Effect of carbohydrate hydrolytic enzyme complex in combination with energy feed on rumen digestion, metabolism, productivity and fatty acid composition of milk fat in cows

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Abstract. The research was carried out on 2 groups of dairy cows of 30 heads on the farm with the formation of technological groups according to the timing of calving and milking on the unit with individual accounting of milk yield. Cows of the experimental group in the period two weeks before calving and for 2 months after calving were added to the diet, respectively, 150 and 300 ml / head / day energy feed based on propylene glycol and glycerin with a complex of enzymes cellulase, xylase, beta-gluconase and glucoamylase activity (EFE). The main diet consisted of traditional feed and was designed to receive an average daily milk yield of 25 kg of milk. EFE had practically no effect on the pH and ammonia content in the rumen chyme, but increased the content of VFA in it by 3.6%. It had no effect on the total microbial mass, but reduced the population of infusoria by 23.5% and increased the bacterial population by 27.8%. EFE had a positive effect on protein-nitrogen metabolism, liver function, antioxidant activity, hormonal status and hematology. As a result, during 3 months of lactation, the average daily milk yield in cows of the experimental group was 19.9, 20.9 and 21.1 kg of milk (P<0.001), which was higher than in the control by 13.0, 9.4 and 16.5%, respectively.

1 Introduction

In recent decades, breeders have significantly increased the genetically determined potential of dairy productivity of cows, created herds with a productivity of 8-12 thousand kg of milk per lactation. However, along with this, there were also problems associated with ensuring the increased needs of cows for energy and plastic substances. As a result, the number of cases of their diseases with acidosis, ketosis, mastitis, endometritis and other diseases caused, as a rule, by metabolic disorders increased, which in turn led to a significant increase in the culling of cows and a reduction in the period of their productive use, causing economic damage to the industry [1, 2]. The most intense in terms of metabolic intensity for the body is the transition period, which includes prenatal 21-0 days, calving, after calving 0-21 days, in addition, the peak phase of lactation is 22-120 days. At this time, there is a significant

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change in homeostasis in the body due to the involution process, physiological bloating, and changes in the hormonal status of the body. Before calving and immediately after it, the cows' appetite worsens. All this requires significantly more energy and plastic costs than can be obtained from the feeding diet, even if it is maximally balanced in terms of nutrients and biologically active substances. As a result, during the first months after calving, a negative energy balance is formed, which the cow compensates by mobilizing the energy resources of the body, which leads to a loss of body weight, and causes many diseases [3-6]. The main direction in meeting the needs of the body of cows in energy and nutrients is to improve the structure and quality of feed ration, balancing the sugar-protein ratio, increasing the dry matter content, the use of protected fats, protein, starch, as well as the use of feed additives such as propionate, propylene glycol, glycerin, as well as exogenous enzymes of various hydrolytic action [7-12]. All these measures are primarily aimed at maintaining a high physiological function of the rumen by controlling enzymatic and microbiological processes, which determines both the amount of milk yield and milk quality, and the productive health of cows [13-15].

2 Materials and methods

The studies were carried out on cows of Holmogor x Holstein crossbreeds during the transit period with loose maintenance with the formation of production and technological groups in the winter and spring months. According to the principle of analogues, two groups of cows of 30 heads each were formed, one of which was control, the other experienced. In the period two weeks before calving and for 2 months after calving, 150 and 300 ml/head/day of liquid energy feed based on propylene glycol and glycerin with the Fekord-2004 C enzyme complex (EFFC) were added to the diet, respectively, in a concentration of 2 liters per 1 ton of EF. The main feeding ration was designed to receive an average daily milk yield of 25 kg / head and consisted of corn silage -15 kg, haylage of perennial grasses 20 kg, hay or oat straw 1 kg, sunflower cake 100 g / l milk, molasses 500 g. compound feed with mineral and vitamin premix 500 g / l milk. Additionally, salt 100 g and chalk 200 g /head / day. Milkanizer liquid energy feed, produced by Eurokorm LLC, is an optimally balanced energy feed and is designed to feed dairy cows before and after calving. [10]. When used in nutrition, it compensates for energy imbalance due to glycoforming non-acidogenic components, providing energy supply for a long period of time. Eliminates the causes of ketosis (acetoneemia).1 kg of product contains 23 MJ of exchange energy. The Fekord 2004 enzyme complex includes four enzymes: cellulase, glucoamylase, xylanase and β-glucanase (Table 1).

