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O IMPACTO DA PECUÁRIA INTENSIVA DE NOVILHAS HOLSTEIN AUSTRALIANAS NAS PROPRIEDADES FÍSICO-QUÍMICAS DO LEITE DE VACA

THE IMPACT OF INTENSIVE RAISING OF AUSTRALIAN HOLSTEIN HEIFERS ON THE PHYSICOCHEMICAL PROPERTIES OF COW MILK

ВЛИЯНИЕ ИНТЕНСИВНОГО ВЫРАЩИВАНИЯ ГОЛШТИНСКИХ ТЕЛОК АВСТРАЛИЙСКОЙ СЕЛЕКЦИИ НА ФИЗИКО-ХИМИЧЕСКИЕ СВОЙСТВА МОЛОКА КОРОВ

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RESUMO

Antecedentes: O estudo das características de crescimento, desenvolvimento e produtividade do gado importado da Austrália para o Território Krasnodar é de grande interesse científico e prático, que é tópico. Uma vez que as características produtivas dos genótipos importados não foram estudados suficientemente, não apenas nas fazendas do Território Krasnodar, mas também em outras regiões da Rússia, eles requerem um estudo mais aprofundado e melhoramento sob as novas condições de alimentação e alojamento. **Objetivo:** a pesquisa teve como objetivo estudar a influência da criação intensiva de novilhas de reposição da raça Holandesa de seleção australiana sobre os indicadores de qualidade do leite de vaca. **Métodos:** Os estudos foram conduzidos na Artex-Agro LLC, distrito de Kushchevsky do Território de Krasnodar, sobre os descendentes do gado holandês importado da Austrália. Sessenta e quatro novilhas foram selecionadas para o estudo. As novilhas de substituição experimentais foram criadas usando as taxas de alimentação de leite aumentadas e o produto probiótico Cellobacterin. Eles foram criados intensivamente e inseminados aos 14 meses de idade. A produtividade do leite das vacas e as propriedades físico-químicas do leite foram estudadas durante três lactações após o parto. **Resultados e Discussão:** A produtividade de leite das vacas dos grupos experimentais excedeu um de seus companheiros de rebanho do grupo controle em 450 - 1.360 kg. Os melhores indicadores das propriedades físico-químicas do leite foram determinados no leite das vacas criadas a partir de novilhas alimentadas com 450 kg de leite integral durante os primeiros 50 dias até os seis meses de idade. **Conclusões:** Os autores acreditam que a criação intensiva de novilhas de reposição afetou positivamente o grau de desenvolvimento do trato gastrointestinal e melhorou o nível de produção de leite e seus parâmetros físico-químicos.
Palavras-chave: *novilhas de reposição, pecuária, produção de leite, acidez, densidade, composição de aminoácidos.*

ABSTRACT

Background: The study of growth, development and productivity features of imported cattle from Australia to the Krasnodar Territory is of great scientific and practical interest, which is topical. Since the productive features of the imported genotypes have not been studied sufficiently, not only in the farms of the Krasnodar Territory but also in other regions of Russia, they require further, more in-depth study and improvement under the new conditions of feeding and housing. **Aim:** The research aimed to study the influence of intensive rearing of replacement heifers of the Holstein breed of Australian selection on cow milk quality indicators. **Methods:** The studies were conducted at Artex-Agro LLC, Kushchevsky District of the Krasnodar Territory, concerning the descendants of the imported Australian Holstein cattle. Sixty-four heifers were selected for the study. The experimental replacement heifers were raised using the enhanced milk feeding rates and the Cellobacterin probiotic product. They were raised intensively and inseminated when they were 14 months old. The milk productivity of cows and the physicochemical properties of milk were studied during three lactations after calving. **Results and Discussion**: The milk productivity of the cows from the experimental groups has exceeded one of their herd mates from the control group by 450 – 1,360 kg. The best indicators of the

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physicochemical properties of milk have been determined in the milk of the cows raised from the heifers fed with 450 kg of the whole milk during the first 50 days until they were six months old. **Conclusions:** The authors believe that intensive rearing of replacement heifers positively affected the degree of development of their gastrointestinal tract and improved the level of milk yield and its physicochemical parameters.

Keywords: *replacement heifers, raising, milk production, acidity, density, amino acid composition*.

АННОТАЦИЯ

Предпосылки: Изучение особенностей роста, развития и продуктивности импортированного скота из Австралии в Краснодарский край представляет большой научный и практический интерес, что является актуальным. Поскольку продуктивные особенности завезенных генотипов недостаточно изучены, не только в хозяйствах Краснодарского края, но и в условиях других регионов РФ, они требуют дальнейшего более углубленного изучения и совершенствования в новых условиях кормления и содержания. **Цель:** Целью наших исследований являлось изучение влияния интенсивного выращивания ремонтных телок голштинской породы австралийской селекции на качественные показатели молока коров. **Методы:**Исследования проводились в ООО “Артекс-Агро” Кущевского района Краснодарского края, на потомках импортного скота голштинской породы австралийской селекции. Для исследований было отобрано 64 телочки. Подопытные ремонтные телки были выращены с использованием повышенных норм выпойки молока при добавлении пробиотического препарата целлобактерина. Они выращивались интенсивно, и были осеменены в 14 месячном возрасте. После отела на протяжении трех лактаций изучена молочная продуктивность коров и физико-химические свойства молока. **Результаты и Обсуждение:** По уровню молочной продуктивности коровы опытных групп превышали сверстниц контрольной на 450-1360 кг, лучшие показатели по физико-химическим свойствам молока были у коров, выращенных из телок, которым скормили до 6-месячного возраста за первые 50 дней – 450 кг цельного молока. В результате исследований установлено, что коровы III группы превосходили сверстниц контрольной и опытных групп по физико-химическим показателям молока. **Заключение:** Интенсивное выращивание ремонтных телок (которым за первые 50 дней выращивания было скормлено – 450 кг цельного молока, а с 50-ти и до 110-ти дневного возраста они потребили – 600 кг обезжиренного молока) положительно повлияло на степень развития их желудочно-кишечного тракта и позволило улучшить уровень молочной продуктивности коров и физико-химические показатели молока.

Ключевые слова:*ремонтные телки, молочная продуктивность, кислотность, плотность, аминокислотный состав.*

1. INTRODUCTION:

Holstein cattle of the USA, Canada, and Israel are the most highly-productive breed in the world. Its milk production is the highest compared to other breeds (Kargar, Habibi, and Karimi-Dehkordi, 2019; Ma *et al*., 2019; Rodrigues *et al*., 2019; Stepurina *et al*., 2019).In the Russian Federation, dairy cattle breeding remains one of the most important branches of agriculture (Gorlov *et al*., 2018; Ratoshny *et al*., 2018). Improving milk production and quality is one of the top priority problems of the agricultural sector of the country (Gorlov *et al*., 2019; Tuzov *et al*., 2018).

To considerably improve the milk production and dairy cattle and promote the Holstein breed, purebred Holstein cattle were imported (Haselmann *et al*., 2019). Holstein cows are characterized by the high genetic potential for dairy productivity (Anisimova, Koshchaev, and Eremenko, 2019; Gurtsieva *et al*., 2018; McKay *et al*., 2019). Cattle of this breed are well adapted to modern milk production (Gorlov *et al*., 2019; Karatunov, Tuzov, and Zelenkov, 2014). Cows of the Holstein breed are distinguished by high milk yield with a high milk constituents content; cows recompense well the fodder with milk yield (Ferreira *et al*., 2019; Krasnova, Batanov and Lebengarts, 2018; Weiss, 2019).

Under the conditions of loose housing and balanced feeding, the milk yield of Holstein cows in breeding herds reaches more than 8,000 kg when produced industrially, with the mass fraction of fat in milk being 3.5-4.1% on average.(Ferreira *et al*., 2019; Krasnova, Batanov, and Lebengarts, 2018; Reynolds *et al*., 2019). To obtain optimal milk yield, the diet of lactating cows should contain raw fiber constituted 18-28% of dry matter (A. S. Gorelik, O. V. Gorelik, and Kharlap, 2016; Gorelik *et al*., 2016; Olagarav *et al*., 2019). It is known that an insufficient amount of raw fiber in the diet leads to serious disorders of cicatricial digestion and negatively affects the level of milk yield (Deen *et al*., 2019; Haskell, Simm, and Turner, 2014).

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The development of replacement heifers during the rearing period is the basis on which the organism is formed with all its physiological and adaptive characteristics. In the first months of the life of young animals, the cardiovascular, respiratory, and digestive systems develop intensively. Therefore, the rearing of replacement heifers should be carried out with complete and balanced feeding during all animal growth (Knoblock *et al*., 2019; Raza and Kim, 2018; Sun, Plastow, and Guan, 2019).

The feeding of animals should be organized properly to ensure the conditions for effective fodder and regulation of microbiological digestion processes. The normal microflora of the gastrointestinal tract of an animal is of great importance, along with a balanced diet. The deficiency of normal microflora negatively affects various body functions. Various factors of modern livestock industry technologies, such as limited contact of young animals with their mothers, excessive density of livestock per unit area, unsanitary conditions of farms, antibiotic treatment, inadequate and unbalanced feeding rations, further lead to disruption of intestinal microflora. Against the foregoing background, the normal intestinal microflora in animals is violated, which leads to dysbiosis, a decrease in natural resistance, and productivity. One solution to this problem is the inclusion of probiotics in the animal feed (Kok *et al*., 2019; Sehested *et al*., 2019; Wang and Kadarmideen, 2019).

In cattle diets, Cellobacterin combines functions of two feed additives: a feed enzyme and a probiotic. Cellobacterin is a cellulolytic probiotic, a collection of living microorganisms *Enterococcus faecium* isolated from the rumen of cattle. It is used for feeding animals as a separate supplement or as part of a compound feed. When calves consume this product, the maturation of the cicatricial microflora is accelerated, and the digestive system is normalized. In addition, the Cellobacterin destroys the fiber and increases the digestibility of grain feed (30).

