

The effect of dry cranberry meal extract on the adaptive ability of calves of Holstein black-and-white breed

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Abstract. Due to the imperfection of industrial technology, stress is the most important factor reducing productive potential of young animals. One of the ways to cope with stress is to increase adaptive abilities and resistance of the body to adverse factors. It can be achieved by optimizing metabolism, in particular, with the introduction of biologically active substances of plant origin. One of these substances is ursolic acid, which is contained in large quantities in cranberry meal extract. In this regard, the aim of the research was to study the effect of different doses of dry cranberry meal extract (containing 40% ursolic acid) on the metabolic status and growth indicators of calves of Holstein black-and-white breed. **Keywords:** early postnatal period, calves, technological stress, phytoadaptogens, hormones, metabolism.

1 Introduction

Getting healthy young animals is a prerequisite not only for herd replacement but also for increasing the production of dairy products, which ultimately affects the food security of the country.

Stress is one of the important factors negatively affecting the growth, development, and resistance of young animals. It is completely impossible to neutralize the effect of stress factors technologically, in industrial animal husbandry. This is especially important for the organism of young animals raised in industrial technology, whose hormonal status changes as a result of exposure to technological stresses (vaccination, formation of new groups, change of diets, etc.), which in animals with low adaptive potential can lead to metabolic disorders, resulting in reduced resistance, viability and safety of young animals and leads to economic losses.

Adaptation of animals is manifested in their ability to maintain vital parameters of homeostasis when environmental conditions change.

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An important role in the adaptation of cattle to technological factors is played by hormones of the pituitary-adrenal axis, which regulate the intensity of metabolic processes and take part in the regulation of general nonspecific resistance of the body. At the same time, calves, not having a sufficiently formed endocrine system, are more often exposed to stress. The strongest stress for the young stock is regrouping and moving, which, on the one hand, lead to a change in behavior which reflected in the loss of appetite and a decrease in the time for eating and rest, while increasing the time for aggression and building hierarchical relationships in the group. On the other hand, the resistance of the organism decreases and the sensitivity to non-infectious diseases increases, which ultimately leads to productivity losses, a decrease in average daily gains and economic losses [1, 2].

Currently, synthetic drugs of the phenothiazine and benzodiazepine series are most widely used for preventive purposes, which are administered intramuscularly or subcutaneously, contributing to the relief of stress syndrome and reducing the loss of live and slaughter weight in young animals.

The main disadvantages of using synthetic drugs (tranquilizers and neuroleptics) is the accumulation of their metabolites in the body of animals. In addition, they are mainly used for the prevention of transport stress, while the growth technology of young animals has a fairly wide range of critical points that are a source of stress factors for calves (from regrouping to vaccination and changing feeding phases), which cannot be avoided.

One of the ways to increase resistance to adverse technological factors and improve the adaptive ability of young animals is to optimize metabolic processes by introducing biologically active substances of plant origin into the diet, for example, ursolic acid, which is contained in large quantities in cranberry meal extract.

Currently, terpene compounds (including ursolic acid) are isolated from the leaves of eucalyptus, rosemary, marjoram, lavender, thyme and organum, schizandra, fruits (peel of green apples), cowberries, cranberries, sea buckthorn, etc. [3, 4, 5, 6]. The great interest of researchers in ursolic acid is due to the fact that it has a wide range of pharmacological activity: anti-inflammatory [7], antioxidant [8], cardioprotective [9], neuroprotective [10], hepatoprotective [11], antiatrophic effect of skeletal muscles [12], can stimulate the differentiation of osteoblasts with the potential to prevent resorption during treatment time [13].

However, these drugs are used in sports and medicine, are manufactured abroad and have a high cost. The opportunity to use medicinal plants containing ursolic acid in animal husbandry practice has not been studied to date.

In this regard, the aim of the research was to study the effect of different doses of dry cranberry meal extract (containing 40% ursolic acid) on the adaptive potential of calves of Holstein black-and-white breed.

