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"EnzActive mix" probiotic preparation effect on some metabolism sites and antioxidant defense parameters of sows and their piglets organisms

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PTY: Conceptualization; Investigation; Methodology; Project administration; Supervision.
SKB: Conceptualization; Data curation; Formal analysis; Investigation.

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Taking into consideration the relevance and significant scientific and practical interest in the issue of probiotic preparations effect on various metabolism sites and productivity of farm animals, we have studied the probiotic feed additive "EnzActive mix" based on live *Saccharomyces cerevisiae* yeast on some metabolism sites, the content of lipid peroxidation products and the antioxidant defense activity in sows and piglets obtained from them. The actuality of such studies is explained by the significant interest in the use of probiotic preparations not only in human medicine, but also in the practice of animal husbandry. The aim of the work was to study the effect of the specified feed additive on some metabolism sites of the farrowing sows and the piglets obtained from them organism, on the lipid peroxidation products (LPP) content, as well as the activity of antioxidant enzymes, in particular, superoxide dismutase and glutathione peroxidase in their body. It was established that feed additive "EnzActive mix", based on *Saccharomyces cerevisiae* yeasts, administration decreased the lipid peroxidation products (LPP) content (TBARS and lipids hydroperoxides) in sows and their progeny blood, in the result of the study. The reason for such LPP content decrease was the antioxidant defense enzyme — superoxide dismutase increase in the sows' and their progeny's RBC (red blood cells). However, it was admitted also feed additives' positive effect on sows' parameters of productivity.

Key words: "EnzActive mix", sows, piglets, *Saccharomyces cerevisiae,* superoxide dismutase, glutathione peroxidase, TBARS (Thiobarbituric acid reactive substances), lipid hydroperoxides

Introduction

In recent years, a large number of probiotics that are used to normalize intestinal microflora and increase animal resistance have appeared [7]. Various yeast strains that can be used as either probiotics or prebiotics (nutritional ingredients that selectively stimulate microbiota growth) are in particular attention. The usage of prebiotics, such as inactive yeasts (*Saccharomyces cerevisiae*) and carbohydrates, isolated from their cell wall (mannan-olygosaccharides-MOS and β-glucans), and also autolytic yeast is the focus of research in poultry and livestock production [2–6, 8].

Benefits of autolytic yeasts usage include improvement of milk and meat productivity, improvement of laying and egg quality; increase of feed efficiency and nutrition digestibility; rumen medium improvement and its pH stabilization in highly concentrated diet; β-glucans boost immune system, mannans bind pathogens and balance intestine microflora; they have long shelf life period, and are thermostable, can be granulated and extruded; functional peptides and flavor nucleotides improve appetite and feed taste, that allows to use them in diets for young animals. The research, development and testing of the efficacy of such products is a promising area for the development of the agricultural sector [3].

Taking into consideration the relevance and significant scientific and practical interest in the issue of probiotic preparations effect on various sites of farm animals metabolism and productivity, we conducted the study of corresponding probiotic preparation effect on several metabolism sites, lipid peroxidation substances content and antioxidant defense enzymes activity in sows' and their piglets' organism — mentioned parameters should additionally characterize metabolic and productive efficacy of researched preparation. "EnzActive mix" is a unique combination of live *Saccharomyces cerevisiae* yeasts with activity not less than $1,5\times10^{10}$ CFU/g and enzyme complex including: protease, cellulase, xylanase, α-amylase, β-glucanase, phytase, under the layer of inactivated yeast cells.

Materials and Methods

During the study, ethical requirements for the use of animals in experimental research were fully respected (Strasbourg, 1986; Kyiv, 2002), and the research methodology was approved by the Bioethics Committee at the Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine (protocol no. 93-01 from June 3, 2021).

The study was conducted on the base of industrial complex "Barkom" LLC, Lviv region. Two groups of large white breed sows (3 litters) of 10 animals each were formed — control and experimental, by the principle of analogues. The animals were kept in the same box under the same conditions. The control group received a standard diet balanced by biologically active substances. The experimental group received standard diet, same with control, though to the diet the "EnzActive mix" feed additive was additional included in the dosage 0.3 kg/t of the finished feed. The study started on the 85th day of sow gestation or in 30 days before the expected farrowing. Blood samples for biochemical analysis were taken in sows before the experiment. The sows were transferred to the farrowing ward in 5 days before farrowing where they were fed lactating sows diet, and the experimental group continued to include additionally the specified feed additive. Gestating sows were fed twice a day at a rate of 3.5 kg/day, lactating sows were fed in individual troughs four times a day in the amount of 7 kg. Blood samples for research were taken three days before expecting farrowing and on the 21st day of lactation. The piglets of the experimental group were additionally fed investigated feed additive in the amount of 0.5 kg/t of the finished feed. The finished compound feed was fed to piglets from 5 days after birth till weaning. Blood samples for biochemical analysis were taken from piglets on the 5th, 14th, and 28th day after birth from cranial *vena cava*.