Table 1. Composition and activity of the Fekord 2004 enzyme complex

| The indicator                        | Guaranteed specifications | The actual |
|--------------------------------------|---------------------------|------------|
| pH value 4.5-6.0                      | 4.5-6.0                   | 5.4        |
| Cellulase activity, units/cm³        | Not less than 200         | 899        |
| Cellulase activity, units/cm³        | Not less than 2200        | 6940       |
| β-glucanase activity, units/cm³³     | Not determined            | 3904       |
| Glucoamylase activity, units/cm³     | Not less than 500         | 2980,2     |

To monitor the metabolic and clinical health of cows at the 1st and 2nd month of lactation, blood samples were taken in which the following hematological parameters (leukocytes, erythrocytes, hemoglobin and hematocrit) were determined on the ABZ VET analyzer (Horiba ABZ, France);- Biochemical parameters of blood serum (total protein, albumin, urea, activity of ALAT, ASAT, AIP, Ca, P, Mg, Fe, chlorides, total cholesterol, total bilirubin, glucose, NEFA, triglycerides, phospholipids on the automatic biochemical analyzer Chem
Well (Awareness Tehnology, USA); TBA AP using a biochemical kit - "Agat-Med", cortisol and thyroxine - by enzyme immunoassay, the content of ceruloplasmin in blood serum by the Ravin method. In the second month of lactation, chyme samples were taken from 3 cows from each group using a gastric probe in which the amount of biomass of protozoa and bacteria was determined by differentiated centrifugation, pH, VFA by steam distillation on the Margham apparatus and ammonia nitrogen by the Conway microdiffusion method; amylolytic activity-photometric method of accounting and evaluation of dairy productivity of cows in the experiment was carried out by daily milking. In milk, the following were determined: the mass fraction of fat, protein, including casein and whey proteins, lactose, the number of somatic cells, the content of urea, ketone bodies and fatty acid composition of milk fat on the analyzer Milko ScanTM, 7RM, FossomaticTM 7DC. The digital material was processed biometrically with the determination of the Student-Fisher reliability criterion using the Microsoft Office Excel 2007 program.

3 Results

The inclusion of EFEC in the diet of dairy cows of the experimental group had an impact on the indicators of cicatricial digestion. In the chyme of cows of the experimental group, there was a slight increase in pH, which was 7.3 versus 7.2 in the control, at the recommended 6.5-7.4. In the chyme of the experimental group of cows, there was a slight increase in the content of VFA and a decrease in ammonia at the VFA/ammonia index of 0.7 versus 0.649 in the control, which may indicate a better use of feed protein.

| Indices                      | Groups                  | Experience to the control: |
|------------------------------|-------------------------|---------------------------|
|                              | Control                 | Experience               | ±       | %      |
| pH                           | 7.20±0.0671             | 7.30±0.064               | +0.1    | 101.3  |
| VFA, mmol/l                  | 6.82±0.396              | 7.07±0.464               | +0.25   | 103.6  |
| Ammonia, mmol/l              | 10.5±0.7                | 10.1±2.41                | -0.4    | 96.2   |
| VFA/ammonia index            | 0.649                   | 0.700                    | +0.051  | -      |
| Microbial mass, chyme, g/100 ml | 0.2961±0.0263          | 0.2936±0.0220            | -0.0025 | 99.1   |
| including bacteria          | 0.1305±0.0007           | 0.1668±0.0147**          | +0.0363 | 127.8  |
| protozoa                     | 0.1656±0.0185           | 0.1267±0.0220            | -0.0389 | 76.50  |

** P<0.02;

The main changes in the chyme under the action of EFEC occurred in the number and species composition of microorganisms with a relatively equal biomass. Thus, the number of bacteria in the chyme of cows of the experimental group increased in relation to the control by 27.8% (P<0.02), and the protozoa decreased by 23.5%. (Table 2).

The addition of EFEC to the diet had a positive effect on protein-nitrogen metabolism. So, at the 1st month of lactation, the total protein content in the blood serum of cows of the experimental group was higher by 7.2% due to both fractions with an A/G ratio of 46.4 versus 41.5 in the control, and at the 2nd month these indicators in cows of the experimental group were lower by 1.8-1.9%, which may indicate the anabolic orientation and intensity of protein metabolism in the body of cows of the experimental group. These data are consistent with the indicators of urea content in blood serum, its intake from the blood into the rumen and creatinine content (Table 3).

