The effect of protein supplement from skin wastes on hematological, biochemical metabolic indices and dairy productivity of highly yielding cows

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Abstract

This study was aimed to establish the effect of a fodder protein supplement from minced subcutaneous epithelium of farm animals on hematological, biochemical parameters of metabolism and milk productivity of high-yielding cows during the early lactation. For this, three groups of cows, six animals each, were formed according to the principle of analogous pairs. The control group received the main diet, the second and third experimental groups were daily fed with 300 g and 500 g of protein supplement for 60 days in addition to the main diet, respectively. The effect of prolongation of the supplement was studied over the next 30 days. Hematological and biochemical blood parameters, qualitative and quantitative composition of milk were studied in all animals. The animals of the experimental groups showed an increase in metabolic and redox processes during the experiment. An increase in the metabolism of nutrients in the liver has been found. Due to the sufficient intake of protein in the organism of animals, there was an increase in milk productivity and milk quality indicators.

Key words: protein supplement; leather industry wastes; high-yielding cows; metabolism; milk productivity

Introduction

One of the main conditions for the realization of the genetic potential of animals is the organization of their adequate nutrition, taking into account the level of productivity while maintaining a normal physiological state. Proteins directly influence the increase in milk production of animals by supplying them with all the necessary amino acids, increasing the amount of available energy and changing the efficiency of utilizing the nutrients contained in the main diet (Brun-Lafleur et al., 2010; Patton et al., 2014; Lazzarini et al., 2016). The organism of highly productive cows needs to receive a large amount of energy in the first weeks of lactation, as there are significant losses of nutrients along with the milk (Leroy et al., 2008, Amanlou et al., 2017). Therefore, the urgent task of modern intensive livestock breeding, is to provide the organism of lactating cows with the necessary amount of protein.

The rational use of feedstuffs containing a large amount of proteins should not lead to a significant increase in the cost of the basic diet. One of the possible solutions for this problem is the use of protein concentrates obtained during the processing of secondary raw materials of animal husbandry, since it is a widely available product and contains a complete protein. (Jayathilakan et al., 2011; Potapov et al., 2017; Popov et al., 2019). According to the number of studies, parts of the subcutaneous epithelium of farm animals, which remain in tanneries in large quantities after processing the hides, are a source of large amounts of protein in a form easily digestible for animals (Pati et al., 2014). This residual organic material can be used to produce protein feed supplements. In addition, the problem of processing and rational use of secondary raw materials in leather production to prevent environmental pollution in Russia, Ukraine, Bangladesh, China, the United States and some countries of South America and Europe, remains urgent (Jiang et al., 2016; Rigueto et al., 2020). Therefore,

the production of a high-protein feed supplement from the subcutaneous epithelium of farm animals could represent an economically feasible way to obtain the required product, as well as reduce the environmental damage caused by solid waste from leather production. Currently, various technological approaches have been developed to obtain safe feed from such waste (Kantarli and Yanik, 2009; Jiang et al., 2016). Despite the fact that such supplements have been produced for a long time, most studies are devoted to the effect of these products on certain physiological indicators of farm animals (Lee et al., 2015; Awawdeh, 2016; Amanlou et al., 2017; Leondro et al., 2019). Comprehensive studies on the effect of protein supplement from minced subcutaneous epithelium of farm animals on indicators of natural resistance, metabolism and milk productivity have not been performed. All of the studied parameters clearly demonstrate the physiological state of highly yielding cows in the early stages of lactation when fed a protein supplement from leather making wastes.

Therefore, the aim of our research was to study the effect of protein supplement made from minced subcutaneous epithelium of farm animals, on hematological, biochemical parameters of metabolism and milk productivity of highly yielding cows during the early lactation.

Materials and methods

To conduct research using the analogue pair method in the first months of lactation, a control (I) and two experimental (II, III) groups of Ayrshire cows, 6 animals each, were formed. The cows were kept fastened in the winter-stall period (Table 1).

Animals of the control and experimental groups received a daily ration, which included (kg/animal/day): corn silage - 22, cereal-legume silage - 17, mixed feed (40 %

Table 1. *Characteristics of animals participating in the experiment (mean ± standard deviations)*

Indices	Groups		
	I control	II experiment	III experiment
Average live weight (kg)	559.2±20.5	543.0±26.8	585.0±23.8
Average daily milk yield (kg)	33.5±1.1	33.1±0.8	31.5±0.7
Mass fraction of fat in milk (%)	3.92±0.31	3.75±0.17	4.60±0.62
A milk yield standardized on 4% of milk fat (kg)	33.0±1.3	32.3±1.6	35.4±3.6

Table 2. *Experiment design*

Groups	Period		
	1-30 day	main (31-60 day)	final (61-90 day)
I	Main ration (MR)	MR	MR
II	MR + protein supplement (300 g/animal/day)	MR + protein supplement (300 g/animal/day)	MR
III	MR + protein supplement (500 g/animal/day)	MR + protein supplement (500 g/animal/day)	MR

soybean meal) - 9.5, grain mixture - 2, crimped grain - 3, fodder molasses - 1.2, barley straw - 0.5, premix - 0.15, glycerin - 0.3, fodder chalk - 0.13, disodium phosphate - 0.15, soda - 0,15, table salt - 0.1, urea - 0.05, fodder sulfur - 0.035. The nutritional value of the main diet was 258.7 MJ. The diets were consistent with the norm in this age period. In addition to the main diet, the cows of the experimental groups were fed protein fodder meal made from minces subcutaneous epithelium of farm animals for 60 days. The aftereffect of the protein feed supplement on the organism of the animals of the experimental groups was studied during the next 30 days (Table 2).

Blood from the jugular vein was taken for biochemical parameters' determination on the 1st, 60th and 90th day, before morning feeding in the control and experimental groups. The activity of alkaline phosphatase, the amount of urea, magnesium, phosphorus, total protein, cholesterol, albumin, the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the level of glucose in the blood serum was determined on a biochemical analyzer Stat Fax 3300 (Awareness Technology, USA) with reagent kits "Diacon" (Russia) and "Vector-Best" (Russia). Serum calcium was determined using a complexometric method with an indicator fluorexon according to Vichev, Karakashev, reserve alkalinity in blood serum by a diffuse method using double flasks, carotene in blood serum according to Karp and Preuss in Yudkin's modification, the total number of ketone bodies in the blood by iodometric method (Kondrahin, 2004), protein index by calculation.

