid bedding and fed TMR. The average milk year yield was 9000 kg. At the first visit the cows with subclinical laminitis were identified (LS group). The occurrence of the sole ulcer was checked at the following visits in all the cows. Statistical analysis was performed by running a chi-squared test to test a difference between sole ulcer incidence in LS and control (healthy) group.

Out of the 220 dairy cows examined during autumn 2016 10 cows were affected by the subclinical laminitis (LS group; 4.55 %). 69 dairy cows were free of claw diseases (control group; 31.4 %). In the following orthopaedic controls the sole ulcer was detected in two cows from the LS group (20 %) and only in one cow in the control group (1.45 %). The difference in incidence of the sole ulcer between both groups was significant ($P<0.05$).

The results of this study indicate that there is an association between subclinical laminitis and prevalence of sole ulcer in dairy cows. Therefore, a dairy farm management should pay more attention to avoid all known risk for subclinical laminitis development on the farm to prevent higher incidence of lameness.

**Keywords:** COWS, SOLE ULCER, SUBCLINICAL LAMINITIS

# CLINICAL TRIAL OF TRADITIONAL CHINESE HERBAL PRESCRIPTION *CHANFUKANG* ON PREVENTION OF CLINICAL ENDOMETRITIS

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The study was aimed to investigate the effect of traditional Chinese herbal medicine *Chanfukang* on reducing postpartum dairy cow uterine infection.

Primary trial involved 66 animals, group 1 (200 g/day/cow, 22 animals), group 2 (300 g/day/cow, 22 animals) and control one (0 g/day/cow, 22 animals). Powder of *Chanfukang* were added to daily food of the animal in group 1 and group 2, from 1 day before calving and to 6 days after calving, while control group feed with normal TMR food. In the expending experiment, 352 animals from 6 different farms were involved. *Chanfukang* was given to 182 animals with 200 g/cattle/d, the other 170 cows served as control group, received normal TMR food. Related data were collected, including time of expelling fetal membrane; animals suffer retained fetal membrane, days to first estrus, days to first service, the rate of pregnancy on day 85, incidence of clinical endometritis.

The primary trial showed the indexes in group 1 and group 2 were similar, and the incidence of retained fetal membrane, clinical endometritis were much lower than the control group, which indicated that 200 g/cattle/day was sufficient for clinical use. In the expending experiment, morbidity of retained fetal membrane was 7.14 % in *Chanfukang* group compared with 17.06 % in the control group. Days to first estrus and days to first in *Chanfukang* group and control group were 47.68±7.3 and 59.9±8.8 vs. 59.5±12.9 and 68.1±13.3. Rate of pregnancy on day 85 was 87.91 % and 77.06 % in experimental group and control group. And the incidence of clinical endometritis was 8.24 % and 26.47 % in *Chanfukang* group and control group. By using *Chanfukang*, days to first estrus and days to first service were ahead for about 10 days than the control animals.

The Chinese herbal prescription *Chanfukang* was effective in promoting uterine evolution, contribute to reduce the days to first service, increase the pregnancy rate and prevent the animals suffering from clinical endometritis.

**Keywords:** DAIRY COWS, ENDOMETRITIS, *CHANFUKANG*, PREVENTION

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# COMPARATIVE STUDY OF IMMUNOGLOBULIN CONCENTRATION BETWEEN HEALTHY COWS AND ANIMALS WITH CLINICAL ENDOMETRITIS (CE COWS)

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The study was designed to investigate the dynamic changes of IgA, IgGs and IgM in serum and uterine secretions of postpartum dairy cows.

Involved animals were limited to these aged from 3 to 8, with similar calving time and without other peripartal diseases. The animals were evaluated by eye view and confirmed *per rectum* on d21, 9 CE cows were enrolled in the evaluation and another 9 healthy animals were served as the control group. Serum samples and uterine secretions were collected on d21, d28, d35 and d42. All samples were kept on ice till laboratory handling or kept at –80 °C freezer. The enzyme-linked immunosorbent assay (ELISA) was adopted for concentration analysis.

The results showed that:

1) The uterine mucus IgA concentration in healthy animals were consecutively decreases from d21 to d42, while in the CE cows reduced from d28 to d35, but significantly rose from d35 to d42. Serum IgA in healthy animals followed a decreasing manner and in CE cows the changes were fluctuated.

2) Uterine mucus IgGs in CE cows down regulated from d28 to d35, but significantly rose from d35 to d42, while in healthy animals the IgGs rises sharply from d28 to d35 and goes even higher on d42. But the serum IgGs in both healthy and CE cow generally down-regulated and did not changed much from d35 to d42. This significant difference between the serum IgGs and the uterine mucus IgGs concentration indicated an independent immune response of the uterus.

3) The serum IgM in the two groups showed a same decreasing manner, but in the uterine mucus, IgM in the CE cows maintained around 1.5 g/L while in the healthy animals less than 0.5 g/L during the period.

In summary, the uterine mucus IgA, IgG and IgM showed a different changing manner than the serum concentration, which indicated an independent immune response in the uterus environment. In the CE cows, IgGs and IgM plays the major role in defending invasiveness from outside, and the IgGs responded a week earlier than the IgA. The higher concentration of IgM in the uterus might serve as an indicator of CE cows, further studies were required to confirm the theory.

**Keywords**: DAIRY COWS, CLINICAL ENDOMETRITIS, IMMUNOGLOBULIN CONCENTRATION

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# RUMEN HEALTH CONSEQUENCES OF HIGH-CONCENTRATE FEEDING IN CATTLE: MORE THAN A SIMPLE DROP IN RUMINAL pH

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The intensive feeding of high-yielding milking cows with grain-rich diets causes a drop in ruminal pH and can impact cow health and production with long term consequences, known as subacute ruminal acidosis (SARA). Results from new analytical methods, such as next generation sequencing and gene expression, have shown that feeding high-grain diets is not just a matter of acidosis but of a dysbiosis of the whole rumen ecosystem. We hypothesized that high-concentrate feeding impacts the microbial diversity differently in the rumen digesta compared to the epithelium, shifts fermentation end products, as well as host epithelial metabolism-, and barrier function gene expression.

Eight rumen cannulated Holstein cows were fed a 100 % roughage diet (RD, 1 week), followed by an intermittent 65 %-high-concentrate-diet (HC1 and HC2; 1 and 2 weeks, respectively, interrupted by 1 week roughage only). The feeding protocol was conducted in 2 consecutive runs, with a 3 weeks washout period. Reticular pH was measured continuously; rumen sampling was performed in RD, HC1, and HC2. Ruminal pH, short-chain fatty acids (SCFA), lactate, and ammonia were analysed in the rumen digesta and fluid. Particle-associated rumen microbiota (PaM) and epithelial microbiota (EpM) were analysed using *Illumina MiSeq* sequencing of the 16S rRNA gene, and epithelial gene expression using RT-qPCR to target barrier function-, cellular transport, pH, and metabolism genes. Bioinformatic analysis was performed using *QIIME 1.0*, and statistical analysis using *PROC MIXED of SAS 9.4*.

The drop of reticular pH was more severe in HC1 with 370 min spent <pH 6.0 vs. 164 min <pH 6.0 in HC2. Microbial diversity in PaM decreased the most in HC1 ($P<0.01$), whereas EpM increased in diversity in HC1 and HC2 ($P<0.05$). Distance matrix analysis revealed that PaM in RD clustered more tightly and away from HC samples compared to the EpM. There was a significant decrease of the highest abundant phylum *Firmicutes*, and an increase of *Bacteroidetes*, and *Actinobacteria* in PaM ($P<0.05$), and a decrease of the highest abundant phylum *Proteobacteria* and an increase in *Bacteroidetes* in EpM with HC ($P<0.05$). SCFA shifted with decreasing acetate and increasing propionate in both rumen digesta and fluid ($P<0.01$), lactate increased ($P=0.07$), and ammonia decreased ($P=0.02$) with HC feeding in comparison to RD. Nutrient transport genes such as MCT1 and MCT4, cellular metabolism target BDH1, and barrier function gene CLDN4 were all downregulated in HC1, whereas the barrier function gene DSG1was upregulated in HC1 ($P<0.05$), and cellular pH regulation gene DRA was upregulated in HC2 ($P<0.05$).

In our study HC1 had greater impact on reticular pH and PaM, suggesting that the microbiota that are located in the rumen digesta are more susceptible to all types of nutritive changes. Most targeted genes showed either a significant or numeric decrease in HC1, with recovered levels in HC2, speaking for an adaptation in HC2. EpM also stabilized in HC2, but was generally less impacted by high-concentrate feeding. In summary, our findings show an impact of the high-concentrate feeding not only on pH but both PaM and EpM communities, their metabolic products, and host gene expression.

**Keywords:** HIGH-GRAIN FEEDING, RUMEN MICROBIOTA, EPITHELIAL-GENE EXPRESSION, RUMEN DYSBIOSIS

# ULTRASONOGRAPHIC IMAGING OF EPIPHYSEAL GROWTH PLATES IN CALVES — PRELIMINARY FINDINGS

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Calves suffer frequently from septic haematogenous arthritis, oftentimes associated with a concurrent osteomyelitis of the epiphyseal growth platesor of the subchondral articular bone. An accurate and early diagnosis is the key for a successful treatment. Radiography is the diagnostic imaging method of choice for the evaluation of bones and joints, however it is inadequate for detecting early stages of septic arthritis and even of early stages of osteomyelitis. Furthermore, many bovine practitioners are not equipped with a radiographic unit, therefore making an accurate diagnosis of disorders of the epiphyseal growth plates difficult or impossible. In contrary, many bovine practitioners are equipped with 5–8 MHz linear rectal probes.

The objective of this study was to describe the ultrasonographic appearance of the epiphyseal and apophyseal growth plates of the front and the rear limbs in young calves from the age of 1 week to 3 months, and to establish an examination protocol which can be applied as a reference for their ultrasonographic examination in calves with suspected pathology.

An ultrasonographic examination of the epiphyseal growth plates of the distal metacarpus/metatarsus, distal radius/ulna, proximal radius, distal and proximal humerus, distal and proximal tibia, distal and proximal femur, of the apophyses of olecranon tuber, major tuberculum, tuber calcis, tibial tuberosity and of the major trochanter was carried out in 12 Simmental calves at 5 time-points from the first and 12$^{th}$ week of life. The calves were examined in a standing position using a 7.5 MHz (5–8 MHz) linear probe. All growth plates were scanned in longitudinal planes from all sides by moving the probe always from dorsal/cranial in a circumferential 360 ° course over the lateral, caudal to the medial aspect, if possible in the particular anatomical situation. At each time point, all these growth plates were imaged and ultrasonographic measurements of their proximo-distal width were taken.

The indicated cartilaginous growth plates were imaged in all calves at the subsequent time-points appearing as short anechoic interruptions (a few millimeters to less than 1 mm in older calves) of the adjoining hyperechoic bone surface in longitudinal planes. The indicated cartilaginous apophyses in these calves were depicted as large heterogeneous hypoechoic 5 to about 17 mm thick structures bordered distally by the hyperechoic contour of the ossified bone. The time needed for ultrasonography of one particular epiphyseal growth plate was about 7 minutes for a trained operator.

It can be concluded that ultrasonography enables good imaging of the cartilaginous growth plates of all long bones in the front and rear limbs of calves. Therefore, this non-invasive diagnostic imaging technique is well suited for examination of these particular areas in young calves with swollen joint regions, suspected septic arthritis and the history of haematogenous spread leading to a possible concurrent infection of the adjoining epiphyseal growth plate, in particular when a radiographic unit is not available.

