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Physicochemical parameters of the longest back muscle of bulls of different breeds when using humic acids-based additives in their feeding

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Abstract. The feed additive based on humic acids used in our studies in feeding Kalmyk, Kazakh White-headed and Aberdeen-Angus bulls convincingly proved its effect on the qualitative parameters of the longissimus dorsi muscle. In the experimental groups, the dominance of the area of the "muscle eye" was confirmed in the bulls of the VI experimental group (Aberdeen-Angus) by 10.62 cm2 ($P < 0.01$) relative to the V control group, the II experimental group (Kalmyk) - by 6.87 cm2 ($P < 0.01$), in relation to the control (I group), in the IV experimental group (Kazakh White-headed) - by 4.66 cm2 ($P < 0.05$) against the background of the control (II group). A decrease in moisture by 0.81% ($P<0.05$), 0.87% ($P<0.05$), 0.89% ($P<0.05$) and an increase in protein by 0.66 ($P<0.05$), 0.62 ($P<0.05$) and 0.75% ($P<0.05$) were recorded. The protein value increased due to the optimization of the amino acid composition. The quality of intramuscular fat increased due to an increase in unsaturated fatty acids. The process of meat maturation after slaughter of animals in the experimental groups was more effective. Monitoring the quality of meat products indicates its high nutritional and energy value. However, the best indicators were shown by bulls of the Aberdeen Angus and Kalmyk breeds.

1 Introduction

Many scientists continue to conduct research on obtaining biostimulants of natural origin suitable for use in feeding beef cattle to obtain higher quality food products [2]. Thanks to phytogenic biostimulants, such as humic, fulvic and amino acids, amide bond peptides, various enzymes, and polysaccharides, the active processes of animal life are increased [3]. Humic acids have a very wide range of actions, relative to many other natural forms, which distinguishes them as one of the strongest sources of chelate complexes. An additional

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prevailing factor is their sorption capacity, superior to other adsorbents of inorganic compounds, reducing the activity and toxicity of heavy metals such as lead and cadmium [4].

Humic acids, as the most common form of organic carbon, play an important role in natural processes [5]. They also have a beneficial effect on the intestinal microbiome (reduction of mycotoxins) due to the inhibition of mold and bacterial spores due to the presence of bioflavonoids [6].

The use of preparations containing humic acids contributes not only to an increase in live weight, but also to meat characteristics, including the chemical composition and biological value of meat [7-9].

It is reported that humic acid compounds ensure optimal pH formation in the digestive tract, suppress harmful types of bacteria, reduce mycotoxin levels and promote intestinal health. It was also emphasized that they significantly reduce digestive disorders and have an antiviral and antibacterial effect [10].

Research on humic substances has mainly focused on poultry, and research on the use of humic acids as performance enhancers in ruminants is limited.

The purpose of our research was to test a humic acid-based supplement for fattening bulls of different breeds (Kalmyk, Kazakh white-headed, Aberdeen-Angus), as a result of which an examination of the physicochemical parameters of the longest back muscle of bulls of the corresponding breeds was carried out.

2 Materials and methods

Experimental studies were conducted in the peasant farm of B.N. Gekhaev (Savdya settlement, Zavetinsky district, Rostov region) on bulls of the Kalmyk, Kazakh and Aberdeen-Angus breeds, using the following design (Figure 1).

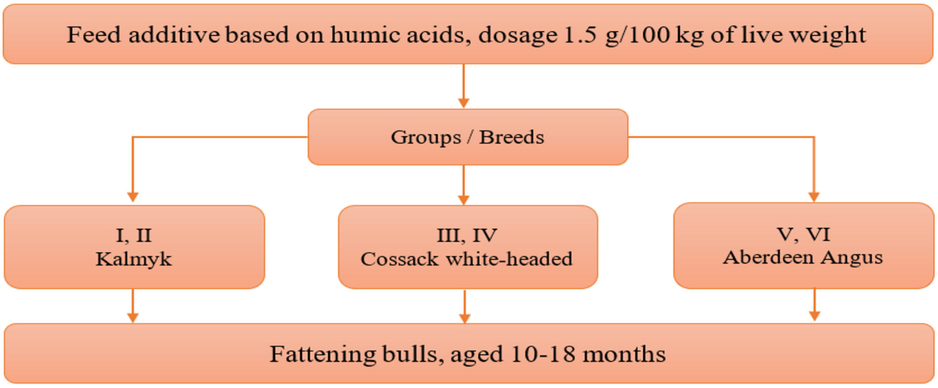


Fig. 1. Experience Design.