Under the influence of EFEC, the hormonal status and the state of lipid peroxidation in the blood of the experimental group changed. The content of cortisol, as a factor that includes gluconeogenesis in the energy metabolism, in the blood serum of cows of the experimental
group at the 1st month of lactation was 5% less than that of the control group and it decreased by 21.3% at the 2nd month, while in cows of the control group, on the contrary, there was an increase in its content more than twice. As a result, the difference between the experimental and control group was 65.6% Thyroxine is the main regulator of basal metabolism and has a direct effect on the mobilization of energy resources in the body. At the 1st month of lactation, its serum concentration in cows of the experimental group was 25.5% higher, and at the 2nd month these differences decreased and amounted to only 2.8%. Cows of both groups had a decrease in thyroxine concentration by the 2nd month of lactation. Changes in the concentration of hormones during the observation period were reflected in the value of the cortisol/thyroxine index, which in the cows of the experimental group was 4.29 and 4.44 versus 7.45 and 16.62 in the control, respectively, at the 1st and 2nd month of lactation, which characterizes the direction and intensity of energy metabolism in the body. The indicators of lipid peroxidation and antioxidant protection are consistent with these data. Thus, in the cows of the experimental group, the content of peroxidation products in the blood serum at the 1st month of lactation was 18.2% less than in the control, but at the same time the content of ceruloplasmin was also lower. At the 2nd month, the cows of both groups had a decrease in the indicator of antioxidant protection, but it was more significant in the control group, as a result, it was 3.8% higher in the cows of the experimental group (Table 4).

### Table 3. Protein and nitrogen metabolism (n=9, ±SEM).

| Indices                        | Month | Groups          | Experience to the control: |
|-------------------------------|-------|-----------------|---------------------------|
|                               |       | Control         | Experience               | ±   | %   |
| Total protein, g/l            | 1     | 72.93±1.41      | 78.18±2.23               | +5.25 | 107.2 |
|                               | 2     | 77.63±2.36      | 76.22±1.72               | -1.41 | 98.2 |
| Albumin, g/l                  | 1     | 29.17±0.72      | 31.75±0.49***            | +2.58 | 108.8 |
|                               | 2     | 33.59±0.94      | 32.95±0.66               | -0.64 | 98.1 |
| Globulin, g/l                 | 1     | 41.53±1.82      | 46.43±2.11               | +4.9  | 111.8 |
|                               | 2     | 44.03±1.95      | 43.24±2.35               | -0.79 | 98.2 |
| A/G ratio                     | 1     | 0.69±0.035      | 0.71±0.02                | +0.02 | 102.8 |
|                               | 2     | 0.77±0.05       | 0.87±0.05                | +0.10 | 112.9 |
| Urea, mmol/l                  | 1     | 4.10±0.337      | 3.62±0.46                | -0.48 | 88.3 |
|                               | 2     | 3.88±0.36       | 4.15±0.17                | +0.27 | 106.9 |
| Urea from blood in the rumen, g| 1     | 9.13±0.64       | 8.17±1.04                | -0.96 | 89.5 |
|                               | 2     | 8.99±0.76       | 8.37±0.38                | -0.62 | 106.9 |
| Creatinine, µmol/l            | 1     | 81.63±4.38      | 79.18±2.08               | -2.45 | 96.9 |
|                               | 2     | 87.32±2.90      | 99.33±4.53*              | +12.01 | 113.8 |

*** P<0.01
Table 4. Hormonal and antioxidant status (n=9, ±SEM).

| Indices                      | Mon th. | Groups                        | Experience to the control: |
|------------------------------|---------|-------------------------------|---------------------------|
|                              |         | Control                       | Experience                |
| Cortisol, nmol/l             | 1       | 114.25±28.98                  | 108.66±33.70              | -5.54  | 95.0  |
|                              | 2       | 248.2±72.60                   | 85.64±50.04               | -162.5 | 34.4  |
| Thyroxine, nmol/l            | 1       | 19.6±2.56                     | 24.6±2.98                 | +5.00  | 125.5 |
|                              | 2       | 18.72±2.31                    | 19.25±2.05                | +0.53  | 102.8 |
| Cortisol/thyroxine index     | 1       | 7.45±2.61                     | 4.29±1.40                 | -3.16  | 57.6  |
|                              | 2       | 16.62±5.87                    | 4.44±2.05                 | -12.14 | 26.75 |
| TBA AP, mmol/l               | 1       | 3.64±0.32                     | 2.98±0.19                 | -0.66  | 81.8  |
|                              | 2       | 3.84±0.115                    | 4.06±0.268                | +0.22  | 105.7 |
| Ceruloplasmin, mg/l          | 1       | 200.7±22.11                   | 184.1±16.70               | -16.6  | 91.7  |
|                              | 2       | 167.7±13.86                   | 174.1±12.50               | +6.4   | 103.8 |

