KAPITEL 12 / *CHAPTER 12* [12]
CHARACTERISTICS OF THE FEED ADDITIVE GRINAT AND ITS EFFECT ON THE PRODUCTIVITY OF CALVES AND HEIFERS

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Introduction

The increase in the production of safe and high-quality meat products is a pressing issue today, as it is directly related to the population's need for complete protein. It is well known that insufficient consumption of high-quality animal proteins negatively affects body growth, human mental and physical development, adaptation to the environment, and reduces the body's protective functions [1].

In world practice, the current problem of animal protein deficiency is solved by increasing the number of farm animals and poultry [2] or by intensification of livestock farming. In world practice, there is a transition from extensive cultivation technology to intensive one through the use of various substances to improve metabolism in the body of farm animals and poultry (anti-stress components, complex additives, etc.) [3–5].

High meat productivity of cattle and high-quality meat products require a new approach to animal feeding [3, 6, 7]. The use of domestically produced feed additives to increase the meat productivity of cattle is relevant for the agro-industrial complex of Ukraine.

The basis of these feed additives is to increase the body weight gain of animals by stimulating metabolic processes and improving feed conversion. However, it should be noted that many of the preparations are of synthetic origin, and sometimes their bioavailability is not high enough.

This reduces the cost of beef, as well as the costs of purchasing and importing similar feed additives from abroad [8]. In recent years, there has been growing interest in humic substances in animal diets. Humic substances are natural organic substances formed in the soil during the humification of dead organic matter.

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Their main components are humic acids, fulvic acids and humins. Humic substances are a rich source of easily digestible minerals. They are considered natural and safe feed additives with many positive effects, including improving animal welfare and the quality of livestock products [3, 4].

The aim of the work was to determine the effect of an organic feed mixture based on humic acids on the productivity and blood parameters of calves of different age groups.

Materials and methods of the study. The work was carried out on the basis of LLC "Pechenizke" of Kharkiv region, Pechenigy settlement and the Expert Center for Diagnostics and Laboratory Support "Biolights",

Laboratory studies were performed on the basis of the scientific research laboratory for diagnostics of animal diseases of the Department of Propaedeutics and Medicine of Internal Diseases of Animals and Poultry named after V.I. Levchenko and at the Department of Veterinary and Sanitary Expertise, Hygiene of Animal Products and Pathoanatomy named after Y.S. Zagaevsky of the Bila Tserkva National University.

The effect of organic feed mixture on the weight of calves of different age groups was determined by individual weighing of the animals when they were placed in the experiment and after its completion after 50 days. The results of the weighings were used to determine the absolute and average daily weight gain of the animals.

Dairy calves were kept in individual houses. The body weight of the animals was determined immediately after birth (3 to 5 calves were born per day): the experimental group was formed within 10 days.

Calves in the experimental group were given an organic feed mixture made on the basis of humic acids at the rate of 20 g / 100 kg of weight, and the daily requirement was determined individually for each animal.

Once a day, dairy calves were given an organic feed mixture produced by Greenat Ecology LLC. The mixture recipe was developed by Ukrainian and Austrian scientists and is a complex of biologically active substances of natural origin, made from organic natural raw materials (peat, restructured water, leonardite).

The mixture has the appearance of a dark brown liquid with a light specific aroma. The content of low molecular weight organic humic acids and fulvic acids is 44.4 and 24.2%, respectively.

Before the start of the experiment, the animals were divided into two groups - control and experimental, 10 animals each, according to the principle of pairs of analogues, during the selection of which the date of birth, body weight and general clinical condition were taken into account. The experiment was conducted on newborn dairy calves and heifers 3-4 months old of the Ukrainian black and white breed.

Dairy calves were kept in individual plastic houses-cages in an open area. Newborn calves were given 3 liters of colostrum through a tube, and later plastic bottles with nipples were used. Milk was drunk from buckets. For 50 days, dairy calves in the experimental group received, along with milk (colostrum), an organic feed mixture made on the basis of humic acids at the rate of 20 g/100 kg of animal body weight. Heifers for fattening were kept loose in cages of 10 heads. Organic feed mixture was added to the water intended for drinking animals.

All animals of the experimental and control groups of different age groups were kept in the same conditions with observance of sanitary and hygienic standards. The animals were observed daily and weighed on the first and 50th day of the study. The total body weight gain of the animals and the average daily body weight gains were calculated.

Blood samples were collected from the jugular vein taking into account the "General Ethical Principles for Animal Experiments" and in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Purposes".

Blood for the study was collected before drinking the organic feed mixture (beginning of the experiment) and after the last feeding (end of the experiment).