The experiment involved three meat breeds of animals (Kalmyk, Kazakh white-headed and Aberdeen Angus), which during the fattening period received an additive containing humic acids in a dosage of 1.5 g/100 kg of live weight as part of their diet. In order to most objectively evaluate the effect of the experimental additive, it was decided to create a control group for each breed. As a result, six completed groups of bulls were distributed as follows: three of which (II, IV and VI) were experimental, and (I, III and V) served as a control for them. The number of livestock in each group was 10 heads, the fattening period for the bulls was 8 months.

Upon completion of the experiment, an analysis of the physical and chemical parameters of the longest muscle of the back of the experimental bulls was carried out, characterizing the quality of beef under the influence of the studied additive.

3 Research results and discussion

Factors affecting meat quality can be grouped into three periods: before, during and after slaughter. The post-mortem assessment of meat productivity of young bulls primarily includes the development of animal muscle tissue and, in particular, the longest back muscle.

Kosilov V.I., Yuldashbaev Yu.A. et al. [6] revealed a relationship between the area of the muscle eye and the total amount of meat in the carcass (correlation 0.4–0.7).

In this regard, we studied, first of all, the measurements of the longest back muscle (Table 1).

Table 1. Measurements of the longest back muscle of experimental young bulls (n=3).

Group/breed		Depth, cm	Width, cm	Area, cm^2	depth / width x 100%
Kalmyk	I - C	6.0±0.05	10.9±0.13	64.81±1.59	55.05±1.15
	II - E	6.3±0.07*	11.4±0.09*	71.68±1.67*	55.26±1.07
Kazakh white-headed	III - C	5.8±0.04	10.7±0.08	62.04±1.21	54.21±1.14
	IV - E	6.1±0.08*	11.0±0.10	67.10±1.12*	55.45±1.18
Aberdeen Angus	V - C	6.8±0.05	12.3±0.15	83.43±1.29	55.29±1.21
	VI - E	7.3±0.09**	12.9±0.11*	94.05±1.36**	56.59±1.19

The effect of the experimental additive on the area of the longissimus dorsi muscle of the bulls in the experimental groups compared to the control groups is obvious. The variation in the absolute indicators of the area of the longissimus dorsi muscle of the bulls among the studied breeds is explained by the genotype. Based on the fact that the quality of beef directly depends on its chemical composition, we studied, first of all, its component composition (the presence of moisture, protein, fat and ash) in the longest back muscle of experimental bulls (Figure 2).

The obtained results indicate a decrease in moisture in the samples of the longest back muscle of all experimental groups: in the II experimental group (Kalmyk) – by 0.81% ($P<0.05$) in a comparative aspect with the control (group I of a similar breed), in the IV experimental group (Kazakh white-headed) – by 0.87% ($P<0.05$) relative to group III, in the VI experimental group (Aberdeen-Angus) – by 0.89% ($P<0.05$) against the background of V control group for this breed. As can be seen, the experimental additive affected the moisture reduction in the experimental groups almost equally, relative to the control groups of the corresponding breeds. The protein content in all experimental samples exceeded that in the control groups of similar breeds by 0.66 ($P<0.05$), 0.62 ($P<0.05$) and 0.75% ($P<0.05$), respectively. The highest fat level was recorded in the longissimus muscle samples of Kalmyk bulls (II experimental), which reached 4.05%, Aberdeen Angus (VI experimental) - 3.14%, and Kazakh White-headed (IV experimental) - 2.98%, which is primarily due to the genotype, and the studied additive had an insignificant effect on this indicator, since the difference with the control groups was only 0.13; 0.12 and 0.24%. The content of ash residue varied depending on both the genotype and the experimental additive, which corrects the mineral composition of the muscle for the better. Based on the parameters of the chemical composition of the muscle, its energy value was calculated, which was the highest in the Kalmyk breed bulls (II experimental) and amounted to 498.81 mJ, exceeding the same indicator in the control (I group) by 16.4 mJ, in the Aberdeen-Angus breed (VI experimental) the energy value reached 486.55 mJ, ahead of this indicator in the compared group (V control) by 17.56 mJ and in the Kazakh white-headed (IV experimental) - 463.32 mJ, where the difference with the control (III group) was 19.99 mJ. Assessing the obtained results, it can be stated that the effect of the experimental supplement was almost the same for all breeds, and the difference in the energy value of the longest back muscle within breeds can be explained by the genotype of the animals being compared.