The research aimed to study the impact of intensive raising of replacement heifers of the Australian Holstein breed on the qualitative indices of cow milk.

The following tasks were set:

1. To develop methods of intensive breeding of Holstein young stock, using increased doses of milking, contributing to the early introduction of the herd of replacement heifers into use;

2. To determine the features of lactation activity of cows in three lactations.

2. MATERIALS AND METHODS:

2.1. General information

The studies were carried out at Artex-Agro LLC in the Kushchevsky District of the Krasnodar Territory concerning imported Australian Holstein cattle descendants.

2.2. Samples

For the experiment, calves were obtained from the Reflection Showering line cows, and four groups of experimental animals were formed (n = 64). Sixteen heifers were selected for each group: I – the control group, and II, III, and IV – the experimental groups. Table 1.

All groups were formed according to the principle of analogs. The groups differed by the raising technology: the animals of the control group I – up to six months old – were raised using traditional technology. They received 200 kg of whole milk (for 50 days) and 400 kg of skimmed milk (from 50 to 110 days). The heifers received concentrated fodder, including pre-feed fodder – 50 %, and corn grains – 50 %. The heifers from the experimental groups received the same percentage of fodder. The heifers of the II experimental group were fed with 200 kg of whole milk for 25 days, and at the age between 25 and 60 days, they consumed 400 kg of skimmed milk. During the first 50 days, the animals from the III experimental group ate 450 kg of whole milk, and at the age of 50 and 110 days, they consumed 600 kg of skimmed milk. The herd mates of the IV experimental group consumed 450 kg for 60 days, and at the age between 60 and 120 days, they were fed with 600 kg of skimmed milk.

Simultaneously, the heifers of all experimental groups were fed with 3 g of the Cellobacterin probiotic product (purchased from BIOTROF LLC) (Biotrof, n.d.) per day for each animal.

After the dairy period, the heifers of all groups were raised intensively. After the fruitful insemination of the heifers and their transfer to bred heifers, they had the ordinary farm diet. The daily diet consisted of the following feeds: corn silage (11 kg), alfalfa hay (2 kg), gramineous hay (0.7 kg), grass haylage (2.5 kg), brewing grains (3.8 kg), forage molasses (0.25 kg), a mixture of concentrates (1.5 kg), monocalcium phosphate 0.038 kg). The daily nutritional structure of the diet was as follows: roughage - 30%, succulent fodder - 45%, concentrated - 25%. The

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experimental animals were kept under identical conditions.

2.3. Materials and reagents

The calcium content in milk was determined according to the titrimetric method (ISO 12081-2013).

Consumables and reagents specified in the method were used.

1. Application area: a titrimetric method for determining the calcium content in milk.

2. Terms and definitions: the following term was used in this method, and an appropriate definition was given:

2.1. Calcium content in milk: mass fraction of substances determined by the method. The calcium content was expressed as a percentage by weight.

3. Principle: protein substances were precipitated in the analyzed sample with trichloroacetic acid and then filtered. Calcium was precipitated in the filtrate in the form of calcium oxalate and separated by centrifugation. The washed and dissolved precipitate was titrated with potassium permanganate.

4. Reagents and Materials: unless otherwise specified, only reagents of recognized analytical grade were used, as well as distilled or demineralized water or water of equivalent purity.

4.1. Trichloroacetic acid solution I ($C_2HCl_3O_2$), concentration 200 g/dm^3.

4.2. Trichloroacetic acid solution II, concentration 120 g/dm^3.

4.3. Ammonium oxalate ($C_2H_8N_2O_4$), saturated cooled solution.

4.4. Methyl red solution

Methyl red ($C_{15}H_{15}N_3O_2$) in the amount of 0.05 g was dissolved in100 ml^{3}of ethanol (96% by volume).

4.5. Acetic acid solution ($C_2H_4O_2$), 20% by volume.

4.6. Ammonia solution I

Equal volumes of ammonia (NH_3) solution (25% by a mass fraction) and water were mixed.

4.7. Ammonia II solution

Ammonia solution in the amount of 2 cm^3 (25% by a mass fraction) was diluted with water to 100 cm^3.

4.8. Sulfuric acid (H_2SO_4)

20 cm^3 of sulfuric acid (98% by a mass fraction) was added to 80 ml of water.

4.9. Titrated solution of potassium permanganate, $c(KMnO_4)$=0.004, mol/dm^3 ± 0.0001 mol/dm^3

The titer was checked according to the normal laboratory practice with oxalic acid or sodium oxalate.

Reagents and equipment: potassium permanganate solution ("0.05 n); 2 n sulfuric acid solution; standard 0.05 n oxalic acid solution (M_e=1/2M($H_2C_2O_4$)); burette; glass funnel; 100 ml conical flasks; Mohr pipettes; 50 ml glass cylinder; electric hotplate. Potassium permanganate solution is poured into a clean burette and prepared for titration. In a conical flask for the titration 10.00 ml of oxalic acid solution is selected with a Mohr pipette, 10 ml of 2 n sulfuric acid is added, the solution in the flask is heated to 70-80 °C (without boiling, in which the oxalic acid decomposes) and the hot solution is titrated with a solution of potassium permanganate. Potassium permanganate solution should be added slowly, drop by drop, while continuously shaking the solution. Each next drop is added only after the previous one has been discolored. At first, the decolorization of potassium permanganate will be slow, but as manganese (II) is formed, it will accelerate.

The titration is stopped when an excessive drop of potassium permanganate gives the solution a pale crimson color that does not disappear within 1-2 minutes. It is convenient to count by the upper edge of the burette because the lower one is hardly visible. The titration is repeated 2-3 times. From the converging counts, take the average titration result and calculate the normality (C_E) of potassium permanganate solution by the Equation 1:

$$N = C_E(KMnO4) = \frac{V(H_2C_2O_4)\times C_E(H_2C_2O_4)}{V(KM_nO_4)};\text{mol/l} \quad \text{(Eq.1)}$$

V – volumes of the corresponding solutions, ml.

5. Equipment and materials: common laboratory equipment was used, including the following.

5.1. Analytical balance capable of weighing with an accuracy of 0.01 g, with the possibility of reading to 0.001 g.

5.2. Volumetric flask with one mark with a capacity of 50 cm^3, according to A.S. Gorelik, O.V. Gorelik, and S.Y. Kharlap (2016), class A.

5.3. Pipette, 20 cm^3, according to Deen and co-authors (2019), class A.

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5.4. Centrifuge providing radial acceleration of 1,400 g.

5.5. Centrifuge tubes, cylindrical, with a round bottom, the capacity of approximately 30 ml^3, graduated to 20 cm^3.

5.6. Pipettes, with a capacity of 2 cm^3 and 5 cm^3, according to Deen and co-authors (2019), class A.

5.7. Suction device with a capillary tube.

5.8. Water bath capable of maintaining water at boiling point

5.9.Graduated burette with the graduation of 0.02 cm^3, according to Anisimova, Koshchaev, and Eremenko (2019), class A.

5.10. Ashless filter paper for slow filtration.

6. Sampling: sampling was not included in the method specified in this standard. The recommended sampling method was given in Ferreira *et al*. (2019). It was important to deliver a truly representative sample to the laboratory that had not been damaged or altered during transport or storage.

7. Preparation of the test sample: the test sample for milk or reconstituted milk was brought to a temperature of (20 ± 2)°C and mixed thoroughly. If homogeneous dispersion of fat was not formed, the sample was slowly heated to 40°C, then gently mixed by repeated overturn and cooled to a temperature of (20 ± 2)°C.

8. Procedure of analysis:

8.1.Test sample for analysis:

Approximately 20 g of the prepared test sample (see 7) was transferred into the volumetric flask (5.2) using the pipette (5.3). The sample was weighed to the nearest 0.01 g.

8.2. Definition:

8.2.1. Precipitation of protein substances: the trichloroacetic acid solution I (4.1) was gradually added, with shaking, to the test sample (8.1) until the volume of 50 $cm^3$was obtained. The test sample was shaken vigorously for a few seconds and left at rest for 30 min. The filter paper (5.10)was used for filtering; the filtrate had to be clear.

8.2.2. Precipitation of calcium as oxalate and separation of oxalate: 5 cm^3 of clear filtrate (8.2.1), 5 cm^3 of trichloroacetic acid solution II (4.2), 2 cm^3 of ammonium oxalate solution (4.3), two drops of methyl red solution (4.4) and 2 cm^3 of acetic acid solution (4.5)were added with a pipette (5.6) into a centrifuge tube (5.5) and stirred with shaking.

Ammonia solution I (4.6) was added dropwise to the solution obtained in the test tube until the color turned pale yellow. Then a few drops of acetic acid solution (4.5) were added until the color turned pink. The solution was left for 4 hours at room temperature. The contents of the centrifuge tube were diluted with water to 20 cm^3. The tube was centrifuged at 1,400 g for 10 min. The clear supernatant was removed from the centrifuge tube using the suction device (5.7). Next, the centrifuge tube walls were flushed with 5 cm^3 of ammonia II solution (4.7) to not affect the calcium oxalate precipitate. The tube was centrifuged at 1,400 g for 5 min. The supernatant was removed from the centrifuge tube using a suction device (5.7). The washing procedure was repeated twice.

8.2.3. Titration: 2 cm^3 of sulfuric acid (4.8) and 5 cm^3 of water were added to the calcium oxalate precipitate (8.2.2). The test tube was placed in a boiling water bath (5.8) to dissolve the calcium oxalate precipitate completely. Then, the dissolved calcium oxalate was titrated with the potassium permanganate solution (4.9) until the pink color remained. During the titration, the temperature of the solution should remain above 60°C. The volume (in cm^3) of potassium permanganate solution consumed was recorded to the nearest 0.01 cm^3.