**Keywords:** DIAGNOSTIC ULTRASOUND, RECTAL PROBE, EPIPHYSEAL GROWTH PLATE, HAEMATOGENOUS OSTEOMYELITIS, CALF

# EFFECT OF CHROMIUM (III) ON MICROBIAL BIOMASS AND HYDROLYTIC ACTIVITIES IN THE BULLS RUMEN

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Despite the proven positive effect of chromium as an essential micronutrient, the mechanisms of its action have not been studied sufficiently, and its recommended content in the animal diet is not yet standardized. Chromium affects glucose and fatty acid metabolism, immune resistance, antioxidant status, and performance of cattle. However, chromium has antimicrobial properties and can cause damage to the plasmid DNA and violation of protein metabolism. Because the diet of ruminant is previously fermented in the rumen, it is important to know the influence of dietary chromium to the rumen microbiota. Therefore, the study of metabolic action of chromium for ruminants needs not only investigations of effects on the animal body but actions on the rumen microbiota too.

The content of the rumen from the fattening bulls of Ukrainian dairy black-and-white breed, with a body weight of 330–340 kg, at the age of 24 months was taken. All animals received similar nutritionally balanced diet. The incubation of the rumen filtrates were performed in anaerobic conditions at a temperature of 38 °C for 24 hours. Chromium chloride (III) was added to the incubation in the amount of 0.5; 1.0; 1.5 and 2.5 μM. The amylolytic and cellulolytic activities and the mass of rumen microorganisms were determined.

Important parameters that characterize the processes of digestion in the rumen and the degree of provision of ruminant with the microbial protein is the quantity and mass of microorganisms. *In vitro* studies, we found that the addition to an incubation medium with a rumen content of chromium chloride (III) in a dose of 1.0 μM had a pronounced stimulating effect on the proliferation of microbial cells and metabolic activity, and led to increasing of microbial mass and elevation of activities of the hydrolytic enzymes in the rumen. Our results have shown that the addition of chromium chloride (III) to the incubation medium has led to the activation of anabolic processes in microorganism cells, resulting in an increase in their mass. The most intense growth of rumen microorganisms after 24 hours of incubation *in vitro* observed when chromium chloride (III) was added to the incubation medium for 0.5 μM concentration. Adding to the medium low doses of chromium (III) stimulated the enhancement of cellulolytic activity of the rumen microorganisms. Under the influence of inorganic chromium (III) in doses of 0.5 and 1.5 μM, the amylolytic activity of the rumen microorganisms increased also. The highest investigated concentration of chromium chloride (III) at a dose of 2.5 μM did not change the rate of growth of microorganisms and somewhat suppressed the cellulolytic activity of rumen microorganisms.

Chromium chloride (III) added to the rumen content in amount of 0.5 μM, positive affects some microbial enzymes what lead to increase in the microbial mass and higher hydrolytic activity in the rumen. So, chromium chloride (III) is an activator of metabolic processes in microbial cells of the rumen microbiota.

**Keywords:** BULLS, RUMEN, CHROMIUM CHLORIDE (III), MICROBIAL MASS, CELLULOLYTIC AND AMYLOLYTIC ACTIVITIES

# THE T- AND B-CELL SPECIFIC IMMUNITY OF CALVES UNDER THE INFLUENCE OF COMPLEX LIPOSOMAL DRUG

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There are contradictions between traditional and modern views on the immunobiology of pregnancy. In the last month of pregnancy certain changes occur, which are accompanied by a decrease in cellular and humoral protection and activation of lipid peroxidation. These disorders in cows' body are caused by physiological immunosuppression, which increases in pregnant animals due to unsatisfactory conditions for the maintenance at this period. So, the elaboration of complex immunotropic drugs with the immunorehabilitation effect, which will be achieved by the presence of components that provide optimization of critical biochemical mechanisms for maintaining metabolic homeostasis is relevant.

Studies were conducted on cows of the last gestation period. Animals of the control group received intramuscular isotonic solution of sodium chloride, cows of the 1st and 2nd experimental groups — vitamins A, $D_3$, E, lecithin, L-methionine, L-arginine, sodium selenite intramuscularly 20- and 10-days before calving as liposomal emulsion, at a dose of 0.04 ml/kg of body weight. Calves born from cows of the 2nd experimental group — vitamins A, $D_3$, E, lecithin, L-methionine, L-arginine, sodium selenite in the form of a liposomal emulsion were administered intramuscularly at 3-day age. Calves received from cows of the 1st experimental and control group, respectively, were injected with isotonic sodium chloride solution. The material for researches was blood of calves at 3-, 7-, 14- and 21-day-old age.

The studies have shown that the introduction to cows at the last month of pregnancy of the liposomal drug causes an immunoregulatory effect on the cellular link of specific immune protection. This is evidenced by the greater number of T-lymphocytes (common, active, theophylline-resistant) in the blood of calves in both experimental groups throughout the study period ($P<0.05$-$0.001$). At the same time, the number of theophylline-sensitive T-lymphocytes in the blood of calves in both experimental groups on the 7th day of life was lower ($P<0.05$). At the same time, the number of T-suppressors in the blood of calves in the 1st experimental group increased at 14 and 21 days of age ($P<0.05$; $P<0.001$), and in animals of the 2nd experimental group at the 14th day of life ($P<0,001$).

During the study period, a higher level of T-lymphocyte induction to blast transformation with phytohemagglutinin, as well as a greater number of B-lymphocytes in the blood of calves in both experimental groups was observed for the action of the liposomal drug ($P<0.05$–$0.001$). The increase in the number and functional activity of T- and B-lymphocytes in blood of calves is probably due to both the direct and/or indirect effects of vitamins A, $D_3$, E, lysine, methionine, arginine and sodium selenite on the expression of T- and B- lymphocytes on the plasma membrane. Thus, the positive effect of the elaborated liposomal drug on the state of the T- and B-cells immunity of calves which will increase their immune potential has been confirmed.

Parenteral administration to cows in the last month of the pregnancy of the complex liposomal drug which includes: vitamins A, $D_3$, E, lecithin, L-methionine, L-arginine, sodium selenite, causes an increase in the number of T-lymphocytes (common, active and theophylline-resistant), and B-lymphocytes in the blood of calves born of them and increases the functional activity of immunocompetent cells due to the redistribution of the receptor apparatus of T- and B-lymphocytes in the direction of increasing their avidity. In this case, an increase in the functional activity of T-lymphocytes in the reaction of ballast transformation of lymphocytes with phytohaemagglutinin was noted.

**Keywords**: IMMUNITY, BLOOD, CALVES, VITAMINS, LYMPHOCYTES

# IRON SUPPLY STATUS OF BREEDING CALVES IN AUSTRIA

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Veal production is important branch of the Austrian beef industry. Many consumers still prefer pale veal over darker meat although it is produced from iron deficient beef calves. However, optimal iron supply is of importance for growth and health, especially in female heifer calves raised for breeding.

The aim of the study was to assess iron supply in female breeding calves in Austria.

Twenty one dairy farms located in the Innviertel (Upper Austria) were visited to take blood samples from 118 heifer calves. Calves were excluded from the study if they were older than 16 weeks of age or had received a treatment with iron containing drugs within 7 days before collection of blood samples. Hematocrit, hemoglobin and plasma iron concentration were measured. On each farm a questionnaire concerning housing, feeding, health and medical treatment of the calves was completed.

Results showed the presence of iron deficiency anemia in a substantial proportion of the heifer calves. In 43.2 % and 17.8 % of the calves hematocrit and hemoglobin were either moderately or severely decreased below physiological values. Calves showed a deficiency of iron in blood plasma in 44.9 % of the cases.

The highest prevalence of anemic calves was found between the 5$^{th}$ and the 8$^{th}$ week of life. After this age the measured parameters in sampled calves steadily increased until 16 weeks of age.

The feeding of milk replacer instead of whole milk resulted in a positive effect on hematocrit, hemoglobin and plasma iron concentration. The same effect was observed if hay or grain was added to the diet as early as day eight of age. A positive effect caused by prophylactic application of iron supplements was evident: calves which received such supplements showed significantly higher blood hematocrit, hemoglobin and iron concentration than calves without any prophylactic measures.

Iron deficiency anemia plays an important role in heifer calves and needs to be considered in veterinary practice. Additional to therapeutic measures in animals suffering from iron deficiency prophylactic measures should become part of the general prophylactic concept for the herd.

**Keywords:** IRON DEFICIENCY, CALVES, IRON SUPPLEMENTATION

# TESTICULAR WEIGHT, OCCURRENCE OF TUBULES WITH ELONGATED SPERMATIDS AND SERTOLI CELL COUNT IN ABATTOIR CALVES. PRELIMINARY RESULTS

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It is important to know when male calves become potentially fertile in mixed suckler herds in order to avoid unwanted pregnancies. The occurrence of tubules with elongated spermatids and especially Sertoli cells is important for the development of sperms as it supports spermatogonia and its successors. The aim of this study was to investigate the appearance time of these markers during testicular development in calves slaughtered at various ages.

Carcass weight and age of 5- (n=5), 6- (n=10), 7- (n=10) and 8-month-old (n=7) male beef calves were documented and their testicles examined. After removal of their sheaths and adnexa, pure testicles were weighted. Left and right testicles were dissected and tissue samples were taken from 3 different localizations of each testis. After collection, all samples were fixed in Bouin's solution. Consecutively, these tissue samples were embedded in paraffin and finally stained with hematoxylin-eosin. Tubules with elongated spermatids and Sertoli cell count per tubule cross section for each of the six locations were histologically examined. The preliminary results include correlation and regression analyses.

Correlation between the means of testicular weight of left and right testicles and carcass weight ($R^2$=0.32, P<0.0001) was markedly higher than that between testicular weight and age ($R^2$=0.03, P=0.0152). With advancing age and slaughter weight, the P-value is slightly above 0.05 and is therefore not significant. Histological evaluation showed that increasing total testicular weight only slightly interacted with the number of tubules with elongated spermatids ($R^2$=0.10, P<0.0001) and even less with increasing age and Sertoli cell count in the testicles ($R^2$=0.02, P=0.0017). In 14 of 32 calves, 5- (n=5), 6- (n=5) and 7-month-old (n=4), elongated spermatids could not be detected. The number of Sertoli cells per tubule cross-section shows a large individual variation. A weak trend towards slightly more Sertoli cells can be detected with increasing age. This supports the hypothesis that Sertoli cell formation is almost complete at the age of five months and that only minor changes occur thereafter. Due to the absence of elongated spermatids, five months young calves still seem to be prepubertal.

Male and female calves should be separated at the latest shortly before they reach 6 months to avoid the presence of potentially fertile young males along females and, thereby, unwanted pregnancies. However, these data should be further analysed, and a larger number of samples might make the recent findings even more reliable.

**Keywords:** BOVINE, TESTIS, HISTOLOGY, PUBERTAL DEVELOPMENT

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# THE OCCURRENCE OF OSTERTAGIOSIS IN POLAND

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The aim of this study was to estimate the prevalence *Ostertagia ostertagi* antibodies in bulk tank milk (BTM) in dairy cattle herds in the all voivodeships in Poland.

BTM samples were collected from dairy farms in Poland. The BTM samples, collected by veterinarians and then were transported directly to the Diagnostic Laboratory at the Faculty of Veterinary Medicine, Wroclaw. The *Ostertagia ostertagi* antibody levels in milk were determined using a semi-quantitative indirect ELISA (*Svanovir® O. ostertagi-Ab*, Svanova, Sweden), according to the manufacturer's instruction. According to the manufacturer's data, the ODR exceeding 0.5 could be associated with a reduction in milk yield.