The total number of erythrocytes and leukocytes in the blood was counted (dilution by the test tube method according to Nikolaev), hemoglobin concentration was determined (hemiglobin cyanide method), and hematocrit value (microcentrifugation).

The indices of "red" blood were calculated mathematically - color index (CI), hemoglobin content in erythrocytes (HbC) and mean erythrocyte volume (MV). In blood serum, total protein (biuret reaction), urea (with diacetylmonooxime), creatinine (by Jaffe color reaction), mineral metabolism – by studying the concentration of total calcium (with the calcium-Arsenazo-III reagent), inorganic phosphorus (by the VIS variant in the reaction with triethanolamine). All of the listed methods were performed with reagents from the "Filisit-diagnostics" NGO using a semi-automatic biochemical analyzer Stat Fax 1904+ (serial number 1904-5040).

For all samples, the arithmetic mean value and the mean square error (M±m) were calculated. Statistical processing of the obtained experimental results was performed using the Statistica 6.0 program (StatSoft Inc., USA).

12.1 The effect of humic acids on calf and heifers growth

In raising young cattle, the most important period is the lactation period, since during this period the skeletal muscles and axial skeleton grow most intensively, tissues and organs change, and the functions of the body are formed and improved.

The body weight of dairy calves in the experimental group at the beginning of the study was slightly lower (1.1 times) compared to the control group, with an average value for the group of 39.9±1.62 and 43.4±1.42 kg, respectively (Table 1).

Table 1 – Body weight of dairy calves when using humic acids

Biometric indicators	Body weight of calves, kg		Growth, kg	Average daily growth, g
	the beginning of the experiment	completion of the experiment		
Research group				
M±m	39,9 ± 1,62	75,7 ± 2,44**	35,8 ± 2,23*	741,2 ± 24,03**
Lim	34–51	57–85	21–45	520,0–918,0
Control group				
M±m	43,4 ± 1,42	73,4 ± 1,79	30,1 ± 1,51	600,0 ± 30,25
Lim	35–49	61–80	25–41	480,2–740,1

*Notes: *p<0,05;** p<0,001*

At the end of the experiment (after 50 days), it was found that the body weight of calves in the experimental group exceeded the corresponding indicator of calves in the control group. Thus, the body weight of calves in the experimental group increased by 35.8±2.23 kg versus 30.1±1.51 kg, which is on average 5.7 kg more ($p<0.05$) than in the control group.

The average daily gains were significantly higher in dairy calves of the experimental group ($p<0.001$) and averaged 741.2 ± 24.03 g for the group with fluctuations in values from 520.0 to 918.0 g versus 600.02 ± 30.25 (480.2–740.1) g.

Studies have also been conducted on the effectiveness of using humic acids to increase body weight gain in heifers aged 3 to 4 months (Table 2).

According to the results of weighing the heifers of the experimental group, it was found that their body weight at the end of the study increased by an average of 41.4 kg ($p<0.001$) and exceeded the value of the control group ($p<0.01$).

The average daily weight gain of heifers in the experimental group exceeded the value of the control group by 284.5 g ($p<0.05$), which indicates a positive effect of humic acids on the body weight gain of calves by stimulating metabolic processes and improving the digestibility of nutrients in the diet [9, 10].

Table 2 – Results of growth of heifers 3-4 months of age with the use of humic acids

Biometric indicators	Body weight of heifers, kg		Growth, kg	Average daily growth, g
	the beginning of the experiment	completion of the experiment		
Research group				
M ± m	86,9 ± 9,4	128,3 ± 5,24***	41,4 ± 5,68**	822,0 ± 113,7
Lim	41–126	116–158	28–69	560,0–1380,0
Control group				
M ± m	78,0 ± 6,06	107,4 ± 6,83** *	29,4 ± 4,47	537,5 ± 58,98*
Lim	41–107	80–151	17–63	333,3–843,1

*Notes: *p < 0,05;**p < 0,01; ***p < 0,001*

12.2 The effect of humic acids on the blood parameters of calves and heifers

According to the results of the studies, changes in hemoglobin concentration were detected in dairy calves of the experimental group from 96.0±11.9 to 118.86±10.4 g/l ($p<0.1$) at the end of the study (with a norm of 95–125 g/l), however, these changes were not statistically significant.

However, this trend can be regarded as a positive effect of humic acids on hemoglobin synthesis in the red bone marrow. In calves of the control group, the amount of hemoglobin did not change significantly and was 109.5±12.12 g/l at the beginning of the experiment and 110.4±11.18 g/l at its completion (Table 3).