8.2.4. Control experiment: in parallel with the sample testing, a control experiment was carried out using 20 cm^3 of water instead of a sample for analysis. The volume of potassium permanganate solution consumed was recorded, in cm^3, to the nearest 0.01 cm^3.

Milk productivity of cows was monitored using Afimilk milking equipment with a subsequent calculation for 305 days of lactation.

Average milk samples were taken from five cows from each experimental group using Afimilk software (https://www.afimilk.com/) devaluation milking equipment.

Physicochemical indicators (acidity, density, NFMS, fat, protein, ash, calcium, phosphorus) of milk were studied once a month.

In the average milk sample, the content of NFMS, fat, protein, and density were determined by the milk quality analyzer Laktan 1-4 model 220. The analyzer allowed determining the six most important parameters – protein, fat, NFMS,

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density, temperature, and mass fraction of added water – in a sample of fresh, canned, pasteurized, normalized, skimmed, reconstituted, and long-term storage milk without the use of chemical reagents for 130 seconds.

Acidity was determined by the titrimetric method. The method was based on titration of milk with an alkali solution (sodium or potassium hydroxide) in the presence of phenolphthalein indicator. It was necessary to measure 10 ml of milk and add 20 ml of distilled water and 3 drops of 1% phenolphthalein solution. Water was needed in order to more clearly see the pink tint during titration. The resulting mixture was stirred and titrated with a solution of 0.1 N sodium hydroxide until a faint pink color appeared, which did not disappear within 1 min. The analysis results were obtained by calculating the amount of alkali left for titration multiplied by 10. If milk had just been milked, it had an acidity index of 16-18 ºT. The acidity rose after two hours if milk had not been refrigerated. As microorganisms developed, the fermentation process took place, respectively, the acidity also increased. An increase in acidity leads to the fact that proteins became less resistant to heating. Therefore milk with the acidity of 21 ºT was off-grade, and with the acidity of 22 ºT, it was already on the verge of fresh and sour and could not be sold to processing plants.

Phosphorus in milk was determined by the spectrometric method (ISO 9874:2006).

1 The field of application.

This standard specifies a spectrometric method for the determination of the mass fraction of total phosphorus in milk.

2 Mass fraction of total phosphorus: Mass fraction of total phosphorus in milk as measured by the method specified in this standard and expressed as a percentage.

3 Essence of the method. The method was based on the destruction of organic substances in a milk sample under the action of sulfuric acid and hydrogen peroxide (wet mineralization) or under the action of high temperature (dry mineralization), the addition of a solution of sodium molybdate in ascorbic acid, the spectrometric determination of the optical density of the formed molybdenum blue at the wavelength of 820 nm and determination of the mass fraction of total phosphorus in milk according to the calibration graph.

4 Measurement instrumentation: Molecular absorption spectrometer, allowing measurements at a wavelength of 820 nm, permissible measurement error of transmission coefficient ± 1%, equipped with a cuvette with an optical path length of 10 mm; laboratory liquid non-mercury thermometer with a measurement range from 0°C to 150 °C and scale interval 0.5 °C; an electric circulating muffle kiln, capable of holding tests at temperatures between 500°C and 550°C; a thermostatically controlled water bath, capable of holding temperatures of (100±2)°C; a desiccator, capable of holding temperatures of (100±2)°C. An electric hotplate.

5 Utensils

Flasks, pipettes, cylinders, beakers, glass beads, 5 mm diameter; Kjeldahl flask for mineralization or test tubes of 50 cm^3 capacity.

6 Reagents: Monopotassium phosphate (KH_2PO_4), ascorbic acid, a solution of mass concentration 50 g/dm^3, sulphuric acid, a solution of mass fraction 50%; hydrochloric acid, a solution of mass concentration 36 g/dm, hydrogen peroxide in a solution of mass concentration 300 g/dm^3, free from phosphorus compounds; sodium molybdate dihydrate, mass fraction of basic substance - not less than 99,5%, insoluble substances - not more than 0,005%, phosphate - not more than 5 mln^{-1}; distilled water

7 Measurement conditions.

The following conditions are to be observed during measurements in the laboratory

ambient air temperature (20±5) °C

relative humidity (55±25)%;

(95±10)kPa atmospheric pressure.

8 Preparation for measurements

8.1 Before taking a sample for analysis, the milk shall be heated slowly to (40±2) °C, stirred gently, and cooled to (20±2) °C.

8.2 All glassware shall be washed thoroughly with phosphorus-free detergent and then with distilled water before use.

8.3 Preparation of reagents

8.3.1 A solution of sulphuric acid with a mass fraction of 50%: 278 cm^3 of concentrated sulphuric acid is carefully added, stirring the solution constantly, to 722 cm^3 of distilled water. The solution should be kept for no longer than 1 month at (20±5) °C in a dark glass container.

8.3.2 A solution of hydrochloric acid with a mass concentration of 36 g/dm^3 (intended for dry mineralization): 83 cm^3 of concentrated

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hydrochloric acid (1.19 g/cm^3) is placed in a 1000 cm^3 volumetric flask and carefully add distilled water.

The volume of the solution is topped up with distilled water to the mark. The solution is stored for no more than 1 month at (20±5) °C in a dark glass vial.

8.3.3 Sodium molybdate solution with mass concentration of 25 g/dm^3 - in a volumetric flask of 100 cm^3 contain (2.5000±0.0001) g of sodium molybdate dihydrate ($Na_2M_oO_4 \times 2H_2O$), add a solution of sulfuric acid (8.3.1) in an amount sufficient to dissolve the sodium molybdate crystals, stir it and then the volume of the solution is topped up to the mark with the same acid solution. The solution is stored in the refrigerator for not more than 7 days.

8.3.4 Ascorbic acid solution with mass concentration of 50 g/dm^3 - in a 100 cm3 volumetric flask, place (5.0000±0.0001) g of ascorbic acid and dissolve in a small amount of distilled water. The volume of the solution is brought to the mark with distilled water.

The solution shall be used freshly prepared.

8.3.5 Solution of sodium molybdate in ascorbic acid: The solution is prepared immediately before use. Pour 10 cm^3 of ascorbic acid solution (see 8.3.4) into a 100 cm^3 volumetric flask and add 25 cm3 of sodium molybdate solution (see 8.3.3) small amount of distilled water, and mix. Add distilled water to the mark.

8.3.6 Standard solution of phosphorus A, containing 100 mg of phosphorus in 1 dm^3: In a 50 cm^3 beaker, weigh (1.0±0.1) g of monosodium phosphate, place in a desiccator, and dry for at least 48 hours. In a 1000 cm^3 volumetric flask, add (0.4394±0.0001) g of dried potassium phosphate, and add a small amount of distilled water and mix.

The volume of the solution is topped up to the mark with distilled water. The mass concentration of phosphorus in solution A is 100 μg/cm^3.

The solution shall be used freshly prepared.

8.3.7 A standard solution of phosphorus B containing 10 mg of phosphorus in 1 dm^3:

Into a 100 cm^3 volumetric flask, add 10 cm^3 of phosphorus A standard solution (see 8.3.6) by pipetting, add a small amount of distilled water, and mix. The volume of the solution is topped up to the mark with distilled water. The mass concentration of phosphorus in solution B is 10 μg/cm^3. The solution is to be used freshly prepared.

9 Conducting the measurements

9.1 Wet saline method

9.1.1 Place (1,500±0,001) g of the milk prepared according to 8.1 into a flask for mineralization. Add three glass beads and 4 cm^3 of concentrated sulfuric acid.

9.1.2 Place the flask in an inclined position in a well-ventilated fume cupboard and heat it over an electric hotplate. Keep the foaming in the flask to a minimum during heating.

Keep the flask at a gentle boil. Local overheating and heating of the flask above the liquid level is not allowed.

9.1.3 Once the flask has stopped foaming, and it shall be cooled in the air to a temperature of (20 ± 2)°C. Carefully add 2 cm^3 of hydrogen peroxide solution and heat again. Repeat this procedure until the contents of the flask become clear and colorless. During heating, periodically stir the flask contents by gently turning the flask to avoid local overheating.

9.1.4 Cool the mixture in the air to (20±2) °C and wash the neck of the flask with about 2 cm^3 distilled water. Heat the contents of the flask again until the water has evaporated. Boil the liquid for (30±1) min to eliminate all traces of hydrogen peroxide. Local overheating is not allowed.

9.1.5 Cool the mixture in the air to a temperature of (20±2) °C. Next, quantify the mixture in a 100 cm^3 volumetric flask and add distilled water to the volume of solution in the flask.

9.1.6 With a pipette, take 2 cm^3 of the mixture into a 50 cm^3 volumetric flask and add about 25 cm^3 of distilled water. Then add 2.0 cm^3 of sodium molybdate solution in ascorbic acid (see 8.3.5) to the flask, stir and bring the volume of the solution in the flask to the mark with distilled water.

9.1.7 Boil the contents of the flask for 15 min in a water bath.

9.1.8 Cool the flask with the mixture to a temperature of (20±2) °C in cold water. Then carry out the tests according to 9.5. The mixture shall be testable for 1 hour.

9.2 Dry mineralization method

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9.2.1 Place (10,000±0.001) g of the milk prepared according to 8.1 in a platinum or quartz crucible.

9.2.2 Evaporate the sample to dryness in a desiccator at (100±2)°C or in a water bath.

9.2.3 The test sample is calcined in a muffle furnace at 500 °C to 550 °C until white (or almost white) ash is formed.

Preferably, before placing the crucible in the muffle furnace, heat it on an electric cooker to burn off the flammable components.