EFEC had a positive effect on the functional state of the liver and on carbohydrate-lipid metabolism, but it was observed only in the 1st month of lactation. So, during this period, the bilirubin content in the blood serum of cows of the experimental group was 7.71 mmol/l versus 9.24 in the control group, which was 16.4% lower. ALT activity was 5.6% higher with close values in AST activity, which is consistent with an increased level of protein, and, in particular, albumin. The glucose content was 15.2% higher (P<0.02), and the NEFA and cholesterol were 26.9% lower and 11.9% with a higher NEFA/cholesterol index, which characterizes the intensity of the flow of NEFA from the liver into the body tissues used for energy purposes. The content of triglycerides and phospholipids in blood serum was also higher than in the control by 12.9% (P<0.01) and 8.5% (P<0.05), which also indicates an

Table 5. Functional state of the liver and indicators of carbohydrate-lipid metabolism (n=9, ±SEM).

| Indices                        | Mon th. | Groups                        | Experience to the control: |
|-------------------------------|---------|-------------------------------|---------------------------|
|                               |         | Control                       | Experience                |
| Total bilirubin, µmol/l       | 1       | 9.24±0.34                     | 7.71±0.90                 | -1.53  | 83.4  |
|                               | 2       | 7.35±0.41                     | 8.83±0.44**               | +1.48  | 120.1 |
| Activity: ALT, IU/l           | 1       | 19.03±2.50                    | 20.11±1.26                | +1.08  | 105.6 |
|                               | 2       | 22.50±1.34                    | 26.63±1.56*               | +4.13  | 115.6 |
| Activity:AST, IU/L            | 1       | 68.53±3.94                    | 67.66±3.87                | -0.87  | 98.7  |
|                               | 2       | 61.82±1.62                    | 68.29±2.52*               | +6.47  | 110.4 |
| De-Rittes Index (AST/ALT)     | 1       | 4.68±1.41                     | 3.50±0.16                 | -1.18  | 74.4  |
|                               | 2       | 2.81±0.20                     | 2.59±0.09                 | -0.22  | 92.1  |
| Glucose, mmol/l               | 1       | 3.29±0.104                    | 3.79±0.07**               | +0.5   | 115.2 |
|                               | 2       | 4.16±0.08                     | 3.79±0.05****             | -0.37  | 91.1  |
| NEFA, mmol/l                  | 1       | 0.67±0.05                     | 0.49±0.03                 | -0.18  | 73.1  |
|                               | 2       | 0.34±0.03                     | 0.52±0.01****             | +0.18  | 152.9 |
| Total cholesterol, mmol/l     | 1       | 6.21±0.35                     | 5.47±0.12                 | -0.74  | 88.1  |
|                               | 2       | 6.47±0.33                     | 6.56±0.44                 | +0.09  | 101.4 |
| Index: NEFA/, cholesterol     | 1       | 0.1126±0.011                  | 0.1716±0.071              | +0.059 | 152.3 |
|                               | 2       | 0.053±0.0005                  | 0.080±0.004****           | +0.026 | 149.9 |
| Triglycerides, mmol/l         | 1       | 0.31±0.001                    | 0.35±0.01****             | +0.04  | 112.9 |
|                               | 2       | 0.10±0.02                     | 0.13±0.01                 | +0.03  | 130.0 |
| Phospholipids, mmol/l         | 1       | 3.06±0.09-                    | 3.31±0.10*                | +0.25  | 108.5 |
|                               | 2       | 3.56±0.08                     | 3.64±0.22                 | +0.08  | 102.2 |

*P<0.05; ** P<0.02; *** P<0.01; ****P<0.001
increased energy metabolism in the body of cows of the experimental group. The second month of lactation, according to the state of metabolism, differs significantly in cows of both groups in relation to the first month, which in cows of the control group may be associated with adaptive processes in connection with milking and, in particular, with increased gluconeogenesis, and in cows of the experimental group both in connection with milking and due to additional energy of the diet (Table 5).

There were also some differences in mineral metabolism between groups of cows. At the 1st month of lactation, the activity of alkaline phosphatases in the blood serum of cows of the experimental group was 20.3% higher, which may be due to the resorption of minerals from bone tissue due to increased milk secretion and its excretion from the body. At the 2nd month of lactation in cows of both groups, there was a decrease in its activity by 14.3 and 32.7%, respectively, in cows of the control and experimental groups, as a result of which its values were close. Differences and changes in the activity of alkaline phosphatase in cows did not affect the content of calcium and phosphorus in the blood serum of both groups of cows. However, the calcium content at the same time was at the level of the lower the reference value of the physiological norm (2.5-3.13 mmol/l), and phosphorus is higher than the upper value (1.45-1.94 mmol/l). As a result, the calcium-phosphorus ratio was 1:1 against 0.7 recommended by physiological norms. The magnesium content in the blood serum of cows of both groups was below the lower reference value of the physiological norm (0.82-0.123 mmol/L). At the same time, its saturation by the 2nd month of lactation was observed by 20.3 and 58.3%, respectively, but in cows of the experimental group, its content was lower by 18.7% (P<0.02) at the 1st month of lactation, and by 7.0% higher at the 2nd month, which can be explained both by the stress state of cows at the beginning of lactation and the influence of EFEC on its absorption in the intestine and resorption from bone tissue.