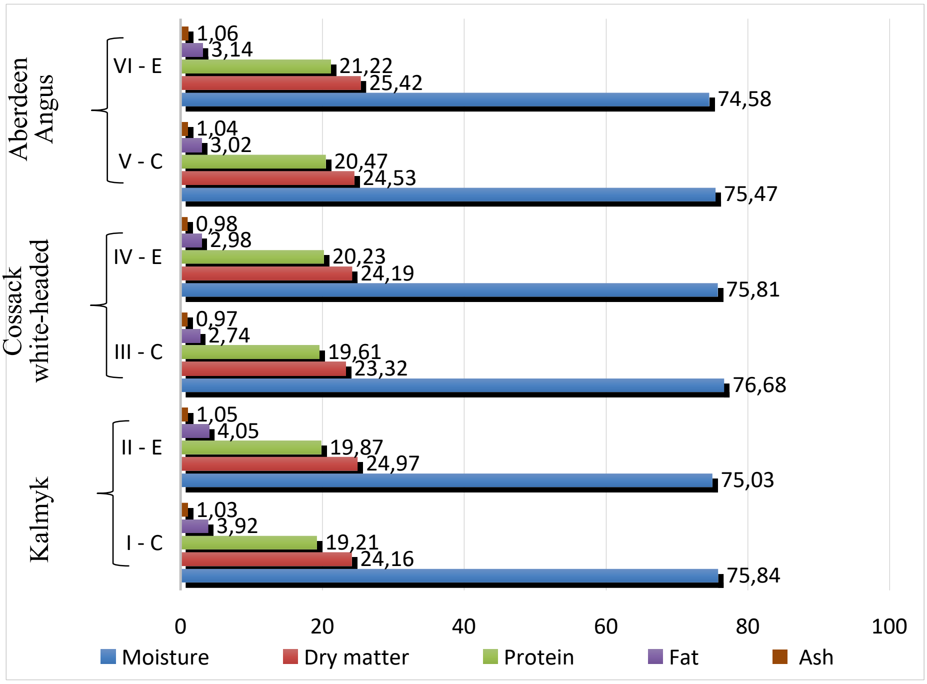
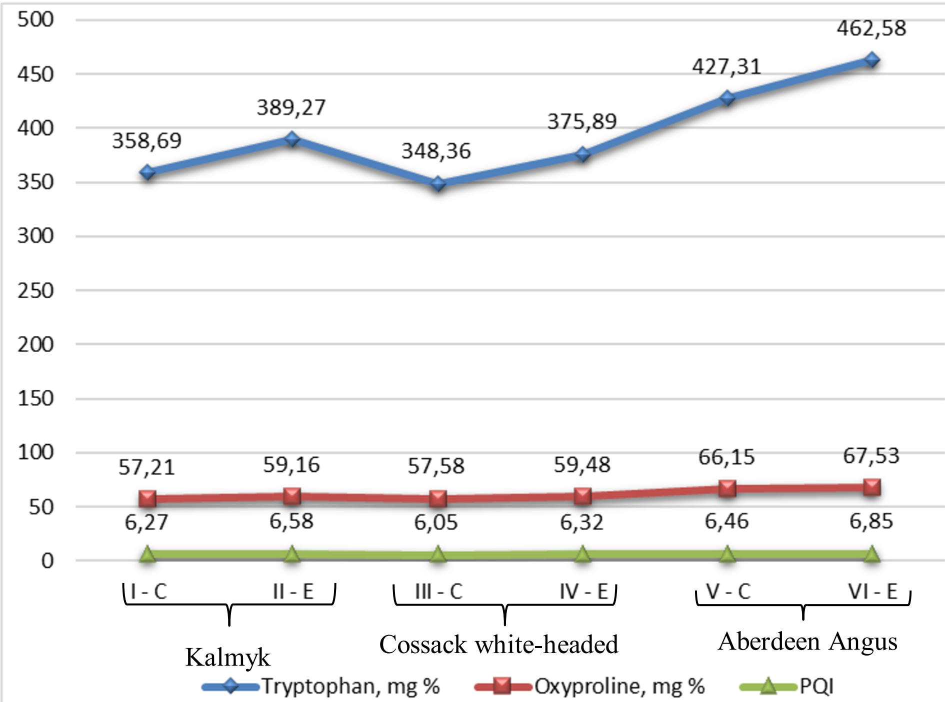


Fig. 2. Component composition of the longest back muscle, %.