2 Materials and Method

Our research was carried out on the production base of the breeding plant of AO Uchkhov Prigorodnoye of the Altai Territory in 2022 on calves of Holstein black-and-white breed. To conduct the experiment, we formed 4 groups of calves (n=40) — a control group and 3 experimental ones with 10 animals each, according to the principle of analogous pairs. When forming analogues, gender (heifers), age (2 months), live weight (49.6 kg ± 5%) and origin were taken into account. Experimental animals were clinically healthy, kept in group cages, under the same conditions, and received the basic diet adopted by the farm.

The calves of the experimental groups were injected with dry cranberry meal extract containing 40% ursolic acid (experimental chemical production of Novosibirsk Institute of Organic Chemistry of the Siberian Branch of Russian Academy of Sciences, Patent RU 2414234C1), respectively, with the study scheme (Table 1).

Table 1. Experimental design

Group	Animals	Feed conditions
Control	10	Base diet (BD)
Experimental 1	10	BD+ cranberry meal extract at a dose of 100.0 mg per day per 1 animal for 10 days before transfer to the main group and 10 days after
Experimental 2	10	BD+ cranberry meal extract at a dose of 250.0 mg per day per 1 animal for 10 days before transfer to the main group and 10 days after
Experimental 3	10	BD+ cranberry meal extract at a dose of 400.0 mg per day per 1 animal for 10 days before transfer to the main group and 10 days after

Laboratory research on the study of morphological, biochemical parameters, and hormonal status were conducted in the educational and scientific laboratory of immunogenetics, immunity, and product quality control of the Department of General Biology, Biotechnology, and Animal Breeding of the Federal State Budget Educational Institution of Higher Education Altai State Agrarian University.

Blood sampling for the study was carried out from the jugular vein at the same time, in the morning before feeding in vacutainer tubes with anticoagulant (ethylenediaminacetic acid (EDTA). Serum for biochemical research was obtained by taking blood using vacuum tubes with a coagulation activator and placing it in a thermostat at 37 °C for 1 hour.

Morphological parameters of blood were studied using veterinary hematological analyzer MicroCC-20Plus: the calculation of blood corpuscles — by the impedance method, hemoglobin concentration — by the hemichromic method, the analysis of erythrocyte indices — by the calculation method.

The study of biochemical parameters of blood was carried out on a BioChemSA analyzer using diagnostic kits from AO Vital Development Corporation for the quantitative determination of total protein, albumins, urea, glucose, cholesterol, and triglycerides, AST, ALT, alkaline phosphatase, calcium, phosphorus, following the instructions.

The content of cortisol, thyroxine, and triiodothyronine in blood serum was determined by immunoenzymatic methods (EIA) using ChemaMedica kits (Moscow) on a multichannel spectrophotometer of Bio-Rad 680 model.

Adaptability coefficient was calculated by the formula:

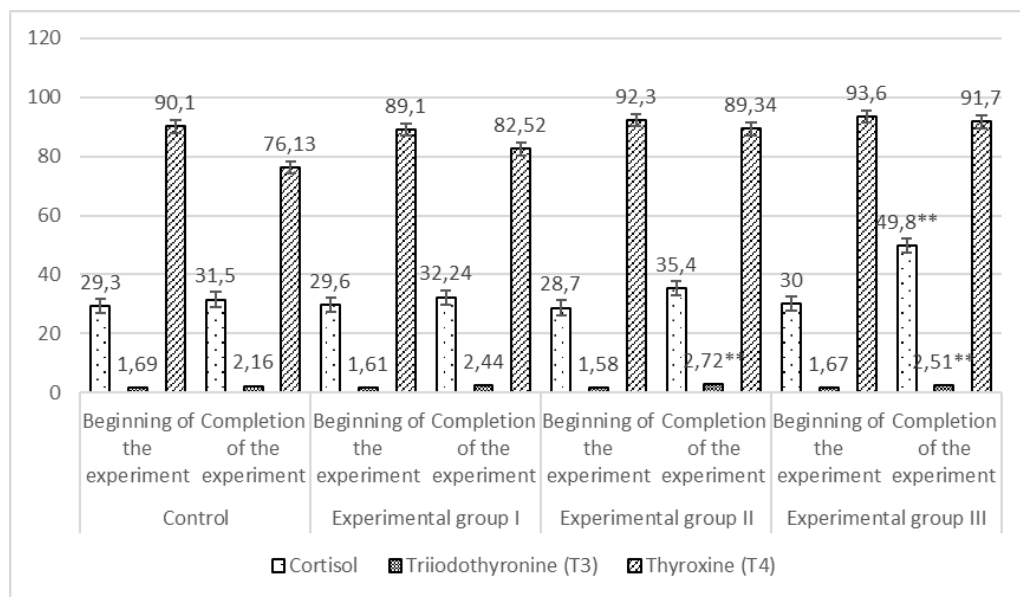
$$G = \sum |r_{ij}|, |r_{ij}| \geq \alpha$$

