УДК: 636.034:619:612.018.

# SOME INDICATORS OF PROTEIN METABOLISM IN BLOOD OF COWS UNDER KETOSIS

*M. R. Simonov, V. V. Vlizlo*
msimonov@inenbiol.com.ua

Institute of animal biology of NAAS, V. Stus str., 38, Lviv, 79034, Ukraine

*During the first weeks of post-partum period in high-yield dairy cows is observed negative energy balance caused by discordance between inadequate diet energy supply and high consumption of glucose for metabolic processes and milk production. In order to compensate it animals use own reserves by means of carbohydrates, lipids and proteins. Results, which characterize the level of protein catabolism in ketotic cows are presented in the paper. The study was performed on ten cows (5 clinically healthy, with negative results of test for ketone bodies in urine and 5 with positive ketone tests) of Ukrainian black and white dairy breed at age from 2 to 5 years, with productivity above 5500 kg of milk for previous lactation. Blood samples were taken in March, two or three weeks after calving. Blood was withdrawn from jugular vein before morning feeding. Conducted research has shown that under hypoglycemia and hypoinsulinemia an increased activity of compensatory mechanisms in organism of high-yielding cows is registered. Particularly, significant ($p<0.001$) increase of plasma cortisol level is revealed. This allows to increase amino acid release from muscle proteins (proteolysis) and to provide necessary starting compounds for gluconeogenesis. As a result, an significant increase of 3-methylhistidine (by 78.9 %; $p<0.001$) and creatinine (by 26.8 %; $p<0.01$) is registered in blood of cows with ketosis in comparison with healthy animals. Herewith the ratio between creatinine and 3-methylhistidine rose almost 4-fold from 5.5 to 19.1. Considering obtained results, and that 3-methylhistidine after entering circulation does not undergo further metabolism and is excreted via urine, it might be suggested that the plasma content of 3-methylhistidine in dairy cows is an informative indicator of destructive changes of contractile proteins, and that determination of ratio creatinine/3-methylhistidine gives an information about relative rate of catabolism in muscle tissue.*

**Keywords:** COWS, KETOSIS, CORTISOL, 3-METHYLHISTIDINE, CREATININE, AMINO ACIDS, GLUCOSE, INSULIN, PROTEIN METABOLISM, CARBOHYDRATE METABOLISM

# ДЕЯКІ ПОКАЗНИКИ КАТАБОЛІЗМУ БІЛКІВ У КРОВІ КОРІВ ЗА УМОВИ КЕТОЗУ

*М. Р. Сімонов, В. В. Влізло*
msimonov@inenbiol.com.ua

Інститут біології тварин НААН, вул. В. Стуса, 38, м. Львів, 79034, Україна

*У перші тижні після отелення у високопродуктивних молочних корів існує негативний енергетичний баланс як результат невідповідності поступлення енергії з кормом і витрат глюкози на метаболічні процеси та синтез молока. Для його покриття тварина використовує власні резерви тіла за рахунок вуглеводів, жирів та білків. У роботі представлені результати, які характеризують рівень катаболізму білка у хворих на кетоз корів. Дослідження проводились на десяти коровах (5 клінічно здорових, з негативним тестом на наявність кетонових тіл у сечі та 5 з позитивним тестом), української чорно-рябої молочної породи, у віці від 2 до 5 років, продуктивністю понад 5500 кг молока за попередню лактацію. Проби крові відбирали у березні через два-три тижні після отелення корів. Кров відбирали з яремної вени перед ранішньою годівлею. Проведені дослідження показали, що за умови гіпоглікемії та гіпоінсулінемії відбувається підвищення активності компенсаторних механізмів організму високопродуктивних корів. Зокрема, реєструється вірогідне ($p<0,001$) зростання вмісту кортизолу у плазмі крові, що дозволяє*

*підвищити мобілізацію амінокислот з м'язових білків (протеоліз) та забезпечити глюконеогенез необхідними вихідними сполуками. У результаті цього у крові корів, хворих на кетоз, порівняно зі здоровими тваринами, реєструється вірогідне зростання вмісту 3-метилгістидину (на 78,9 %; p<0,001) та креатиніну (на 26,8 %; p<0,01). При цьому, у хворих корів зросло співвідношення між креатиніном та 3-метилгістидином майже у чотири рази від 5,5 до 19,1. Враховуючи отримані результати, а також те, що 3-метилгістидин після вивільнення у кров далі не метаболізується і повністю екскретується із сечею, можна зробити висновок про те, що вміст 3-метилгістидину у плазмі крові молочних корів є інформативним показником деструктивних змін скоротливих білків, а дослідження індексу креатинін/3-метилгістидин дає уяву про відносну швидкість катаболізму м'язової тканини.*