Table 3 – Dynamics of changes in blood parameters when humic acids are applied to dairy calves

List of indicators	Stages of the experiment	Groups of animals	
		research (n=10)	control (n=10)
Erythrocytes, T/l	beginning	6,8±0,91	6,9±1,63
	end	6,7±0,72	7,2±0,93
Leukocytes, G/l	beginning	8,6±1,6	7,1±0,47
	end	7,4±0,9	8,3±0,4
Hemoglobin, G/l	beginning	96,0±11,9	109,5±12,12
	end	118,4±9,4*	110,4±11,18
Hematocrit value, %	beginning	33,8±2,86	37,2±4,6
	end	35,8±1,26	36,3±2,40
Average erythrocyte volume, μm3	beginning	50,5±6,01	55,6±11,8
	end	52,7±5,18	50,4±6,8
Average hemoglobin content in one erythrocyte, pg	beginning	15,9±3,30	16,8±2,96
	end	18,1±2,24	15,3±1,98

*Notes: *p < 0,1*

The number of erythrocytes in the blood of calves of both the experimental and control groups (at the beginning and at the end of the experiment) was within physiological values. However, an important indicator is not only the number of erythrocytes, but also the ratio between the number of erythrocytes and hemoglobin, that is, calculate the so-called indices of "red" blood – color index (CP) and average hemoglobin content (AHC) in one erythrocyte.

HEV in dairy calves of the experimental group probably increased slightly and amounted to 18.1±2.24 pg compared to 15.9±.30 at the beginning of the study (with a norm of 15–20 pg), which is evidence of improved saturation of erythrocytes with hemoglobin.

In the blood of calves from the control group, a slight decrease in the average hemoglobin content in one erythrocyte was detected compared to the beginning of the experiment, but the values remained within the physiological norm. Therefore, adding humic acids to the milk of dairy calves in the experimental group activates metabolic processes in the animal's body.

The results of experimental studies of hematological blood parameters of experimental calves 3–4 months of age are given in Table 4. As can be seen from the table, the addition of a feed mixture based on humic acid to a certain extent affects the morphological parameters of blood.

Hemoglobin, a respiratory blood pigment involved in the transport of oxygen and carbon dioxide, in 3–4-month-old heifers of the experimental group slightly increased from 98.7 ± 9.61 at the beginning of the experiment to 121.7 ± 7.9 g/l at its completion, but these changes were not significant ($p<0.1$).

Along with this, at the end of the experiment, the heifers of the experimental group showed an increase in erythrocytes from 5.5±0.64 to 7.1±0.60 T/l ($p<0.1$), but these changes were not statistically significant.

The total volume of red blood cells contained in the blood is characterized by the hematocrit value, which depends on two indicators: the number of red blood cells and their volume.

Table 4 – Morphological blood parameters after application of humic acids to calves

List of indicators	Stages of the experiment	Groups of animals	
		research (n=10)	research (n=10)
Erythrocytes, T/l	beginning	5,5±0,64	6,1±1,5
	end	7,1±0,60*	6,1±0,46
Leukocytes, G/l	beginning	7,2±1,2	6,2±0,74
	end	6,6±0,9	7,6±0,81
Hemoglobin, G/l	beginning	98,7±9,6	107,95±8,42
	end	121,7±7,9*	112,0±10,41
Hematocrit value, %	beginning	32,6±5,0	34,2±2,40
	end	35,4±2,78	36,4±3,40
Average erythrocyte volume, μm3	beginning	45,7±2,61	49,32±11,8
	end	52,0±2,43*	51,2±5,6
Average hemoglobin content in one erythrocyte, pg	beginning	13,8±3,30	14,6±2,66
	end	16,5±2,8	16,8±2,3

*Notes: *p<0,1*

In the blood of heifers of 3–4 months of age at the beginning of the experiment, the values ranged from 28 to 36% with an average value for the group of 32.6 ± 5.980%. The reason for the decrease in this indicator may be a decrease in the number of erythrocytes and their volume (Table 4). At the end of the experiment, the average values of the hematocrit value corresponded to physiological limits and on average for the group were 35.4 ± 2.78%.

Along with this, in heifers of the experimental group at the beginning of the experiment, a decrease in the average volume of erythrocytes was detected, the average value for the group was 45.7 ± 3.61 pg, which indicates the development of microcytosis. At the end of the experiment, the respiratory surface of erythrocytes did not significantly increase, the average value for the group was 52.0 ± 2.43 pg (Table 4).