The number of leukocytes and erythrocytes was counted in a Goryaev chamber under a MIKMED-6 microscope (LOMO, Russia). To count leukocytes, 0.02 ml of blood was stained with 0.4 mL of a 3 % solution of acetic acid and methylene blue; to count erythrocytes, 0.02 ml of blood was mixed with 8 mL of 0.9 % sodium chloride solution. The composition and ratio of leukocytes was studied on blood smears, which were fixed with ethyl alcohol. To determine the ratio of leukocytes, peripheral blood smears were stained with azure-eosin according to Romanovsky (1 drop of the basic solution per 1 cm^3 of distilled water with a pH of 6.8-7.0). Under the MIKMED-6 microscope, 200 cells were counted on each blood smear, isolating lymphocytes, monocytes, stab and segmented neutrophils, eosinophils, and basophils. Leukocyte identification was carried out according to Nikitin (1949).

The registration and assessment of milk productivity in animals in all groups was carried out by calculating the daily milk yield according to the monthly control milkings carried out during the experiment. Physicochemical properties of milk: dry matter, content of fat, protein, urea and the number of somatic cells were determined on the analyser "BentleyCombi 150" (Bentley Instruments Inc., USA).

Chemical analysis of protein supplement made from subcutaneous epithelium of farm animals aimed at determining: total moisture - by a two-stage method; crude protein according to the Kjeldahl method with a VELP UDK139 (Velp Scientifica, Italy) semi-automatic steam still (Association of Official Analytical Chemists, 1990);

rumen degradable protein by extraction in borate-phosphate buffer (Åkerlind et al., 2011).

Statistical processing of the obtained results was carried out in Statistica program (Stat Soft Inc., USA). For the evaluation of the surveyed parameters we used statistical methods. We calculated the mean arithmetic value and the standard error. The differences between the values in the control and experimental groups were determined using ANOVA, where the differences were considered significant at P<0.05. The results in the tables are presented as mean ± standard error (x ± SE).

Results and discussion

According to the results of the chemical analysis of the composition of protein supplement, it was found to contain the following: 10.3 % moisture and 89.7 % dry matter. It was found that the studied protein supplement contains rumen undegradable protein. The dry matter of the supplement contains 81.9 % crude protein, of which 42.6 % is rumen undegradable protein (RUP). The safety of the additive used is confirmed by the Declaration of Conformity dated November 23, 2018 (Test Report No. 5059 dated November 23, 2018, Test Report No. 5286-1 dated December 07, 2018).

Indicators of protein metabolism in lactating cows of the control and experimental groups were generally within the physiological norm (Table 3).

Table 3. Indicators of protein metabolism

Indicators	Groups			P-value
	I	II	III	
In the 1st day				
Total protein (g/L)	81.27±1.37	78.93±3.01	80.62±1.17	0.828
Albumin (g/L)	27.50±0.54	26.98±1.04	27.70±0.41	0.524
Globulin (g/L)	53.77±1.59	51.95±3.03	52.92±0.81	0.779
Protein index	0.511±0.025	0.528±0.038	0.524±0.005	0.548
Urea (mmol/L)	4.07±0.39	4.33±0.50	4.05±0.54	0.653
In the 60th day				
Total protein (g/L)	81.55±4.99	85.20±2.47	81.18±1.35	0.402
Albumin (g/L)	26.93±2.00[b]	31.92±1.03[a]	29.88±0.47[ab]	0.035
Globulin (g/L)	54.62±3.98	53.28±1.89	51.30±1.52	0.371
Protein index	0.499±0.039	0.601±0.023	0.585±0.024	0.109
Urea (mmol/L)	5.25±0.40	5.52±0.29	5.05±0.24	0.430
In the 90th day				
Total protein (g/L)	81.38±2.24	88.82±1.77	86.54±2.61	0.069
Albumin (g/L)	26.70±2.54	29.86±0.66	27.92±0.96	0.968
Globulin (g/L)	54.68±1.32	58.96±1.47	58.62±2.37	0.061
Protein index	0.491±0.053	0.507±0.014	0.479±0.026	0.527
Urea (mmol/L)	6.50±0.23	6.32±0.47	6.96±0.28	0.527

a, b - values within a row with different letters differ at P<0.05 (where "a" is a higher value)

On the 60th day, there was a 4.5 % increase in the total protein in the blood serum of the animals, which received 300 g of the feed protein supplement in addition to the main diet. This increase remained on the 90th day of the experiment and was 9.1 % compared to that of animals in the control group. The increase in the total protein content in cows of the II experimental group occurred as a result of albumin content increase on the 60th and 90th days by 18.5 % (P<0.05) and 11.8 %, respectively, compared with animals that did not receive a protein supplement. As a result, on the 60th day, the protein index in animals of the second experimental group exceeded this indicator in cows of the control by 20.4 %.

There was also an increase in total protein on the 90th day of the experiment by 6.3 % compared to the control in animals that received 500 g of the feed additive in addition to the main diet. The increase in the albumin fraction of the blood serum in the animals of the III experimental group, which was 11 % in comparison with the control on the 60th day of the experiment led to an increase in the protein index during this period of the experiment by 17.2 %. An increase in whey proteins was found in animals that received a protein supplement containing RUP, which indicates the absence of a violation of their metabolism (Szterk et al., 2017). An increase in the protein index or albumin-globulin ratio in cows from the experimental groups was shown, which was noted on the 60th day of the experiment, probably indicating a more intensive course of protein metabolism in the organism of cows (Gorlov et al., 2015). The increase in the total protein content in the blood of animals receiving the protein supplement may be associated with the enzymatic activity of rumen microorganisms, which leads to a slight increase in the amount of ammonia in the rumen fluid (Hassan and Saeed, 2012; Özsoy et al., 2013).

The level of urea in the blood serum of animals between the groups differed insignificantly, some increase was noted during the experiment, however, it did not exceed the normal values. According to some researchers, a significant increase in the content of urea in the blood and milk of animals may be the result of degradation of protein in the rumen due to its excessive intake along with the feed. Excess protein, which is not converted into milk as a result of metabolism, is converted into ammonia during catabolism, which in turn is converted into urea. Thus, an excess of urea appears as a significant increase in its content in blood and milk (Broderick and Reynal, 2009; Guliński et al., 2016; Zhang et al., 2019; Walkenhorst et al., 2020). Slight increase of urea in the blood of animals of the experimental groups within the acceptable values with the physiological norm of the remaining biochemical parameters may indicate a good level of absorption of dietary protein (Amanlou, 2017).