The biological value of the muscle, assessed by the presence of the essential amino acid tryptophan and its ratio with connective tissue (incomplete) proteins, represented by oxyproline, is demonstrated in Figure 3. The participation of tryptophan in the formation of serotonin, melatonin, choline and niacin gives this amino acid special significance.

The most effective effect of humic acids of the studied additive was on the tryptophan level in the back muscle of Aberdeen Angus breed (VI experimental group), which exceeded the similar indicator in the control (V group) by 35.27 mg% (8.25%; $P < 0.01$). In the longissimus dorsi muscle of Kalmyk breed bulls (II experimental group), the tryptophan content exceeded the control (I group) by 30.58 mg% (8.53%; $P < 0.01$), and in Kazakh white-headed (IV experimental group) relative to the control (III group) - by 27.53 mg% (7.90%; $P < 0.05$). In this regard, the protein-quality indicator dominated in all experimental groups and reached 6.85 in group VI (Aberdeen Angus breed) against 6.46 in the control (group V), in the II experimental group (Kalmyk breed) - 6.58 against 6.27 in the I control group, in the IV experimental group (Kazakh white-headed breed) - 6.32 against 6.05 in the control (group III). Meat, especially beef, is an indispensable high-protein, the safest and most popular product in the nutrition of the population [11]. However, the requirements for the quality of beef are rapidly increasing and tender and biologically complete meat of bulls of meat breeds is becoming more and more in demand. The presence of amino acids in the longest back muscle of experimental bulls is shown in Figure 4).



* PQI – Protein Quality Indicator

Fig. 3. Biological value of the longissimus dorsi muscle.

As a result of the experiment, a significant effect of the humic acid-based supplement in the diets of young bulls on the concentration of amino acids in the longissimus dorsi muscle of all beef breeds included in the experiment was revealed, but to varying degrees. The most significant changes were recorded in the muscle of Aberdeen-Angus bulls (Group VI), where among the essential amino acids the level of lysine increased reliably by 0.52% ($P<0.05$), histidine - by 0.73% ($P<0.01$), the total content of leucine and isoleucine - by 0.68% ($P<0.05$), threonine - by 0.62% ($P<0.05$) compared to the V control group. Experimental samples of the Kalmyk (II experimental) and Kazakh white-headed (IV experimental) breeds also had a reliable advantage over the control groups of comparable breeds (I and III): for lysine - by 0.49 ($P < 0.05$) and 0.42% ($P < 0.05$), for histidine - by 0.60 ($P < 0.05$) and 0.54% ($P < 0.05$), for the total content of leucine and isoleucine - 0.57 ($P < 0.05$) and 0.51% ($P < 0.05$), for threonine - by 0.54 ($P < 0.05$) and 0.49% ($P < 0.05$). From the list of replaceable amino acids, a reliable difference was achieved between the experimental and control groups for glutamine, asparagine and alanine: in experimental group VI (Aberdeen Angus) - 0.73 ($P < 0.01$), 0.44 ($P < 0.05$) and 0.41% ($P < 0.05$), in experimental group II (Kalmyk) - 0.69 ($P < 0.01$), 0.47 ($P < 0.05$) and 0.37% ($P < 0.05$), in experimental group IV (Kazakh White-headed) - 0.63 ($P < 0.01$), 0.42 ($P < 0.05$) and 0.36% ($P < 0.05$) relative to the control groups of the corresponding breeds (V, I, III). As a result, the total amount of amino acids in the experimental groups dominated over the control by 5.09 ($P<0.01$), 4.46 ($P<0.01$) and 3.85% ($P<0.05$) relative to the control, with the ratio of essential amino acids to replaceable ones equal to 0.99 in the VI experimental group against 0.96 in the V control, 0.96 in the II experimental group against 0.93 in the I control, and 0.94 in the IV experimental group against 0.92 in the III control.