The studied herds were assigned to two areas of Poland, corresponding to the colloquial and conventional division of the country into two zones "A" and "B". In the area of "traditional agriculture" still dominates, there is an increased migration of the young generation to cities and the highest percentage of people in the post-working age. In the area "A" — where the level of development is high only in the vicinity of large cities, which affects the labor market and income, increase the profits of rural areas. The ODR rates obtained in studies for ostertagiosis are as follows: Area A (Warmia-Masuria, Podlasie, Lublin, Masovia, Świętokrzyskie and Łódź) mean ODR and SD was 0.421/0.157. In the area "B" (Pomerania, West Pomerania, Kuyavia-Pomerania, Greater Poland, Lubusz, Lower Silesia, Opole, Silesia, Subcarpathian) was 0.483/0.236.

There were no differences in ODR in the studied regions of Poland. ODR values are similar, but differences in ODR rates between voivodships were observed.

**Keywords:** DAIRY CATTLE HERDS, OSTERTAGIOSIS, *OSTERAGIA OSTERAGI*, BULK TANK MILK, POLAND

# USE OF HOP CONES AND VITAMIN E TO PREVENT METABOLIC DISORDERS IN TRANSITION DAIRY COWS

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After calving cows get into negative energy balance accompanied by glucose deficiency and excessive release of fatty acids from adipose tissue. In assessing the metabolic status of cows during this period, the focus is on the violation of carbohydrate and lipid metabolism, but such important aspect as the ammonia intoxication, what is one of the factors of liver degeneration remains often out of attention. The main contribution to the formation of ammonia in the rumen is performed by gram-positive hyper ammonia producing bacteria (HAB). The activity of these bacteria is inhibited by ionophore antibiotics that are prohibited for use as a feed supplement in the EU. The substitute for antibiotics may be hop cones contained substances that selectively affect gram-positive bacteria, including HAB. However, ionophores inhibit cellulolytic bacteria activity too. High doses of dietary vitamin E can stimulate fiber degradation in the rumen. The purpose of our study was the possibility of use hops cones and vitamin E as a complex for prevention of metabolic disorders in the transition cows.

The experiment used twenty Ukrainian dairy black-and-white breed cows; milk yield 6000–7000 kg for previous lactation; divided into two groups 10 animals each. The 1st group is control. Diet of the 2nd group was supplemented with (per kg DM) 1 g of dry hop cones and 300 mg of α-tocopherol acetate as a 0.6 g of *Rovimix E-50* (NRC 2001 recommends 80 mg/kg for dry cows and 30 mg/kg for lactating cows). Experiment lasted during transition period (from 3 wk prepartum until 3 wk postpartum).

Supplementation the diet with hop cones and vitamin E has affected rumen fermentation. In particular, the feed additive stimulated cellulosolytic and suppressed proteolytic activities ($P<0.01$). As a result, the concentration of ruminal volatile fatty acids was increased. Reduced proteolytic activity led to a decrease in ammonia concentration in the rumen ($P<0.05$). At the same time, the amount of microbial nitrogen in the rumen of the experimental group of cows has moderately increased, what indicates the absence of depress effect of the additive on the rumen microbiota in general. The feed supplement reduced the concentration of lipid oxidation products ($P<0.05$) in the blood of dry cows, without affecting other parameters. After calving, changes that are more significant were detected. In the blood of cows of the experimental group an increase in the concentration of glucose ($P<0.05$), triacylglycerols ($P<0.05$), cholesterol esters ($P<0.05$), and a decrease in the concentration of NEFA ($P<0.05$), TBARS ($P<0.05$), and beta-hydroxybutyrate ($P<0.05$) were found.

Consequently, supplementation the diets of transition cows with 300 mg of α-tocopherol acetate and 1 g of dry hop cones per kg of DM stimulates the synthesis of glucose by the liver, reduces the intensity of release of fatty acids from adipose tissue, suppresses peroxide oxidation and reduces the concentration of ketone bodies in blood. Proposed feed supplement can be used to prevent metabolic disorders in cows.

**Keywords**: TRANSITION COWS, HOP CONES, VITAMIN E, RUMEN, BLOOD

# POLYARTHRITIS CAUSED BY *ERYSIPELOTHRIX RHUSIOPATHIAE* IN THREE AUSTRIAN SHEEP FLOCKS — DIAGNOSIS, TREATMENT AND MANAGEMENT MEASURE

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Between December 2017 and March 2018 lambs from 3 different farms were presented at the University Clinic for Ruminants because of lameness. First clinical examination at the clinic revealed different swollen joints. By anamnestic questionnaire the farmer reported that all animals with lameness are lambs of twin or multiple births. Orthopaedic examination revealed swollen and painful carpal and tarsal joints and in some of these lambs a slight to moderate abnormal flexion of the carpal joints. Ultrasonographically, a mild to moderate anechoic to hypoechoic effusion with and without flow-phenomena, and raw articular bone surfaces were assessed in affected joints. Radiological examination confirmed the ultrasonographic findings showing mild subchondral osteolysis and mild periosteal bone proliferation of the affected joints. Blood analysis revealed that the blood count was inconspicuous and calcium, phosphorus, iron and magnesium were within the physiological range.

Samples for bacteriology were taken from the incriminated joints by arthocentesis. The bacteriological examination revealed an infection with *Erysipelothrix rhusiopathiae*. In addition an antibiotics-resistance test was carried out.

Severely infected animals were euthanized and a standard necropsies were undertaken with special emphasis on the joints, showing moderate to severe cartilage damage of subchondral osteolysis.

Slightly infected sheep were treated with antibiotics (ampicillin) and non-steroidal anti-inflammatory drugs and had a successful outcome. A herd-specific autogenous vaccine was produced from isolated *Erysipelothrix rhusiopathiae*, which was administered to pregnant sheep and lambs in the affected farms.

**Keywords**: SHEEP, POLYARTHRITIS, *ERYSIPELOTHRIX RHUSIOPATHIAE*, AUTOGENOUS VACCINE

# CASE REPORT: EXTRACTION OF INCISORS AND LOWER JAW RECONSTRUCTION IN LLAMA

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Fractures of the incisor part of mandibular may be caused by clinching in the box, kicking by another animal or, for example, after a fence impact, as it was in this case. The mental canal in camelids makes this area more weak and susceptible to fractures.

The male llama was brought to the clinic in age of 2 years. It was refered due to a mouth injury. After clinical examination and x-ray imaging, the alveolar fracture and the dislocation of the incisors were found on both sides — I2, I3. The nutritional condition of the animal was poor. It was obvious that the llama could not properly eat, because the teeth were partially dislocated in direction to the upper hard palate and mechanically prevented the complete closure of the mouth. Given that this was an older injury and that the ossification already occurred in a dislocated position, it was not possible to carry out the incisors reposition. Therefore, it was approached to extraction of the incisors under the total injection anesthesia. Following the extraction of the incisors, debridement in fracture line was performed. Then intraoral cerclage was used to retract the caudal wall of the alveolar remains back to the physiological position along with the mucosa and bone base. Cerclage was performed using a 1.2 mm diameter osteosynthetic wire. After recovering from the anesthesia, llama started to eat without any complications. Post operative medical therapy included systemic antibiotics and NSAID. In 14 days cerclage was removed and the healing continued with Healing by secondary intention of the wound. The wound was daily flushed and food residue was manually removed.

Intraoral cerclage was successful for reconstruction of lower jaw. The animal was able to eat properly and improved the nutritional status.

Fractures of incisor part of mandibular are common within camelids. Intraoral cerclage is one of the option how to deal with this type of fractures. It was successful in this case, where we had to deal with older jaw injury.

**Keywords:** LLAMA, INTRAORAL CECRLAGE, INCISORS

# PREVALENCE OF FOOTROT IN BILOGORA REGION IN CROATIA

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The aim of this study was to investigate prevalence of the ovine footrot (FR) in Bilogora region in Croatia.

A cross-sectional survey was conducted in areas of Bilogora region in Croatia from May of 2016 to July of 2017. History of the farms and management as well as previous outbreaks data were collected. Data of interdigital lesions with lameness, foul odor and discharges were recorded as positive cases. The study area of the region comprised 5 villages. A total of 1344 randomly selected sheep from 5 farms were selected as the study population. The FR in this population was not studied previously in details.

The overall prevalence of ovine FR was 14.6 %. The prevalence of the FRis reported to be 8 to 10 % in the United Kingdom and 12 % to 15 % in India. The prevalence of FR among the 5 villages in Croatia was not significantly different ($P>0.05$). The owners of sheep in the study areas do not practice footbath, foot trimming or paring of hoof, which are commonly practiced in developed countries. Most farmers did not ask veterinary care when sheep showed lameness.

This is the first study to report the prevalence of FR lesions in a random sample of sheep flocks in Bilogora region. The knowledge on the etiology of the disease and the development of effective management practices may be key facts to control the FR. The prevalence of FR depends on the environmental conditions and pasture because the regular management practices prevent the colonization of bacteria in the interdigital spaces. It decreases the chance of skin integrity damage due to trauma, wetness or mud deposition, which are essential for the FR causative agents to colonized the interdigital space. These data may be helpful for advising farmers of potential environmental events and preventive management practices that may control the probability of sheep develop FRs.

**Keywords:** OVINE, FOOT ROT, PREVALENCE

# CYTOLOGICAL EVALUATION OF BONE MARROW SMEARS IN GOATS DURING MASTITIS

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Mastitis occurs in sub-clinical and clinical form with visible and easily perceptible changes within the mammary gland. Mastitis is often a result of bacterial infections caused by *Streptococcus aureus*, *Staphylococcus agalactiae* and *Escherichia coli* which mainly occur at the beginning of lactation and during dry period. Several factors, including: conditions in which animals are kept, feeding, milking hygiene and stress generating factors, may have a huge impact on the development of mastitis.

The aim of this study was to determine the effect of mastitis on the results of cytological evaluation of bone marrow smears in goats during mastitis.

For obtaining bone marrow samples 63 mm long 16 G biopsy needles were used. The animals were premedicated with xylazine prior to bone marrow sampling. The site of sampling was prepared in accordance with standard surgical procedures. Due to the very fast coagulation of the tested material, smears were made on previously prepared microscope-slides. The bone marrow smears were stained with the May-Grünwald-Giemsa method with shorter staining times than used for staining peripheral blood smears. The hematological counter SH-96/24D was used to count the bone marrow cells. Hematological analyses were performed using the ADVIA 2120i apparatus.

Results of the experiment showed an increase in the number of white blood cells and platelets above the reference values specific to goats. In peripheral blood smears, a significant increase in the number of neutrophilic granulocytes with segmented nucleus was noted. The increase in the number of leukocytes above the reference values was mainly caused by the increase in the number of neutrophilic metamyelocytes (MYE) and segmented granulocytes. In the erythroblastic cell line a decrease in the number of polychromatic and orthochromatic erythroblasts (EPOL and EORT) with a decrease in the number of reticulocytes was observed. Cytological evaluation of bone marrow smears revealed an increase in the number of neutrophilic and eosinophilic metamyelocytes. Also significant decrease in the number of megakaryocytes should be highlighted.

The decrease in the number of megakaryocytes and consequently the number of platelets may suggest the occurrence of disturbances in blood clotting tests.