The study of mineral metabolism in animals during the experiment did not reveal significant deviations of the studied parameters from the physiological norm (Table 4). It should be noted that the phosphorus content in the blood of animals that received 300 g of feed protein supplement in addition to the main diet, statistically significi-

Table 4. *Indicators of mineral metabolism*

Indicators	Groups			P-value
	I	II	III	
In the 1st day				
Mg (mmol/L)	1.13±0.05	1.13±0.06	1.06±0.09	0.303
P (mmol/L)	1.50±0.12	1.38±0.07	1.50±0.15	0.728
Ca (mmol/L)	2.25±0.09	2.36±0.05	2.19±0.08	0.328
Reserve alkalinity (%)	52.86±1.33	51.58±0.73	53.24±0.75	0.355
In the 60th day				
Mg (mmol/L)	1.01±0.06	1.00±0.04	1.05±0.06	0.751
P (mmol/L)	1.58±0.12	1.60±0.05	1.68±0.09	0.843
Ca (mmol/L)	2.27±0.10	2.46±0.09	2.24±0.10	0.261
Reserve alkalinity (%)	49.36±1.26	49.38±1.79	48.53±1.25	0.765
In the 90th day				
Mg (mmol/L)	1.05±0.06	1.05±0.03	1.24±0.25	0.403
P (mmol/L)	1.13±0.07[b]	1.52±0.08[a]	1.25±0.06[ab]	0.018
Ca (mmol/L)	2.90±0.15	3.26±0.11	3.15±0.13	0.221
Reserve alkalinity (%)	50.62±1.31	51.47±1.96	54.66±1.01	0.138

a, b - values within a row with different letters differ at P<0.05 (where "a" is a higher value)

cantly increased on the 90th day of the experiment by 0.39 mmol/L or 34.5 % (P<0.05) in compare to control values.

There was an increase in the level of phosphorus by 10.6 % and magnesium by 18.1 % in the blood serum of lactating cows of the III experimental group on the 90th day of the experiment, in comparison with the analogous indicators of animals of the I group. A slight increase in the level of calcium in animals of group II was observed on the 60th (0.19 mmol/L (8.4 %)) and 90th (0.36 mmol/L (12.4 %)) day of the experiment. The value of this indicator on the 30th day after the end of feeding the additive in animals of group III exceeded the control one by 0.25 mmol/L or 8.6 %. Alkaline reserve during the entire experiment in all groups was relatively stable, and there was a 4.0 % increase in this indicator in animals of group III compared to the control on the 90th day.

Cows have an increased demand for calcium at the early stage of lactation, since a large amount of it is excreted from the animals' body along with milk, which is especially typical of high-yielding cows (Jawor et al., 2012; Goff, 2014). An increase in calcium in the blood of animals from experimental groups while feeding a supplement with RUP helps reducing the risk of hypocalcaemia, which can lead to endometritis in highly productive animals (Priest et al., 2013). In addition, cows from the experimental groups showed an increase in phosphorus content on the 90th day of the experiment, which indicates an increase in the metabolism of this macro-element in the organism (Grünberg et al., 2015).

There were no deviations from the values of the physiological norm of the activity indicators of the studied enzymes in the blood serum of the animals of the experimental and control groups (Table 5).

Table 5. *Enzyme activity indicators*

Indicators	Groups			P-value
	I	II	III	
In the 1st day				
AST (U/L)	73.33±5.17	79.70±4.46	74.12±4.22	0.519
ALT (U/L)	22.52±2.57	23.90±2.10	22.32±1.20	0.612
Alkaline phosphatase (U/L)	123.27±28.34	99.33±26.13	108.03±10.59	0.421
In the 60th day				
AST (U/L)	72.72±10.50	72.78±3.87	69.60±2.22	0.531
ALT (U/L)	23.02±2.56	27.65±2.72	27.75±0.74	0.114
Alkaline phosphatase (U/L)	144.82±34.73	114.93±28.09	135.03±23.75	0.587
In the 90th day				
AST (U/L)	85.86±8.47	102.52±8.30	93.50±10.39	0.310
ALT (U/L)	32.78±4.22	33.22±1.54	35.70±1.96	0.691
Alkaline phosphatase (U/L)	155.32±45.39	105.66±18.59	137.20±23.38	0.472

AST - aspartat transaminase, ALT - alanine aminotransferase

Table 6. *Indicators of carbohydrate, lipid and vitamin metabolisms*

Indicators	Groups			P-value
	I	II	III	
In the 1st day				
Glucose (mmol/L)	2.26±0.16	2.22±0.19	2.27±0.16	0.697
Cholesterol (mmol/L)	4.38±0.46	4.21±0.48	3.83±0.33	0.587
Total lipids (mg%)	514.00±34.86	498.00±55.97	436.17±36.71	0.376
Ketone bodies (mg%)	2.73±0.34	2.39±0.22	2.77±0.34	0.328
Carotene (mg%)	0.577±0.047	0.510±0.098	0.480±0.058	0.493
Vitamin A (µg%)	34.93±3.08	38.22±1.31	34.39±2.87	0.071
In the 60th day				
Glucose (mmol/L)	2.25±0.06	2.29±0.15	2.24±0.13	0.261
Cholesterol (mmol/L)	5.03±0.32	4.96±0.29	5.00±0.35	0.519
Total lipids (mg%)	569.83±27.07	622.83±30.76	566.00±30.48	0.314
Ketone bodies (mg%)	1.51±0.19	1.96±0.23	1.96±0.23	0.261
Carotene (mg%)	0.751±0.091	0.709±0.087	0.634±0.057	0.209
Vitamin A (µg%)	28.03±3.09[b]	36,42±5.04[a]	25.81±3.23[ab]	0.024
In the 90th day				
Glucose (mmol/L)	2.27±0.15	2.33±0.07	2.35±0.17	0.251
Cholesterol (mmol/L)	4.44±0.35	4.76±0.56	4.71±0.45	0.887
Total lipids (mg%)	509.80±14.81	548.40±31.22	543.00±23.79	0.457
Ketone bodies (mg%)	3.12±0.81	2.28±0.35	2.06±0.60	0.403
Carotene (mg%)	0.697±0.058	0.681±0.077	0.571±0.088	0.527
Vitamin A (µg%)	37.96±3.05	36.69±8.49	36.95±1.25	0.898

a, b - values within a row with different letters differ at P<0.05 (where "a" is a higher value)

On the 60th and 90th day of the experiment, an excess of ALT activity level was observed in the experimental groups of lactating cows, in comparison with the values of this indicator in the control: in the II experimental group the increase was 4.63 U/L or 20.1 % and 0.44 U/L or 1.3 %, in III experimental group - 4.73 U/L or 20.5 % and 2.92 U/L or 8.9 %, respectively. On the 30th day after the end of feeding the feed protein supplement in cows of groups II and III, the AST activity indices in comparison with the control were higher by 16.66 U/L or 19.4 % and 7.64 U/L or 8.9 % respectively. Nevertheless, the activity of the studied enzymes in both experimental groups was at the optimal level.