9.2.4 The crucible with its contents is cooled together with the muffle furnace and then covered with a watch glass. Dissolve the ashes in a 2 to 3 cm^3 solution of hydrochloric acid (see 8.3.2) and add about 3 cm^3 of distilled water.

9.2.5 Transfer the ash solution to a 100 cm^3 volumetric flask by rinsing the watch glass and crucible with distilled water and discarding the washing water in the flask. Add the volume of the solution in the flask to the mark with distilled water. Filter the solution through filter paper.

9.2.6 Using a pipette, pour 10 cm^3 of the filtrate into a 100 cm^3 volumetric flask. Add the volume of the solution in the flask to the mark with distilled water.

9.2.7 Pour 2 cm^3 of the filtrate solution into a 50 cm^3 volumetric flask and add 25 cm^3 distilled water. Then add 2.0 cm^3 sodium molybdate in ascorbic acid (see 8.3.5). Add the volume of the solution in the flask to the mark with distilled water.

9.2.8 Boil the contents of the flask in a water bath for 15 min.

9.2.9 Cool the flask with the mixture by placing it in a bath of running water to a temperature of (20±2)°C. Then carry out the tests according to 9.5. The mixture is suitable for measurements for 1 hour.

9.3 Control measurement

Simultaneously with the measurement of the sample, a control measurement is carried out using the same procedure as for the test sample (see 9.1 or 9.2) but with 1.5 cm^3 or 10 cm^3 respectively of phosphorus-free distilled water instead of the test sample.

9.4 Drawing a graduation chart

9.4.1 Pour 0, 1, 2, 3, and 5 cm^3 respectively of phosphorus B standard solution into five 50 cm^3 volumetric flasks.

9.4.2 Add 2.0 cm^3 of sodium molybdate solution in ascorbic acid to the contents of each volumetric flask (see 8.3.5). Next, add the volume of each solution in the flask to the mark with distilled water. The prepared solutions contain 0, 10, 20, 30, and 50 μg of phosphorus, respectively.

9.4.3 The contents of the flasks are boiled in a water bath for (15±1) min.

9.4.4 Cool the solutions to a temperature of (20±2)°C in cold water. For 1 h, measure with a spectrometer equipped with a cuvette at 820 nm the optical density of each calibration solution compared to the phosphorus-free solution (see 9.4.2). If the optical density of the phosphorus-free solution in 50 cm^3 of the solution is high, check the reagents.

9.4.5 Plot the chart of the corresponding value of the optical density on the mass in micrograms of phosphorus contained in the calibration solutions (see 9.4.2).

9.5 Spectrometric measurement

Measure the optical density of the cooled mixtures according to 9.1.8 and 9.2.9 on a spectrometer at a nominal value of 820 nm relative to the control sample (see 9.3).

10 Processing of measurements

10.1 The mass of phosphorus corresponding to the measured optical density of the test solution shall be determined from the calibration chart.

The mass fraction of total phosphorus (W) in the sample, %, is calculated using equations 2 and 3:

a) wet mineralization method

$$W = \frac{m_1 100}{200 m_0}, \qquad \text{(Eq. 2)}$$

b) dry mineralization method

$$W = \frac{m_1 100}{20 m_0}, \qquad \text{(Eq. 3)}$$

where W - mass of phosphorus determined from the calibration curve, μg;

200 and 20 - sample dilution factor;

m_0 - sample weight, g.

The final result is the arithmetic mean of the results of two parallel measurements, rounded to the third decimal place.

The water content in milk was determined by Equation 5:

$$B = 100 - C, \qquad \text{(Eq. 5)}$$

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where C was the dry matter (%), and B was the water content in milk (%). Determination of the presence of the studied indicators. Fluctuations of the dry matter in milk in cows were in the range of 11.3-14.5%. A glass weighing bottle with 20-30 g of well-washed and calcined sand and a glass rod that did not protrude beyond the edges of the bottle was placed in the drying cabinet and kept at (102 ± 2)^{0}C for 30-40 minutes. After that, the weighing bottle was removed from the drying cabinet, covered with a lid, cooled in a desiccator for 40 minutes, and weighed with an error of not more than 0.001 g. 10 cm^3 of milk was added to the same weighing bottle with a pipette, covered with a lid and immediately weighed. The contents were thoroughly mixed with a glass rod, and the open weighing bottle was heated in a water bath, with repeated stirring of the contents until a crumbling mass was obtained. Then the open weighing bottle and the lid were placed in the drying cabinet with a temperature of (102 ± 2) ºC. After 2 hours, the weighing bottle was removed from the drying cabinet, covered with the lid, cooled in the desiccator for 40 minutes, and weighed. Subsequent weighings were carried out after drying for 1 hour until the difference between two successive weighings was equal to or less than 0.001 g. If an increase in mass was found during one of the weighings after drying, the previous weighing results were taken for calculations. The mass fraction of dry matter (c) in percent was calculated by Equation 4:

$$C = \frac{m_1 - m_0}{m - m_0} \times 100 \qquad \text{(Eq. 4)}$$

where $m_0$was the mass of a weighing bottle with sand and a glass rod, g;

m was the mass of a weighing bottle with sand and a glass rod and a weighed portion of the test sample before drying, g;

$m_1$was the mass of a weighing bottle with sand and a glass rod and a test sample after drying, g;

The discrepancy between parallel determinations had to be no more than 0.1%. The arithmetic mean of two parallel determinations was taken as the final result.

11. Determination of ash in milk.

Determination technique.

1. Temper the porcelain crucible in a muffle furnace, cool it in a desiccator, and weigh it on an analytical scale. Then weigh about 25 g of milk into the crucible or measure this quantity with a pipette. In the latter case, correct the density of the milk.
2. Evaporate the milk to dryness in a water bath or desiccator and char the dry residue over a low flame. At the end of the emission of smoke, intensify the heating and continue until a dark grey residue is obtained.
3. Cool the crucible, add 15 mL distilled water, and heat gently. Then filter through an ash-free filter. Treat the residue in the crucible 2-3 times with small portions (5-6 mL each) of hot water and transfer to a filter.
4. After filtration, the filter is transferred into a crucible with the undissolved residue, dried in the desiccator and further, calcined in a muffle furnace or on high heat until greyish white ash is obtained in the crucible.
5. Pour the filtrate obtained by washing the precipitate into a crucible with the ash, evaporate to dryness, and incinerate over low heat.
6. Cool the crucible in a desiccator; weigh it on an analytical scale, and calculate the amount of ash in the milk according to the formula 6:

$$A = \frac{c - a}{b - a} \times 100 \qquad \text{(Eq. 6)}$$

where A is the amount of ash in the milk (%); a is the mass of the empty crucible (g); b is the mass of the crucible with the milk (g); c is the mass of the crucible with the ash (g).

Preparation of samples for analysis:

1. Using a microdoser, take 5 ml of milk into a vial and add 5 ml of HCl hydrochloric acid (36%).
2. Close the vial tightly with an iron lid and place it in a desiccator at t=+110ºC for 16-20 hours for hydrolysis.
3. The resulting hydrolysate should be filtered through a paper filter.
4. Evaporate 0.5 ml of filtered hydrolysate with a hairdryer to a dry residue.
5. Add 1 ml of buffer-2,2 pH to the dry residue to dilute the sample.
6. The dilution occurs twice. Transfer the tube (Eppendorf) filled sample to the amino acid analyzer for analysis.

Collection and processing of the chromatographic data were done using the software Multichrome (https://multichrom.ru/).

2.4. Experimental Procedures

The NFMS, fat, protein, and density were

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determined in an average milk sample using a Lactan 1-4 model 220 milk quality analyzer (https://sibagropribor.ru/en/).

Acidity was determined by titration of milk with alkaline solution (sodium or potassium hydroxide) in the presence of phenolphthalein indicator.

The essence of the ash determination method is to burn a sample of milk and weigh the resulting ash.

The spectrometric method of determining the mass fraction of total phosphorus is based on the destruction of organic substances of milk sample under the influence of sulfuric acid and hydrogen peroxide (wet mineralization) or under the influence of high temperature (dry mineralization), adding sodium molybdate solution in ascorbic acid, spectrometric determination of the optical density of the formed molybdenum blue at wavelength 820 nm, and determination of the mass fraction of total phosphorus in milk using the graduation chart (ISO 9874|IDF 42:2006).

The titrimetric method of calcium determination is based on the precipitation of calcium by ammonium oxalate in the filtrate obtained after precipitation of milk proteins by trichloroacetic acid, followed by titrimetric determination of the mass fraction of calcium (ISO 12081|IDF 36:2010).

The amino acid composition of milk was determined by high-performance liquid chromatography (HPLC) using an LC-10 chromatograph (Shimadzu) with a fluorometric detector and precolumn derivatization (DataApex, Clarity, 2020).

2.5. Ethics

The research was approved under the regulations of Kuban State Agrarian University, and the data collected was published for scientific and research purposes only.

2.6. Statistics

The data were statistically processed: (M) - arithmetic mean;σ - sigma, standard deviation; Cv - coefficient of variation; m - error of arithmetic mean; td - criterion of reliability.

Experimental data were biometrically processed using Microsoft Excel, to which the presented formulas were added:

$$M = \frac{\sum V}{n}; \quad \text{(Eq. 7)}$$

$\sum V$ – is the sum of the indicators for the studied animals;

n - number of studied animals.

$$\sigma = \frac{Vmax - Vmin}{K}; \quad \text{(Eq. 8)}$$

K – coefficient of sigma calculation according to the number of studied animals in the group;

$Vmax$ – the highest index;

$Vmin$ – the lowest index.

$$Cv = \frac{\sigma * 100}{M}; \quad \text{(Eq. 9)}$$

$$m = \frac{\sigma}{\sqrt{n}}; \quad \text{(Eq. 10)}$$

$$td = \frac{M1 - M2}{\sqrt{m1^2 + m2^2}}; \quad \text{(Eq. 11)}$$

$M1 - M2$ and $m1$ and $m2$ – the compared values by the group.

