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Editors

Fundamental and Applied Scientific Research in the Development of Agriculture in the Far East (AFE-2021)

Agricultural Innovation Systems, Volume 2

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Abstract	Low-quality protein in the diet of animals leads to excessive formation of a by-product in the rumen in the form of ammonia. Formed ammonia does not participate in the synthesis of microbial protein and excretes in the urine and leads to additional energy costs. The aim of the research is to determine the optimal level of stable protein consumption, which allows to completely provide the growing animal organism with protein, as a guarantee of future health and productivity potential and an barrier to the development of rumen pathologies. Researches were conducted on calves of the Kholmogory breed raised in the VNIIFBIP vivarium from the age of 40 days to 14 months. Milk substitute feeding was carried out until the age of 70 days with free access to compound feed and hay. In the aftermilk period, the bull-calves received compound feed, hay and silage. To study the effect of concentrated feed on metabolic processes and nutrient digestibility, factors of rumen digestion were studied. It was found that the physiological and biochemical parameters fit into the reference values. The proportional intake of concentrated feed together with coarse feed with intensive rearing and fattening of dairy bulls and the normal course of enzymatic processes in the rumen and throughout the body allows achieving an average daily increase of up to 1420 g by the age of 14 months. The results obtained are most significant and cost-effective, especially in regions with a large number of dairy cattle and low cost of concentrated feed.

Keywords (separated by '-')	Rumen fermentation - Cellulolytic activity - Amylolytic activity - Rumen microflora - Protein safety - Feed conversion - Concentrated feed - Bull calves

Assessment of Rumen Digestion Processes and Productivity of Fattening Bull Calves with a High Level of Concentrates in the Diet

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Keywords: Rumen fermentation · Cellulolytic activity · Amylolytic activity · Rumen microflora · Protein safety · Feed conversion · Concentrated feed · Bull calves

1 Introduction

Productivity, disease resistance, as well as the programmed ability to reproduce, which have been planned on the basis of a complete identification of the animal's genetic capabilities, are possible only when all the necessary nutrient, mineral, and biologically active substances are fed into the body and the energy demand is complete. The balanced

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feeding is the provision of all the necessary nutrients in the diet and the delivery of the necessary classes of nutrients including vitamins and minerals in optimal proportions. Depending on the combination, primarily of the protein components of the feed, a protein component is provided, which is the main material in the constructive metabolism for a rapidly developing organism. In the transition to intensive forms of animal husbandry, it is necessary to supply the protein deficit by feeding with protected protein and normalizing the functioning of rumen digestion.

Standardized nutrition provides for the accounting of the required quantity and quality of protein in feed. The problem of low-quality protein in the diet, which is used in animal husbandry, is characterized by a high content of metabolized protein, which leads to the excessive formation of a by-product (ammonia) in the rumen that is not involved in the synthesis of microbial protein and excreted in the urine with additional energy costs. This disturbance leads to an overspending of fodder protein and is accompanied by metabolic disorders, deterioration of the animal's health and leads to higher production costs. An unbalanced diet, especially of young calves, leads to the development of all kinds of rumen pathologies and impedes the implementation of pedigree qualities.

In intensively growing bull calves as well as in cows, one of the factors limiting the intensity of the processes of biosynthesis of meat components is the amount of glucose and amino acids coming from the gastrointestinal tract to the metabolic pool [1, 2]. Apart from that, the problem of not only the profitable beef production, but also its quality should be solved. The result can be achieved if the attitude to the production of beef is not only as the production of by-products of animal husbandry with an average daily gain of 500–550 g and the delivery of bull calves to a meat-processing plant with a live weight of 330–350 kg, but also as a highly efficient and profitable production [3–5].