It was found that the studied parameters of carbohydrate, lipid and vitamin metabolism of lactating cows in the experiment did not have significant deviations from the physiological norm (Table 6).

The level of cholesterol in the blood serum of cows of the II experimental group was 0.32 mmol/L (7.2 %), and in the III experimental group 0.27 mmol/L (6.1 %) higher than that of the control on the 90th day of the experiment. A slight increase in the total lipid index (by 53 mg% and 39 mg% on the 60th and 90th days, respectively) was observed in animals of the II experimental group, compared with the control values; in cows of the III group, this indicator was 34 mg% higher than in the control on the 30th day after the end of feeding the supplement. The content of vitamin A in the serum of cows of group II exceeded the control indicator by 8.39 µg% in comparison with the control group on the 60th day of the experiment. An increase in cholesterol along with an increase in blood glucose levels in animals from the experimental groups is possibly associated with an increase in the production of propionate in the rumen,

since propionate is the main substrate for gluconeogenesis in ruminants, which for the most part actively proceeds in the liver (Aschenbach et al., 2010). The combination of these processes indicates an improvement in the metabolism of nutrients passing through the liver (Schlegel et al., 2012; Hashemzadeh-Cigari et al., 2014; Kessler et al.,

2014). The absence of pathological changes in the liver, as well as the metabolic rate, is to some extent indicated by an increase in the main hepatic enzymes ALT and AST along with increased albumin and cholesterol levels in the blood serum of animals treated with RUP supplement (Mordak et al., 2020).

Hematological indicators of lactating cows of all studied groups at all stages of the experiment were within the physiological norm (Table 7).

Table 7. Blood indicators of animals in the experiment

Indicators	Groups			P-value
	I	II	III	
In the 1st day				
Leukocytes (10^9/L)	8.83±0.19	8.64±0.14	8.81±0.19	0.559
Erythrocytes (10^{12}/L)	5.04±0.04	5.35±0.23	4.94±0.11	0.641
In the 60th day				
Leukocytes (10^9/L)	8.47±0.18	8.68±0.40	9.14±0.29	0.371
Erythrocytes (10^{12}/L)	5.93±0.36	6.40±0.18	6.17±0.23	0.219
In the 90th day				
Leukocytes (10^9/L)	8.10±0.38	8.15±0.52	8.76±0.42	0.512
Erythrocytes (10^{12}/L)	6.90±0.59	7.12±0.26	6.64±0.29	0.435

Table 8. Leukocyte blood count of cows in the experiment

Indicators	Groups			P-value
	I	II	III	
In the 1st day				
Basophils (%)	0.50±0.24	0.67±0.37	0.50±0.37	0.969
Eosinophils (%)	5.83±0.77	3.83±0.52	4.17±0.52	0.256
STN (%)	4.17±0.44	4.67±0.67	4.00±0.28	0.414
SEN (%)	21.17±1.04	21.00±0.69	22.00±1.02	0.676
Lymphocytes (%)	64.83±1.04	65.00±1.41	65.00±1.60	0.128
Monocytes (%)	3.50±0.37	4.83±0.77	4.33±0.67	0.911
In the 60th day				
Basophils (%)	0.33±0.23	0.33±0.23	0.50±0.24	0.531
Eosinophils (%)	5.50±0.73	4.00±0.57	4.33±0.54	0.831
STN (%)	4.00±0.49	4.00±0.40	4.17±0.66	0.927
SEN (%)	21.17±0.72	21.33±0.88	20.83±0.87	0.849
Lymphocytes (%)	64.83±1.04	66.50±1.19	66.17±1.45	0.250
Monocytes (%)	4.17±0.34	3.83±0.44	4.00±0.49	0.854
In the 90th day				
Basophils (%)	1.00±0.50	0.40±0.27	0.60±0.27	0.774
Eosinophils (%)	5.20±0.96	4.40±0.57	4.60±0.67	0.656
STN (%)	3.80±0.54	3.80±0.42	4.00±0.35	0.933
SEN (%)	19.80±0.55	19.60±0.76	20.20±0.89	0.558
Lymphocytes (%)	66.20±1.08	68.20±0.65	67.40±0.45	0.499
Monocytes (%)	4.00±0.50	3.60±0.27	3.60±0.57	0.344

STN - stab neutrophils; SEN - segmented neutrophils

The increase in the number of leukocytes in the blood of animals of the experimental groups in group II was 2.5 % on the 60th day, in III experimental group by 7.9 % and 8.1 % on the 60th and 90th day, respectively. The number of erythrocytes in the cows of the experimental group increased in comparison with the animals of the control group: in the blood of cows of the II experimental group on the 60th day of the experiment by 7.9 %, and this excess remained on the 30th day after the end of feeding additives and amounted 3.2 %; in the blood of cows of the III experimental group by 4.0 % on the 60th day of the experiment. Some increase in erythrocytes within the physiological norm in the blood of cows in the experimental groups may indicate an increase in the rate of redox reactions, which contributes to a better supply of oxygen to tissues and affects an increase in the intensity of metabolic processes. An increase in the number of leukocytes may indicate an increase in the non-specific immunity of the animal organism (Gorlov et al., 2015; Kalyani et al., 2018).

The ratio of individual forms of leukocytes in the control and in the experimental groups of lactating cows was within the physiological norm (Table 8).