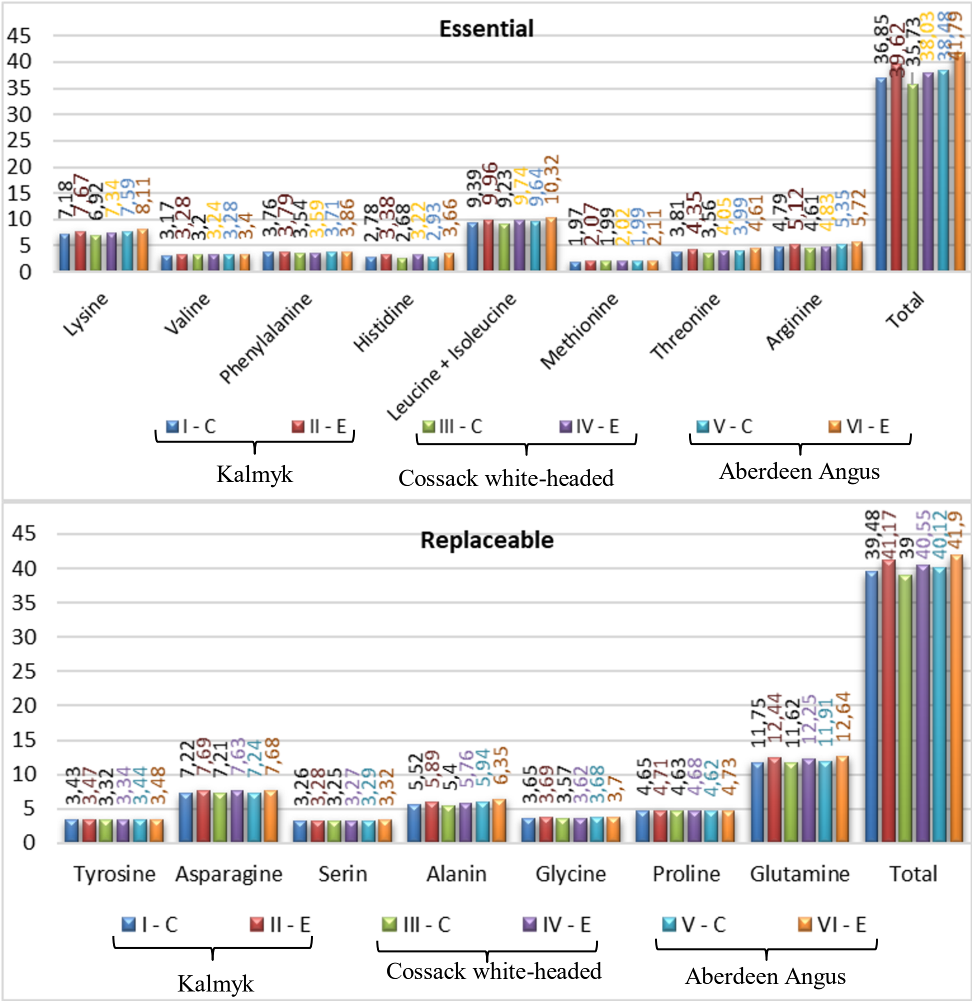
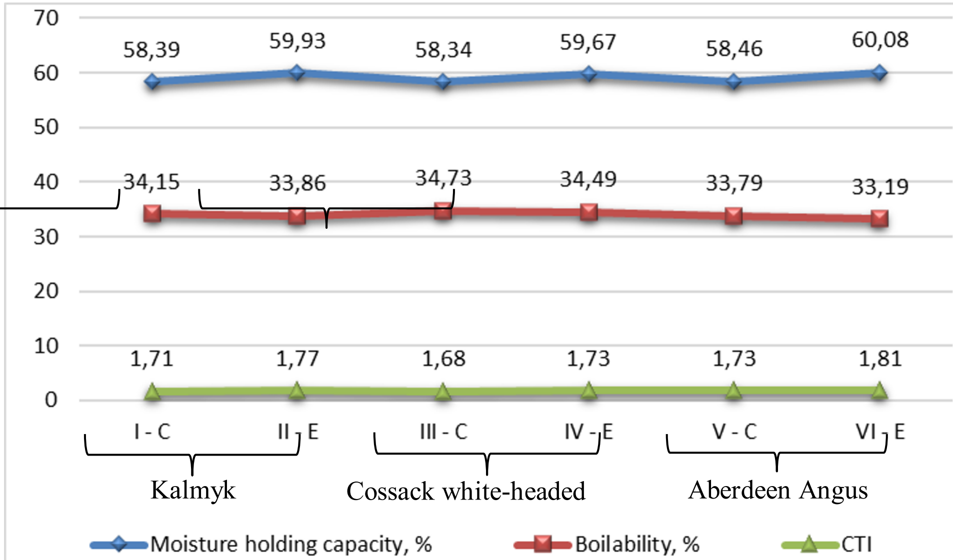


Fig. 4. Amino acid levels in the protein of the longest back muscle of experimental bulls.