In countries with a high development of cattle breeding (USA, Argentina, and European countries), it was proved that technological feeding of Holstein bull calves requires an increase in energy demand of 10% to maintain their body compared with Aberdeen-Angus bulls. However, at the stage of growing-finishing, bull calves of dairy breeds use the energy of the diet for the formation of muscle tissue with less accumulation of fat more efficiently [5, 6]. Thus, dairy bull calves are economically beneficial to be fed with a high energy content (concentrated feed) diets and, to a lesser extent, roughage. A deficiency in protected protein is characterized by the signs of digestive upset, causes atony of the rumen, and leads to a sharp reduction in the number of ciliates. The destruction of amino acids occurs with the formation of harmful proteinogenic amines (histamine, tyramine, and cadaverin), the absorption of which contribute to the development of laminitis. The alkaline reaction of the medium is accompanied by the inhibition of the function of ciliates, symbiontic bacteria, their death, violation of fermentation processes in the pancreas. Putrefactive microorganisms are developing intensively, the concentration of ammonia to 25 mg/100 ml and more (normal 5–20) increases in rumen. A lack of protein is accompanied by a decrease in productivity, immunity, natural and non-specific resistance of the body, and the development of alimentary dystrophy.

When fattening bull calves, the negative consequences of high-protein concentrated feeding should be considered, i.e., consuming high-energy food, animals are at a constant risk of acidosis. Therefore, strict control is necessary in the development of technology and the adherence to the stages of feeding. It is shown that when using the nutrition

system of bull calves of the dairy breeds, taking into account the peculiarities of digestion and metabolism, by 12–14 months of age it is possible to achieve a live weight of 400–450 kg with good quality meat products. Thus, the development and implementation of a nutritional system for growing and fattening bull calves of dairy breeds will make a significant contribution to the fight against rumen pathologies and increase their productivity [7, 8].

The aim of the research is to determine the optimal level of stable protein consumption, which allows to completely provide the growing animal organism with protein, as a guarantee of future health and productivity potential and an barrier to the development of rumen pathologies.

2 Materials and Methods

The research were conducted in the vivarium of the All-Russian research Institute of Physiology, Biochemistry and Nutrition of animals – branch of the Federal Research Center for Animal Husbandry named after Academy Member L. K. Ernst (Borovsk, Kaluga region, Russian Federation) on bull calves under controlled feeding and keeping conditions from 40 days of age to 14 months in accordance with the norms of the Russian Academy of Agricultural Sciences [19] and Recommendations for optimizing the energy and protein nutrition of young cattle during intensive growing and fattening [20, 21].

2.1 Animals and Feeding Management

The study included 7 bull calves of the Kholmogory breed with an average age of 4, 9.5 and 14 months and an average body weight (BW) of 156.0 ± 0.76 kg, 381.9 ± 1.86, 553.8 ± 4.12 kg (mean value ± standard deviation) respectively. Milk substitute feeding was carried out until the age of 70 days with free access to compound feed and hay. In the aftermilk period, the animals, in each age period, received an appropriate diet for 4.5 months of the experimental period consisting of compoud feed, hay and silage, taking into account the eatability (Table 1) based on natural humidity in accordance with the instructions of the Russian Academy of Agricultural Sciences (RAAS) [11, 13, 17] and Recommendations for optimizing the energy and protein nutrition of young cattle during intensive growing and fattening [9, 12].

Compound feed was fed to experimental young animals ad libitum (5% refusal was allowed). The animals were placed in individual stalls and fed twice a day at 08:00 and 18:00. In addition, the bulls in all experimental groups were given water ad libitum. The ingredients and chemical composition of experimental diets are shown in Table 1.

2.2 Data Collection and Sampling

The given feeds and their leavings were collected and recorded each day to calculate the average dry matter intake (DMI). The animals were weighed individually before morning feeding at the beginning and end of the experiment to determine live weight gain.

Table 1. Food intake and nutritional intake (actual eatability).

Item	Age		
	4 months gain in weight 1300 g	9.5 months gain in weight 1340 g	14 months gain in weight 1420 g
Ingredients (kg, natural humidity)			
Hay	1.0	0.5	1.0
Compound feed	4.5	4.3	5.4
Grasssilage	–	6.0	12.0
Thedietcontains			
Metabolizableenergy, MJ	55.0	60.9	89.0
Drymatter, kg	5.1	6.1	9.9
Crudeprotein, g	950	924	1343
Degradableprotein, g	705	655	921
Non-degradableprotein, g	258	269	422
Metabolizableprotein, g	480	502	728
Crudefat, g	155	194	285
Crudefiber, g	470	923	1815
Nitrogen-free extractive substances, g	3270	4059	5848

The average daily gain (ADG) was calculated by dividing the BW gain by the number of experimental days.