**Keywords:** GOAT, MASTITIS, BONE MARROW

# CYSTICERCOSIS OUTBREAK IN FATTENING BOVINES

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Cysticercosis is a parasitic disease of cattle caused by human tapeworm *Taenia saginata*. Cattle are the intermediate hosts and harbour a larval form called *Cysticercus bovis*, which resides primarily in skeletal and cardiac muscle tissues. To prevent completion of the life cycle of *T. saginata* animals for slaughter are routinely inspected for presence of cysticerci. Carcases that contain cysticerci on meat inspection are confiscated or in case of very mild infestation have to undergo a cold treatment at –18 °C for 10 days to be declared fit for human consumption according to regulations (EU Zoonosis Directive 2003/99/EC and Regulation EC 854/2004). Infested animals are asymptomatic and there is no treatment that can clear the cysticerci from muscle tissues, so it is not possible to prevent zoosanitary measures after slaughter in case we suspect infestation. Thus, economic impact of infestation is very significant. The only prevention of the disease is to prevent consumption of human faeces by cattle. Since cattle are known for coprophagy of human faeces, all efforts should be made to prevent human faeces to come into contact with cattle. The aim of the study was to investigate a cysticercosis outbreak in a fattening bovine herd.

A fattening bovine herd of 220 animals was included in the study. Management practices were analysed. Meat inspection for cysticercosis was conducted. Stool samples from persons working at the farm were examined by microscopy, copro-antigen ELISA and copro PCR for *T. saginata*. Basic descriptive statistics were calculated.

In a fattening bovine herd of 220 animals 13 (5.9 %) animals were recognised as having cysticeri at carcass inspection. None of the carcases were confiscated, but all had to be cold treated before selling. All the persons working at the farm tested negative for taeniasis.

This was the first case of cysticercosis in the examined herd. Animals in the herd were divided into boxes with 10 to 30 animals and infested animals came from boxes located at different parts of the barn. Due to such distribution of cases there is a high probability that ova from *T. saginata* were dispersed by total mix ration. Prevalence of cysticercosis in cattle in Slovenia was from 0.003 to 0.05 % according to official meat inspection findings and just 0 to 20 persons per year were diagnosed as having *T. saginata* reported by Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection in the 7 years period before the outbreak and up to year 2018. Even though prevalence of cysticercosis is very low in Slovenia, this case proves that strict meat inspection for cysticercosis is prudent. Possible ways of infestation and strategies to prevent cysticercosis in cattle are discussed.

**Keywords:** *TAENIA SAGINATA*, CATTLE, PARASITIC ZOONOSIS, MEAT INSPECTION, PREVENTION

# SAFETY CULTURE ON A LIVESTOCK FARM AND PREVENTION OF ZOONOSES

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Veterinarians in buiatrics practice are not responsible just for caring for animal health and welfare, but also for protection of personnel assisting them as well as personnel dealing with livestock from zoonoses. So, it is our duty to be knowledgeable about common zoonotic diseases of ruminants, and especially how people can protect themselves from contracting them. Veterinarians should know which zoonotic diseases are present in their country and neighboring countries in order to be prepared in case of an outbreak. Zoonotic diseases of ruminants often do not show typical clinical presentation, so awareness of how to safely behave when dealing with livestock in order to prevent zoonoses is very important. In the paper ruminant zoonoses common in Middle Europe are going to be presented as well as behaviors of people that prevent their transmission.

Analysis of the most common zoonoses of domestic ruminants that are transmitted when handling animals and the ways they are transmitted to humans were studied. European Centre for Disease Prevention and Control database was used for identification of domestic ruminant's zoonoses in Middle European countries, which is a result of EU member state reporting according to Zoonoses Directive 2003/99/EC and peer reviewed literature.

Identified zoonoses that could be transmitted by direct and indirect contact with live animals in Middle Europe are cryptosporidiosis, rabies, infections with parapox viruses, leptospirosis, tuberculosis, listeriosis, brucellosis, Q fever, chlamydiosis, salmonellosis, campylobacteriosis, colibacillosis, clostridiosis, anthrax, staphylococcal infections and dermatomycosis. Use of appropriate personal protective equipment and adequate hygiene can effectively prevent most zoonoses. Especially vulnerable for contracting a zoonosis are persons on immunosuppressive medication, those who have immunosuppressive diseases, children and pregnant women.

The risk of zoonosis cannot be eliminated but can be significantly reduced by following preventive measures. For nearly all the diseases there is a relationship between dose and severity. A threshold dose is required to establish infection, and low doses may cause only mild infections, which can also be asymptomatic. Developing risk control tools for better safety culture and risk management on farms is important. Safety culture when dealing with animals as potential risk for zoonosis has a major effect on preventing them. In order to reduce zoonoses on a farm strict biosecurity plan and systematic surveillance should be implemented as well.

**Keywords**: DISEASE TRANSMISSION, SAFETY, WORKER, RUMINANTS, ZOONOTIC AGENTS

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# BOLA-DRB3 GENE AS A MARKER OF COW'S MAMMARY GLAND STATUS

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In recent years, research on the use of liposomal drugs, which do not contain antibiotics for therapy of mastitis are of a great importance. The research used a liposomal preparation which contains *Hypericum perforatum* extract, vitamins, lecithin, and twin. The aim of this study was to assess the suitability of the BoLA-DRB3 gene polymorphism for determining the phenotypic value of the somatic cells in Ukrainian black-and-white dairy cows and to find out the effect of the "Limanin" on the number of somatic cells in cows with subclinical mastitis.

The spectrum of the BoLA-DRB3.2 gene alleles was studied by PCR in Van Eijk et al. For restriction analysis of exzone 2 of the BoLA-DRB3 gene the endonucleases of restriction RsaI, HaeIII, BstYI (XhoII) were used. For the somatic cell count (SCC) samples of parenchymal milk were taken from cows at day 1, 3 and 9 of the experiment. Somatic cell count (SCC) was carried out by Prescott-Breed's calculation method.

The investigated breed is characterized by a uniform distribution of allelic frequencies of the BoLA-DRB3 gene. 28 alleles (mean frequency P=3.57 %) of the cows of Ukrainian black-and-white are identified from 54 PCR-RFLP and allelic-specific PCR for the BoLA-DRB3.2 gene. In patients with mastitis of cows, 24 alleles were detected (mean frequency 4.17 %). With a frequency of more than 5 %, five BoLA-DRB3.2 alleles were identified: *24, *28, *26, *22, and *03. Alleles *16, *25, *31 and *36 in this group did not show at all. Among healthy cows 27 alleles were detected. Of the 8 alleles BoLA-DRB3.2: *22, *24, *08, *13, *28, *10, *03 and *36, were detected with P(A)≥5%. The allele BoLA-DRB3.2 *41 was never detected. Biometric analysis of the polymorphism of the BoLA-DRB3.2 gene revealed two alleles that affect the morbidity of cows with mastitis: *24 (RR=2.17; P=11.7 %; $\chi_2$=4.33) and *26 (RR=4; 62; P=4.3 %; $\chi_2$=7.13). There are also two alleles that determine the resistance of cows to diseases of the udder: *13 (RR = –5.29; P=5.3 %; $\chi_2$=5.65) and *22 (RR = –2.52; P=1.2 %; $\chi_2$=5.02).

In samples of milk taken in 92 cows, the number of somatic cells varied from 84 to 6926 thousand cells/cm$^3$. Among them, only 27 samples of SCC did not exceed the threshold of 200 thousand cells/cm$^3$. There were 22 animals with subclinical mastitis in which 876 to 4436 thousand cells/cm$^3$ were detected. The SCC value in 5 cows diagnosed with clinical mastitis was from 2264 to 6926 thousand cells/cm$^3$. The number of somatic cells in 65 healthy cows ranged from 84 to 704 thousand cells/cm$^3$. It was found significant association between allele *28 and low level of somatic cells. Treatment of subclinical mastitis of cows with the "Limanin" led to a decrease in SCC in the milk of experimental animals. On the 9$^{th}$ day in experimental group cows the number of somatic cells (388.7+44.97 thousand/cm$^3$) decreased compared to the first day of the experiment (667.9+64.9 thousand/cm$^3$), which shows the normalizing effect of the drug on the content of somatic cells in the milk of cows with subclinical mastitis.

In cows of the Ukrainian black-and-white breed of the allele BoLA-DRB3.2 *24 and *26 determine the morbidity of mastitis. Alleles BoLA-DRB3.2 *13 and *22 indicate the resistance of cows to mammary gland diseases. There is a statistically significant association between the allele *28 and the low level of somatic cells in milk. The normalizing effect of liposomal drug "Limanin" on somatic cell cultures in patients with subclinical mastitis of cows has been established.

**Keywords:** MASTITIS, SOMATIC CELLS, GENE, ALLELE, MAMMARY GLAND, SUBCLINICAL MASTITIS

# THE EFFECT OF SILICON DIOXIDE NANOPARTICLES AS FEED ADDITIVE ON HEALTH CONDITION AND IMMUNOLOGICAL PARAMETERS OF CALVES

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In the initial stage of cattle breeding attention should be paid on immune system of calves. An increased sensitivity to bacterial and viral infections leads in consequence to economic losses in cattle industry. Published data indicate that the use of a silicon dioxide nanoparticles as feed additives have a destructive effect on bacterial cells, which leads to their death. Then the bacterial toxins are selectively bound in the gastrointestinal tract. Additionally a mixture of organic acids acidifying the digestive tract and gives an additional biocidal effect against pH-sensitive bacteria.

In the study six calves in the age 4–8 weeks of life were divided into two equal groups: experimental (E) and control (C). Calves from the E group were given feed additives which contained silicon dioxide nanoparticles with a mixture of protected organic acids which were added to milk replacer at the dose of 3000 mg per calf once a day for 7 weeks. The C group received milk replacer without additives in the same time. Behavioral observations were conducted daily; the amount of feed intake, rectal temperature, overall health and their weekly body weight gains were monitored. The blood samples were collected from animals once a week. White blood cells counts (WBC) with leukocyte differentiation (lymphocytes, monocytes and granulocytes) were examined in peripheral blood using veterinary blood analyzer (*Exigo*, *Boule Medical*, Spånga, Sweden). Immunophenotyping of lymphocyte subsets, i.e. T-cells ($CD2^+$), Th ($CD4^+$), Tc/s ($CD8^+$) according to Beckman Coulter's Guide, phagocytic activity of granulocytes and monocytes and their mean fluorescent intensity (MFI) (*Phagotest*, *Glicotope Biotechnology GmbH*, Berlin, Germany) were analysed with the use of flow cytometer (*Epics XL*, *Beckman Coulter Inc.*, Brea, California, USA).

The overall health condition of the animals was good, they had good appetite and it was better expressed in the group E. The body weight of animal was on average higher by 11 kg in the group E. The leukocyte subpopulation counts were similar in the both groups. The percentage of $CD2^+$, $CD4^+$ and $CD8^+$ in the group E was similar to the control group. A mean percentage of phagocytic monocytes was similar in the C and E groups. In the 0 day of experiment, i.e. before the beginning of feed additives administration, the value for the E group was lower than for the C group ($P<0.05$). In E groups the values increased by 7 % and in C decreased by 4 % on 7 weeks of the study. The MFI of monocytes was similar for the both groups. The mean percentage of phagocytic granulocytes decreased by 5 % and by 14 % in E and C group respectively. The MFI of granulocytes decreased by 10 % and 20 % in the E and C group respectively on 7 weeks of the study.

There were examined overall body condition and nonspecific immunological parameters of calves. The obtained results were similar in the experimental and control group. Although the experimental animals were in better health condition and higher body weight gains. Further studies are needed to assess the protective role of examined feeding additives against bacteria on calves.