**Table 6. Indices of mineral metabolism. (n=9, ±SEM)**

| Indices                  | Month | Groups            | Experience to the control |
|-------------------------|-------|-------------------|---------------------------|
| Alkaline phosphatase, U/l | 1     | 88.60±7.79        | 106.58±18.32              |
|                         | 2     | 76.26±6.12        | 71.83±5.98                |
| Calcium, mmol/l         | 1     | 2.43±0.10         | 2.47±0.09                 |
|                         | 2     | 2.41±0.06         | 2.38±0.06                 |
| Phosphorus, mmol/l      | 1     | 2.50±0.21         | 2.56±0.09                 |
|                         | 2     | 2.38±0.11         | 2.32±0.07                 |
| Ratio: Ca: P            | 1     | 1:1.02            | 1:1.03                    |
|                         | 2     | 1:0.98            | 1:0.97                    |
| Magnesium, mmol/l       | 1     | 0.59±0.03         | 0.48±0.03**               |
|                         | 2     | 0.71±0.03         | 0.76±0.05                 |
| Ratio: Ca: P: Mg        | 1     | 1:1.02:0.24       | 1:1.03:0.19               |
|                         | 2     | 1.09:0.29:0.31    | 1.09:0.31                 |
| Ferrum, µmol/l          | 1     | 21.70±1.76        | 23.25±0.95                |
|                         | 2     | 30.78±1.09        | 21.24±0.75****            |
| Chlorides, mmol/l       | 1     | 87.78±1.73        | 99.94±1.16****            |
|                         | 2     | 104.05±0.78       | 107.3±0.40****            |

**P<0.02; ***P<0.01, ****P<0.001**

The iron content in the blood serum of cows of both groups during the observation period was higher than the physiological norm equal to 17.91 mmol/l, and amounted to 23.25 in the 1st month of lactation in cows of the experimental group, and 21.70 mmol/l in control cows. At the 2nd month, the cows of the control group had an increase in its content by 41.8%, while the cows of the experimental group, on the contrary, it decreased by 8.7%, as a result of which its content was lower than that of the control cows during this period by 30.2%
(P<0.01), which can be explained by increased redox processes in the body of the experimental group due to lactogenesis. The content of chlorides in the blood serum of cows of the experimental group b at both the 1st and 2nd month of lactation was higher than in the control by 13.8 (P<0.01) and 3.1% (P<0.01). Chlorides in the blood, gastric juice and intercellular fluid take part in a large number of biochemical reactions that have a key impact on ensuring the vital activity of the whole organism. Maintaining the normal concentration of chemical compounds based on chlorine ions is regulated by thyroid and steroid hormones (Table 6).

Changes in metabolism under the influence of EFEF had a significant impact on the dairy productivity of cows. In the cows of the control group, the average daily milk yield varied in accordance with the regularity of the lactation curve and amounted to 17.6, 19.1 and 18.1 kg, respectively, at the 1st, 2nd and 3rd month. In the cows of the experimental group, the average daily milk yield continued to increase after the 2nd month and amounted to 19.9, 20.9 and 21.1 kg, which was more than in the control by 13.0, 9.4 and 16.5%, respectively (P<0.001), (Table 7).

Table 7. Milk productivity of cows (n=32 average daily milk yield, n=9 others indices, ±SEM).