**Ключові слова:** КОРОВИ, КЕТОЗ, КОРТИЗОЛ, 3-МЕТИЛГІСТИДИН, КРЕАТИНІН, АМІНОКИСЛОТИ, ГЛЮКОЗА, ІНСУЛІН, БІЛКОВИЙ ОБМІН, ВУГЛЕВОДНИЙ ОБМІН

# НЕКОТОРЫЕ ПОКАЗАТЕЛИ КАТАБОЛИЗМА БЕЛКА У КРОВИ КОРОВ ПРИ КЕТОЗЕ

*М. Р. Симонов, В. В. Влизло*
msimonov@inenbiol.com.ua

Институт биологии животных НААН, ул. В. Стуса, 38, г. Львов, 79034, Украина

*В первые недели после отела у высокопродуктивных молочных коров существует отрицательный энергетический баланс как результат несоответствия поступления энергии с кормом и расходов глюкозы на метаболические процессы и синтез молока. Для его покрытия животное использует собственные резервы тела за счет углеводов, жиров и белков. В данной работе представлены результаты, характеризующие уровень катаболизма белка у больных кетозом коров. Исследования проводились на десяти коровах (5 клинически здоровых, с отрицательным тестом на наличие кетоновых тел в моче и 5 с позитивным тестом), украинской черно-пёстрой молочной породы, в возрасте от 2 до 5 лет, продуктивностью более 5500 кг молока за предыдущую лактацию. Пробы крови отбирали в марте месяце через две-три недели после отела коров. Кровь отбирали из яремной вены перед утренним кормлением. Проведенные исследования показали, что при гипогликемии и гипоинсулинемии происходит повышение активности компенсаторных механизмов организма высокопродуктивных коров. В частности регистрируется достоверное (p<0,001) повышение содержания кортизола в плазме крови, что позволяет повысить мобилизацию аминокислот из мышечных белков (протеолиз) и обеспечить глюконеогенез необходимыми исходными соединениями. В результате этого в крови больных кетозом коров, по сравнению со здоровыми животными, регистрируется достоверное увеличение содержания 3-метилгистидина (на 78,9 %, p<0,001) и креатинина (на 26,8 %, p<0,01). При этом, у больных кетозом коров выросло соотношение между креатинином и 3-метилгистидином почти в четыре раза с 5,5 до 19,1. Учитывая полученные результаты, а также то, что 3-метилгистидин после высвобождения в кровь дальше не метаболизируется и полностью выводится с мочой, можно сделать вывод о том, что содержание 3-метилгистидина в плазме крови молочных коров является информативным показателем деструктивных изменений сократительных белков, а исследования индекса креатинин/3-метилгистидин дает представление об относительной скорости катаболизма мышечной ткани.*

**Ключевые слова:** КОРОВЫ, КЕТОЗ, КОРТИЗОЛ, 3-МЕТИЛГИСТИДИН, КРЕАТИНИН, АМИНОКИСЛОТЫ, ГЛЮКОЗА, ИНСУЛИН, БЕЛКОВЫЙ ОБМЕН, УГЛЕВОДНЫЙ ОБМЕН

Ketosis occurs in dairy cows primarily as a result of negative energy balance caused by discordance between high requirements for milk production and inadequate diet energy

supply. Metabolism in the cattle organism is organized in such a way as to save glucose in case of energy deficiency, because the brain can use for nourishment only glucose. At the beginning of lactation, when there is high demand in glucose for synthesis of lactose, and the supply of glucose precursors with feed is low, activation of gluconeogenesis is a vital compensatory mechanism. Available literature data [1, 2] provide information that demand in energy and protein on the fourth day after calving in healthy cows exceeds their consumption by 25 %. In high-yielding dairy cows milk production demands significant quantity of nutritional substances and energy. Thus, in cows with daily milk production rate 25, 35 and 45 kg per cow, demand in glucose is 2.6, 3.5 and 4.2 kg respectively [3]. During the period of intensive lactation it cannot only be covered with feed, therefore in cows energy deficiency occurs. In order to cover it, animal uses its own reserves by means of carbohydrates, lipids and proteins. In particular, amino acids released from protein catabolism are used in citric acid cycle or are converted in pyruvate and may directly turn into glucose [4]. On the one hand in helps to smooth the energy deficit to somewhat degree, but on the other hand, intensive use of own reserves, in particular muscle proteins, without sufficient exogenic compensation causes quick decrease in body weight and productivity, and finally deep metabolic disorders. By the reference to this, the objective of our study was to determine the level of protein catabolism in cows with ketosis and to work out an index, which describes the process.