**Keywords:** CALVES, SILICON DIOXIDE NANOPARTICLES, IMMUNOPHENOTYPING, PHAGOCYTIC ACTIVITY

# PREGNANCY LOSSES IN BOVINE SINGLETON AND TWIN PREGNANCIES

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Twin pregnancy in dairy cattle is affecting reproductive performance as an unwanted phenomenon. In practice, pregnancy loss due to embryonic/fetal mortality is the main factor affecting the results of pregnancy diagnoses; therefore the nature of the phenomenon must be taken into consideration when evaluating any diagnostic method.

In total, 1253 positive pregnancy diagnoses on three farms [farm A: n=304 (24.3 %), farm B: n=674 (5 3.8%) and farm C: n=275 (21.9 %)] were made between days 29 to 42 of gestation and followed up until calving. The prevalence of twin gestations diagnosed between days 29 to 35 (73/866, 8.4 %) and days 36 to 42 (32/387, 8.3 %) were similar. There were one CL in 957 (83.4 %) and two CLs in 191 (16.6 %) singleton pregnancies, respectively.

In twin-carriers only one CL was found in three cases (2.9 %), and all other twin cows had two (n=99) or three CLs (n=3). Cavitary CL occurred in one twin-carrier (1.0 %) and in 58 singleton pregnancies (5.1 %).

The rate of pregnancy loss diagnosed between days 29–42 and 57–70 was altogether 4.6 % (53/1148) in singleton and 4.8 % (5/105) in twin pregnancies (P=0.95), respectively. Differences in pregnancy loss at drying-off were also not significant between singleton and twin pregnant animals (P=0.99). Based on logistic regression analysis, in any time points total losses were not different in singleton and twin pregnancies (P=0.94, OR=1.04 and P=0.96, OR=0.98, respectively), and we could not detect any farm effect (P=0.36, OR=0.83 and P=0.08, OR=0.79, respectively). Pregnancy loss was also evaluated on the basis of laterality in cases of singleton and twin pregnancies. In singleton gestations, the rate of right-side pregnancy losses (35/670; 5.2 %) did not differ significantly (P>0.05) from those of the left-side pregnancy losses (18/478; 3.8 %) between days 29–42 and 57–70, respectively. This difference was also not significant at drying-off pregnancy check (P>0.05). Based on logistic regression analysis in twin gestations neither the difference of the pregnancy losses at days 57–70 (4/57; 7 % vs. 1/48; 2.1 %), nor the differences at the time of drying off (4/57; 7 % vs. 2/48; 4.2 %) were significant (P>0.05) between unilateral and bilateral pregnancies.

When analysing the pregnancy losses of twin pregnancies in dairy cattle there was no differences between singleton- and twin-carrying cows at the confirmation of pregnancy between days 57–70 of gestation, moreover, at drying-off also a non-significant difference was detected between singleton and twin carrying groups. In singleton pregnancies, presence of a cavity in the *corpus luteum* effected pregnancy loss. Between days 57–70 of gestation and drying-off this difference between cavitary vs. non-cavitary CL was still significant, while it was non-significant between cows with one CL vs. double CLs.

**Keywords:** DAIRY CATTLE, TWIN PREGNANCY, SINGLETON PREGNANCY, CAVITARY *CORPUS LUTEUM*

# IMPORTANCE TO PREDICT THE ONSET OF CALVING TO DECREASE STILLBIRTH AND STRESS RELATED UTERINE DISEASES

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Successful genetic selection for higher milk production has caused a dramatic decline in the reproductive performance of dairy cows all over the world. Achievement of optimum herd reproductive performance requires concentrated management activities especially during calving and during the first 100 DIM. The following management activities are needed to pursue during early postparturient (p.p.) period to reach or approach the optimal reproductive performance such as careful surveillance and assistance at calving, prevention of pp metabolic diseases, early diagnosis and treatment of p.p. uterine diseases, accurate detection of oestrus, correct timing of insemination, reducing the effect of heat stress and early pregnancy diagnosis. Among these main activities only careful surveillance and assistance at calving and their effects on milk production, reproductive performance as well as on newborn calves will be discussed.

Due to the fact that the cause of stillbirth with a non-infectious aetiology is likely to be multifactorial and difficult calving may explain only about half of them therefore it is very important to examine the risk factors of stillbirth especially in large-scale dairy farms. While it is not possible to eliminate dystocia, adequate management of growing heifers and close observation during calving are essential for reducing stillbirth rate. Since in many cases there are no visible clinical signs of the onset of calving, therefore especially in large dairy farm it is difficult to recognize it. The aim of our study was to evaluate an intelligent control system and test its effectiveness in predicting calving.

Two hundred fifty-seven Holstein-Friesian dairy cows were monitored by inserting a vaginal thermometer into the vagina (*Vel'Phone*, *Medria*, Châteaugiron, France) from day 5 before expected calving while 116 cows served as control. Once the thermometer has been placed into the vagina, the *Vel'Phone* was going to inform via SMS about the imminence of calving, and breaking of the allantoic sac.

Our results indicate the effectiveness of such instrument to control the onset of parturition in dairy cows because in case of heifer calvings the stillbirth rate was 1.7 % in the monitored group vs. 10.5 % in the control group, while in case of cow calvings it was 2.5 % (monitored group) vs. 10.3 % (control group), respectively. The differences in both groups were statistically significant (P=0.029 and P=0.003 respectively).

According to Heinrichs & Radostits (2001) the target prevalence rate of perinatal mortality would be 1 to 3 %, and it seems that it can be reached in large dairy farms by using *Vel'Phone* to predict the onset of calving. On the other hand it has been recently confirmed by our group that inappropriately timed obstetrical assistance can significantly increase the prevalence of stillbirth, the injuries of the soft birth canal, retained fetal membranes and clinical metritis.

**Keywords**: DAIRY COW, MONITORING CALVING, STILLBIRTH, VAGINAL THERMOMETER

# SHEDDING OF *COXIELLA BURNETII* IN DAIRY CATTLE AND POSSIBILITY OF TRANSMISSION VIA ALIMENTARY ROUTE

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Estimation of herd-level prevalence of *C. burnetii* shedding in the Polish dairy cattle and identification of the pathogen's genotypes and STs using multiple-locus variable number tandem repeat analysis (MLVA) and multispacer sequence typing (MST) methods. Moreover, the possibility of transmission of the pathogen via alimentary route was evaluated.

In total 2635 bovine serum samples from 969 cattle herds were tested by ELISA/CFT test. Moreover 1439 specimens such as: individual milk samples (n=897), bulk tank milk (n=101), vaginal swabs (n=409), placenta (n=32); were subjected to *C. burnetii* specific qPCR. The qualitative real-time PCR, detecting IS1111 element was performed. The 49 samples with the lowest Ct values were selected for genotyping by MLVA-6 and MST methods. MLVA was performed using 6 variable loci. Amplification products were run on *ABI 3500 Genetic Analyser* and electropherograms were evaluated with *GeneMapper* software. MST was performed as previously described by Glazunova et al. (2005). Ten different intergenic spacers: Cox 2, 5, 18, 20, 22, 37, 51, 56, 57 and 61 were amplified and after purification products were subjected to sequencing. Moreover, to assess the possibility of transmission of the pathogen via alimentary route, an experiment on guinea pigs was conducted.

Average seroprevalence for bovine herds was 24.46 % (969/237). Molecular analysis by real-time PCR revealed the presence of *C. burnetii* DNA in 88 (31.54 %) of tested cattle herds. Positive results were obtained for placenta specimens as well as for swabs from reproductive tract, however the most common was shedding in milk. Five previously described MLVA genotypes: I, J, BG, BE, NM and two novel PL1 and PL2 were identified in 31 out of 49 samples. Two sequence types: ST16 and one newly discovered, named ST61, were identified in field samples using MST technique. After *per os* administration of the pathogen, one guinea pig developed seroconversion and the presence of *C. burnetii* DNA was detected using real-time PCR in testicles and intestine of some animals.

The research confirmed that level of prevalence of *C. burnetii* in dairy cattle herds in Poland is significant and similar to other European countries. It should be highlighted that MLVA and MST profiles identified in this research were different from profiles of the strain involved in the Q fever outbreak in The Netherlands as well as from genotypes of the outbreak strains isolated in Poland the 20th century. However, some of them as genotype I, J, NM and ST16 have been previously recorded in humans, therefore zoonotic threat cannot be ruled out. The results of conducted experiment did not exclude the possibility of infection by alimentary route.

**Keywords:** *COXIELLA BURNETII*, MILK, CATTLE

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# EFFECT OF PEGYLATED GRANULOCYTE COLONY-STIMULATING FACTOR ON HEALTH OF MAMMARY GLAND IN DAIRY COWS

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The granulocyte colony-stimulating factor (gCSF) from a family of glycoprotein cytokines-stimulates the production of granulocytes and stem cells in the bone marrow.Their release into the bloodstreamis the most important cellular defense against different pathogens. Pegylated granulocyte colony-stimulating factor (PEG-gCSF) has been shown to significantly increase the number ofcirculating neutrophils, level of phagocytosis, myeloperoxidasis release and oxidative burst in dairy cows. Clinical effect of PEG-gCSF on occurrence of clinical mastitis during the first month postpartum as well as on disease severity, bacterial count or reduction of milk yieldwas reported. However, available studies are not numerous and confirmation of published results is necessary. The aim of the study was to evaluate the effect of PEG-gCSF on health of mammary gland postpartum in dairy cattle.

Cows in experimental group (n=119) were treated by PEG-gCSF (*Imrestor*, *Elanco*, treatment 7 days before expected parturition and 1 day after parturition s.c.). Cows in control group (n=125) remained without treatment. Incidence of clinical mastitis and subclinical mastitis was observed during 3 months postpartum. Bacteriological examination of milk was performed in 3rd and 8th week (1st sampling, 2nd sampling) postpartum in 55 cows from experimental and control group.

Incidence of clinical mastitis was 26.9 % and 21.6 % during 1 month postpartum, 31.1 % and 29.6 % during 2 months postpartum and 37 % and 33.6 % during 3 months postpartum in experimental and control group, respectively. Incidence of subclinical mastitis was 18.1 % and 18.8 % during 1 month postpartum, 32.8 % and 37.6 % during 2 months postpartum and 46.1 % and 56.4 % during 3 months postpartum in experimental and control group, respectively. Proportion of bacteriologically positive milk samples was 14.5 % and 16.4 % at 1st sampling, 10.9 % and 10.9 % at 2nd sampling and 23.6 % and 20 % at both samplings in experimental and control group, respectively. There were no significant differences between groups.

Results of the study did not confirm positive effects of PEG-gCSF onthe occurrence of clinical and subclinical mastitis as well as on bacteriological findings in milk in dairy cows.

**Keywords:** COW, PEGYLATED GRANULOCYTE COLONY-STIMULATING FACTOR, MASTITIS

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# BILE-SYNTHESIZING FUNCTION OF LIVER ON ENTROPATHOLOGY OF NEWBORN CALVES

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The presence of a close anatomical and functional connection between the liver and the intestine causes the possibility of simultaneous destruction of these organs caused by the development of diseases of the gastrointestinal tract of newborn calves, which reduces the clinical effect of traditional therapy and leads to complications. The issue of bile-synthesizing function of liver on enteropathology of newborn calves is still insufficiently studied. The purpose of this work was to investigate changes in the bile-acid spectrum of bile and liver in calves caused by the development of neonatal enteropathology.