The content of lipid hydroperoxides and TBC-active products, activity of glutathione peroxidase and superoxide dismutase were determined in the blood. The research data is highlighted and related to works [9, 10].

Biochemical parameters of the sows and piglets blood were determined with *Humalyzer 2000* biochemical analyzer. We also evaluated sows behavior, feed consumption, live weight loss during the experiment, quantity of piglets born. For piglets behaviour, live weight gain and average daily weight gain on days 5–14–28 of life, pre-starter feed consumption, and the presence of diarrhoea were evaluated. The results were processed by standard methods of mathematical statistics using *Microsoft Excel* software. Statistical significance was determined by the Student's test.

Results and Discussion

The data on certain biochemical parameters changes in the blood serum of sows treated with "EnzActive mix" feed additive in addition to the diet are shown in the table 1. Based on the results we saw in this study, we have determined that in general the use of this additive did not cause significant changes in biochemical blood parameters of the studied sows, in particular the content of macronutrients calcium and phosphorus, triacylglycerol fats, cholesterol and enzymes, which would indicate a certain toxic effect. There was also a tendency to an increase in albumin content during the study period and a decrease in urea on the 21st day of lactation ($P<0.05$), which may indicate a positive effect of the feed additive on the protein metabolism intensification in their bodies.

Table 1. Biochemical blood parameters in sows (M±m, n=5)

Parameters	Groups	Study periods		
		85 days in gestation	112 days in gestation	21 day in lactation
Albumines, g/l	C	32.8±2.46	35.0±2.39	30.8±2.44
	T	34.9±1.82	39.6±1.97	32.4±1.29
Calcium, mmol/l	C	2.27±0.13	2.12±0.23	2.48±0.11
	T	2.26±0.07	2.36±0.09	2.4±0.04
Phosphorus, mmol/l	C	2.2±0.12	3.32±0.14	1.80±0.16
	T	2.1±0.16	3.62±0.39	1.96±0.11
Triglycerides, mmol/l	C	0.6±0.07	1.40±0.15	0.45±0.04
	T	0.63±0.02	1.31±0.30	0.72±0.28
Cholesterol, mmol/l	C	1.46±0.15	2.18±0.12	2.24±0.15
	T	1.73±0.08	2.23±0.167	2.1±0.29
Urea, mmol/l	C	4.97±0.09	4.4±0.38	7.26±0.32
	T	4.7±0.23	4.28±0.18	6.12±0.32*
ALT, MO/l	C	49.2±2.76	50.98±4.73	42.38±2.72
	T	55.2±2.25	57.06±2.59	38.44±1.32
AST, MO/l	C	22.73±1.85	38.6±2.99	35.72±2.85
	T	27.46±1.61	31.3±2.24	32.6±4.16

Table 2. Biochemical blood parameters in piglets (M±m, n=5)

Parameters	Groups	Study periods		
		5 days	14 days	28 days
Albumines, g/l	C	25,02±2,35	23,46±2,08	19,24±1,49
	T	29,6±1,97	26,28±1,81	28,4±2,15**
Calcium, mmol/l	C	2,12±0,23	2,3±0,13	2,7±0,15
	T	2,36±0,09	2,68±0,02*	2,96±0,13
Phosphorus, mmol/l	C	3,32±0,14	2,36±0,08	3,78±0,30
	T	3,62±0,38	2,54±0,08	3,88±0,25
Triglycerides, mmol/l	C	1,41±0,16	1,61±0,15	1,34±0,05
	T	1,31±0,30	1,61±0,15	1,39±0,12
Cholesterol, mmol/l	C	2,18±0,12	3,90±0,30	2,90±0,22
	T	2,23±0,17	3,62±0,25	2,46±0,20
Urea, mmol/l	C	4,40±0,38	4,56±0,31	4,40±0,38
	T	4,28±0,18	6,18±1,09	4,28±0,18
ALT, MO/l	C	57,06±2,59	39,28±2,97	51,24±5,50
	T	51,24±5,50	57,24±7,27	57,06±2,59
AST, MO/l	C	51,48±11,81	70,72±17,01	51,48±12,99
	T	31,24±2,23	140,46±25,46	31,24±2,23