where r_{ij} — correlation coefficient between the i -th and j -th parameters (hormone levels, biochemical and morphological parameters of blood), α — r_{ij} confidence level [14].

Biometric analysis of morphological biochemical and hormonal blood status indicators was carried out using StatSoft STATISTICA 10.0.1011 Eneterpise analytical program [En].

3 Results

Thyroid and adrenal hormones are one of the main regulators of adaptation processes, they influence the productive indicators and metabolism direction, therefore, analysis of the dynamics of thyroid hormones concentration and corticosteroid-cortisol makes it possible to assess the degree of adaptation capabilities to new technological conditions. The results of hormonal status assessment in calves are shown in Figure 1.



* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ — the difference is statistically significant in comparison with the control group.

Fig. 1. Hormonal status in calves of Holstein black-and-white breed, nmol/l. *Source:* “Compiled by the authors”.

Studies have found that the use of cranberry meal extract in the calves’ diet contributes to a change in the functional activity of the endocrine glands that determine the metabolism direction and hematopoiesis intensity in a dose-dependent manner.

A month after the transfer of calves (at the end of the experiment), we noted an increase in cortisol level in the blood of animals of all experimental groups, the minimum — in the control and first experimental groups by 7.5 and 8.9%, and the maximum — in groups 2 and 3 by 23.3 and 66%, respectively, compared with the beginning of the experiment. At the same time, the highest level of cortisol by the end of the experiment was noted in the experimental groups 2 and 3, which turned out to be 12.3 and 58.1% higher compared to the animals of the control group. The dose-dependent nature of the dynamics and cortisol content in the blood of calves established by us is consistent with the studies [15].

It should be noted that a higher basal cortisol concentration is not a sufficiently informative criterion for assessing the body challenge degree. Thus, when introducing dry cranberry meal extract into the diet, we noted some trends in the concentration of thyroid hormones, which indicate that the calves of the experimental group 2 had the best adaptive potential. It is manifested in the fact that with an increase in the functional activity of the adrenal cortex, a higher level of thyroid hormone metabolism was noted, which is reflected in a higher

concentration of thyroxine and triiodothyronine by 14.7 and 20.5%, as well as a higher ratio of T3/T4, respectively, in comparison with the control group of animals.

At the same time, the lowest T3/T4 ratio, amid the increase in cortisol levels, was recorded in the experimental group 3, which indicates the functional tension of the endocrine system and adaptation processes. No significant differences in hormonal status between the experimental group 1 and control group were observed.

To identify the effect of cranberry meal extract on the health of experimental animals, we studied the morphological parameters of calves' blood (Table 2).