Experiments were carried out at the Velikosnitynske Training and Research Farm in the Fastovsky District of the Kyiv Region. Black-and-white calves of 2-day age were assigned to two groups: control and experimental, 5 animals in each. The control group included clinically healthy animals. The experimental group included the calves with acute digestive disorders of non-contagious etiology. On the fifth day of life, calves were sampled with bile and liver. The bile acids in the biological material were investigated by thin-layer chromatography (Veselsky S. P., 1991). The content of individual bile acids was determined using a refractometer DO-1 densitometer (λ 620 nm) and calibration graphs. The results of the research were subject to statistical analysis (Kucherenko M. E. et al., 1985).

Due to the chromatographic analysis of extracts from bile and liver tissues of newborn calves, 7 fractions of conjugated and free bile acids were identified. In the bile of diseased calves there are deviations both in the ratio of individual bile acids, and in a significant decrease in their overall content. In particular, the total content of cholates in cystic bile of the calves with enteropathology decreased to 1353.4±88.1 mg%. The concentration of TCA in bile decreased by 38.4 %, TChDxCA + TDxCA by 36.7 %, GCA by 62.7 %, and GChDxCA + GDxCA by 67.6 %. At the same time, the level of free bile acids in bile significantly increased. Thus, the CA content increased by 70.1 %, ChDxCA + DxCA by 69.2 %, and LiCA by 10 times. Increasing the level of free bile acids, together with a significant decrease in concentrations of conjugated taurine and glycine bile acids, caused a significant decrease in the conjugation rate compared to control, indicating inhibition of the biosynthesis and conjugative liver function of sick calves. In the analysis of extracts from liver tissues in calves, there is a significant decrease in the total content of bile acids (by 35.4 %), and in all fractions of conjugated bile acids compared with the control. Among the free bile acids, only the concentration of LHC significantly increased 4 times compared with the control.

In the bile of sick calves, compared to healthy ones, there are differences both in the ratio of individual bile acids, and in a significant decrease in their total content. However, against this backdrop of a decrease in bile acid concentrations conjugated to taurine and glycine, the level of free representatives increased significantly, indicating a decrease in the synthesis and conjugative liver function of diseased calves. In the liver of sick calves, the content of all fractions of conjugated bile acids was significantly lower than control values. Attention is drawn to the fact that the level of free bile acids decreases, but the concentration of toxic LHC increases. The established facts are important to consider when designing therapeutic schemes.

**Keywords**: NEWBORN CALVES, BILE ACIDS, BILE, LIVER, ENTEROPATHOLOGY

# EFFECT OF DIETARY PROPYLENE GLYCOL, VITAMIN E, METHIONINE AND CARNITINE SEPARATELY AND AS COMPLEX SUPPLEMENT ON PERFORMANCE OF TRANSITION DAIRY COWS

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Despite the significant range of drugs and supplements regulating rumen metabolism, glucose synthesis and fatty acids utilization in the liver, approximately 40 % of high-yielding cows exhibit subclinical form of ketosis and fatty liver syndrome. Propylene glycol is widely used as glucose precursor for the prevention and treatment of ketosis. Inadequate intake of methionine reduces the synthesis of phospholipids and lipoproteins in the liver. As a result, the elimination from the liver to the bloodstream of triacylglycerols by the very low-density lipoproteins slowed down. The addition of high-dose vitamins E to the diets of transition cows reduces the somatic cells count in milk, lower the frequency occurrence of mastitis and placenta retention. Furthermore, some researchers suggest increasing the content of vitamin E in diets of cows, since rumen bacteria respond positively to high doses of this vitamin. Carnitine transports fatty acids into the mitochondria for oxidation, and therefore contributes to less accumulation of lipids in the liver. The purpose of our work was to investigate the effect of adding to the diet of cows at the end of the dry period and after calving, the complex feed supplement to prevent ketosis and steatosis and stimulate next milk yielding.

Six groups of cows were used for the experiment, 5 animals each. The 1st group received a standard balanced diet. To the diet of the 2nd, 3rd, 4th, 5th and 6th groups cows added (per animal per day): dry propylene glycol — 200 g, 50 % vitamin E (*Rovimix-50*) — 5 g, of rumen protected methionine (MHA 86 %) — 20 g, rumen protected carnitine — 1.0 g (5 g of *Carnipass*), and all these additives in the complex. The trial lasted three weeks before and the three weeks after calving. The milk yields of cows were monitored during the first three months of lactation.

Propylene glycol increased amylolytic, vitamin E — cellulosolitic, and methionine — proteolytic activities in the rumen. As a result, in the rumen of cows receiving propylene glycol the concentration of propionate and lactate, and in rumen of cows receiving vitamin E the total volatile fatty acids concentration were higher ($P<0.05$). Propylene glycol, vitamin E and methionine reduced the blood concentration of acetoacetate and beta-hydroxybutyrate. The total amount of ketone bodies in the cows received propylene glycol, vitamin E or methionine were 2.49; 1.64 and 1.23 times less than in control group ($P<0.01$). The addition of propylene glycol increased glucose concentration ($P<0.05$) and reduced the concentration of triacylglycerols ($P<0.01$); methionine increased urea concentration ($P<0.05$); higher amount of vitamin E reduced the concentration of lipid peroxidation products ($P<0.01$) in blood plasma of cows.

All studied feed additives have reduced the concentration of un-esterified fatty acids in plasma, what is important for the prevention of ketosis. Propylene glycol and complex feed supplement with the same effectiveness reduce the concentration of ketone bodies in the blood of cows. Propylene glycol increased milk yield, but decreased milk fat content. Vitamin E did not increase milk yields, but elevated the fat content in milk. Methionine and carnitine did not affect milk productivity. Adding to the diet of cows the complex supplement did not affect milk yield, but increased milk fat.

**Keywords:** COWS, PROPYLENE GLYCOL, VITAMIN E, METHIONINE, CARNITINE

# THE INTENSITY OF PROTEIN EXCHANGE AND THE CONTENT OF GLUCOPROTEINS IN THE BLOOD OF COWS UNDER THE CONDITION OF FEEDING IODINE CITRATE

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A balanced and complete feeding of cows during the dry period is an important condition for their preservation and health, as well as the growth of dairy productivity in the period of the future lactation. The purpose of the work was to study the parameters of protein metabolism and the content of glycoproteins in the blood of cows after calving under the condition of feeding iodine citrate in various doses.

The research was conducted on the 15 full-age cows of Ukrainian black-and-white milk breed, analogues by age (3–5 lactation), body weight, lactation period (1st month after calving) in the winter-stool period under the condition of anchoring the cows. The cows of I (control) group received the basic diet (BD), which was normalized according to the physiological state, productivity and body weight. The animals of the II experimental group from 18 to 78 days of lactation received iodine citrate in the feed daily at a rate of 0.6 mg I/kg of dry matter of the diet and the animals of the III experimental group received BD and iodine citrate at the rate of 0.06 mg I/kg of dry matter of the diet. For biochemical studies, the selected samples of venous blood were used in the preparatory and experimental (60 days of feeding of iodine supplements) periods. The state of protein exchange was estimated by the content of urea, total protein, albumin, the activity of AlAT and AsAT sialic acids, and hexose-bound proteins in the blood serum.

It has been found that the level of total proteins increased by 13.5 % ($P<0.05$) in the blood serum of animals of group III, which fed iodine citrate in the amount of 0.06 mg/kg of dry matter of the diet. The feeding of animals with iodine citrate contributed to 16.7 % increase in albumin content in these animals and 16.6 % decrease in the activity of AsAT ($P<0.05$). Increasing the total protein and albumin in the blood of animals in the III experimental group may indicate a stimulating effect of iodine citrate in this concentration, on the intensity of protein synthesis in the liver of the cows. In the blood of cows of the II experimental group, which received 0.6 mg I/kg of dry matter of the diet, the content of albumin was not increased to be 15.1 % and the total protein to 2.43 % and the activity of AsAT was decreased by 16.6 % ($P<0.05$). AlAT did not undergo significant differences in the cows of experimental groups. It is worth noting that the probable changes in the activity of AlAT due to the activity of iodine citrate in cows of experimental groups were not detected. Obviously, the feeding of iodine in the form of citrate increases the protein exchange rate and activates the processes of transamination in the liver. In the blood of cows of experimental groups, no probable differences were found between the main final protein metabolism product — urea and creatinine. Obviously, the use of these doses of iodine citrate did not affect the course of the protein exchange and the energy processes in the myocytes and it did not cause the functional and structural changes in the muscle and excretory systems of the organism.

Studies of the level of glycoproteins in the blood of control and experimental cows indicate the corrective effects of iodine citrate on the immunobiological reactivity of their organism. This is evidenced by the higher level of glycoproteins and individual monocycles of their carbohydrate components in the blood of animals in experimental groups compared with the control that remained within the physiological norm. However, there is a more pronounced effect of adding 0.6 mg I/kg of dry matter of iodine citrate to the animal diet, used in the II group and was accompanied by a probable increase in the concentration of ceruloplasmin by 10,2 % ($P<0,01$), hexose bound to proteins by 15 % ($P<0,01$), sialic acids — by 24 % ($P<0.01$). Whereas in the blood of animals of the III group, the tendency to increase the concentration of ceruloplasmin and hexose bound to proteins and the probable increase in the content of sialic acids was maintained by 11.4 % ($P<0.05$). Thus, the biological effect of iodine citrate is more pronounced in cows of group II, which received a higher concentration of mineral additive.

**Keywords:** COWS, IODINE, GLYCOPROTEINS, PROTEIN, TRANSAMINASES

# DESIGN, SYNTHESIS AND ACTIVITY EVALUATION OF NOVEL HOMOSERINE LACTONES DERIVATIVES WITH PHENYLUREA GROUPS AS BACTERIAL QUORUM SENSING INHIBITORS

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We design, synthesis and activity evaluation of novel homoserine lactones derivatives with phenylurea groups as bacterial quorum sensing inhibitors.

We designed and synthesized a series of novel quorum sensing inhibitors 2a~i. Through *chromobacterium* CV026 instruction strains and *Pseudomonas aeruginosa las* system quorum sensing detection model, extracellular virulence factors (pyocyanin, elastase, rhamnolipid), swarming motility and biofilm formation regulated by QS system of PAO1 compared with brominated furanone C-30. And we used Autodock molecular simulation software to simulate the combination of the active compounds to the receptor protein LasR.

AHLs linked different phenylurea groups expressed more outstanding anti-QS activity than brominated furanone C-30. Compound 2f significantly reduced extracellular virulence factors (pyocyanin, elastase, rhamnolipid), swarming motility and biofilm formation regulated by QS system of PAO1 in a lower concentration and formed two hydrogen bonds by interacting with Arg-61 to exert more outstanding anti-QS activity than C-30. So, Arg-61 is very important in QSI than other amino acid residues.

It is possible that this work could be a precursor compound for further study of novel QSIs based on structural modification and introduce new methods for developing anti-QS active products.

**Keywords:** QSI, PHENYLUREA, BACTERIOSTATS, AHLS

# THE INFLUENCE OF RUMINAL BOLUS WITH SHORT-DISSOLVING TIME ON SELECTED BLOOD PARAMETERS IN HOLSTEIN-FRIESIAN COWS AFTER PARTURITION — PRELIMINARY RESULTS

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The aim of this preliminary study was to determine the influence of ruminal bolus with short-dissolving time on selected blood parameters in Holstein-Friesian cows after parturition.