| Indices                     | Month | Groups       | Experience to the control: |
|-----------------------------|-------|--------------|----------------------------|
|                             |       | Control      | Experience                 | ±   | %   |
| Average daily milk yield, kg| 1     | 17.6±0.13    | 19.9±0.08**                | 2.3 | 113.0 |
|                             | 2     | 19.1±0.10    | 20.9±0.07***               | 1.8 | 109.4 |
|                             | 3     | 18.1±0.11    | 21.1±0.01***               | 3.0 | 116.5 |
| Mass fraction:              |       |              |                            |     |     |
| fat, %                      | 1     | 4.34±0.13    | 3.60±0.08***               | -0.74 | - |
|                             | 2     | 4.35±0.07    | 3.35±0.07                 | +0.14 | - |
| protein, %                  | 1     | 2.84±0.03    | 2.63±0.018**               | -0.21 | - |
|                             | 2     | 2.97±0.03    | 2.80±0.023***              | -0.17 | - |
| including true, %           | 1     | 2.68±0.03    | 2.43±0.019***              | -0.25 | - |
| casein, %                   | 2     | 2.76±0.03    | 2.63±0.023***              | -0.13 | - |
| lactose, %                  | 1     | 4.94±0.02    | 4.94±0.018                | ±    | ± |
|                             | 2     | 4.85±0.023   | 4.78±0.036                | -0.07 | - |
| dry skim matte, %           | 1     | 8.85±0.06    | 8.36±0.032***             | -0.49 | - |
|                             | 2     | 8.55±0.055   | 8.37±0.087***             | -0.18 | - |
| dry skim matte, %           | 1     | 13.01±0.149  | 11.85±0.080***            | -1.16 | - |
|                             | 2     | 11.78±0.11   | 11.77±0.083               | ±    | ± |

** P<0.02; *** P<0.01; **** P<0.001

The changes also affected the chemical composition of milk. Thus, the fat content in the milk of cows of the experimental group at the 1st month of lactation was less by 0.74% (P<0.001), and at the 2nd higher by 0.14% as a result of the fact that cows of both groups did not have an adequate decrease in it and, in particular, in cows of the control group it was 1.13%, and in the experimental 0.25%. The cows of the experimental group also had a lower protein content in relation to the control. At the 1st month of lactation, it was lower by 0.21% (P<0.02), and at the 2nd by 0.17% (P<0.02). This decrease was due to both true protein and casein. In terms of lactose content, the milk of cows of both groups practically did not differ. Changes in fat and protein content directly they affected the value of dry skim milk and the content of dry matter in milk, which in the cows of the experimental group at the 1st month of lactation was less by 1.16% (P<0.001) and equal at the 2nd month (Table 7). The milk of the experimental cows was characterized by a lower urea content, which indicates a better supply of energy to them.
While in the milk of the control group of cows they increased by 0.085 (P<0.05) and 0.217 mg/100 g (P<0.01, ****P<0.001). The content of β-hydroxybutyrate, especially at the 1st month of lactation, was 140 mmol/l, at the 2nd 43 mmol/l, which may be due to the large formation of butyrate in the rumen. So, at the 1st month of lactation, the acetone content in the milk of cows of the experimental group decreased by 0.140 mg (P<0.001) and 0.366 mg (P<0.001) per 100 g of milk, respectively, and at the 2nd month increased by 0.085 (P<0.05) and 0.217 mg/100 g (P<0.02), respectively. There were fewer transisomers in the composition of fat in the cows of the experimental group, at the 1st month of lactation by 0.03 mg (P<0.001), and at the 2nd by 0.041 mg/100 g (P<0.001) (Table 9).

### Table 8. Chemical, physical and sanitary-hygienic indicators of milk quality (n=9 others indices, ±SEM).

| Indices                        | Month | Groups                              | Experience to the control |
|-------------------------------|-------|-------------------------------------|---------------------------|
|                               |       | Control                             | Experience               | ±         | %         |
| Freezing point, °C            | 1     | -0.538±0.001                        | -0.538±0.001              | ±         |           |
|                               | 2     | -0.539±0.001                        | -0.538±0.001              | ±         |           |
| Urea, mmol/l                  | 1     | 7.75±0.177                         | 6.32±0.109****           | -1.43     | 81.5      |
|                               | 2     | 7.12±0.04                          | 5.41±0.08****            | -1.71     | 75.9      |
| Acidity, pH                   | 1     | 6.49±0.014                         | 6.58±0.006****           | +0.09     | 101.3     |
|                               | 2     | 6.52±0.01                          | 6.47±0.007****           | 0.05      | 99.2      |
| Acetone, mmol/l               | 1     | 24±5                               | 140±4*                   | +137      | 583.3     |
|                               | 2     | 14±2                               | 43±5****                 | +29       | 307.1     |
| β-HB, mmol/l                  | 1     | 9±2                                | 80±3                     | +7        | 888.8     |
|                               | 2     | 3±1                                | 24±3****                 | +21       | 800.0     |
| Somatic cells, u/ml           | 1     | 43±4                               | 125±22***                | +81.6     | 290.6     |
|                               | 2     | 44±6                               | 144±38**                 | +100      | 327.3     |
| including lymphocytes ,PMN %  | 1     | 76.5                               | 65.4                     | -11.1     | 85.3      |
|                               | 2     | 79.4                               | 59.3                     | -20.1     | 74.6      |
| macrophages, %                | 1     | 13.5                               | 34.6                     | +21.1     | 256.3     |
|                               | 2     | 20.6                               | 40.7                     | +20.1     | 197.5     |