An increase in the proportion of lymphocytes in the blood of animals of the experimental groups on the 60th and 90th day of the experiment in relation to the control values was observed: in the II experimental group by 1.67 % and 2.00 %, in the III experimental group by 1.34 % and 1.20 %, respectively. The content of basophils in the blood of lactating cows of groups II and III on the 30th day after the end of feeding the additive was reduced by 0.60 % and 0.40 %, respectively, in comparison with animals in the control group. At all stages of the experiment, the number of basophils in the blood of cows of all groups was within the normal range, which indicates the absence of allergic reactions in the body, including the inclusion of a supplement from leather industry wastes (Otter, 2013).

In this way, the stimulating effect of a feed supplement containing RUP on the erythro- and leukopoiesis of lactating cows, as well as a shift in the leukocyte formula towards an increase in the relative number of individual forms of leukocytes, was established.

It is known that high-yielding cows often experience liver dysfunction due to inflammatory processes as a result of postpartum stress, unbalanced nutrition, metabolic disorders in the first months of lactation (Trevisi et al., 2012). Inflammatory processes in the liver negatively affect the synthesis of many proteins, including albumin (Osorio et al., 2014). An increase in the amount of albumin in the blood of animals of the experimental groups, together with haematological parameters, which are within the physiological norm, indicates the absence of pathological changes in the liver.

It was found that on the 90th day of the experiment there was an increase in the mass fraction of fat in the milk of animals of all groups, however, in cows of group II, the value of this indicator was higher by 0.14 %, in cows of group III by 0,72 % compared to the control group (Table 9). On the 30th day of the experiment there was an almost twofold decrease in urea in the milk of cows compared to

the beginning of the experiment, while this indicator in the milk of animals of groups II and III statistically significantly exceeded the control value by 2.00 mg % and 2.32 mg % respectively. On the 60th day, an increase in the amount of urea in the milk of cows of all groups was found, and in the milk of animals of the II group, there was 1.86 mg% more urea than in the milk of cows in the control.

The somatic cells count (SCC) in the milk of animals of all groups at the beginning of the experiment did not exceed the permissible standard values. There was a significant increase in this indicator in the milk of cows of the I control group on the 30th day of the experiment, while the value of this indicator remained within the normal range in the milk of cows of the II and III experimental groups. On the 60th and 90th days of the study, the SCC in the milk of cows receiving the protein supplement also did not have significant fluctuations from the earlier established values in these experimental groups. The value of this indicator was lower than the control in groups II and III by 44 x 10^{3} cells and 39 x 10^{3} cells on the 60th day and 210 x 10^{3} cells and 121 x 10^{3} cells in 30 days after the end of feeding the supplement, respectively.

On the 30th day, the experimental groups showed an increase in the daily milk yield with 4 % fat content; group II exceeded the control value by 31.4 % (P<0.05) and group III by 13.1 %. On the 60th day, cows of all groups showed a slight decrease in the average daily milk yield, however, the value of this indicator was higher than in the animals of the control groups, and when converted to 4 % milk, the difference in the average daily milk yields of animals in groups II and III with control was 33.9 % and 21.6 % respectively. This trend continued on the 30th day after the end of feeding the protein supplement containing the RUP. The productivity in terms of 4 % fat content milk in the cows of the II group was higher by 27.2 %, in the III group - by 19.5 % compared to the control.

The study showed that protein supplement from minced subcutaneous epithelium of farm animals containing RUP helped to supply high-yielding cows with the necessary amount of protein, which is especially deficient in animals during the early lactation. This had a stimulating effect on increasing the milk productivity of cows and the biosynthesis of milk constituents. According to the data of milk control, an increase in milk productivity as well as quality indicators of milk was established along with a decrease in the number of somatic cells in the milk of cows in the experimental groups. It should be noted that during the entire period of the experiment, mastitis was not detected in the cows of the first experimental group. An increase in the number of SCC in cows at the beginning of lactation may be the result of the accumulation of macrophages in the secretion prior to milking, which is not the cause of udder disease. The activation of defence mechanisms caused by exogenous and endogenous factors is a likely cause. In addition, the value of this indicator depends on the technological methods of milking, udder hygiene, the presence of stress in the animal (Auldist et al., 1995; Græsbøll et al., 2016; Alhussien and Dang, 2018). The stimulating effect of the protected protein on the body of high-yielding

Table 9. Indicators of milk production

Indicators	Groups			P-value
	I	II	III	
In the 1st day				
Protein (%)	3.10±0.09	2.89±0.08	2.91±0.08	0.436
Fat (%)	3.92±0.31	3.75±0.10	4.60±0.39	0.702
Dry matter (%)	13.13±0.61	12.39±0.21	13.24±0.43	0.647
SCC (c x 10³/cm³)	151±62	250±159	170±69	0.999
Urea (mg%)	22.00±1.35	21.10±1.00	26.38±3.32	0.300
Daily yield (kg)	33.5±1.1	33.1±0.8	31.5±0.7	0.598
Milk with 4% fat content (kg)	33.0±1.3	32.3±1.6	35.4±3.6	0.409
In the 30th day				
Protein (%)	3.28±0.09	3.30±0.17	3.27±0.11	0.436
Fat (%)	3.38±0.27	3.82±0.31	3.50±0.41	0.702
Dry matter (%)	11.29±0.46	12.04±0.38	11.83±0.58	0.562
SCC (c x 10³/cm³)	803±608	155±59	269±193	0.231
Urea (mg%)	10.37±0.52[b]	12.37±0.63[a]	12.69±0.43[a]	0.021
Daily yield (kg)	29.4±1.0	36.0±1.1	33.0±1.5	0,010
Milk with 4% fat content (kg)	26.7±1.42[b]	35.1±2.5[a]	30.2±1.0[ab]	0.038
In the 60th day				
Protein (%)	3.62±0.19	3.57±0.42	3.40±0.07	0.923
Fat (%)	4.22±0.37	3.93±0.20	3.87±0.18	0.537
Dry matter (%)	11.99±0.27	11.85±0.52	11.77±0.32	0.934
SCC (c x 10³/cm³)	231±108	187±81	192±90	0.648
Urea (mg%)	13.34±1.65	15.20±0.77	13.64±0.78	0.408
Daily yield (kg)	24.6±2.9	34.3±1.6	31.9±3.7	0.128
Milk with 4% fat content (kg)	25.5±3.4	33.9±1.4	31.0±3.2	0.171
In the 90th day				
Protein (%)	3.26±0.13	3.13±0.16	3.24±0.08	0.923
Fat (%)	4.14±0.10	4.28±0.40	4.86±0.80	0.537
Dry matter (%)	11.48±0.52	11.41±0.41	11.82±0.57	0.934
SCC (c x 10³/cm³)	403±307	193±58	282±101	0.648
Urea (mg%)	18.40±0.84	17.49±1.21	17.51±0.67	0.408
Daily yield (kg)	25.2±1.3	31.4±1.6	26.8±1.8	0.077
Milk with 4% fat content (kg)	25.7±1.2	32.7±2.6	30.7±5.3	0.369