Suppose td criterion is equal to or greater than 2 (td ≥ 2), corresponding to infallible probability P (probability significance level) equal or greater than 95% (P ≥ 95%). In that case, the difference should be considered credible (significant), i.e., conditioned by some factor that would be valid in general. If td < 2, the probability of error-free prediction P < 95% means that the difference is unreliable, random, i.e., not due to any regularity (not due to the influence of any factor).

3. RESULTS AND DISCUSSION:

3.1 RESULTS

The study aimed at researching the effect of various doses of dairy feed on the growth and development of replacement heifers, subject to adding 3 g of the probiotic product Cellobacterin per heifer from the experimental groups since the age of ten days on an everyday basis. The positive effect of the enhanced doses of whole milk and the probiotic product Cellobacterin was determined.

The cows from the experimental groups gave more milk than those from the control group during lactation III: II and Ist groups – by 700 kg, III and I – by 1860 kg, and IV and I – by 1560 kg (Table 2).

According to three lactations, the dynamics of changes in the milk production of the cows and the physicochemical composition of

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milk were studied. Table 1 shows the change in the milk yield and the physicochemical properties of milk.

The milk yield of the experimental cows for the I lactation in the control group was 6,590 kg. Milk yield per lactation was determined using Afimilk milking equipment. In the experimental groups, the milk yield was as follows: II – 7,120 kg, III – 7,860 kg, and IV – 7,720 kg, respectively. Moreover, the milk yield was higher by 7.4 % in the II experimental group than in the control group, by 16.2 % in the III one, and by 14.6 % in the IV one; accordingly, for the II lactation – by 8.4 %, by 18.5 % in the III group, by 16.9 % in the IV group, and similarly for the III lactation – by 8.8 %, by 20.4 % in the III group, and by 17.7 % in the IV group.

The milk productivity of the cows from the control group increased during the II lactation when compared with the I lactation – by 260 kg (3.8 %), II – by 360 kg (4.8 %), III – by 540 kg (6.4 %), and IV – by 520 kg (6.3 %); respectively, during the III lactation – by 680 kg (9.4 %), II – by 850 kg (10.7 %), III – by 1,270 kg (13.9 %) and IV – by 1,110 kg (12.6 %).

When studying the physicochemical composition of milk by lactation, it was determined that the acidity of the milk of the animals from the experimental groups was within the standard. The acidity norm of whole milk was 16-21ºT. The statistical differences by the acidity of milk from lactation I to III in groups were not significant – $P < 0.95$.

The water content in the milk of the cows from the experimental groups was slightly less than the control one. The water content in the milk of the cows from lactation I to III decreased. The fluctuations were within 0.1 %. These differences from lactation I to III with the animals from the experimental groups were not significant – $P < 0.95$.

During the lactation, the dry matter amount changed and depended on feeding, age, and other factors. The nutritional content of milk depends on the content of dry matter and dry skimmed milk rest. The content of fat in milk slightly increased in the experimental groups as compared to the control one.

The content of fat in milk slightly increased in the experimental groups as compared to the control one. The fat content in the milk of the cows from group III was higher than the herd mates. The differences in fat content in the milk from lactation I to III were not significant in the groups – $P < 0.95$.

The protein content was slightly higher with the cows from group IIIthan the herd mates from groups I, II, and IV. The differences in protein content in the milk from lactation I to III were not significant – $P < 0.95$.

The indicators for the cows from group III were a bit higher as compared to their herd mates. The calcium and phosphorus content differences in the milk from lactation I to III were not significant – $P < 0.95$.

The cows from the experimental groups had slightly better indicators of the chemical composition of milk as compared to the control group.

Table 3 shows the change in fat content in the milk of the experimental cows by months of lactation.The qualitative composition of the milk of the experimental cows made it possible to determine the changes in fat content in the milk of the experimental cows during the lactation.

The cows from groups II, III, and IV surpassed their herd mates from control group I by the fat content in the milk during three lactations. There was an increase in the content of fat in the milk from lactation I to III in the experimental groups. As compared to the control group, the difference between the groups during lactation I was as follows: II and I – by 0.03 %; III – I – by 0.08 %;IV – I – by 0.06 %. In the III lactation, the difference was, respectively: II and I – by 0.04 %, III – I – by 0.11 %, and IV – I – by 0.09 %

The cows from group III had a slightly higher fat content than their experimental herd mates. This was because the intensive heifer breeding methods positively affected both the milk production of cows and its properties.

Table 4 contains the data on the protein content in the milk by months of lactation. The provided data indicate that the protein content in the milk differs both between the groups and between the lactations. The protein content in the milk for lactation I between the groups of the cows from the experimental groups (II, III, and IV) was higher compared to control group I, but the differences were insignificant – $P < 0.95$.

The decrease in the average value of protein for lactation I with the experimental cows between groups is different as compared to the fat content of milk: III – I – by 2.5 %, III-II – by 1.5 %, IV – III and IV – I – by 1.2 %, IV-II – by 0.3 %, and II – I – 0.9 %. Analyzing the protein content in the milk during lactation II and III, it was

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determined that the cows from group III surpassed their herd mates from the control group. There was an enhanced protein content in the milk from lactation I to III in the experimental groups compared to the control group. The difference for lactation III was as follows: I and II – by 0.05 %, I – III – by 0.11 %, and I – IV – by 0.09 %.

The cows from group III raised using intensive technology slightly surpassed their herd mates from the experimental groups in protein content for III lactations. However, the differences were not significant – P < 0.95.

The fat in the milk of cows was a thin emulsion with a considerable amount of tiny fat globules. The fat globules were surrounded by a protein-lecithin shell distributed evenly among the aqueous portion of the milk. After the milk gravity separation, the fat part was located on the top because the specific density of fat was less than water. Table 5 shows the data on the content of milk fat.

It was determined that the fat content in the milk of the cow differed between groups and in lactation. In terms of the fat content in milk, in lactation, I the cows from experimental groups II, III, and IV surpassed their herd mates from the control group.

The milk fat content in lactation II increased in groups I and II for up to three months and in groups III and IV for up to four months. Then the milk fat decreased by the 10th month of lactation. However, the differences were not significant – P < 0.95. Thus, in terms of milk fat content, the cows from the experimental groups surpassed the control one.

It was determined for lactation I to III by groups that the milk fat content in the milk of the cows from group I had increased by 26.2 kg (9.5 %) and 26.0 kg (8.9 %) for the herd mates from group II. The highest difference was with the cows from group III. It was 39.2 kg (11.9 %). The analogs of group IV, according to the indicator, understudy took the intermediate position between groups I, II, III – by 34.1 kg (10.6 %).

The obtained data indicate that in terms of fat content in the milk, the cows from the experimental groups in III lactations surpassed their herd mates from the control group, which indicates the efficiency of the methods used when raising heifers.

The determined differences in the milk protein content in the cow's milk for the analyzed lactations differ both between groups and by lactations (Table 6).

The cows from the experimental groups exceeded their herd mates from the control group by milk protein content. It was determined that the milk protein content in the milk had increased from lactation I to III. The difference betweengroups was as follows: I – by 30.4 kg (12.4 %), II – by 32.3 kg (12.3 %), III – by 47 kg (15.2 %), and IV – by 42.9 kg (14.4 %). The milk protein content in the milk from lactation I to II increased in the experimental groups compared to the control group of lactation I: I and II – by 18.3 %, I – III – by 30.4 %, and I – IV – by 27.6 %.

The cows from the experimental groups that received the enhanced daily milk and the probiotic product when raised surpassed their herd mates from the control group by the milk protein content in III lactations. It was determined that the control group cows were inferior to the analogs from the experimental groups by the content of milk fat and milk protein.

The data presented in Table 5 indicate the superiority of the animals from the experimental groups over the control one by the indicator under study. This was a consequence of the efficient breeding of heifers using intensive technology involving the Cellobacterin probiotic product.

Tables 7 and 8 show the composition of amino acids in the milk by groups of cows. The essential amino acids cannot be synthesized in mammals and must come from food. When raising young mammals, there are metabolic disorders if at least one essential amino acid is missing.

Calculation and expression of results: Calculation: the calcium content, W_{Ca}, wt%, was calculated according to Equation12:

$$W_{Ca} = 0.0004(V - V_0) \times \frac{1000f}{m} = 0.4(V - V_0) \times \frac{f}{m}$$ (Eq. 12)

where V was the volume of potassium permanganate solution consumed during the titration of a sample for analysis (see 8.2.3); V_0 was the volume of potassium permanganate solution consumed in the control experiment (see 8.2.4); m was the mass of the sample for analysis, g; ʃ was the correction factor given in Table 9 for the volume of sediment obtained by precipitation with trichloroacetic acid.

Table 9. *Correction factor ʃ as a function of fat content in a sample*

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Content of fat in a sample,wt%	Correction factor
3.5-4.5	0.972
3	0.976
2	0.980
1	0.985
<0.1	0.989

Expression of results: the result was expressed to the third decimal place.

3.2 DISCUSSION

Considering the total content of nonessential amino acids in the milk of the cows from lactation I to III, it was determined that by the content of replaceable amino acids in their milk, the cows in group III surpassed their herd mates in the I control group, as well as from the II and IV experimental groups.

Regarding the content of essential amino acids in the milk, the control group cows were inferior to the herd mates from the experimental groups for all lactations under analysis.

The provided data indicate a consistent increase in the total number of essential and nonessential amino acids in all experimental groups of cows from lactation I to III.