Table 3. The content of lipid peroxidation products in the blood plasma of sows (M±m, n=5)

Parameters	Groups	Study periods		
		85 days in gestation	112 days in gestation	21 day in lactation
TBARS, μmol/ml	C	4.31±0.103	4.37±0.111	4.26±0.143
	T	4.33±0.118	3.52±0.132**	3.51±0.185*
HPL, Units. E/ml	C	2.58±0.110	2.60±0.045	2.42±0.035
	T	2.34±0.075	2.41±0.087	2.22±0.069*

Table 4. The content of lipid peroxidation products in the blood plasma of suckling piglets (M±m, n=5)

Parameters	Groups	Study periods		
		5 days	14 days	28 days
TBARS, μmol/ml	C	4.8167±0.05	4.3323±0.03	3.9424±0.13
	T	4.4071±0.06**	4.2201±0.08	3.4829±0.04**
HPL, Units. E/ml	C	0.53±0.02	0.52±0.01	0.49±0.02
	T	0.45±0.03	0.39±0.02**	0.35±0.007***

Table 5. Activity of glutathione peroxidase and superoxide dismutase in blood erythrocytes of sows (M±m, n=5)

Parameters	Groups	Study periods		
		85 days in gestation	112 days in gestation	21 day in lactation
Glutathione peroxidase, nM NADPH/ min/mg Hb RBS	C	10.55±0.21	7.39±0.25	9.73±0.09
	T	9.51±0.32	7.13±0.34	10.51±0.14
Superoxide dismutase, units Act/mg protein×min	C	21.54±3.61	19.91±1.84	22.15±1.57
	T	23.01±1.53	24.92±1.79	27.02±1.24*

The data of biochemical processes dynamics in piglets (obtained from the sows fed with “EnzActive mix”) organism are shown in the table 2. We can see from the table, that on 28th day of life in the piglets blood a significant increase of albumin content is observed. In particular, the albumin content in the blood serum of the experimental group piglets was statistically higher by 47.61% (P<0.01) comparing with the control.

It is known that albumin performs, among other things, antioxidant and transport functions in the body, including and for macro- and microelements, which may indicate a positive stimulating effect of the feed additive on the albumin fraction of proteins metabolism [6]. Analyzing the results of the blood serum test of suckling piglets of both groups, we see a gradual increase in the Potassium content throughout the entire experiment period. However, in experimental group piglets of 14 days of age, the Potassium content was significantly higher by 16.50% (P<0.05) than in the control group. The level of inorganic Phosphorus in the experimental group slightly decreased on the 14th day of piglet life, but compared to the control group it was still slightly higher.

Table 3 shows the data on changes in the content of lipid peroxidation products in the blood of sows receiving the “EnzActive mix” feed additive included in the diet. As a result of the studies, it was determined the concentration of TBARS and lipid hydroperoxides in the blood serum, which characterizes the intensity of lipid peroxidation processes in the body and serves as a marker of the endogenous intoxication rate [9, 10]. The studies have shown that including the “EnzActive mix” to sows diet of the experimental group had an inhibitory effect on the lipid peroxidation processes intensity, as indicated by a statistically lower content of TBARS in the blood plasma three days before farrowing and on the 21st day of lactation 19.45% and 17.6% (P<0.01; P<0.05) and lipid hydroperoxides on the 21st day of lactation 8.26% (P<0.05) compared to the same indicators in the control group.

As for the piglets obtained from these sows, it was found that the inclusion of “EnzActive mix” feed additive into the piglet diet caused a decrease in the lipid peroxidation products content in the blood plasma. Specifically, we found a significantly lower level of TBARS on the 5th and 28th day of suckling piglets’ life and lipid hydroperoxides on the 14th and 28th day (table 4). The obtained results indicate the inhibitory effect of the studied preparation on the intensity of the lipid peroxidation processes.

In the table 5 we can see that these changes occurred because of increased superoxide dismutase activity in red blood cells of sows and their piglets. The results of the study of the antioxidant defense system by the activity of glutathione peroxidase and superoxide dismutase in sow blood erythrocytes show significantly higher activity rate of superoxide dismutase on the 21st day of lactation in the experimental group comparing to the control.