Rumen fluid sampling: at the end of the experiment, samples of rumen fluid were collected from each bull after morning feeding using a flexible oral gastric pump. Samples of rumen fluid obtained over three consecutive days were combined to represent a single feeding phase. The samples were filtered using two layers of gauze.

Quantitative indicators of rumen microbiocenosis was determined in the hemocytometer during 15–20 min after taking, counting the number of ciliates and the total number of bacteria in 1 ml content, enzymatic activity, motility and acidity (pH) of the medium by pH meter OHAUS Starter ST2100-B.

Glass capillaries were used for laboratory studies (*in vitro*) of the enzymatic activity of the rumen microflora of animals. The study of the splitting ability of microorganisms in relation to "pure" nutrients was carried out according to the following indicators: cellulolytic and amylolytic activity. As sources of nutrients there were taken: cotton thread No. 10 and 20% solution of potato starch. The value of enzymatic activity was judged by the decrease in the length of the column in the capillary of the sources of nutrients, they were expressed as a percentage. Cellulolytic activity was evaluated according to the method of V. I. Georgievsky (1976) by the difference in thread weight before and

after incubation. The incubation time was 36 h. At the same time, we tried to recreate as much as possible the natural conditions for the development of microorganisms (nutrient intake, constant temperature of 38–39 °C, anaerobic conditions, imitation of rumen motility, pH control).

VFA (volatile fatty acids) were determined in accordance with the requirements of the state standard GOST 33819-2016 (determination of VFA composition by gas chromatography) using a TSVET 500 Mgas chromatograph.

VFA analysis were performed by gas chromatography (GC) (6890 N, Agilent, Santa Clara, CA, USA), equipped with an HP – INNOWax column and a flame ionization detector. To analyze the VFA, 5 ml of slurry sample, 25% phosphoric acid solution and 1 ml of saturated mercury solution (Sigma-Aldrich, St. Louis, Mo., USA) were taken into a 15 ml tube and then the solution was centrifuged at 3134 × g for 20 min. Thereafter, 1 ml of the supernatant was centrifuged at 13.800 × g for 10 min and filtered through a 0.2 μm filter (Whatman, Uppsala, Sweden). The filtrates were placed in 2.0 ml GC vials (Agilent, Santa Clara, Calif., USA) to measure the concentration of volatile fatty acids by GC. 0.2 μL was the sample injection volume with a split ratio of 10: 1.The temperature of the oven started from 80 °C, and then initially increased by 20 °C per minute and kept at 120 °C for 2 min, then the temperature was upgraded to 205 °C by increasing 10 °C per minute, finally, it was maintained at 205 °C for 2 min. The injection and detection ports were maintained at 250 °C.

Ammonia was determined by the microdiffusion method in Conway dishes.

Chemical Analysis

The energy level and feed composition were determined using generally accepted physiological, biochemical, and zootechnical methods.

Feed samples were taken in accordance with GOST ISO 6497-2014. Chemical analysis of feed and metabolic products was carried out according to the ɪeneral zootechnical analysis scheme: initial, hygroscopic and total moisture (GOST R 54951-2012); nitrogen, crude protein (GOST 13496.4-93), crude fiber (GOST 31675-2012), crude fat (GOST 13496.15-97), crude ash (GOST 26226-95).

The effective disintegration of feed protein was calculated from the data of determining the relative disintegration in the rumen *in sacco* and the rate of evacuation from the rumen [14, 16].

The metabolic protein was determined by the sum of digestible non-decayed feed protein and digested microbial protein evaluated by the measured indicators of rumen fermentation (kg of fermented organic matter of feed × 24 g of microbial protein nitrogen × 0.7 [5].

2.3 Statistical Analysis

Statistical analysis of the obtained values of the studied indicators was evaluated using the Wilcoxon-Mann-Whitney U-test. The significance of differences was assessed at $p < 0.05$ by the corresponding age.