Considering the data from Tables 6 and 7, there is an increase in essential amino acids in lactations. Compared to their experimental herd mates, the enhanced content of essential amino acids in the milk of cows from group III was determined. This is because they had been raised intensively from their birth till 18 months and had been given the probiotic product.

The determined superiority of the cows from group III by the total content of essential and nonessential amino acids as compared to their herd mates confirms that the intensive growth of young animals had a positive effect on the amino acid composition of the milk of the cows from the experimental groups as compared to the control one.

The amino acid index determines the ratio of essential amino acids to nonessential ones. The higher it is, the higher the nutritional value of milk is. It characterizes the nutritional and biological usefulness of milk. The highest amino acid index was determined for groups III and IV. Statistically, these differences are of low quality – $P < 0.95$.

4. CONCLUSION:

The cows from group III have exceeded their herd mates from the experimental groups and the control one regarding the indicators under study.

During the I lactation, higher milk yield was obtained from the III group cows compared to the cows from the other experimental groups.

The replacement heifers from the experimental groups surpassed their herd mates from the control group by growth and development. They reached economic maturity earlier.

It has been established that intensive rearing of replacement heifers using Cellobacterin has positively impacted the development of their gastrointestinal tract, which is confirmed by the results of conducted research. It has been found that cows of the III experimental group surpassed the cows of the other experimental groups in terms of milk productivity and the physicochemical properties of milk.

The study of the physicochemical properties of milk makes it possible to conclude that intensive raising of replacement heifers using enhanced milk feed rates and the Cellobacterinprobiotic product allowed obtaining milk with better physicochemical properties from the cows in the experimental groups as compared to the ones in the control group.

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Table 1. *Scheme of the experiment*

Group	n	Scheme of technology for raising Holstein cattle
I control	16	Up to 6 months of age - traditional (T): 200 kg of milk (in 50 days) and 400 kg of swill (from 50 to 110 days); pre-starter (50%) with maize (50%). From 7 to 16 months of age - rearing, insemination.
II experimental	16	Up to 6 months of age - increased milking rate (in 25 days) of 200 kg and 400 kg of swill (from 25 to 60 days); pre-starter (50%) with maize (50%); probiotic "Cellobacterin" - 3 g/goal daily (before insemination). From 7- to 15-months of age - intensive rearing (IR), insemination.
III experimental	16	p to 6 months of age, increased rate of milking (in 50 days) - 450 kg and milk (from 50 to 110 days) - 600 kg of swill; pre-starter (50%) with maize (50%); probiotic "Cellobacterin" - 3 g/goal daily (before insemination). From 7 to 14 months of age - (IR), insemination/
IV experimental	16	Up to 6-months of age increased milking rate (in 60 days): 450 kg of milk (60 to 120 days); heifers: 600 kg, steers: 800 kg; pre-starter (50%) with maize (50%); "Cellobacterin" probiotic: 3 g/head daily (before insemination). From 7 to 14 months of age - (IR), insemination.

Table 2. *Cow Productivity and Physicochemical Composition of Milk for 305 Lactation Days (n = 5)*

Indicator	Group			
	I	II	III	IV
Lactation I				
Milk yield, kg	6590 ± 200.0	7120 ± 144.8	7860 ± 334.2	7720 ± 281.7
Acidity, °T	16.82 ± 0.2	16.79 ± 0.3	16.49 ± 0.2	16.53 ± 0.2
Density, °A	30.08 ± 0.4	29.87 ± 0.5	29.83 ± 0.5	29.85 ± 0.5
Water, %	88.21 ± 0.3	87.93 ± 0.4	87.25 ± 0.4	87.44 ± 0.5
Dry matter, %	11.79 ± 0.2	12.07 ± 0.2	12.75 ± 0.3	12.56 ± 0.2
Nonfat milk solids, %	9.05 ± 0.3	8.95 ± 0.3	8.87 ± 0.4	8.89 ± 0.3
Fat, %	3.75 ± 0.1	3.74 ± 0.1	3.65 ± 0.09	3.70 ± 0.1
Protein %	3.22 ± 0.2	3.25 ± 0.1	3.29 ± 0.1	3.27 ± 0.1
Ash, %	0.68 ± 0.09	0.73 ± 0.07	0.79 ± 0.06	0.76 ± 0.05
Calcium, %	0.145 ± 0.004	0.146 ± 0.005	0.149 ± 0.005	0.148 ± 0.004
Phosphorus, %	0.1045 ± 0.001	0.1047 ± 0.001	0.1048 ± 0.001	0.1048 ± 0.0008
Lactation II				
Milk yield, kg	6850 ± 129.5	7480 ± 151.4	8400 ± 348.2	8240 ± 329.1
Acidity, °T	16.78 ± 0.2	16.72 ± 0.3	16.47 ± 0.3	16.50 ± 0.2
Density, °A	30.01 ± 0.4	29.83 ± 0.2	29.80 ± 0.3	29.82 ± 0.4
Water, %	88.15 ± 0.5	87.86 ± 0.4	87.19 ± 0.4	87.35 ± 0.4
Dry matter, %	11.85 ± 0.3	12.14 ± 0.3	12.81 ± 0.3	12.65 ± 0.2
Nonfat milk solids, %	9.01 ± 0.2	8.90 ± 0.2	8.81 ± 0.2	8.83 ± 0.3
Fat, %	3.72 ± 0.1	3.70 ± 0.2	3.64 ± 0.1	3.69 ± 0.1
Protein %	3.25 ± 0.2	3.28 ± 0.2	3.31 ± 0.2	3.30 ± 0.1
Ash, %	0.69 ± 0.07	0.73 ± 0.08	0.80 ± 0.07	0.79 ± 0.05
Calcium, %	0.146 ± 0.005	0.147 ± 0.003	0.151 ± 0.003	0.150 ± 0.006
Phosphorus, %	0.1047 ± 0.002	0.1049 ± 0.002	0.1052 ± 0.003	0.1051 ± 0.002
Lactation III				
Milk yield, kg	7270 ± 184.6	7970 ± 208.3	9130 ± 407.9	8830 ± 330.5
Acidity, °T	16.72 ± 0.2	16.68 ± 0.2	16.43 ± 0.2	16.47 ± 0.3
Density, °A	29.95 ± 0.5	29.76 ± 0.5	29.72 ± 0.4	29.75 ± 0.4
Water, %	88.11 ± 0.4	87.81 ± 0.5	87.15 ± 0.4	87.31 ± 0.5
Dry matter, %	11.89 ± 0.3	12.19 ± 0.3	12.85 ± 0.2	12.69 ± 0.3
Nonfat milk solids, %	8.98 ± 0.2	8.88 ± 0.3	8.78 ± 0.2	8.80 ± 0.2
Fat, %	3.71 ± 0.2	3.69 ± 0.1	3.62 ± 0.1	3.65 ± 0.1
Protein %	3.29 ± 0.1	3.33 ± 0.08	3.39 ± 0.05	3.37 ± 0.07
Ash, %	0.70 ± 0.03	0.74 ± 0.03	0.84 ± 0.02	0.83 ± 0.03
Calcium, %	0.147 ± 0.006	0.149 ± 0.003	0.153 ± 0.006	0.152 ± 0.007
Phosphorus, %	0.1048 ± 0.001	0.1051 ± 0.002	0.1054 ± 0.001	0.1053 ± 0.002

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Table 3. *Content of Fat in Milk of the Experimental Cows, % (n = 5)*

Group	Lactation month										Average fat content per lactation
	1	2	3	4	5	6	7	8	9	10	
Lactation I											
I	3.64 ± 0.02	3.65 ± 0.05	3.70 ± 0.04	3.74 ± 0.03	3.77 ± 0.03	3.59 ± 0.02	3.54 0.02	3.53 ± 0.02	3.50 ± 0.02	3.35 ± 0.02	3.60
II	3.69 ± 0.02	3.70 ± 0.03	3.71 ± 0.04	3.76 ± 0.02	3.80 ± 0.02	3.64 ± 0.02	3.60 ± 0.02	3.54 ± 0.02	3.51 ± 0.02	3.32 ± 0.02	3.63
III	3.74 ± 0.02	3.89 ± 0.02	3.88 ± 0.01	3.83 ± 0.02	3.78 ± 0.02	3.70 ± 0.02	3.62 ± 0.02	3.59 ± 0.02	3.50 ± 0.02	3.31 ± 0.01	3.68
IV	3.72 ± 0.02	3.81 ± 0.03	3.84 ± 0.02	3.86 ± 0.02	3.75 ± 0.02	3.67 ± 0.02	3.59 ± 0.02	3.58 ± 0.01	3.51 ± 0.02	3.31 ± 0.02	3.66
Lactation II											
I	3.67 ± 0.02	3.71 ± 0.03	3.76 ± 0.03	3.78 ± 0.02	3.67 ± 0.03	3.62 ± 0.02	3.59 ± 0.02	3.55 ± 0.02	3.52 ± 0.02	3.31 ± 0.02	3.62
II	3.73 ± 0.02	3.80 ± 0.02	3.86 ± 0.02	3.86 ± 0.02	3.75 ± 0.02	3.67 ± 0.03	3.60 ± 0.02	3.58 ± 0.02	3.51 ± 0.02	3.26 ± 0.02	3.66
III	3.76 ± 0.02	3.84 ± 0.02	3.90 ± 0.01	3.89 ± 0.02	3.79 ± 0.02	3.71 ± 0.02	3.62 ± 0.02	3.61 ± 0.02	3.56 ± 0.02	3.27 ± 0.02	3.70
IV	3.74 ± 0.02	3.81 ± 0.02	3.88 ± 0.02	3.85 ± 0.02	3.76 ± 0.02	3.67 ± 0.02	3.62 ± 0.02	3.59 ± 0.02	3.52 ± 0.02	3.35 ± 0.02	3.68
Lactation III											
I	3.69 ± 0.02	3.73 ± 0.04	3.78 ± 0.03	3.80 ± 0.02	3.69 ± 0.03	3.63 ± 0.02	3.61 ± 0.02	3.56 ± 0.02	3.52 ± 0.02	3.28 ± 0.02	3.63
II	3.75 ± 0.02	3.79 ± 0.03	3.84 ± 0.02	3.85 ± 0.02	3.76 ± 0.02	3.69 ± 0.03	3.67 ± 0.03	3.59 ± 0.02	3.54 ± 0.02	3.24 ± 0.03	3.67
III	3.80 ± 0.02	3.90 ± 0.01	3.86 ± 0.01	3.88 ± 0.02	3.83 ± 0.02	3.75 ± 0.02	3.69 ± 0.02	3.64 ± 0.02	3.60 ± 0.02	3.40 ± 0.02	3.74
IV	3.81 ± 0.02	3.85 ± 0.03	3.88 ± 0.02	3.87 ± 0.02	3.85 ± 0.02	3.72 ± 0.02	3.70 ± 0.02	3.61 ± 0.02	3.60 ± 0.02	3.27 ± 0.02	3.72