*P<0.05; ** P<0.02; *** P<0.01, ****P<0.001
β – β-hydroxybutyrate
PMN – polymorphous neutrophils

So, at the 1st month of lactation, the urea content in the milk of cows of the experimental group was 18.5% less (P<0.001), and at the 2nd by 24.1% (P<0.001). The acidity of the milk between the groups of cows was practically the same. At the same time, the milk of cows of the experimental group contained more acetone and β-hydroxybutyrate, especially at the 1st month of lactation, which may be due to the large formation of butyrate in the rumen. So, at the 1st month of lactation, the acetone content in the milk of cows of the experimental group was 140 mmol/l, at the 2nd 43 mmol/l, while in the milk of the control group of cows they were equal to 24 and 14 mmol/l (P<0.001). The content of β-HB in the milk of cows of the experimental group at the 1st month of lactation was 43 mmol/l, and at the 2nd 43 mmol/l versus 9 and 3 mmol/l in the control (P<0.001). The increased content of ketone bodies in the milk of cows of the experimental group may indicate a subclinical form of ketosis. The result of which may be an increased content of somatic cells in milk. Thus, in the milk of cows of the experimental group, the content of somatic cells was 125 (P<0.01) at the 1st month of lactation and 144 units/ml (P<0.01) at the 2nd month against 43 and 44 units/ml, respectively, in the control. (Table 8).

A decrease in the fat content in the milk of cows of the experimental group in the 1st month of lactation, as well as an increase in its content in the 2nd month, was reflected in its fatty acid composition. Basically, these changes were associated with stearic and oleic acids. So, at the 1st month of lactation, the content of stearic and oleic acid in the milk of cows of the experimental group decreased by 0.140 mg (P<0.001) and 0.366 mg (P<0.001) per 100 g of milk, respectively, and at the 2nd month increased by 0.085 (P<0.05) and 0.217 mg/100 g (P<0.02), respectively. There were fewer transisomers in the composition of fat in the cows of the experimental group, at the 1st month of lactation by 0.03 mg (P<0.001), and at the 2nd by 0.041 mg/100 g (P<0.001) (Table 9).
The analysis of hematological studies in cows showed a positive effect of adding EF EC to the diet. As a result, cows of the experimental group had 39.1% fewer white blood cells in the blood at the 1st month of lactation compared to the control. At the 2nd month, the number of leukocytes in cows of both groups decreased by 58.6% in the control and by 26.9% in the

Table 9. Fatty acid composition of milk fat, mg/100 g (n=9; ±SEM)

| Indices         | Groups         | Experience to the control | Content, % | Experience |
|-----------------|----------------|---------------------------|------------|------------|
|                 |                | ± | %      | Control | Experience |
| Individual      |                |   |         |         |            |
| 1- st month of lactation | Myristic         | 0.329±0.013 | 0.337±0.006 | +0.008 | 102.4 | 9.90 | 12.4 |
|                 | Palmitic       | 0.990±0.039 | 0.945±0.016 | -0.054 | 95.4 | 29.8 | 34.8 |
|                 | Stearic        | 0.500±0.014 | 0.360±0.014**** | -0.140 | 72.0 | 15.0 | 13.3 |
|                 | Oleic acid     | 1.501±0.042 | 1.135±0.040**** | -0.366 | 75.6 | 45.2 | 41.8 |
| 2 - nd month of lactation | Myristic         | 0.328±0.013 | 0.303±0.007 | -0.025 | 93.2 | 12.15 | 11.05 |
|                 | Palmitic       | 0.877±0.038 | 0.848±0.023**** | -0.029 | 96.7 | 32.5 | 30.9 |
|                 | Stearic        | 0.269±0.011 | 0.351±0.012* | +0.082 | 130.5 | 9.96 | 13.0 |
|                 | Oleic acid     | 1.023±0.024 | 1.240±0.030** | +0.217 | 121.2 | 37.8 | 45.9 |
| Chain Length Distribution | Long-chain | 1.946±0.058 | 1.407±0.051**** | -0.539 | 72.3 | 49.6 | 44.8 |
|                 | Medium-chain | 1.406±0.064 | 1.267±0.027* | -0.139 | 90.1 | 35.8 | 40.3 |
|                 | Short-chain   | 0.572±0.019 | 0.465±0.009**** | -0.107 | 81.3 | 14.6 | 14.8 |
| 2-nd month of lactation | Long-chain | 1.231±0.034 | 1.412±0.044**** | +0.181 | 114.7 | 41.2 | 45.6 |
|                 | Medium-chain | 1.302±0.067 | 1.238±0.035 | -0.064 | 95.1 | 43.5 | 40.0 |
|                 | Short-chain   | 0.466±0.021 | 0.439±0.011 | -0.027 | 94.2 | 15.6 | 14.2 |
| Saturation distribution | 1- st month of lactation |          |            |         |         |         |         |
|                 | Monounsaturated | 1.392±0.040 | 1.076±0.036**** | -0.316 | 77.3 | 31.5 | 29.0 |
|                 | Polynsaturated | 0.165±0.004 | 0.117±0.003**** | -0.048 | 70.9 | 3.73 | 3.10 |
|                 | Saturated      | 2.735±0.09 | 2.410±0.078**** | -0.324 | 88.3 | 61.9 | 64.9 |
|                 | Trans-isomers  | 0.137±0.004 | 0.107±0.002**** | -0.03 | 78.1 | 3.1 | 2.9 |
| 2-nd month of lactation | Monounsaturated | 0.927±0.021 | 1.016±0.028** | +0.089 | 109.6 | 27.7 | 29.8 |
|                 | Polynsaturated | 0.123±0.004 | 0.1097±0.002 | -0.013 | 89.2 | 3.68 | 3.2 |
|                 | Saturated      | 2.214±0.105 | 2.247±0.061 | +0.033 | 101.4 | 66.3 | 63.9 |
|                 | Trans-isomers  | 0.080±0.006 | 0.039±0.002**** | -0.041 | 48.7 | 2.4 | 1.1 |

