Microbiome and its association with nutrient metabolism in farm animal nutrition

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Abstract. The article shows the effect of amino acid preparations on metabolism, nutrient digestibility, and calf development. Essential amino acid mixes in different concentrations (per 1 kg of feed) were introduced into the diet of calves from 9 to 18 months of age: I experimental group - 2 g of lysine + 2 g of methionine + 3 g of threonine + 1 g of tryptophan, II experimental group - 3 g of lysine + 3 g of methionine + 4 g threonine + 2 g tryptophan. Calves were calculated on pure amino acids, in % of feed dry matter (per head per day). In calves of the II experimental group there was a significant increase in live weight by 11.7 % (p≤0.05) due to better utilisation of bacterial nitrogen by 8.3 % (p≤0.05), amino acids in duodenum by 3.46 % (p≤0.05) than in the I experimental group and by 8.83 % (p≤0.05) than in the control group. The growth rate of tissues and their protein composition changed with the growth of animals. The obtained data indicate the positive effect of a mixture of amino acids with a higher concentration in the diet of growing animals due to the enhancement of protein biosynthesis processes in muscle tissue. Thus, for improvement of intestinal microbiota, metabolism, digestive processes, and maintenance of calf health, the most comfortable concentration of amino acids was the ratio of 3 g lysine + 3 g methionine + 4 g threonine + 2 g tryptophan. The higher the level of essential amino acids in the feed composition, the more they increase the digestibility of nitrogenous substances in the gastrointestinal tract.

1 Introduction

The gut microbiota is considered to be the most important for the maintenance of animal health. Intestinal bacteria perform several functions such as fermentation of food, defence against pathogens, stimulation of immune response and production of vitamins, amino acids. Nutrients such as amino acids are now known to fulfil functional roles beyond their use as building blocks for proteins and have immunomodulatory properties and interact through common biochemical pathways [1]. At the same time, the issues of prolongation of biological age of conditional “youth”, first of all, of valuable individuals of farm animals...
and poultry, still require the solution of separate problems within the framework of studying the methods of intestinal microbiota correction discussed below. The dominant role in the direct impact on the intestinal microbial world is played by animal nutrition [2].

The rumen is a unique part of the gastrointestinal tract in ruminants. As the rumen develops and becomes colonised by microorganisms, the calf physiologically transitions from a pseudomonogastric to a functioning ruminant. Rumen development in calves can directly affect feed intake, nutrient digestibility, and final calf growth. Any changes in early feeding and nutritional regimes can affect rumen development and therefore lead to long-term consequences for subsequent growth, health and productivity [3].

The gastrointestinal tract of ruminants contains a variety of microbes that ferment the different feeds consumed by the animals to produce various fermentation products such as volatile fatty acids. Fermentation products can affect the productivity, health and well-being of animals. Within gastrointestinal microbes, rumen microbes are highly diverse, contribute significantly to fermentation, and are the most important in ruminant nutrition [4].

Protein is considered a key nutrient in ruminant nutrition, not only providing amino acids to the animal but also being a source of nitrogen (N) for microbial protein synthesis. The final supply of protein in the small intestine is formed by dietary protein (pre-gastric protein) and microbial protein. Microbial protein synthesised in the pre-gut can supply more than 50% of the amino acids absorbed by ruminants and is considered to be a protein of high biological value. Therefore, optimising microbial synthesis is one of the main objectives sought by ruminant nutrition researchers [6-7].

2 Methods and Materials

2.1 Livestock and feed

The experimental part of the work was carried out in accordance with the protocols of the Geneva Convention and the principles of good laboratory practice (National Standard of the Russian Federation GOST R 53434-2009), the rules of laboratory practice for preclinical research in the Russian Federation (GOST 3 51000.4-96) and The experimental research on animals was conducted according to instructions recommended by the Russian Regulations, 1987 (Order No.755 on 12.08.1977 the USSR Ministry of Health) and "The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)".

The studies were carried out in the conditions of the Laboratory of Biological Tests and Expertise of the Federal Scientific Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences. Calves were divided into 3 groups (n=3 calves in each group). The control group received OR1, I-experimental group OR1+KA1 (amino acid complex per 1 kg of feed: 2 g lysine+2 g methionine+3 g threonine+1 g tryptophan), II-experimental group OR1+KA2 (with addition of 3 g lysine+3 g methionine+4 g threonine+2 g tryptophan). Calculation was made for pure amino acids, in % of feed dry matter (per head per day).