3 Results and Discussion

A study of metabolic indices in the rumen shows that intensively growing bull calves are characterized by a high level of enzymatic and microbiological processes (Table 2).

Table 2. Indicators of enzymatic and microbiological processes in the rumen of bull calves of different ages (M ± m, $n = 5$).

Indicators	Age		
	4 months	9.5 months	14months
Weightindicators, kg	156.0 ± 0.76	381.9 ± 1.86	553.8 ± 4.12
Daily gain, g	1300 ± 43	1340 ± 64	1420 ± 87
pH, unit	6.3 ± 0.14	6.8 ± 0.02[1]	7.1 ± 0.04[1,2]
Ammonia, mg%	12.3 ± 0.78	6.4 ± 0.98[1]	7.8 ± 0.55[1]
VFA, mmol/100ml	16.0 ± 1.79	8.6 ± 0.38[1]	8.9 ± 0.27[1,2]
Acetate, %	58.2 ± 1.52	68.5 ± 0.44[1]	71.3 ± 1.01[1,2]
Propionate, %	30.7 ± 2.02	16.8 ± 0.31[1]	15.5 ± 0.55[1]
Butyrate, %	11.0 ± 1.93	14.6 ± 0.18[1]	13.0 ± 0.57[2]
The number of bacteria, billion/ml	8.3 ± 0.32	9.73 ± 0.06	9.7 ± 0.18
The number of ciliates, thousand/ml	429 ± 6.6	688 ± 17.7	615 ± 22.51,2
Amylolytic activity, units/ml	30.3 ± 0.96	28.7 ± 0.65	34.3 ± 0.352
Cellulolyticactivity, %	5.3 ± 0.39	5.0 ± 0.27	10.4 ± 0.391,2
Hereinafter, a significant difference is $p < 0.05$ to the corresponding age			

The study determined no significant deviations from physiological norms indicating that a high-concentrate type of feeding does not cause disturbances in the microbiocenosis of the rumen during the growing of bulls. Towards the end of the feeding period, an increase in cellulolytic and amylolytic activity is observed, which correlates with the total increase in microbiota activity.

Biological features of the young organism are rapid growth and lower consumption of nutrients per unit of increase in live weight [9, 10]. With age, the efficiency of the use of nitrogen and energy expectedly decreases but remains 1.5–2 times higher than not only in extensive, semi-intensive, or intensive growing, but also at a more mature age.

Ruminant animals are known to have fundamental differences in the physiology of digestion and metabolism. Due to the enzymatic activity of microorganisms, not only the quantitative, but also the qualitative characteristics of almost all feed components change. Microbiological processes in the pancreas modify the amount and composition of the amino acids of the feed, and the carbohydrates of the feed turn into VFA. Higher fatty acids are synthesized from non-lipid components, and significant changes occur in the fatty acid composition of feeds [11, 12, 15]. Anaerobic organisms have the ability to hydrolyze cellulose and other nutrients. An important feature of metabolism of ruminant

animals is the processes of protein breakdown and synthesis in the pancreas, which have a decisive influence on the provision of their body with protein and amino acids. Depending on the quantity and quality of the protein in the diet, the pancreatic microorganisms can turn a significant part of the protein into ammonia, and it will be excreted from the body in the form of urea, or a biologically complete microbial protein can be synthesized from the non-protein part of the protein. However, the high ability of ruminants to symbiotic digestion, like any other, makes them easily vulnerable not only to environmental stresses but also to fluctuations in the nutritional value of the diet, which is especially noticeable in the early periods of the formation of rumen microbiota. Microbial protein synthesis can satisfy the needs of only low-productivity animals (in dairy cows, it is up to 3000 kg of milk per lactation). In highly productive bull calves with a high productivity potential and growth rate, the part of the complete fodder protein should avoid decay in the rumen and enter the small intestine [14, 15]. Strict adherence to the technology of fattening dairy bull calves allows physiologically competent formation of the microbiocenosis of the rumen, which in the future will allow the efficient use of concentrated feed energy and give high gain weight.