a, b - values within a row with different letters differ at P<0.05 (where "a" is a higher value); SCC - somatic cells count

cows has been convincingly proven by many researchers (Dijkstra et al., 2013; Yatsko et al., 2013; Matyaev and Andin, 2015; Zhou et al., 2016). Its presence in the main diet is one of the key points that have a beneficial effect on the synthesis of milk and milk components (Batistel et al., 2017; Wang et al., 2017). Obtained results indicate that the effect of the proposed protein supplement containing RUP is consistent with the effect of similar feed products (Lee et al., 2015; Awawdeh, 2016; Leondro et al., 2019).

This study demonstrates that the use of a feed supplement containing protein from subcutaneous epithelium of farm animals, in the amount of 300 g and 500 g per animal per day for 60 days, in feeding cows for milk production,

promotes the activation of metabolic processes in the organism of cows.

It should be noted that within 30 days after the end of feeding the protein supplement from leather industry wastes, the animals of the experimental group experienced a prolonged effect of the supplement, which consists in higher values of blood biochemical parameters, an increase in milk productivity compared to same indicators of the control.

Conclusions

It was found that the use of a protein supplement from minced subcutaneous epithelium of farm animals, in an amount of 300 g and 500 g per animal per day in feeding lactating cows for 60 days, provides a more balanced protein nutrition of animals at the beginning of lactation, which has a beneficial effect on their productive performance. According to the obtained data, it can be assumed that the use of RUP as part of an additive in feeding high-yielding cows in the first months of lactation improved the physiological state of animals due to an increase in the indicators of all types of metabolism within the physiological norm. However, further research is needed in order to recommend this protein supplement from leather industry wastes, containing RUP, as means to balance the protein nutrition of high-yielding cows at the beginning of lactation and to accurately analyse its effect on metabolism, liver health, digestion processes and milk production of animals.

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Učinak dodatka proteina iz otpada kože na hematološke i biokemijske pokazatelje metabolizma i mliječnost visokoproduktivnih krava

Sažetak

Cilj je ovog istraživanja utvrditi učinak dodatka proteina iz mljevenog potkožnog epitela domaćih životinja, na hematološke i biokemijske parametre metabolizma te proizvodnost visokoproduktivnih krava tijekom rane laktacije. Za to su prema principu analognih parova formirane tri skupine krava, svaka po šest životinja. Kontrolna skupina hranjena je osnovnim obrokom, a druga i treća eksperimentalna skupina svakodnevno su uz osnovnu hranidbu hranjene s 300 g i 500 g proteinskog dodatka tijekom 60 dana. Učinak dodatka proučavan je tijekom sljedećih 30 dana. U svih životinja proučavani su hematološki i biokemijski parametri krvi, te kvalitativni i kvantitativni sastav mlijeka. Životinje pokusnih skupina pokazale su porast metaboličkih i redoks procesa tijekom pokusa. Utvrđen je porast metabolizma hranjivih sastojaka u jetri. Zbog dovoljnog unosa proteina u organizam životinja eksperimentalnih skupina došlo je do povećanja pokazatelja mliječne proizvodnosti i pokazatelja kvalitete mlijeka.

Ključne riječi: proteinski dodatak; otpad od kožne industrije, krave s visokom proizvodnjom, metabolizam, mliječna proizvodnost

References

1. Åkerlind, M., Weisbjerg, M., Eriksson, T., Tøgersen, R., Udén, P., Ólafsson, B.L., Harstad, O.M., Volden, H. (2011): Feed analyses and digestion methods. *NorFor-The Nordic feed evaluation system*, 41-54.
https://doi.org/10.3920/978-90-8686-718-9_5

2. Alhussien, M.N., Dang, A.K. (2018). Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. *Veterinary World* 11 (5), 562-577.
https://doi.org/10.14202/vetworld.2018.562-577

3. Amanlou, H., Farahani, T.A., Farsuni, N.E. (2017): Effects of rumen undegradable protein supplementation on productive performance and indicators of protein and energy metabolism in Holstein fresh cows. *Journal of Dairy Science* 100 (5), 3628-3640.
https://doi.org/10.3168/jds.2016-11794

4. Aschenbach, J.R., Kristensen, N.B., Donkin, S.S., Hammon, H.M., Penner, G.B. (2010): Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. *IUBMB life* 62 (12). 869-877.
https://doi.org/10.1002/iub.400

5. Association of Official Analytical Chemists. (1990): Official Methods of Analysis: Changes in Official Methods of Analysis Made at the Annual Meeting. Supplement. *Association of Official Analytical Chemists* 15, 725.

6. Auldist, M.J., Coats, S., Rogers, G.L., McDowell, G.H. (1995): Changes in the composition of milk from healthy and mastitic dairy cows during the lactation cycle. *Australian Journal of Experimental Agriculture* 35 (4), 427-436.
https://doi.org/10.1071/EA9950427

7. Awawdeh, M.S. (2016): Rumen-protected methionine and lysine: effects on milk production and plasma amino acids of dairy cows with reference to metabolisable protein status. *Journal of Dairy Research* 83 (2), 151-155.
https://doi.org/10.1017/S0022029916000108

8. Batistel, F., Arroyo, J.M., Bellingeri, A., Wang, L., Saremi, B., Parys, C., Trevisi, E., Cardoso, F.C., Loor, J.J. (2017): Ethyl-cellulose rumen-protected methionine enhances performance during the peripariturient period and early lactation in Holstein dairy cows. *Journal of Dairy Science* 100 (9), 7455-7467.
https://doi.org/10.3168/jds.2017-12689

9. Broderick, G.A., Reynal, S.M. (2009): Effect of source of rumen-degraded protein on production and ruminal metabolism in lactating dairy cows. *Journal of Dairy Science* 92 (6), 2822-2834.
https://doi.org/10.3168/jds.2008-1865

10. Brun-Lafleur, L., Delaby, L., Husson, F., Faverdin, P. (2010): Predicting energy× protein interaction on milk yield and milk composition in dairy cows. *Journal of Dairy Science* 93 (9), 4128-4143.
https://doi.org/10.3168/jds.2009-2669