Comparable values of moisture loss in the studied meat samples prove the absence of a negative effect of humic acids for beef, poultry and pork, while the authors suggest that humates reduce lipid peroxidation, and this has a positive relationship with the indicators of water-holding capacity [13]. In our studies (Figure 5), the water-holding capacity of the longissimus dorsi muscle increased in the experimental groups (VI, II, IV) relative to the control groups (V, I, III): for the Aberdeen Angus breed - by 1.62% ($P < 0.05$), for the Kalmyk breed - by 1.54% ($P < 0.05$), for the Kazakh white-headed breed - by 1.33% ($P < 0.05$).



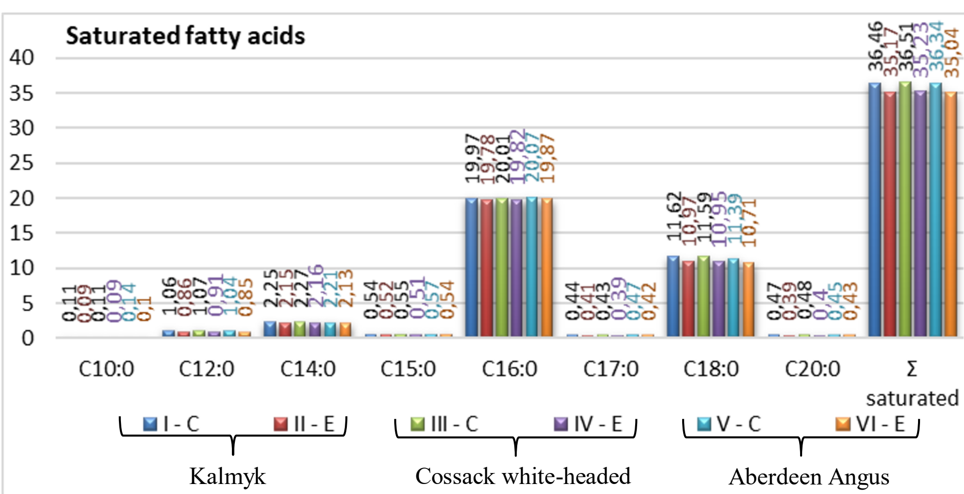
* CTI - Culinary technological indicator

Fig. 5. Culinary and technological parameters of the longissimus dorsi muscle.

With a decrease in the cooking ability, the culinary and technological indicator increased in the experimental groups by 0.06; 0.05 and 0.08 compared to the control samples.

Red meat differs from white meat, such as pork and chicken, in the content of iron, zinc, vitamin B12 and fatty acid composition. A number of researchers have shown that intramuscular fat concentration is associated with beef quality, including tenderness, juiciness, taste and fatty acid composition [14]. The results presented by Mokotedi N.P. et al. [15] showed that cattle fed humate produced beef with higher intramuscular fat content, with increased C20:2n-6 concentration and omega-6 to omega-3 fatty acid ratio (n-6:n-3).

Our studies confirmed the effect of humic acids on long-chain carboxylic acid concentrations in the longissimus dorsi muscle of experimental bulls (Figure 6).