In many farms, it is not always possible to comply with the required technology for the preparation and storage of feed and to balance the diets properly; therefore, it is difficult to provide animals with complete feeds. This causes the disturbance of the established balance of symbiotic microorganisms (suppliers of nutrients) and, as a result, a decrease in productivity.

When attached to food substrates, symbiont bacteria of the rumen secrete enzymes that destruct the plant fragments, disrupting the cellulose molecule, separating side chains and hydrolyzing the remaining oligosaccharides. Cellulolytic bacteria are sensitive to pH changes (in this regard, an increase in the content of starch and sugars leads to a decrease in pH to 5.8 units, and fiber fermentation is inhibited). During the studies, it was found that the addition of hay and silage to concentrated feeds does not make it possible to pH decrease with the active breakdown of concentrates and the release of a great amount of oligosaccharides [10, 17–19]. This balance helps to provide the body of bull calves with energy not only to maintain metabolism, but also to super-maintain the growth. Throughout the feeding, the pH level was within the physiological norm. The reaction of the medium containing the rumen is an important factor determining the state of enzymatic processes, the formation of metabolites, their absorption and use in the body. It should be noted that the pH value of the scar content depends on many factors, including the fractional composition of the protein. In our studies, it was found that with a decrease in protein cleavability in diets, the indicator of the concentration of hydrogen ions in the scar content of animals of the experimental groups tended to increase the acidity. Concentrated feed containing a high level of protected protein becomes inaccessible to rumen proteolytic microorganisms, which is accompanied by a decrease in protein breakdown and leads to less formation of its breakdown products and increases bioavailability for the macroorganism.

Amylolytic bacteria, hydrolyzing starch, do not break the cellulose down, but they ferment dextrins and maltose; however, these bacteria are not able to use most mono- and disaccharides as a substrate. They are less sensitive to pH although they significantly affect the ratio of VFA. The rate of starch degradation in the rumen depends on the type of

feed and its processing [20, 21]. Saccharolytic bacteria also ferment simple soluble sugars (unlike hydrolysis of starch grains and fiber, the adhesion mechanism is not involved). Starch undergoes hydrolytic cleavage with the formation of various dextrins, from which maltose is formed, and then glucose. In the rumen, starch is easily fermented with the formation of volatile and non-volatile fatty acids. An increase in amylolytic activity during the experiment indicates a high intensity of metabolic processes and an increase in gross energy, which is one of the main factors of growth intensification.

Fermentation of soluble sugars can occur regardless of bacterial growth. Some types of rumen bacteria are proteolytic and break down the soluble proteins, amino acids and peptides with the formation of ammonia [21]. The concentration of ammonia formed in the rumen is determined primarily by the quantity and quality of feed protein and nitrogen-containing non-protein compounds, as well as the intensity of its absorption and use for de novo protein synthesis. A decrease in ammonia in the rumen indicates an intensification of the reactions of direct amination of keto acids with ammonia as the main way of microbial amino acid synthesis [18].

Thus, strict coordination of the complex processes of digestion and metabolism can lead to a change in the degree of feed nutrients transformation into any type of product. Only in this way real opportunities open up for intensification of growing and fattening, improvement of product quality, active intervention in saving feed costs and reducing the cost of production of livestock products.

4 Conclusions

Ascertainment of the optimal level of stable protein consumption allows providing the growing animal organism with protein, laying the future potential for health and productivity, and preventing the development of rumen pathologies.

The analyzed physiological and biochemical parameters fit into the reference values. The highly concentrated type of feeding does not cause disturbances in the rumen microbiocenosis during the growing of bull calves. By the end of stage 3, an increase in cellulolytic and amylolytic activity was observed, which correlates with the total increase in microbiota activity and contributes to improved health and, as a result, daily weight gains. The use of concentrated feed for intensive growing and fattening of bull calves of dairy breeds makes it possible to reach an average daily increase of up to 1420 g by the age of 14 months and effectively pay for the feed with the products upon the normal course of enzymatic processes in the rumen and throughout the body. The results obtained are most significant and profitable, especially in regions with a large number of dairy cattle and low cost of concentrated feed.

Conflicts. The authors declare that they have no conflict of interest.

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