11. Dijkstra, J., Reynolds, C.K., Kebreab. E., Bannink, A., Ellis, J.L., France, J., Van Vuuren, A.M. (2013). Challenges in ruminant nutrition: towards minimal nitrogen losses in cattle. *Energy and Protein Metabolism and Nutrition in Sustainable Animal Production* 134, 47-58.
https://doi.org/10.3920/978-90-8686-781-3_3

12. Goff, J.P. (2014): Calcium and Magnesium Disorders. *Veterinary Clinics: Food Animal Practice* 30 (2), 359-381.
https://doi.org/10.1016/j.cvfa.2014.04.003

13. Gorlov, I.F., Levakhin, V.I., Radchikov, V.F., Tsai, V.P., Bozhkova, S.E. (2015): Effect of feeding with organic microelement complex on blood composition and beef production of young cattle. *Modern Applied Science* 9 (10), 8-16.
http://dx.doi.org/10.5539/mas.v9n10p8

14. Græsbøll, K., Kirkeby, C., Nielsen, S.S., Halasa, T., Toft, N., Christiansen, L.E. (2016). Models to estimate lactation curves of milk yield and somatic cell count in dairy cows at the herd level for the use in simulations and predictive models. *Frontiers in Veterinary Science* 3, 115.
https://doi.org/10.3389/fvets.2016.00115

15. Grünberg, W., Scherpenisse, P., Dobbelaar. P., Idink, M.J., Wijnberg, I.D. (2015): The effect of transient, moderate dietary phosphorus deprivation on phosphorus metabolism, muscle content of different phosphorus-containing compounds, and muscle function in dairy cows. *Journal of Dairy Science* 98 (8), 5385-5400.
https://doi.org/10.3168/jds.2015-9357

16. Guliński, P., Salamończyk, E., Młynek, K. (2016): Improving nitrogen use efficiency of dairy cows in relation to urea in milk–A review. *Animal Science Papers and Reports* 34 (1), 5-24.

17. Hashemzadeh-Cigari, F., Khorvash, M., Ghorbani, G.R., Kadivar, M., Riasi, A., Zebeli, Q. (2014): Effects of supplementation with a phytobiotics-rich herbal mixture on performance, udder health, and metabolic status of Holstein cows with various levels of milk somatic cell counts. *Journal of Dairy Science* 97 (12), 7487-7497.
https://doi.org/10.3168/jds.2014-7989

18. Hassan, S., Saeed, A. (2012): Effect of feeding different levels of dietary protein with high or low rumen degradable: undegradable dietary nitrogen on Awassi lambs performance 3-selected biochemical parameters. *KSU Journal of Natural Sciences* 15 (3), 36-45.

19. Jawor, P.E., Huzzey, J.M., LeBlanc, S.J., Von Keyserlingk, M.A.G. (2012): Associations of subclinical hypocalcemia at calving with milk yield, and feeding, drinking, and standing behaviors around parturition in Holstein cows. *Journal of Dairy Science* 95 (3), 1240-1248.
https://doi.org/10.3168/jds.2011-4586.

20. Jayathilakan, K., Sultana, K., Radhakrishna, K., Bawa, A.S. (2011): Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology* 49 (3), 278-293.
https://doi.org/10.1007/s13197-011-0290-7

21. Jiang, H., Liu, J., Han, W. (2016): The status and developments of leather solid waste treatment: A mini-review. *Waste Management & Research* 34 (5), 399-408.
https://doi.org/10.1177/0734242X16633772

22. Kalyani, P., Aswani, K.K., Haritha, Y., Srinivas, B., Kanaka, D.A. (2018): Comparative blood haemato-biochemical variations in Indian Zebu cattle breeds during early summer. *Biological Rhythm Research* 49 (6), 811-818
https://doi.org/10.1080/09291016.2018.1424772.

23. Kantarli, I.C., Yanik, J. (2009): Use of waste sludge from the tannery industry. *Energy & fuels* 23 (6), 3126-3133.
https://doi.org/10.1021/ef8011068

24. Kessler, E.C., Gross, J.J., Bruckmaier, R.M., Albrecht, C. (2014): Cholesterol metabolism, transport, and hepatic regulation in dairy cows during transition and early lactation. *Journal of Dairy Science* 97 (9), 5481-5490.
https://doi.org/10.3168/jds.2014-7926

25. Kondrahin, I.P. (2004): *Methods of veterinary and clinical laboratory diagnostics.* Moskva: KolosS, 520. (in Russian)

26. Lazzarini, Í., Detmann, E., de Campos Valadares Filho S., Paulino, M.F., Batista, E.D., de Almeida Rufino, L.M., William Lima Santiago dos Reis, de Oliveira Franco M. (2016): Nutritional performance of cattle grazing during rainy season with nitrogen and starch supplementation. *Asian-Australasian Journal of Animal Sciences* 29 (8), 1120-1128.
https://doi.org/10.5713/ajas.15.0514

27. Lee, C., Giallongo, F., Hristov, A.N., Lapierre, H., Cassidy, T.W., Heyler, K.S., Varga, G.A., Parys, C. (2015): Effect of dietary protein level and rumen-protected amino acid supplementation on amino acid utilization for milk protein in lactating dairy cows. *Journal of Dairy Science* 98 (3), 1885-1902.
https://doi.org/10.3168/jds.2014-8496

28. Leondro, H., Widyobroto, B.P., Agus, A. (2019): Effects of undegradable dietary protein on milk production and composition of lactating dairy cows. In *IOP Conference Series: Earth and Environmental Science* 387 (1), 012004.
https://doi.org/10.1088/1755-1315/387/1/012004

29. Leroy, J., Vanholder, T., Van Knegsel, A., Garcia-Ispierto, I., Bols, P. (2008): Nutrient Prioritization in Dairy Cows Early Postpartum: Mismatch Between Metabolism and Fertility? *Reproduction in Domestic Animals* 43, 96-103.
https://doi.org/10.1111/j.1439-0531.2008.01148.x

30. Matyaev, V.I., Andin, I.S. (2015): Splitting of Crude Protein of Feed in Rumen of Highly Productive Dairy Cows. *Vestnik of Ulyanovsk State Agricultural Academy* 1. 102-105. (In Russian)