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Table 4. *Content of Protein in Milk of the Experimental Cows, % (n = 5)*

Group	Lactation month										Average content of protein in milk per lactation
	1	2	3	4	5	6	7	8	9	10	
Lactation I											
I	3.25 ± 0.02	3.48 ± 0.04	3.39 ± 0.03	3.39 ± 0.03	3.33 ± 0.02	3.20 ± 0.04	3.22 ± 0.03	3.21 ± 0.03	2.79 ± 0.03	2.67 ± 0.03	3.19
II	3.21 ± 0.02	3.29 ± 0.03	3.50 ± 0.04	3.40 ± 0.03	3.41 ± 0.02	3.35 ± 0.02	3.26 ± 0.03	3.25 ± 0.03	2.83 ± 0.02	2.69 ± 0.03	3.22
III	3.30 ± 0.02	3.31 ± 0.03	3.33 ± 0.02	3.39 ± 0.03	3.44 ± 0.02	3.42 ± 0.04	3.31 ± 0.03	3.28 ± 0.02	2.99 ± 0.03	2.88 ± 0.03	3.27
IV	3.30 ± 0.02	3.29 ± 0.03	3.29 ± 0.02	3.33 ± 0.02	3.40 ± 0.02	3.41 ± 0.04	3.30 ± 0.03	3.24 ± 0.03	2.91 ± 0.03	2.82 ± 0.02	3.23
Lactation II											
I	3.33 ± 0.02	3.35 ± 0.03	3.44 ± 0.02	3.43 ± 0.02	3.35 ± 0.02	3.26 ± 0.02	3.14 ± 0.02	3.13 ± 0.02	3.10 ± 0.02	2.72 ± 0.02	3.23
II	3.34 ± 0.02	3.37 ± 0.03	3.46 ± 0.02	3.45 ± 0.02	3.36 ± 0.03	3.28 ± 0.02	3.19 ± 0.02	3.15 ± 0.03	3.14 ± 0.02	2.77 ± 0.04	3.25
III	3.36 ± 0.03	3.39 ± 0.03	3.46 ± 0.03	3.48 ± 0.03	3.38 ± 0.03	3.32 ± 0.02	3.24 ± 0.02	3.23 ± 0.02	3.17 ± 0.02	2.91 ± 0.03	3.29
IV	3.36 ± 0.02	3.38 ± 0.02	3.45 ± 0.02	3.47 ± 0.02	3.37 ± 0.03	3.28 ± 0.02	3.20 ± 0.02	3.21 ± 0.02	3.15 ± 0.02	2.87 ± 0.03	3.27
Lactation III											
I	3.37 ± 0.02	3.38 ± 0.02	3.39 ± 0.02	3.39 ± 0.03	3.38 ± 0.02	3.37 ± 0.02	3.21 ± 0.02	3.19 ± 0.03	3.16 ± 0.02	2.80 ± 0.04	3.26
II	3.41 ± 0.02	3.42 ± 0.02	3.44 ± 0.03	3.47 ± 0.02	3.40 ± 0.02	3.39 ± 0.02	3.23 ± 0.03	3.22 ± 0.02	3.18 ± 0.02	2.89 ± 0.02	3.31
III	3.42 ± 0.03	3.43 ± 0.02	3.43 ± 0.03	3.51 ± 0.03	3.52 ± 0.03	3.41 ± 0.03	3.38 ± 0.03	3.33 ± 0.02	3.19 ± 0.02	3.10 ± 0.04	3.37
IV	3.40 ± 0.02	3.42 ± 0.03	3.42 ± 0.03	3.50 ± 0.03	3.52 ± 0.03	3.40 ± 0.02	3.36 ± 0.02	3.27 ± 0.02	3.18 ± 0.02	3.00 ± 0.07	3.35

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Table 5. *Milk Fat in the Cow's Milk, kg (n = 5)*

Group	Lactation month										Milk fat obtained per 305 days of lactation
	1	2	3	4	5	6	7	8	9	10	
Lactation I											
I	26.4 ± 0.7	29.6 ± 0.6	28.5 ± 0.9	27.9 ± 0.9	27.8 ± 0.9	26.3 ± 0.6	24.9 ± 0.6	22.6 ± 0.6	20.4 ± 0.5	16.5 ± 0.4	250.9
II	29.0 ± 0.7	30.6 ± 0.6	31.5 ± 0.7	31.2 ± 0.9	30.8 ± 0.7	26.2 ± 0.8	25.8 ± 0.5	24.4 ± 0.6	20.8 ± 0.4	16.0 ± 0.3	266.3
III	31.9 ± 1.0	33.3 ± 0.7	34.7 ± 0.9	35.1 ± 0.7	32.9 ± 0.8	30.3 ± 0.8	27.8 ± 0.8	25.2 ± 0.6	22.8 ± 0.6	17.3 ± 0.5	291.3
IV	32.2 ± 0.9	32.9 ± 0.7	34.2 ± 0.8	34.4 ± 0.7	32.6 ± 0.8	30.8 ± 0.9	28.7 ± 0.8	25.6 ± 0.5	20.7 ± 0.5	16.1 ± 0.4	288.2
Lactation II											
I	27.4 ± 0.8	29.5 ± 0.9	31.0 ± 0.8	30.2 ± 1.0	28.8 ± 0.7	26.9 ± 1.0	25.7 ± 0.8	23.2 ± 0.7	19.7 ± 0.8	16.1 ± 0.8	258.5
II	29.6 ± 1.0	31.2 ± 0.9	33.5 ± 0.8	32.8 ± 0.8	30.9 ± 0.7	29.3 ± 0.6	26.9 ± 0.9	25.3 ± 0.7	20.6 ± 0.7	16.7 ± 0.7	276.8
III	33.5 ± 0.9	36.1 ± 1.0	36.3 ± 0.9	37.2 ± 0.9	34.7 ± 0.9	31.8 ± 0.9	29.5 ± 0.9	26.3 ± 0.7	23.0 ± 0.6	17.4 ± 0.8	305.8
IV	33.0 ± 0.9	35.8 ± 1.0	36.7 ± 0.9	37.5 ± 0.9	34.2 ± 0.8	32.0 ± 0.9	29.3 ± 1.0	26.2 ± 0.7	22.6 ± 0.6	16.8 ± 0.7	304.1
Lactation III											
I	29.5 ± 0.9	33.7 ± 0.8	32.9 ± 0.7	31.4 ± 0.8	31.4 ± 0.6	29.3 ± 0.8	26.7 ± 0.6	25.4 ± 0.7	20.5 ± 0.7	16.3 ± 0.7	277.1
II	31.6 ± 0.8	33.7 ± 0.8	34.8 ± 0.7	36.4 ± 0.8	33.2 ± 0.8	30.2 ± 0.7	27.6 ± 0.7	25.0 ± 0.8	22.5 ± 0.7	17.3 ± 0.9	292.3
III	36.4 ± 0.7	37.3 ± 0.8	37.4 ± 0.9	39.0 ± 0.8	40.6 ± 1.0	34.7 ± 0.8	32.3 ± 0.9	28.1 ± 0.8	24.8 ± 0.8	19.9 ± 1.0	330.5
IV	35.7 ± 0.8	36.1 ± 0.7	37.0 ± 0.7	37.6 ± 0.7	39.3 ± 0.9	35.3 ± 0.8	31.6 ± 0.7	27.8 ± 0.7	23.5 ± 0.8	18.4 ± 0.8	322.3

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Table 6. *Milk Protein in the Cow's Milk, kg (n = 5)*