Amino acid mixtures supplements were used in the experiment scheme: methionine, lysine, histidine, threonine, tryptophan (LLC "Agrosoyuz", Moscow, Russia). The control group of animals received a basic diet (BD) balanced according to the norms (NRC, 2000; www.nap.edu/catalog/9791.html). In carrying out the studies, care was taken to minimise animal suffering and to reduce the number of samples used. Depending on the age and needs of the animals, the nutritional content of the ration was varied during calf rearing.
2.2 Observation, indicators assessed

Observation, indicators assessed included body mass, dry matter intake recorded daily. Feed efficiency was calculated for one period. The amount of amino acids in the feed was estimated by ion exchange chromatography with post-column derivatisation with ninhydrin reagent and subsequent detection at a wavelength of 570 nm (440 nm for proline). Analyses were performed using a YL 9100 HPLC System (Young Lin Instrument Co, Ltd, Korea), which consists of a YL9110 quaternary gradient pump, a YL9101 vacuum degasser, a YL9120 UV/VIS detector, and a YL9150 autosampler (Pinnacle PCX post-column derivatiser, Na+ 4.0½150 mm, 5 μm ion exchange column, Na+ 3.0½20 mm, 5 μm pre-column; “Pickering Laboratories, Inc.”, USA).

Average samples of feed, its residues, and faecal samples were weighed on scales (Electronic scales NPV 1000, Russia). The samples were examined for content of dry matter, crude protein (GOST 13496.4-93), crude fat (GOST 13496.15-97), crude fibre (GOST 31675-2012), crude ash (GOST 26226-95), calcium (GOST 26570-95), phosphorus (GOST 26657-97).

The estimation of feed digestibility in the rumen was carried out according to the method of W. Lampeter. The amount of microbial mass was determined by differential centrifugation (Centrifuge laboratory IEC MicroCL 21, ThermoElectron LED GmbH, Germany) and further drying in a desiccator (Electric dry-air thermostat TS-1/80 SPU, JSC “Smolensk SKTB SPU”, Russia) to constant weight. Then total nitrogen was determined in air-dried preparations by the Kjeldahl method (GOST 13496.4-93). The digestibility coefficient (DC) was calculated. The content of dry matter (DM), crude protein (CP), fat and ash were analysed in litter and feed after freezing, drying, homogenisation in accordance with AOAC recommendations (1995).

2.3 Statistical processing

Statistical processing included calculation of the mean value (M) and standard errors of the mean (±SEM). Reliability of differences between the compared indicators was determined by Student’s t-criterion. The level of significant difference was set at p≤0.05.

3 Results

The need of growing animals in amino acids is determined by the value of body protein synthesis (productivity level), its derivative (meat) and the need to maintain vital processes. Biological activity of functional feed additives is high and is based on more stable preservation and adhesive activity of immobilised microorganisms, and sorbents and hepatoprotective component, in turn, more quickly and effectively relieve intoxication and accelerate the reparative process [8].

The change in the qualitative composition of the diet affected the dry matter digestibility in experimental animals. The maximum amount of amino acids supplied to the duodenum was 3.84 kg in animals of the II experimental group, which is higher by 11.2 and 8.07 % (p≤0.05) relative to animals of the control and I experimental groups. The level of absorbed amino acids increased in I experimental group (5.57 %), II experimental group (8.83 %) (p≤0.05) relative to calves of control group. The level of bacterial nitrogen intake into duodenum of calves of I experimental group by 15,21 % (p≤0.05), II experimental group – by 20.17 % was higher than in control animals (Fig. 1).
Beef cattle are particularly sensitive to the level and quality of protein nutrition [9]. Bacterial nitrogen intake increased in I experimental group by 15.87 % (p≤0.05), II experimental group (21.14 %) relative to animals of control groups. The given scheme of experiment shows that balancing of diets on critical amino acids by means of various biotechnological methods increases the level of protein metabolism in animal organism, which in its turn makes higher demands to the level of other amino acids intake.

Using the results of the N balance study, it is possible to express the N retention reaction on the example of individual amino acids (Fig. 2).