31. Mordak, R., Kupczyński, R., Kuczaj, M., Niżański, W. (2020): Analysis of correlations between selected blood markers of liver function and milk composition in cows during late lactation period. *Annals of Animal Science* 20 (3), 871-886.
https://doi.org/10.2478/aoas-2020-0020

32. Nikitin, V.N. (1949): *Atlas of blood cells of agricultural and laboratory animals*. Moscow: Gosizdat. sel'khoz. lit. publ., 118. (in Russian)

33. Osorio, J.S., Trevisi E.R.M.I.N.I.O., Ji, P., Drackley, J.K., Luchini, D., Bertoni, G., Loor, J.J. (2014): Biomarkers of inflammation, metabolism, and oxidative stress in blood, liver, and milk reveal a better immunometabolic status in peripartal cows supplemented with Smartamine M or MetaSmart. *Journal of Dairy Science* 97 (12), 7437-7450.
https://doi.org/10.3168/jds.2013-7679

34. Otter, A. (2013): Diagnostic blood biochemistry and haematology in cattle. *In Practice* 35 (1), 7-16.
https://doi.org/10.1136/inp.e8719

35. Özsoy, B., Yalçın, S., Erdoğan, Z., Cantekin, Z., Aksu, T. (2013): Effects of dietary live yeast culture on fattening performance on some blood and rumen fluid parameters in goats. *Revue de Médecine Vétérinaire* 164 (5), 263-271.

36. Pati, A., Chaudhary, R., Subramani, S. (2014): A review on management of chrome-tanned leather shavings: a holistic paradigm to combat the environmental issues. *Environmental Science and Pollution Research* 21 (19), 11266-11282.
https://doi.org/10.1007/s11356-014-3055-9

37. Patton, R.A., Hristov, A.N., Lapierre, H. (2014): Protein feeding and balancing for amino acids in lactating dairy cattle. *Veterinary Clinics: Food Animal Practice* 30 (3), 599-621.
https://doi.org/10.1016/j.cvfa.2014.07.005

38. Popov, V.P., Dudorov, V.E., Sidorenko, G.A., Akhmadiyeva, Z.R., Tsyrkaeva, E.A., Sagitov, R.F. (2019): Economic assessment of the need for processing of raw materials of animal origin. In *IOP Conference Series: Materials Science and Engineering* 560 (1), 012199.
https://doi.org/10.1088/1757-899X/560/1/012199

39. Potapov, V., Yevlash, V., Parzhanov. Zh., Tastanbekova, G., Khamzayeva, S., Tolegen, M. (2017): Use of Food Production Waste in Fodder Products. Overview of Problems and Solutions. *Industrial Technology and Engineering* 1 (22), 73-83.

40. Priest, N.V., McDougall, S., Burke, C.R., Roche, J.R., Mitchell, M., McLeod, K.L., Greenwood, S.L., Meier, S. (2013): The responsiveness of subclinical endometritis to a nonsteroidal antiinflammatory drug in pasture-grazed dairy cows. *Journal of Dairy Science* 96 (7), 4323-4332.
https://doi.org/10.3168/jds.2012-6266

41. Rigueto, C.V.T., Rosseto, M., Krein, D.D.C., Ostwald, B.E.P., Massuda, L.A., Zanella, B.B., Dettmer, A. (2020): Alternative uses for tannery wastes: a review of environmental, sustainability, and science. *Journal of Leather Science and Engineering* 2 (1), 1-20.
https://doi.org/10.1186/s42825-020-00034-z

42. Schlegel, G., Ringseis, R., Keller, J., Schwarz, F.J., Eder, K. (2012): Changes in the expression of hepatic genes involved in cholesterol homeostasis in dairy cows in the transition period and at different stages of lactation. *Journal of Dairy Science* 95 (7), 3826-3836.
https://doi.org/10.3168/jds.2011-5221

43. Szterk, P., Dorszewski, P., Grabowicz, M., Podkowka, L. (2017): Health status of cows fed maize silage covered with oxo-biodegradable foil. *Journal of Central European Agriculture* 18 (4), 773-782.
https://doi.org/10.5513/jcea.v18i4.5908

44. Trevisi, E.R.M.I.N.I.O., Amadori, M., Cogrossi, S.I.M.O.N.E., Razzuoli, E., Bertoni, G. (2012): Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. *Research in Veterinary Science* 93 (2), 695-704.
https://doi.org/10.1016/j.rvsc.2011.11.008

45. Walkenhorst, M., Leiber, F., Maeschli, A., Kapp, A.N., Spengler Neff, A., Faleschini, M.T., Garo. E., Hamburger, M., Potterat, O., Mayer, P., Graf-Schiller, S., Bieber, A. (2020): A multicomponent herbal feed additive improves somatic cell counts in dairy cows-a two stage, multicentre, placebo-controlled long-term on-farm trial. *Journal of Animal Physiology and Animal Nutrition* 104 (2), 439-452. https://doi.org/10.1111/jpn.13297

46. Wang, Y.J., Xiao, J.X., Li, S., Liu, J.J., Alugongo, G.M., Cao, Z.J., Yang, H.J., Wang, S.X., Swanson, K.C. (2017): Protein Metabolism and Signal Pathway Regulation in Rumen and Mammary Gland. *Current Protein and Peptide Science* 18 (6), 636-651. https://doi.org/10.2174/1389203717666160627075021

47. Yatsko, N.A., Suchkova, I.V., Letunovich, E.V. (2013): Qualitative characteristics of the «protected» protein of rapeseed feed and their influence on the milk production of cows. *Scientific notes of the Vitebsk Educational Institution of the Order Badge of Honor State Academy of Veterinary Medicine* 49 (1), 206-210. (In Russian)

48. Zhang, Y., Zhao, X., Chen, W., Zhou, Z., Meng, Q., Wu, H. (2019): Effects of Adding Various Silage Additives to Whole Corn Crops at Ensiling on Performance, Rumen Fermentation, and Serum Physiological Characteristics of Growing-Finishing Cattle. *Animals* 9 (9), 695. https://doi.org/10.3390/ani9090695

49. Zhou, Z., Bulgari, O., Vailati-Riboni, M., Trevisi, E., Ballou, M.A., Cardoso, F.C., Luchini, D.N., Loor, J.J. (2016): Rumen-protected methionine compared with rumen-protected choline improves immunometabolic status in dairy cows during the peripartal period. *Journal of Dairy Science* 99 (11), 8956-8969. https://doi.org/10.3168/jds.2016-10986