12.3 Changes in biochemical blood parameters under the influence of humic acids

Proteins serve as the main building blocks for all cells and tissues in the body, making them important objects for research [1]. In addition, the total protein level in the blood serum is also important, as it reflects the body's supply of nutrients and plastic substances.

Blood proteins perform many functions: they maintain constant osmotic pressure and blood pH, play an important role in the formation of immunity, and form complexes with carbohydrates, lipids, and hormones.

In the blood serum of dairy calves of the experimental group, a tendency towards an increase in total protein was observed at the end of the experiment, with an average value for the group of 71.7±4.32 versus 60.5±5.47 g/l ($p<0.1$) at the beginning of the study (Table 5).

Table 5 – Biochemical parameters of blood serum of dairy calves with the use of humic acids

List of indicators	Stages of the experiment	Groups of animals	
		research (n=10)	research (n=10)
Total protein, g/l	beginning	60,5 ± 5,47	69,19±7,97
	end	71,7±4,32*	66,0±4,71
Urea, mmol/l	beginning	2,4±1,6	4,6±1,63
	end	3,4±0,76	3,5±0,22
Creatinine, kmol/l	beginning	98,5±9,28	111,9±21,02
	end	107,0±8,65	109,9±15,73
Total calcium, mmol/l	beginning	2,2±0,13	2,3±0,27
	end	2,5±0,05*	2,3±0,06
Inorganic phosphorus, mmol/l	beginning	2,2±0,15	2,3±0,27
	end	2,3±0,12	2,3±0,06

*Notes: *p<0,1*

In the blood serum of dairy calves of the control group, no significant difference in the amount of total protein was detected at the beginning and end of the experiment, and the average values for the group were 69.2±7.97 and 66.0±4.71 g/l, respectively.

Providing calves with protein is possible only by getting it from the diet. Humic acids do not provide the animal's body with total protein, but they contribute to better absorption of feed nutrients and increased synthesis of blood proteins.

An increase in urea was detected in the blood serum of dairy calves in the experimental group, as it is the end product of protein metabolism. However, the indicators correspond to the physiological value.

The correlation between total protein and urea is also observed in dairy calves of the control group (table 5).

The increase in creatinine in the blood serum of calves of the experimental group, in our opinion, may indicate partial dehydration of the body or muscle damage (damage), possibly due to the muscular activity of the calves.

The level of total calcium in the blood serum of calves in the experimental group at the end of the study tended to increase and was 2.46 mmol/l versus 2.20 mmol/l at the beginning of the study, but these changes were not statistically significant ($p<0.1$).

In the blood serum of calves of the control group, the content of total calcium did not change.

The content of inorganic phosphorus increased after the end of the experiment in the blood serum of calves of the experimental group, but the value was within physiological limits throughout the experiment.

Therefore, the use of an organic feed mixture made on the basis of humic acids has a positive effect on the protein and mineral metabolism of dairy calves.

Total protein in the serum of heifers of the experimental group of 3–4 months of age at the beginning of the study was low and on average for the group was 66.3±6.4 g/l (Table 6). After the application of humic acids, the level of total protein tended to increase to 76.4±2.42 g/l ($p<0.05$), which indicates an improvement in the assimilation of proteins from the consumed feed.

Table 6 – Biochemical indicators of blood serum of heifers with the use of humic acids

List of indicators	Stages of the experiment	Groups of animals	
		research (n=10)	research (n=10)
Total protein, g/l	beginning	66,3±6,4	66,0±6,0
	end	76,4±2,42*	72,7±2,3
Urea, mmol/l	beginning	3,6±1,3	3,9±1,45
	end	4,3±0,4	3,5±0,42
Creatinine, kmol/l	beginning	91,0±8,98	117,5±6,1
	end	102,6±6,13	128,4±6,23
Total calcium, mmol/l	beginning	2,1±0,17	2,1±0,13
	end	2,6±0,13*	2,3±0,08
Inorganic phosphorus, mmol/l	beginning	2,2±0,13	2,0±0,21
	end	2,3±0,14	2,2±0,8

*Notes: *p<0,05*

Along with this, in the blood serum of calves of the experimental group at the end of the experiment, a slight increase in urea, which is the end product of protein metabolism, was detected.

Creatinine in clinically healthy calves is completely filtered by the glomerular apparatus of the nephron and is not reabsorbed in the renal tubules.

Therefore, the determination of creatinine is used to study the filtration function of the kidney glomeruli. According to the results of our studies, creatinine in the blood serum of calves in the experimental group did not exceed physiological limits.

A positive effect of humic acids was found on the state of mineral metabolism, namely on the level of total calcium in the blood serum of calves of the experimental group. An increase in the amount of total calcium by 20.5% ($p<0.05$) indicates an improvement in mineral metabolism.