Table 6. Activity of glutathione peroxidase and superoxide dismutase in blood erythrocytes of suckling piglets (M±m, n=5)

Parameters	Groups	Study periods		
		5 days	14 days	28 days
Glutathione peroxidase, nM NADPH/ min/mg Hb RBS	C	15.1±0.31	13.45±0.19	13.86±0.17
	T	14.15±0.37	13.26±0.22	12.97±0.27
Superoxide dismutase, units Act/mg protein×min	C	22.40±1.18	23.36±1.07	26.30±1.25
	T	23.87±0.71	25.18±1.12	30.11±0.92*

From the data presented in table 6 we can see that the activity of superoxide dismutase, which is one of the first line of enzyme protection against superoxideanion radicals and hydrogen peroxide [5], was 14.5% higher in piglets of the experimental group on day 28 after weaning compared to control (P<0.05). Our results are completely consistent with the literature data on the antioxidant effect of probiotic preparations based on *Saccharomyces cerevisiae*. Thus, it is known that their antioxidant effect relates to the yeast polysaccharides action [11], and our studies show that it is accompanied by an increase of the key antioxidant enzyme — superoxide dismutase activity. No significant changes were observed in glutathione peroxidase activity.

A positive effect of feed additive is shown in the study on young piglets from the 1st day of life till 5 days of age. Based on the study results it was determined that piglets' live weight increased by 7.77% in the experimental group comparing to control. A significant increase in the live weight of piglets in the experimental group on day 14 was 13.72% (P<0.5), and on day 28 it was 8.40% (P<0.01) compared to the control group, respectively. The average daily weight gain of the control and experimental groups during the study period was 210 g and 230 g, respectively, that is statistically significant by 9.53% (P<0.01). Piglets of the experimental group consumed 1.89% more pre-starter feed than the control group. Higher feed intake at an early age in piglets indicates a healthy intestine and increased nutrient absorption, that is confirmed by the average daily gain and body weight in the experimental group [7]. During the lactation period sows of the control group lost 23 kg and those of the experimental group lost 20 kg, which is 1.95% less than in control. The higher feed intake and digestibility by 2.54% (P<0.01) compared to the control group caused the preservation of body weight in experimental group.

Based on the results of the study, we have determined, that administration of the "EnzActive mix" probiotic feed additive, based on *Saccharomyces cerevisiae* yeasts to gestating sows led to a decrease in the lipid peroxidation products (TBARS and lipid hydroperoxides) content in their blood and in the blood of their progeny. Such a decrease in the content of LPP products was due to an increase in the key antioxidant defense enzyme — superoxide dismutase activity in the erythrocytes of sows and piglets obtained from them. Furthermore, it was admitted a positive effect of the feed additive on sow productivity.

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Вплив пробіотичного препарату «ЕнзАктив Мікс» на деякі ланки обміну речовин та показники антиоксидантного захисту в організмі свиноматок та отриманих від них поросят

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Враховуючи актуальність та значний науково-практичний інтерес до питання впливу пробіотичних препаратів на різні ланки метаболізму і продуктивності сільськогосподарських тварин, ми дослідили вплив кормової добавки «ЕнзАктив Мікс» на основі живих дріжджів *Saccharomyces cerevisiae* на деякі ланки обміну речовин, вміст продуктів пероксидації ліпідів і активність антиоксидантного захисту в організмі свиней та отриманих від них поросят. Актуальність таких досліджень обумовлена значним інтересом до застосування пробіотичних препаратів не тільки в гуманітарній медицині, але й у практиці тваринництва. Метою роботи було дослідити вплив вказаної кормової добавки на деякі ланки метаболізму в організмі поросних свиноматок та отриманих від них поросят, на показники вмісту продутів перекисного окислення ліпідів (ПОЛ), а також активність антиоксидантних ензимів, зокрема супероксиддисмутази та глутатіонпероксидази в їх організмі. В результаті проведених досліджень встановлено, що застосування свиноматкам під час поросності пробіотичної кормової добавки «ЕнзАктив Мікс» на основі дріжжів *Saccharomyces cerevisiae* призводило до зниження вмісту продуктів перекисного окиснення ліпідів (ТБК-активних продуктів та гідроперекисей ліпідів) у їх крові та крові отриманих від них поросят. Таке зменшення вмісту продуктів ПОЛ було обумовлене зростанням активності ключового ферменту антиоксидантного захисту — супероксиддисмутази в еритроцитах свиноматок та отриманих від них поросят. Крім того, ми простежили позитивний вплив кормової добавки на показники продуктивності свиноматок.

Ключові слова: «ЕнзАктив Мікс», свиноматки, поросята, *Saccharomyces cerevisiae*, супероксиддисмутаза, глутатіонпероксидаза, ТБК-активні продукти, гідроперекиси

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