Group	Lactation month										Milk protein obtained per 305 days of lactation
	1	2	3	4	5	6	7	8	9	10	
Lactation I											
I	22.6 ± 0.7	26.4 ± 0.8	24.5 ± 0.9	23.1 ± 0.8	24.6 ± 0.9	23.8 ± 1.0	21.7 ± 0.8	19.9 ± 0.7	15.8 ± 0.9	13.0 ± 0.4	215.4
II	25.1 ± 0.8	25.5 ± 1.0	28.5 ± 0.8	27.2 ± 0.9	26.9 ± 0.8	24.0 ± 0.9	22.8 ± 0.8	21.9 ± 0.8	16.3 ± 0.9	13.2 ± 0.3	231.4
III	28.5 ± 0.9	29.6 ± 0.9	30.6 ± 1.0	31.3 ± 1.0	30.7 ± 0.9	28.5 ± 0.8	25.5 ± 0.9	23.2 ± 0.8	19.4 ± 0.9	15.2 ± 0.5	262.5
IV	28.3 ± 0.8	28.5 ± 0.7	29.7 ± 0.8	30.4 ± 0.9	28.5 ± 0.8	28.4 ± 0.8	25.8 ± 0.9	23.1 ± 0.8	17.0 ± 0.9	15.0 ± 0.4	254.7
Lactation II											
I	24.2 ± 0.8	25.7 ± 0.9	27.3 ± 0.9	26.7 ± 0.9	25.4 ± 1.0	23.7 ± 0.9	22.2 ± 0.7	20.1 ± 0.9	17.2 ± 0.9	13.4 ± 0.9	225.9
II	26.5 ± 0.8	27.6 ± 0.8	29.8 ± 0.8	29.8 ± 0.9	27.6 ± 0.8	26.2 ± 0.9	23.7 ± 0.9	22.2 ± 1.0	18.4 ± 0.9	13.8 ± 0.7	245.3
III	30.7 ± 0.9	32.2 ± 0.8	33.6 ± 0.9	33.6 ± 0.7	31.9 ± 0.8	29.2 ± 0.8	26.6 ± 0.9	23.9 ± 0.8	20.7 ± 0.8	15.3 ± 0.9	278.0
IV	29.7 ± 0.9	31.8 ± 0.8	33.0 ± 0.9	33.0 ± 0.9	30.7 ± 1.0	28.6 ± 0.7	26.0 ± 0.9	23.5 ± 0.7	20.3 ± 0.7	14.8 ± 0.8	271.9
Lactation III											
I	26.6 ± 0.9	27.7 ± 0.9	28.7 ± 0.8	29.4 ± 0.8	28.2 ± 0.9	26.7 ± 0.8	23.7 ± 0.9	22.6 ± 0.8	18.4 ± 0.8	13.8 ± 0.8	245.8
II	29.0 ± 0.9	30.4 ± 0.8	31.4 ± 0.8	32.1 ± 0.9	30.1 ± 1.0	27.9 ± 0.8	24.8 ± 0.8	22.5 ± 0.8	20.4 ± 0.8	15.1 ± 0.8	263.7
III	34.3 ± 0.8	35.5 ± 0.7	35.5 ± 0.9	36.8 ± 0.8	36.9 ± 0.8	32.9 ± 0.7	30.8 ± 0.8	26.5 ± 0.8	22.6 ± 0.8	18.2 ± 1.0	309.5
IV	33.0 ± 0.8	34.0 ± 0.9	34.1 ± 0.8	35.2 ± 0.7	35.4 ± 0.7	32.9 ± 0.9	29.5 ± 0.9	25.7 ± 0.6	21.3 ± 0.9	16.5 ± 0.9	297.6

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Table 7. *Amino Acid Composition of Milk in Groups I and II, g/100g (n = 5)*

Amino acid	Group I lactation I	I lactation II	I lactation III	II lactation I	II lactation II	II lactation III
Essential amino acids:						
Lysine	0.205 ± 0.007	0.242 ± 0.005	0.254 ± 0.006	0.268 ± 0.004	0.274 ± 0.003	0.279 ± 0.005
Leucine	0.211 ± 0.008	0.263 ± 0.007	0.278 ± 0.006	0.273 ± 0.007	0.285 ± 0.006	0.290 ± 0.006
Isoleucine	0.170 ± 0.006	0.183 ± 0.007	0.294 ± 0.005	0.197 ± 0.008	0.205 ± 0.006	0.208 ± 0.005
Phenylalanine	0.130 ± 0.004	0.153 ± 0.005	0.161 ± 0.004	0.162 ± 0.003	0.177 ± 0.007	0.185 ± 0.005
Threonine	0.095 ± 0.004	0.129 ± 0.008	0.136 ± 0.007	0.122 ± 0.003	0.128 ± 0.005	0.131 ± 0.004
Methionine	0.152 ± 0.005	0.174 ± 0.005	0.181 ± 0.008	0.174 ± 0.004	0.184 ± 0.008	0.192 ± 0.008
Valine	0.157 ± 0.007	0.161 ± 0.008	0.170 ± 0.006	0.169 ± 0.005	0.173 ± 0.007	0.178 ± 0.006
Essential amino acids, in total	1.140	1.305	1.374	1.365	1.426	1.463
Nonessential amino acids:						
Glycine	0.048 ± 0.003	0.052 ± 0.004	0.053 ± 0.005	0.050 ± 0.004	0.053 ± 0.006	0.055 ± 0.005
Cystine	0.054 ± 0.005	0.055 ± 0.007	0.057 ± 0.008	0.055 ± 0.005	0.057 ± 0.007	0.058 ± 0.008
Serine	0.102 ± 0.007	0.103 ± 0.009	0.104 ± 0.007	0.103 ± 0.006	0.104 ± 0.007	0.106 ± 0.009
Proline	0.101 ± 0.006	0.105 ± 0.008	0.107 ± 0.008	0.102 ± 0.008	0.106 ± 0.006	0.108 ± 0.008
Tyrosine	0.172 ± 0.008	0.176 ± 0.008	0.178 ± 0.007	0.174 ± 0.007	0.178 ± 0.007	0.179 ± 0.008
Alanine	0.103 ± 0.007	0.105 ± 0.008	0.106 ± 0.009	0.104 ± 0.007	0.106 ± 0.005	0.107 ± 0.009
Glutamine acid	0.578 ± 0.04	0.580 ± 0.02	0.589 ± 0.02	0.581 ± 0.02	0.584 ± 0.02	0.593 ± 0.013
Aspartic acid	0.193 ± 0.007	0.196 ± 0.03	0.198 ± 0.012	0.194 ± 0.007	0.198 ± 0.03	0.199 ± 0.012
Arginine	0.128 ± 0.02	0.129 ± 0.007	0.128 ± 0.005	0.129 ± 0.004	0.130 ± 0.006	0.131 ± 0.007
Histidine	0.101 ± 0.005	0.104 ± 0.008	0.103 ± 0.008	0.102 ± 0.007	0.104 ± 0.005	0.104 ± 0.007
Nonessential amino acids, in total	1.580	1.605	1.623	1.594	1.620	1.640
Amino acids, in total	2.720	2.910	2.997	2.959	3.046	3.103
Amino acid index*	0.72	0.81	0.84	0.86	0.88	0.89

Note: *The amino acid index was determined by the ratio of essential to nonessential amino acids.

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Table 8. *Amino Acid Composition of Milk in Groups III and IV, g/100g (n = 5)*

Amino acid	Group					
	I			II		
	lactation			lactation		
	I	II	III	I	II	III
Essential amino acids:						
Lysine	0.280 ± 0.004	0.288 ± 0.005	0.302 ±0.006	0.273 ± 0.005	0.283 ± 0.006	0.295 ± 0.007
Leucine	0.289 ± 0.007	0.298 ± 0.006	0.310 ± 0.005	0.282 ± 0.006	0.291 ± 0.008	0.302 ± 0.006
Isoleucine	0.216 ± 0.005	0.223 ± 0.006	0.236 ± 0.004	0.213 ± 0.006	0.215 ± 0.005	0.230 ± 0.006
Phenylalanine	0.186 ± 0.004	0.191 ± 0.004	0.196 ± 0.006	0.181 ± 0.005	0.187 ± 0.006	0.190 ± 0.006
Threonine	0.135 ± 0.005	0.138 ± 0.007	0.148 ± 0.008	0.134 ± 0.005	0.132 ± 0.006	0.145 ± 0.007
Methionine	0.198 ± 0.006	0.226 ± 0.005	0.249 ± 0.006	0.190 ± 0.006	0.214 ± 0.009	0.238 ± 0.008
Valine	0.184 ± 0.008	0.198 ± 0.007	0.215 ± 0.005	0.182 ± 0.008	0.191 ± 0.006	0.208 ± 0.005
Essential amino acids, in total	1.488	1.562	1.656	1.455	1.513	1.608
Nonessential amino acids:						
Glycine	0.057 ± 0.006	0.061 ± 0.005	0.064 ± 0.006	0.056 ± 0.006	0.059 ± 0.007	0.062 ± 0.005
Cystine	0.061 ± 0.007	0.067 ± 0.007	0.069 ± 0.006	0.059 ± 0.006	0.065 ± 0.008	0.067 ± 0.005
Serine	0.109 ± 0.006	0.114 ± 0.008	0.119 ± 0.007	0.106 ± 0.006	0.111 ± 0.005	0.117 ± 0.006
Proline	0.121 ± 0.003	0.125 ± 0.006	0.129 ± 0.005	0.119 ± 0.003	0.122 ± 0.007	0.125 ± 0.006
Tyrosine	0.183 ± 0.006	0.186 ± 0.005	0.189 ± 0.006	0.181 ± 0.007	0.184 ± 0.008	0.185 ± 0.008
Alanine	0.112 ± 0.004	0.116 ± 0.004	0.118 ± 0.006	0.111 ± 0.005	0.114 ± 0.004	0.116 ± 0.006
Glutamine acid	0.592 ± 0.03	0.608 ± 0.02	0.617 ± 0.005	0.590 ± 0.03	0.604 ± 0.02	0.614 ± 0.009
Aspartic acid	0.212 ± 0.007	0.216 ± 0.013	0.219 ± 0.009	0.210 ± 0.004	0.214 ± 0.03	0.217 ± 0.005
Arginine	0.133 ± 0.007	0.137 ± 0.006	0.139 ± 0.007	0.132 ± 0.007	0.135 ± 0.006	0.136 ± 0.006
Histidine	0.103 ± 0.008	0.106 ± 0.007	0.108 ± 0.006	0.102 ± 0.006	0.104 ± 0.005	0.106 ± 0.006
Nonessential amino acids, in total	1.683	1.737	1.771	1.666	1.712	1.745
Amino acids, in total	3.171	3.299	3.427	3.121	3.225	3.353
Amino acid index*	0.88	0.90	0.94	0.87	0.88	0.92

Note: *The amino acid index was determined by the ratio of essential to nonessential amino acids.

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