Fig. 1. Analysis of nitrogen digestibility in the gastrointestinal tract in calves, p≤0.05

Fig. 2. Analysis of nitrogen digestibility in the gastrointestinal tract in calves, p≤0.05

The lowest values of amino acids excreted with faeces were in the II experimental group (23.4 g), which were 4.56 and 2.34 % (p≤0.05) lower than in the control and I experimental groups of calves. Digested N in I 76.4 g and in II 82.6 g, which was higher compared to the indicators in animals of the control group by 7.52 and 10.87 % (p≤0.05), respectively.
The II experimental group had the lowest index (21.8 g), which was lower by 3.21 and 1.98 \% (p≤0.05) relative to the control and I experimental groups of calves. Nitrogen digestibility in calves was: I experimental group - 76.4 g and II - 77.4 g, which was higher by 7.51 and 6.30 \% (p≤0.05) relative to the peers of the control group. The difference in gastrointestinal nitrogen digestibility coefficient was 2.15 \% (p≤0.05) higher in the experimental groups than the control group (113.5 g). High \% of absorption from duodenal intake was in calves of II experimental group.

4 Discussion

Amino acids absorbed in the intestine are either used productively (for synthesis of tissue proteins) or oxidised, and also contribute to reduction of feed protein consumption per unit of production by 12-15 \%. It has been established that in ruminants substitutable amino acids are used more in energy processes than essential ones. For glucose synthesis 10 to 25 \% of substitutable amino acids and only 2-3 \% of essential amino acids are used [10].

The increase in nitrogen digestibility in I (77.8 g) and II experimental groups (79.9 g) indicates the breakdown of excess protein in the rumen to amino acids and ammonia, which are used for the synthesis of microbial protein, which differs significantly in amino acid composition from that of the eaten feed. Ammonia can be absorbed, which increases the concentration of urea in the blood and is eventually excreted as urea and ammonia. This leads to increased adverse health effects, decreased reproductive and production performance and increased environmental pollution [11].

Our findings are in agreement with the results of a study [12] showed modern protein estimation systems described the actual supply and requirement of protein that is digested and absorbed from the small intestine. The metabolisable protein available for absorption in the small intestine depends on the flux and digestibility of microbial crude protein and dietary ruminal undegradable protein. The rumen of adult ruminants contains a dense and diverse microbiota, whereas the rumen of newborn calves and lambs has a rather simple microbiota. The rumen is inoculated during lactation, ingestion and environmental contact, so that it is gradually colonised by a wide variety of microbes that affect epithelial cell function and the development of gut-associated lymphoid tissue.

Many studies have shown [13] that increased dietary crude protein levels, did not improve the development performance of dairy cattle, due to asynchrony between rumen protein supply and digestible carbohydrates or amino acid imbalance during absorption. In ruminants, as well as monogastric animals, the efficiency of protein utilisation varies considerably depending on its content in diets, on the balance of limiting essential amino acids and on the supply of metabolic energy [14].

Favourable quality and level of feeding insignificantly affects the increase of amino acid composition of individual organs and tissues. This is especially true for muscle tissue [15]. Since the rate of protein synthesis in the whole organism is a sum of synthesis rates in all tissues and organs of the organism, and the rate of protein synthesis in visceral tissues is much higher than in peripheral tissues, the study of the effect of amino acids and insulin on protein synthesis in visceral tissues is important.

The high level of arginine, glycine, alanine deposition in the organism of calves, exceeding the level of their consumption with feed, that, undoubtedly, testifies to the synthesis of these amino acids in the organism of animals of the II experimental group, attracts attention. To assess the ruminants’ amino acid supply, it is necessary to take into account the volume of microbial synthesis of amino acids in the rumen, the amount and amino acid composition of feed proteins not decaying in the rumen, the degree of digestion of microbial and non-digested feed protein and absorption of amino acids contained in them [16].
5 Conclusion

The balance between protein and nitrogen metabolism in the host is critical for efficient utilisation of nutrients from feed. Nitrogen sources in the gut limit microbial competition, influence microbiota assembly, and shape host-microbiome interactions. Protein imbalance in calves can be both deficiency and excess of individual amino acids supplied to the protein synthesising system of the body, indicating the need to establish the optimal amino acid requirements of growing animals, and since the utilisation of any of the amino acids depends on its status in the calf's diet with other amino acids. The higher the level of essential amino acids in the feed composition, the more they increase the digestibility of nitrogenous substances in the gastrointestinal tract.

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