Group of 6 healthy Holstein-Friesian cows during third pregnancy was chosen to participate in the study. The cows included in the experiment had to meet the following criteria: no record of hypocalcemia, single pregnancy and physiological parturition without human assistance before bolus administration. Cows received 2 ruminal boluses: first — up to 2 hours after parturition, second — 24 hours after the first. Blood samples were taken 5 times. First sampling occurred just before first bolus administration (0), second after 5 hours (5$^{th}$ hour), third during second bolus administration (24$^{th}$ hour), fourth 10 hours after second bolus administration (34$^{th}$ hour) and fifth 48 hours after first bolus administration (48$^{th}$ hour). Blood was collected from the tail vein using 1,2 mm needle into *Vacutainer* test-tubes with clot activator. The blood was centrifuged at 3000 rpm for 15 minutes to obtain the serum. The following parameters were determined: ASPAT, ALP, glucose, $Na^{+}$, $K^{+}$, $Cl^{-}$, vitamin $D_3$ (25-OH), Ca, Mg, P. Obtained results were analyzed with *Statistica* software using Student's *t*-test to compare results of first to other samplings and ANOVA to determine the differences between all samplings.

Ruminal bolus administration appeared to significantly ($P \leq 0.05$) affect several analyzed parameters. It increased ($P \leq 0.05$) Ca and P concentrations, the activity of ASPAT — from 1,40 to 1,95 mmol/l, from 1,14 to 1,50 mmol/l and from 81,17 to 129,83 u/l during 48 hours respectively, and decreased ($P \leq 0.05$) the glucose concentration and the ALP activity — from 7,61 to 3,98 mmol/l and from 68 to 60,67 u/l during 48 hours respectively, even though all the obtained results oscillated around reference values for cattle. No statistically significant changes were observed in the concentration of electrolytes and vitamin $D_3$ (25-OH).

Fluctuations in the levels of determined blood parameters oscillating around the reference values may indicate the existence of interactions between the components of the bolus at the stage of absorption in the gastrointestinal tract and interactions between the components of the bolus and environment of the rumen itself. Ruminal boluses with short-dissolving time, due to their beneficial influence on some blood parameters including calcium concentration in serum, might be considered as a preventative measure for hypocalcemia in dairy cows after parturition. However, a more detailed study will be conducted in order to determine the most effective bolus administration protocol which will enable to make the most of the potential of such products.

**Keywords:** DAIRY COW, HYPOCALCEMIA, RUMINAL BOLUS

# THE EFFECT OF EXOGENOUS MELATONIN ON ANTIOXIDATIVE ENZYMATIC ACTIVITY OF FRENCH ALPINE BUCKS SEMINAL PLASMA AND SPERMATOZOA DURING THE NON-BREEDING SEASON

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The aim of this study was to determine the effect of exogenous melatonin on antioxidative protection of bucks ejaculate during the non-breeding season by monitoring of antioxidative enzymatic activity, the ratios of antioxidative enzymes and the concentration of malondialdehyde (MDA) in seminal plasma and spermatozoa.

Twelve clinically healthy bucks of the French alpine breed aged from 1.5 to 4 years were randomly assigned into melatonin (MG) and control (CG) groups, with 6 bucks in each. The experimental period 3 months (March-May) was divided into six periods of 15 days each. The bucks in the MG group received four melatonin implants subcutaneously in the ear basis at the end of March. Two semen samples were taken from the bucks by artificial vagina once per week. The activities of glutathione-reductase (GR), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and concentration of malondialdehyde (MDA) were determined in seminal plasma and spermatozoa.

The MG bucks had significantly lower values of GR in the spermatozoa and the seminal plasma during almost all periods of the experiment. In addition, significantly lower activity of GSH-Px in the spermatozoa and higher in the seminal plasma were observed in the last period of the experiment as well as significantly lower value of SOD in spermatozoa during the last 3 periods of the experiment. The MG bucks had significantly higher values of the ratios: CAT/SOD, GSH-Px/SOD in the seminal plasma and spermatozoa during 6$^{th}$ period of the experiment. In addition, the same group of bucks had significantly lower values of the ratio: GR/GSH-px in the spermatozoa during 6$^{th}$ period and in the seminal plasma during 5$^{th}$ period of the experiment.

According to the obtained results it could be concluded that the exogenous melatonin changed the value of particular antioxidative enzyme activities in certain periods of the experiment, especially of GR and GSH-Px in the seminal plasma and the spermatozoa and SOD in spermatozoa. Also, the exogenous melatonin had an influence on the ratios of antioxidative enzymes in the seminal plasma and the spermatozoa, and thus, the precise determination of these ratios in the future could be considered as a better indicator of oxidative stress which may provide a better insight into adaptation and antioxidative status of the semen in regard to activities of single antioxidative enzymes. In this study the antioxidative status in French Alpine buck spermatozoa was established for the first time.

**Keywords:** EXOGENOUS MELATONIN, SEMINAL PLASMA, SPERMATOZOA, RATIOS OF ANTIOXIDATIVE ENZYMES, BUCKS

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<u>Резюме.</u> Авторське резюме повторює структуру статті і коротко висвітлює мету та завдання, методи, результати, висновки. Подана в резюме інформація не повинна містити матеріал, відсутній в основній частині публікації. Висновки можуть супроводжуватись рекомендаціями, оцінками, пропозиціями, гіпотезами, описаними у статті. Відомості, які є у назві статті, не повинні повторюватися у тексті авторського резюме. Варто уникати зайвих вступних фраз (наприклад, «автор статті розглядає…»). Текст резюме повинен бути лаконічним і чітким, вільним від другорядної інформації, загальних і незначущих формулювань. Скорочення та умовні позначення, крім загальновживаних, застосовують у виняткових випадках або дають їх роз'яснення і визначення при першому вживанні в авторському резюме. У резюме не роблять посилань на джерела використаної літератури. Обсяг тексту авторського резюме визначається вмістом публікації (обсягом відомостей, їх науковою цінністю та/чи практичним значенням), він повинен становити не менше 250 слів.

**НАЗВА СТАТТІ АНГЛІЙСЬКОЮ МОВОЮ (ВЕЛИКИМИ ЖИРНИМИ ЛІТЕРАМИ)**

*Ініціали і прізвище автора англійською мовою (курсивом)*

Назва наукової установи, адреса, яка містить назву міста і країну, повні адресні відомості разом з поштовою та електронною адресами (кегль 12). Усі адресні відомості, крім найменування вулиці (подається транслітерацією), повинні бути подані англійською мовою, у т. ч. місто і країна. Усі ці дані враховуються при формуванні профілів організацій кожного автора статті. Резюме англійською мовою має відповідати українському тексту.

**НАЗВА СТАТТІ РОСІЙСЬКОЮ МОВОЮ (ВЕЛИКИМИ ЖИРНИМИ ЛІТЕРАМИ)**

*Ініціали і прізвище автора російською мовою (курсивом).*

Назва наукового закладу російською мовою (кегль 12). Резюме, назва статті, прізвище, ім'я, по батькові, назва установи і ключові слова російською мовою повинні відповідати українському тексту.

Якщо статтю написано англійською мовою, резюме подаються, відповідно, українською та російською мовами.

Вступ. На початку статті стисло викладається огляд літератури з посиланням на джерела літератури (за абеткою) та обґрунтування мети дослідження.

**Матеріали і методи**

Формують так, щоб за наведеним описом можна було відтворити дослідження. На загальновідомі методи достатньо дати посилання. Необхідно навести назви фірм та зазначити країни-виробники реактивів і матеріалів, вид і кількість піддослідних тварин і обов'язково — методи знеболювання та евтаназії відповідно до Європейської конвенції про захист хребетних тварин, що використовуються для дослідних та інших наукових цілей (Страсбург, 1986).

**Результати й обговорення.**

Не потрібно наводити ті самі результати у таблицях і на рисунках. Якщо є таблиця, у тексті цифровий матеріал не потрібно подавати, треба вказувати лише зміну показників з вірогідними різницями (P<) у разах або відсотках, кореляційні зв'язки (r=). За наявності у статті рисунків у тексті слід дати цифрові дані (середнє арифметичне та відхилення, коливання).

**Висновки** (5–10 речень).

**Перспективи подальших досліджень** (3–4 речення).

Таблиці. Таблиці подають після згадування у тексті. Скорочення слів, наведення абревіатур, за винятком загальновідомих, у таблицях не дозволяється. Не потрібно наводити одні й ті самі результати у таблицях і на рисунках. Всі колонки у таблицях повинні мати назву і бути заповнені відповідними даними. **Назву і графи таблиць потрібно дублювати англійською мовою.**

*Таблиця*

**Показники плазми крові**
**Blood plasma parameters**

| Показники<br>Parameters | Групи<br>Groups | | |
|---|---|---|---|
| | Контрольна<br>Control | I дослідна<br>1st experimental | II дослідна<br>2nd experimental |
| Загальний протеїн, г/л<br>Total protein, g/L | | | |
| Глюкоза, ммоль/л<br>Glucose, mmol/L | | | |
| АСТ, Од/л<br>AST, U/L | | | |

Ілюстрації. Рисунки подають після згадування. Фотографії слід надсилати відсканованими і вставленими в текст після згадування. На фотовідбитках зазначається верх. Заміна фотовідбитків електрофоретичних, хроматографічних та інших досліджень рисунками неприйнятна. Неякісні неконтрастні знімки не приймаються. Підписи до рисунків, фотографій (кегль 10): загальна назва рисунка і пояснення його окремих елементів, зокрема умовних позначень (А, Б, а, б, 1, 2, I, II). **Назву рисунків і фотографій потрібно дублювати англійською мовою.**

Формули. Хімічні й математичні формули повинні бути надруковані латинськими літерами. Потрібно розмітити великі й малі літери, верхній і нижній індекси.

Термінологія та одиниці вимірювання. Усі позначення і найменування фізичних і хімічних одиниць вимірювання наводять у системі SI. Згідно з сучасною номенклатурою, слід використовувати терміни *ензим* (а не фермент), *протеїн* (а не білок), назви хімічних елементів згідно з реформою української хімічної термінології. Якщо в дослідженнях було використано конкретні організми (тварини, мікроорганізми), під час першого згадування їх у тексті статті необхідно зазначити повну видову назву цих організмів *латинською мовою (курсивом)*, дотримуючись сучасної систематики, а за повторного згадування найменування роду наводять скорочено, наприклад, *Staph. aureus*, *Str. lactis*. Цифрові дані необхідно заокруглювати згідно з усталеними правилами, враховуючи середню похибку досліду. Вірогідність відмінностей показників обґрунтовувати статистичним аналізом, посилаючись на конкретні методи.

Література. У списку літератури (за абеткою) мають переважати посилання на роботи останніх десяти років. У посиланні на джерело необхідно вказувати прізвища усіх авторів, індекс DOI (якщо є), назву журналу чи книги подавати курсивом. Посилання на іншомовні джерела наводять мовою оригіналу. Не дозволяється робити посилання на неопубліковані матеріали.

**Приклади оформлення списку літератури:**

**Англомовні публікації:** Kushkevych M. V., Vlizlo V. V. Localization and level of the cellular prion in the jejunum of the rats Wistar line of different age groups. *Biological systems*, 2013, vol. 3, pp. 325–329.