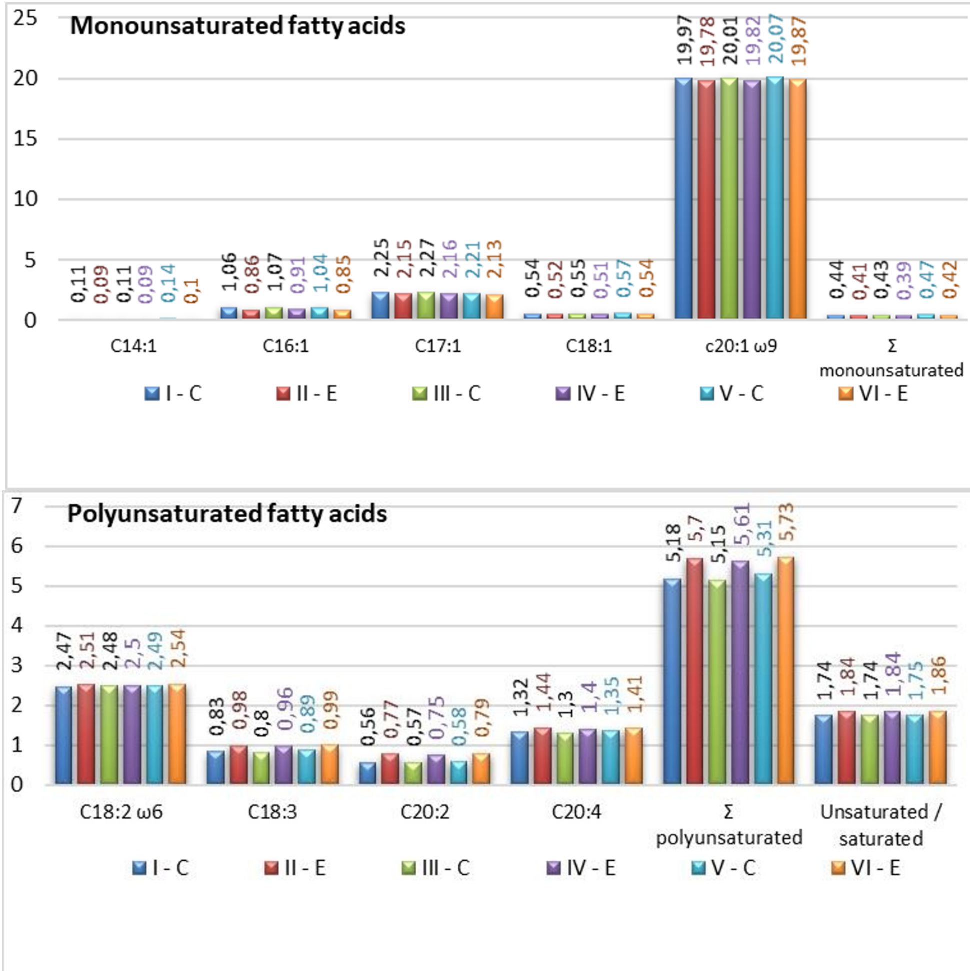


Fig. 6. Presence of carboxylic acids in beef samples of experimental groups (n = 3).

In experimental samples of the longissimus dorsi muscle of bulls of the breeds participating in the experiment (II, IV, VI), a decrease was recorded across the entire spectrum of saturated fatty acids, under the influence of the test additive, relative to the control (I, III, V). At the same time, a reliable difference was achieved only for stearic acid, which was lower than in the control by 0.65 ($P < 0.05$), 0.64 ($P < 0.05$) and 0.68% ($P < 0.05$), respectively. The total reduction in saturated fatty acids reached: in the 2nd experimental group (Kalmyk) – 1.29% ($P<0.01$) in relation to the 1st control group, in the 4th experimental group (Kazakh white-headed) – 1.28% ($P<0.01$) in comparison with the 3rd control group, in the 6th experimental group (Aberdeen-Angus) – 1.30% ($P<0.01$) against the background of the 5th control group.

The content of unsaturated fatty acids in the experimental groups (II, IV, VI) prevailed across the entire spectrum: from the list of monounsaturated, a reliable increase was found for oleic acid in the II experimental group (Kalmyk) - by 0.54 ($P < 0.05$), in the IV experimental group (Kazakh white-headed) - by 0.60 ($P < 0.05$) and in the VI experimental group (Aberdeen-Angus) - by 0.61% ($P < 0.05$); from the list of polyunsaturated carboxylic acids, a tendency to increase was recorded, which, ultimately, affected their total concentration. In the II experimental group (Kalmyk), the total level of monounsaturated carboxylic acids dominated over the control (group I) by 0.77% ($P < 0.05$), polyunsaturated acids - by 0.52% ($P<0.05$), in the IV experimental relative to the III control - by 0.82 ($P<0.05$) and 0.46% ($P<0.05$), in the VI experimental relative to the V control - by 0.88 ($P<0.05$) and 0.42% ($P<0.05$). The ratio of unsaturated fatty acids to saturated fatty acids in muscle samples of Kalmyk and Kazakh white-headed bulls (experimental groups II and IV) was the same and amounted to 1.84 versus 1.74 in control groups I and III, and this indicator in experimental group VI (Aberdeen-Angus) was slightly higher and amounted to 1.86 versus 1.75 in control group V.