*P<0.05; ** P<0.02; *** P<0.01; ****P<0.001
experiment, as a result of which their number equaled and amounted to $10.14 \times 10^9$ in the control and $10.91 \times 10^9$ in the experiment. In the blood of cows of the experimental group during the observation period, the content of erythrocytes was higher by 4.3 and 8.0%, there was also more hemoglobin by 6.7-6.9% ($P<0.05$) and the hematocrit by 1.93 and 0.71%. Blood erythrocytes in cows of the experimental group were more saturated with hemoglobin. (Table 10).

| Table 10. Morpho-hematological parameters, (n= 9, ±SEM) |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Indices                    | Month          | Groups                        | Experience to the control    | ±  | %            |
| White blood cells, 10\(^9\)/l | 1              | Control | 24.48±3.94 | 14.92±1.92 | -9.56 | 60.9 |
|                           | 2              | Experience | 10.14±0.67 | 10.91±0.67 | +0.05 | 107.6 |
| Red blood cells, 10\(^12\)/l | 1              | Control | 7.40±0.15 | 7.72±0.16 | +0.32 | 104.3 |
|                           | 2              | Experience | 7.22±0.11 | 7.37±0.13 | +0.15 | 108.0 |
| Hemoglobin, g/             | 1              | Control | 86.60±1.56 | 92.6±2.46 | +6.0  | 106.9 |
|                           | 2              | Experience | 81.75±2.04 | 87.02±0.77** | +5.24 | 106.7 |
| Hematocrit, %              | 1              | Control | 35.19±0.63 | 37.12±1.09 | +1.93 |            |
|                           | 2              | Experience | 34.47±1.00 | 35.18±0.56 | +0.71 |            |
| Color indicator, units     | 1              | Control | 0.340±0.008 | 0.36±0.007 | +0.013 | -105.8 |
|                           | 2              | Experience | 0.330±0.010 | 0.381±0.004 | +0.05 | 115.4 |

** P<0.02

### 4 Conclusion

The introduction of energy feed into the diet of cows together with a complex of carbohydrate hydrolytic enzymes during the transit period had a noticeable effect on the physiological and microbiological processes in the rumen, which manifested itself primarily in a change in the structure of microbiocenosis. In relation to the control, the number of infusoria in the microbial mass of cows in the experimental group decreased by 23.5%, and bacteria increased by 27.8% ($P<0.02$), which, with a slight increase in the content of VFA, could affect their ratio and, in particular, an increase in the proportion of butyric acid and a decrease in acetic acid, and thus affect lipid metabolism, the fat content in milk and its fatty acid composition. The main changes in which occurred in the content of stearic and linoleic acids, an increase in the content of microbial mass in the rumen chyme with a higher VFA/ammonia index favorably affected the state of protein-nitrogen metabolism. The cows of the experimental group showed an improvement in the functional state of the liver, hormonal status, antioxidant protection and morpho-hematological indicators. As a result, their average daily milk yield was higher by 13.0, 9.4 and 16.5% ($P<0.001$), respectively, at the 1st, 2nd and 3rd month of lactation than in the control.

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