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Antonyak H., Olijnyk Ch., Koval N., Fedyakov R., Dosviadchynska M., Panchuk I. Effects of aflatoxin B1 on lipid peroxidation and activities of antioxidant enzymes in rat organs and erythrocytes. *Visnyk of Lviv University*, Biological series, 2015, vol. 69, pp. 41–48. (in Ukrainian)

**Стаття з DOI:** Kozak M. R., Vlizlo V. V., Kit Y. Y., Stoika R. S. Induction of apoptosis and necrosis in leukemic cells by purified IgG of blood serum of mice which were fed with cattle brain for a long time. *Biopolymers and Cell*, 2008, vol. 24, no 1, pp. 28–34. DOI: 10.7124/bc.00078D. (in Ukrainian)

Fleseriu M. Recent advances in the medical treatment of Cushing's disease. *F1000 — Prime Reports*, 2014, vol. 6, no. 18. DOI: 10. 12703/P6–18. (in English)

Wesoly R., Jungbluth I., Stefanski V., Weiler U. Pre-slaughter conditions influence skatole and androstenone in adipose tissue of boars. *Meat science*, 2015, vol. 99, pp. 60–67. DOI: 10.1016/j. meatsci.2014.08.015.

**Стаття з електронного журналу:** Swaminathan V., Lepkoswka-White E., Rao B. P. Browsers or buyers in cyberspace? An investigation of electronic factors influencing electronic exchange. *Journal of Computer-Mediated Communication*, 1999, vol. 5, no. 2. Available at: http://www. ascusc.org/ jcmc/ vol5/issue2/ (Accessed 28 April 2011).

**Стаття з періодичного видання (збірника праць):** Duh A. I., Vovk S. Changes of lipid content and fatty acid composition of yolk in eggs and liver breeding chickens and embryos depending on the level of carotenoids in the diet. *Ukr. Biochem. J.*, 2010, vol. 82, no. 5, pp. 118–124. (in Ukrainian)

**Матеріали конференцій:** Yukalo A. V., Yukalo V. G., Shynkaryk M. M. Electrophoresis separation of the milk protein. Proceeding of the International Conference on Bio and Food Electrotechnologies, Compiegne (France), 2009, pp. 227–231.

Golovko O. V., Smolyaninova V. K., Severin R. V., Savenko M. M., Gluschenko Y. S., Smolyaninova I. V. Planning and organization of veterinary measures for the prevention and treatment of diseases of domestic animals in the area of private clinic. Problems of zooengineering and veterinary medicine: Preview. Collection of scientific works Kharkiv state zooveterinary Academy, Kharkiv, PBB KSZA, 2013, is. 26, Part 2 "Veterinary sciences", pp. 211–215. (in Ukrainian)

**Книга:** Verbitsky P. I. S*pongioform encephalopathy in cattle and other prion infections*. Kyiv, Vetinform, 2005, 240 p. (in Ukrainian)

Lapovets L. Ye., Lutsyk B. D., Lebed H. B. *Handbook of Laboratory Immunology*. Lviv, 2014, 292 p. ISBN 966-02-2704-3. (in Ukrainian)

**Перекладена книга:** для англомовних (та виданих латиницею) перекладених книг подавати дані лише оригіналу книги.

**Дисертація:** Sharan M. M. Experimental justification and improvement of methods of transplantation and cryopreservation of cattle embryos. Dr. agricultural sci. thesis, Institute of Animal Biology NAAS, Lviv, 2010, 277 p. (in Ukrainian)

**Автореферат:** Major Ch. Ya. The content of physiological prion in peripheral part of rats' prion-replication system under the action of glicoseaminoglycans series drugs. Autoref. of PhD thesis in biol. sci., Institute of Animal Biology NAAS, Lviv, 2010. 16 p. (in Ukrainian)

**Стандарти:** Olive oils and olive-pomace oils — Determination of wax content by capillary gas chromatography. International standard. ISO 12873: First edition 2010(E), LTD.

EN 12823-2. Foodstuffs — Determination of vitamin A by high performance liquid chromatography-Part 2: Measurement of β-carotene. 2000, European Committee for Standardization, Brusels.

**Патент:** Vlizlo V. V., Kushkevych M. V. A method for detection of the cellular prion localization in the tissues. *Patent UA*, no. 108110, 2016. (in Ukrainian)

Ohorodnyk N. Z., Kychun I. V., Vischur O. I. Vitamin and mineral drug with prolonged action «Vitarmin». *Patent UA for utility model*, no. U201501800. From 02.03.2015. (in Ukrainian)

Kosinov M. V., Kaplunenko V. G. Ukrainian patent for utility model number 38391. IPC (2006): C07C 51/41, C07F 5/00, C07F 15/00, C07C 53/126 (2008.01), C07C 53/10 (2008.01), A23L 1/00, B82B 3/00. Method metal carboxylates "Nanotechnology receiving metal carboxylates". Publish. 12.01.2009, Bull. no. 1. (in Ukrainian)

Подані статті скеровуються редакцією на рецензію двом провідним фахівцям у відповідній галузі. Статті з поправками та зауваженнями рецензентів повертаються авторам. Після доопрацювання статті автор повертає надісланий йому примірник рукопису та рецензії до редакції, а також подає два примірники виправленої статті і її електронну версію.

Для контрольного вичитування статті редакція надсилає авторам електронною поштою верстку, яку необхідно повернути не пізніше, ніж через три дні, також електронною поштою. Якщо відповідь від авторів не надійшла вчасно, редакція залишає за собою право затримати публікацію. У випадку негативної рецензії, яка заперечує можливість доопрацювання статті, один її примірник редакція залишає в архіві, а другий разом із рецензією повертає авторам. У випадку відхилення рукопису редакція залишає у своєму архіві один його примірник.

Датою отримання статті вважають дату надходження остаточно опрацьованого автором варіанту рукопису.

***Увага!*** У випадку одержання статті, оформленої з порушеннями наведених «ВИМОГ», редакція залишає за собою право повертати рукопис авторам для доопрацювання, не реєструючи його.

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# ІНСТИТУТ БІОЛОГІЇ ТВАРИН НААН

ПРОВОДИТЬ:

- Дослідження біохімічних показників (аналізатор Humalyzer 2000, Німеччина)
- Гематологічний аналіз (аналізатор Mythic-18Vet, Швейцарія)
- Мікробіологічні дослідження (посів на стерильність, антибіотикограма, склад мікрофлори кишечнику тварин, мікробіологічний аналіз кормів, води, повітря)
- Імуноферментні дослідження (аналізатор Stat Fax 3000, Німеччина)
- Оцінка репродуктивної здатності тварин, штучне осіменіння, трансплантація ембріонів
- Селекційно-генетичні дослідження
- Дослідження кормів
- Дослідження молока
- Дослідження яєць
- Визначення показників якості меду
- Дослідження вовни і волосся
- Атомно-абсорбційний і атомно-емісійний аналіз концентрації хімічних елементів
- Аналіз органічних добрив

Організовує проведення досліджень на лабораторних тваринах і надає кваліфіковану інтерпретацію отриманих результатів.

** можливе проведення інших досліджень*
*** всі лабораторії інституту акредитовані для проведення досліджень*

**Інститут біології тварин НААН**
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# Інститут біології тварин НААН виготовляє та реалізує гранульований корм для годівлі лабораторних щурів і мишей

Гранульований, повнокомпонентний, збалансований за поживними речовинами корм забезпечує біологічну потребу лабораторних тварин (щурів і мишей) поживними речовинами відповідно до норм живлення NRC (*National Research Council*).

Рецептура корму розроблена та апробована в Інституті біології тварин НААН.

Корм відповідає:

*Патент № 115538 від 25.04.2017 «Гранульований корм для лабораторних щурів і мишей».*

*ТУ У 10.9-30995014-010:2017 «Гранульований корм для лабораторних щурів і мишей».*

## Характеристики корму:

**Поживність**

| | | |
|---|---|---|
| сирий протеїн | не менше | 23,5 % |
| сирий жир | не менше | 5,0 % |
| сира клітковина | не більше | 4,5 % |
| зола | не більше | 7,5 % |

**Мінеральні елементи**

| | | |
|---|---|---|
| Кальцій | не менше | 1,20 % |
| Фосфор | не менше | 0,95 % |
| Калій | не менше | 0,80 % |
| Натрій | не менше | 0,33 % |
| Магній | не менше | 0,15 % |
| Хлорид | не менше | 0,05 % |
| Залізо | не менше | 250,0 $10^{-6}$ % |
| Цинк | не менше | 45,00 $10^{-6}$ % |
| Марганець | не менше | 80,00 $10^{-6}$ % |
| Мідь | не менше | 10,00 $10^{-6}$ % |
| Кобальт | не менше | 0,70 $10^{-6}$ % |
| Йод | не менше | 1,80 $10^{-6}$ % |
| Молібден | не менше | 0,15 $10^{-6}$ % |
| Селен | не менше | 0,15 $10^{-6}$ % |

**Амінокислоти**

| | | |
|---|---|---|
| аргінін | не менше | 1,25 % |
| лізин | не менше | 1,20 % |
| метіонін | не менше | 0,50 % |
| цистеїн | не менше | 0,35 % |
| триптофан | не менше | 0,25 % |
| гліцин | не менше | 1,10 % |
| гістидин | не менше | 0,50 % |
| лейцин | не менше | 1,80 % |
| ізолейцин | не менше | 1,10 % |
| фенілаланін | не менше | 1,10 % |
| тирозин | не менше | 0,75 % |
| треонін | не менше | 0,90 % |
| валін | не менше | 1,20 % |

**Вітаміни**

| | | |
|---|---|---|
| Вітамін А | не менше | 10,0 МО/г |
| Вітамін D | не менше | 4,0 МО/г |
| Альфа-токоферол | не менше | 35,0 $10^{-6}$ % |
| Тіамін | не менше | 14,0 $10^{-6}$ % |
| Рибофлавін | не менше | 7,0 $10^{-6}$ % |
| Нікотинова кислота | не менше | 80,0 $10^{-6}$ % |
| Пантотенова кислота | не менше | 20,0 $10^{-6}$ % |
| Холін | не менше | 2000,0 $10^{-6}$ % |
| Піридоксин | не менше | 10,0 $10^{-6}$ % |
| Фолієва кислота | не менше | 3,0 $10^{-6}$ % |
| Біотин | не менше | 0,3 $10^{-6}$ % |
| Вітамін $B_{12}$ | не менше | 60,0 мкг/кг |
| Вітамін К | не менше | 3,0 $10^{-6}$ % |

**Ціна 1 кг корму — 18 грн.**

Контакти:
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Посилюючи здоров'я тварин та підвищуючи ефективність використання стада, наша продукція створює відмінну базу для успішного сільського господарства.

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Ми завжди поряд із Вами, Ми гарантуємо Вам дохід, ріст та успіх завдяки нашому досвіду в інноваційній годівлі.

Ми прагнемо працювати, враховуючи інтереси усіх сторін та дивлячись у майбутнє - не тільки у сфері годівлі тварин, а й у сфері взаємновідносин, інноваційних технологій, якості, надійності та охорони навколишнього середовища.


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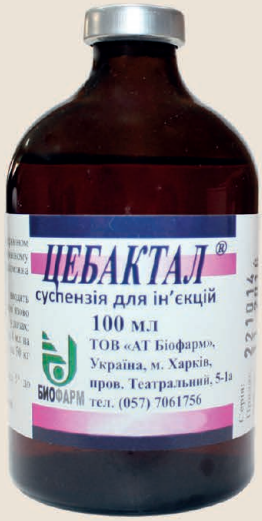


ЦЕБАКТАЛ

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БХФЗ bcpp

*Мистецтво досконалої фармації*

# ЗДОРОВА ТВАРИНА – БЕЗПЕЧНА ПРОДУКЦІЯ



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- екології (**ISO 14001**);
- безпеки здоров'я (**OHSAS 18001**);
- соціальної відповідальності (**SA 8000**)

**тощо**





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