Thus, the demand for safe and high-quality beef worldwide is forcing producers

to seek alternatives to antibiotics and growth promoters to meet consumer needs.

Organic feed mixtures based on humic acids are natural growth stimulants that reduce feed consumption for animal body weight gain, reduce the risk of diseases, increase immunity, and improve meat quality.

Humic acids, having colloidal properties, have the ability to form protective layers on the epithelial layer of the digestive tract mucosa, which helps prevent the penetration of pathogenic bacteria or toxic substances produced by bacteria [8, 14, 15].

This provides an additional level of protection for the gastrointestinal tract of calves and improves the absorption of nutrients from the feed. Our research is consistent with the results of scientists [19], which state an increase in the body weight of calves under the influence of humic acids, which are based on the stimulation of metabolic processes and improvement of the digestibility of nutrients in the diet. According to the results of our studies, the body weight of dairy calves in the experimental group significantly increased ($p<0.05$) after the end of the experiment compared to the control group.

Average daily gains exceeded those of dairy calves in the control group ($p<0.001$), indicating the ability to improve diet digestibility by stimulating microbial activity in the intestine and, thus, improving nutrient absorption.

The body weight of heifers 3-4 months of age at the end of the experiment significantly increased ($p<0.001$) and exceeded the value of the control group ($p<0.01$). The average daily gains of the heifers of the experimental group exceeded the value of the control group ($p<0.05$), which indicates a positive effect of humic acids on the body weight gain of calves due to an increase in the microbial population of the rumen or changes in the intestinal microbiota.

Increased microbial activity, in turn, improves fermentation and nutrient absorption [18, 19]. Humic acids act as natural antibiotics, which leads to increased productivity of farm animals, improve the functioning of the intestines of animals, promoting the growth of beneficial microorganisms, suppressing harmful microflora.

The study of blood parameters confirms the positive effect of humic acids on erythropoiesis. According to the results of the study, the hemoglobin content in the

blood of calves in the experimental group, which received an organic feed mixture made on the basis of humic acids, increased at the end of the experiment, but these changes were not statistically significant.

Our studies are partially consistent with the results of a group of scientists [21] based on studies of morphological indicators of the blood of cows to which humic substances were added to the diet, which contributed to an increase in hemoglobin by 5.5% and erythrocytes by 6.6%, which also allowed the authors to conclude that metabolic processes in the body of cows were activated.

Since hemoglobin supplies oxygen to tissues to ensure the normal course of energy processes in the body, transports carbon dioxide from tissues to the lungs; is part of the hemoglobin buffer system of the blood and participates in the regulation of acid-base balance, and its slight increase ($p<0.1$), indicates the need to continue feeding organic fodder to animals.

Total protein in blood serum, reflecting the provision of nutrients and macronutrients, had a tendency to increase ($p<0.05$), in heifers of the experimental group 3-4 months of age. Based on the studies conducted [20] in the digestive tract, with the participation of hydrolase enzymes, the activation of humic acid macromolecules (both their nuclear part and peripheral functional groups) takes place, and this, in turn, leads to further activation of digestive tract enzymes.

At the same time, the system of intracellular hydrolases is activated in the liver, which leads to an increase in the synthesis of blood proteins.

Organic feed mixtures made on the basis of humic acids stimulate the formation, development and maturation of blood cells (leukocytes, erythrocytes, platelets), synthesis of blood proteins and utilization of glucose by body tissues; as a result, a significant increase in body weight gain in calves is observed [21].

Conclusions

The use of a feed mixture based on humic acids contributes to an increase in the body weight of dairy calves and heifers 3–4 months of age in the experimental group

by an average of 5.7 kg ($p<0.05$) and 12.0 kg ($p<0.001$), respectively, compared to the control groups.

The average daily gains of dairy calves in the experimental group by 141.1 g ($p<0.001$) exceed the values of the control group, and the heifers in the experimental group by 284.5 g ($p<0.05$), which indicates a positive effect of humic acids on the increase in live body weight of calves by stimulating metabolic processes and improving the digestibility of nutrients in the diet.

The tendency to increase the concentration of hemoglobin in the blood of dairy calves and heifers 3–4 months of age in the experimental group ($p<0.1$) may indicate a positive effect of humic acids on the synthesis of hemoglobin in the red bone marrow.

The increase in total calcium in the blood serum of 3–4-month-old heifers ($p<0.05$) and the tendency to increase in dairy calves ($p<0.1$) of the experimental group at the end of the study indicate increased calcium absorption.