## Materials and methods

The study was performed in March on ten cows of Ukrainian black and white dairy breed at age from 2 to 5 years, with productivity above 5500 kg of milk for previous lactation. Animals were kept is analogous conditions and received identical diet. Clinical examination of cows was performed and testing for ketone bodies in urine was done using diagnostic strips (Ketophan, Pliva). During examination it was revealed that some animals (n = 5) are lying up, quick lowering of live weight and yield, they look oppressed, some of them had muscular tremor. After the contact of indicator strips with urine, colour turn to violet, this testifies of the presence of ketone bodies. Animals with positive results, i.e. with the presence of ketone bodies in urine, were selected into a separate group. Blood samples were withdrawn two or three weeks after calving, from jugular vein before morning feeding, into sterile tubes with heparin and immediately centrifuged at 3000 rpm. Obtained plasma was frozen at – 20 °C until analyses were done (up to 1 month). The plasma level of cortisol was measured using enzyme immunoassay technique and test-kits manufactured by «DRG» (Germany), and the content of 3-methylhistidine in blood plasma was detected using amino acid analyzer Biotronik LC 6001. The level of creatinine was determined in blood serum by Jaffe reaction (Popper's method).

Received results were processed in Excel, arithmetical mean (M) was calculated as well as statistical error (m) and significance of the difference between two variation series ($p<$).

## Results and discussion

Conducted laboratory research have shown that in organism of cows with ketosis there is significant energy deficiency, which manifests itself with hypoglycemia (<1.95 mmol/L) and hypoinsulinemia (<6.3 uIU/mL). As adjustment for high energy demand in dairy cows, an increased activity of compensatory mechanisms is registered, oriented to fatty acids mobilization from adipose tissue (lipolysis) as well as a release of amino acids from muscle proteins (proteolysis) that give necessary starting compounds for gluconeogenesis. Glucocorticoids, primarily cortisol, play the key role in this [4]. As it is shown on Figure 1, there is a significant increase (by 41.3 %; $p<0.001$) of plasma cortisol level in ketotic cows in comparison with healthy animals.

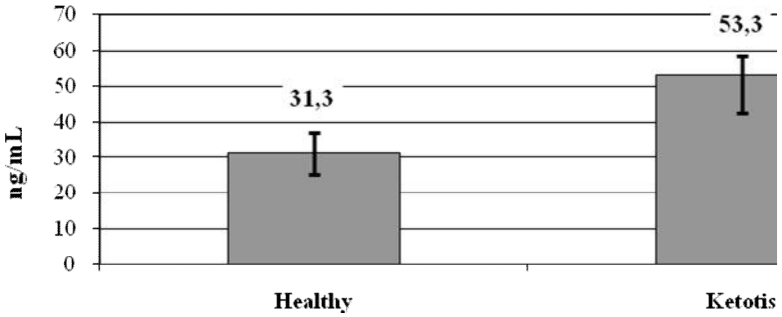


*Fig. 1.* Plasma content of cortisol in cows; (n=5)

Among all free amino acids the greatest informativeness concerning protein catabolism has 3-methylhistidine [5–8]. 3-methylhistidine is an amino acid, which is specific for contractile proteins (actin and myosin). During degradation of these proteins it enters circulation, but due to the absence of specific tRNA, it is not used in synthesis, is not metabolized and without changes is excreted via urine. These properties make measuring of 3-methylhistidine content an informative indicator of muscle protein metabolism. As can be seen on Figure 2, the plasma content of 3-methylhistidine in cows with ketosis is higher (by 78.9 %; $p<0.001$), in comparison with healthy animals.

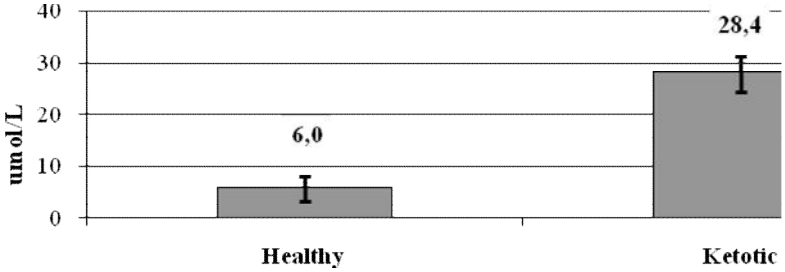


*Fig. 2*. Plasma content of 3-methylhistidine in cows (n=5)

Creatinine is another characteristic parameter, which reveals the level of destructive changes in muscle tissue [9, 10]. Creatinine is produced as a result of muscle metabolism and is also eliminated from organism through kidneys. Contrary to urea, its concentration in blood does not depend on the amount of protein received with feed. Our research has revealed (Fig. 3) that the level of creatinine in ketotic cows is increased by 26.8 % ($p<0.01$) in comparison with such in healthy animals.