The most important quality indicator of meat is the nature of its maturation, which occurs under the influence of tissue enzyme systems. Glycogenolysis is the enzymatic breakdown of glycogen and is the first step in the release of energy by oxidizing glucose. After slaughter, glycogen begins to break down in meat, turning into lactic acid. Accumulating in meat, lactic acid reduces the pH of muscle tissue. The results of the analysis of glycogenolysis in the muscle tissue of young bulls are presented in Table 2.

Table 2. Glycogenolysis indicators during meat maturation (n=3).

Group/breed		pH	Glycogen, mg%	Lactic acid, mg%
3 hours after slaughter				
Kalmyk	I - C	5.79	604.8	279.6
	II - E	5.85	677.2	298.7
Kazakh white-headed	III - C	5.72	621.5	285.1
	IV - E	5.81	691.6	304.4
Aberdeen Angus	V - C	5.77	627.4	305.2
	VI - E	5.83	701.3	324.5
24 hours after slaughter				
Kalmyk	I - C	5.54	285.5	592.8
	II - E	5.61	383.5	594.4
Kazakh white-headed	III - C	5.47	287.7	624.2
	IV - E	5.58	380.2	602.7
Aberdeen Angus	V - C	5.49	301.6	653.4
	VI - E	5.56	394.7	621.9

Muscle acidity 3 hours after slaughter of the bulls was within normal limits, varying in the control groups from 5.72 to 5.79, and in the experimental groups from 5.81 to 5.85. 24 hours after slaughter, the pH in the control groups decreased and fluctuated from 5.47 to 5.54 units, and in the experimental groups it varied from 5.56 to 5.61. The glycogen concentration in the back muscle of the bulls in the experimental groups 3 hours after slaughter was higher than in the control groups: in the II experimental group (Kalmyk) - by 72.4 mg% (11.97%; $P\leq0.01$), in the IV experimental group (Kazakh white-headed) - by 70.1 mg% (11.28%; $P\leq0.01$), in the VI experimental group (Aberdeen-Angus) - by 73.9 mg% (11.78%; $P\leq0.01$). After 24 hours after slaughter, as a result of the onset of the maturation process (glycogenolysis), the glycogen concentration in the meat of the bulls in the control groups (I, III, V) decreased by 2.12; 2.16 and 2.08 times, while in the experimental (II, IV, VI) groups by 76.58; 81.90 and 77.68%. As a result, the difference in glycogen levels between the control (I, III, IV) and experimental (II, IV, VI) groups increased for the Kalmyk breed to 34.32% ($P\leq0.001$), for the Kazakh White-Headed to 32.15% ($P\leq0.001$), and for the Aberdeen-Angus to 30.87% ($P\leq0.001$).

In parallel with the process of glycogen breakdown, lactic acid accumulated in the longissimus dorsi muscle. It should be noted that after 3 hours after slaughter, the variation in lactic acid in the samples of the experimental groups relative to the control was insignificant; for Kalmyk breed - 5.76%, for Kazakh white-headed - 5.02%, for Aberdeen-Angus - 6.32%.

After 24 hours after slaughter, the concentration of lactic acid in the studied samples of the control groups (I, III, V) increased by 2.12; 2.19 and 2.14 times. The accumulation of lactic acid, as a result of the breakdown of glycogen, in the process of glycogenolysis, proceeded more slowly than in the control and the increase was 1.99 times in the II experimental group, 1.98 times in the IV experimental group, 1.92 times in the VI experimental group, which, in our opinion, can explain the lower acidity of the longissimus dorsi muscle of the bulls of the experimental groups.

4 Conclusion

The feed additive based on humic acids used in our studies in feeding Kalmyk, Kazakh white-headed and Aberdeen-Angus bulls convincingly proved its effect on the quality parameters of the longissimus dorsi muscle. In the experimental groups, the area of the "muscle eye" increased; a decrease in moisture and an increase in protein were recorded; the protein value increased due to the optimization of the amino acid composition; the quality of intramuscular fat increased due to an increase in unsaturated fatty acids; the process of meat maturation after slaughter of animals in the experimental groups was more effective. Monitoring the quality of meat products indicates its high nutritional and energy value. At the same time, the longissimus dorsi muscle of Aberdeen-Angus and Kalmyk bulls had the best indicators.

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