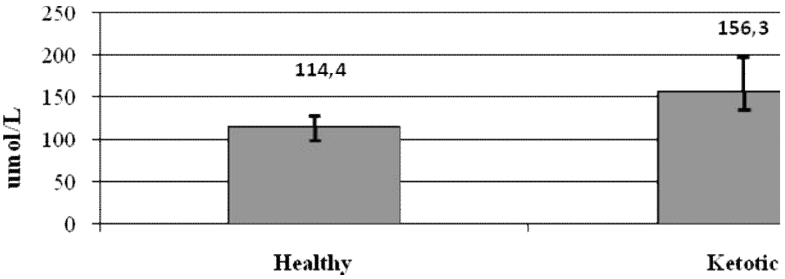


*Fig. 3*. Serum content of creatinine in cows (n=5)

Considering that fact that the amount of excreted creatinine is directly-proportional to the weight of skeletal muscles, the ratio between creatinine and 3-methylhistidine in

cow's blood might reflect relative rate of muscle proteins catabolism. Received figures revealed that the index of relative rate of muscle proteins catabolism in ketotic cows rose almost four-fold and was 19.1 compared with 5.5 in healthy animals.

## Conclusions

In high-yielding dairy cows with ketosis negative energy balance occurs, which activates compensatory mechanisms. In particular, a significant ($p<0.001$) increase of plasma cortisol level is revealed; it allows to increase release of amino acids from muscle proteins (proteolysis) and provide necessary starting compounds for gluconeogenesis. As a result, an significant increase of 3-methylhistidine (by 78.9 %; $p<0.001$) and creatinine (by 26.8 %; $p<0.01$) is registered in blood of cows with ketosis in comparison with healthy animals. Herewith in cows affected with ketosis the ratio between creatinine and 3-methylhistidine rose almost 4-fold (from 5.5 to 19.1). Considering obtained results, and that 3-methylhistidine after entering circulation does not undergo further metabolism and is completely excreted via urine, it might be suggested that the plasma content of 3-methylhistidine in dairy cows is an informative indicator of destructive changes of contractile proteins, and that determination of ratio creatinine/3-methylhistidine gives an information about relative rate of catabolism in muscle tissue.

**Prospects for further research** are in establishing of physiological ranges for 3-methylhistidine and for index creatinine/3-methylhistidine in blood plasma and urine of clinically healthy cattle and in investigating of the changes of these values under different physiological states, during various keeping periods and with different feeding strategies.

1. Levchenko V. I. Ketoz vysokoproduktyvnyh koriv: etiologia, diagnostyka I likuvannia [Ketosis highly productive cows: etiology, diagnosis and treatment]. *Zdorovia tvaryn I liky — Animal health and medicine*, 2009, vol. 2, pp. 14–15 (in Ukrainian).

2. Lee H. H. Slow recovery of blood glucose in insulin tolerance test during the prepartum transition period negatively impacts the nutritional status and reproductive performance postpartum of dairy cows. *J. Vet Med Sci.*, 2011, 47, (1), pp. 95–105.

3. Vlizlo V. V. *Zyrovyi gepatoz u koriv. Dysertacia doktora veterynarnyh nauk* [Steatosis in cows. Dr. veterinary sci. diss.]. Kyiv, 1998. 491 p. (In Ukrainian).

4. Annison E. F. Perspectives on ruminant nutrition and metabolism. *J. Nutr Res Rev.,* 1999, 12, (1), pp. 147–177.

5. Houweling M., van der Drift R. Jorritsma A. G. M. Tielens Technical note: Quantification of plasma 1- and 3-methylhistidine in dairy cows by high-performance liquid chromatography — tandem mass spectrometry. *Journal of Dairy Science,* 2012, 95, pp. 3125–3130.

6. Akamatsu H., Saitoh Y., Serizawa M. Changes of Serum 3-Methylhistidine Concentration and Energy-Associated Metabolites in Dairy Cows with Ketosis. *Journal of veterinary medical science Y.*, 2007, 69, (10), pp. 1091–1093.

7. Bos C., Gaudichon C., Tomé D. Nutritional and physiological criteria in the assessment of milk protein quality for humans. *J Am Coll Nutr.,* 2000, 19, (l2), pp. 191–205.

8. Therkildsen M. Muscle protein degradation in bull calves with compensatory growth. *Livestock Production Science,* 2005, 98, 205–218.

9. Myint T., Fraser G., Lindsted D. et al. Urinary 1-methylhistidine is a marker of meat consumption in black and in white california seventh-day adventists. *American Journal of Epidemiology,* 2000, 152, (8), pp. 752–755

10. Graugnard D., Bionaz M., Trevisi E. et al. Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum. *J Dairy Sci*., 2012, 95, (4), pp. 1749–1758.