Table 2. Morphological parameters of calves' blood

Indicator	Physiological norm	Control group		Experimental 1		Experimental 2		Experimental 3	
		At the beginning of the experience	At the end of the experience	At the beginning of the experience	At the end of the experience	At the beginning of the experience	At the end of the experience	At the beginning of the experience	At the end of the experience
Leucocytes, *10 ⁹ /l	5-16	9.2 ±1.03	9.97 ±0.667	8.93 ±0.91	9.85 ±0.58	9.4 ±0.75	10.38 ±1.072	8.14 ±1.05	12.1 ±0.69*
Erythrocytes, *10 ¹² /l	4.5-10.1	5.09 ±0.638	4.85 ±0.592	4.89 ±0.43	5.11 ±0.62	4.59 ±0.505	6.36 ±0.314*	5.07 ±0.398	5.27 ±0.60
Hemoglobin, g/l	90-139	94.5 ±4.53	99.0 ±6.65	95.0 ±3.89	99.50 ±2.31	94.3 ±2.97	109.0 ±2.42	89.7 ±4.96	101.7 ±2.74
Hematocrit, %	28-46	28.1 ±2.36	28.4 ±2.08	28.0 ±0.63	29.7 ±0.22	25.65 ±2.12	32.3 ±1.10*	27.7 ±1.51	30.4 ±0.29
Thrombocytes, *10 ⁹ /l	120-820	508.6 ±55.43	587.3 ±48.85	532 ±17.4	565 ±17.5	503.5 ±67.75	721.6 ±98.23* *	493.8 ±33.94	617.0 ±16.9*

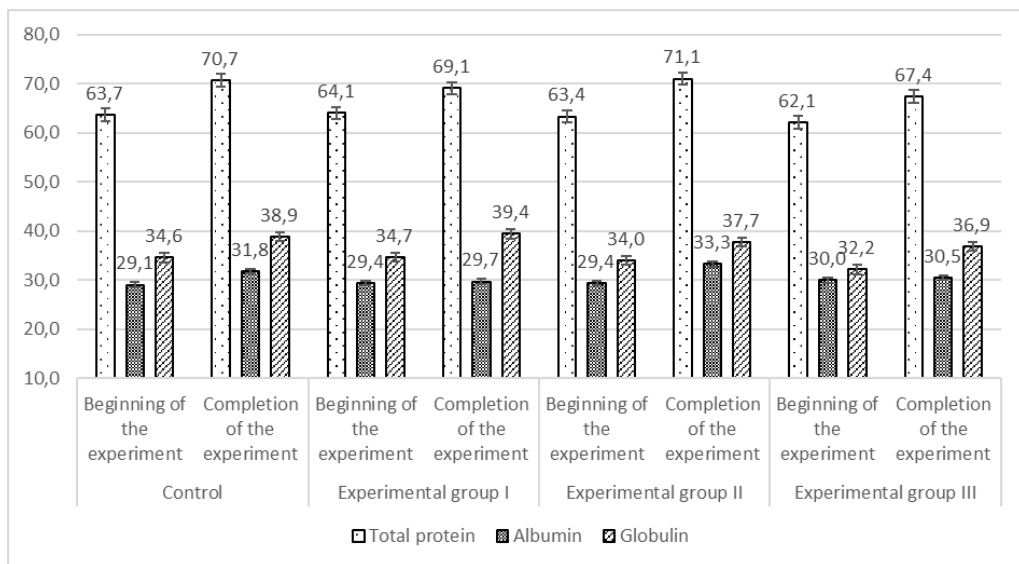
*P ≤0.05; **P ≤0.01; ***P≤0.001 — the difference is statistically significant in comparison with the control group.

The regularities in the influence of different doses of cranberry meal extract on the hormonal status of calves established by us are also confirmed in the dynamics of morphological blood parameters. The most optimal changes, indicating an improvement in the supply of oxygen to the body and a more intensive course of redox processes, were noted in the experimental group 2. Thus, in calves receiving 250.0 mg of cranberry meal extract in addition to the diet, an increase in the number of red blood cells, hemoglobin and hematocrit levels by 23.7 (P<0.05); 9.2 and 12.1 (P<0.05)%, respectively, was observed in comparison with the control group of animals.

A similar effect in calves of the second experimental group is manifested in a higher thrombocyte content by 22.8 (P < 0.05); 21.7; 14.5%, compared with the control and experimental groups 1 and 3, respectively. The established trend is consistent with the results [16], in which it is reported that the use of ursolic acid contributes to an increase in thrombocyte levels depending on the dose.

In the experimental group 3, where the amount of administered cranberry meal extract was the maximum — 400.0 mg, an increase in the level of leukocytes by 14.2% (P < 0.05), was observed compared with the control group which may be an indirect sign of functional stress of the immune system. No significant differences in the morphological status of blood were observed between the experimental group 1 and control group.

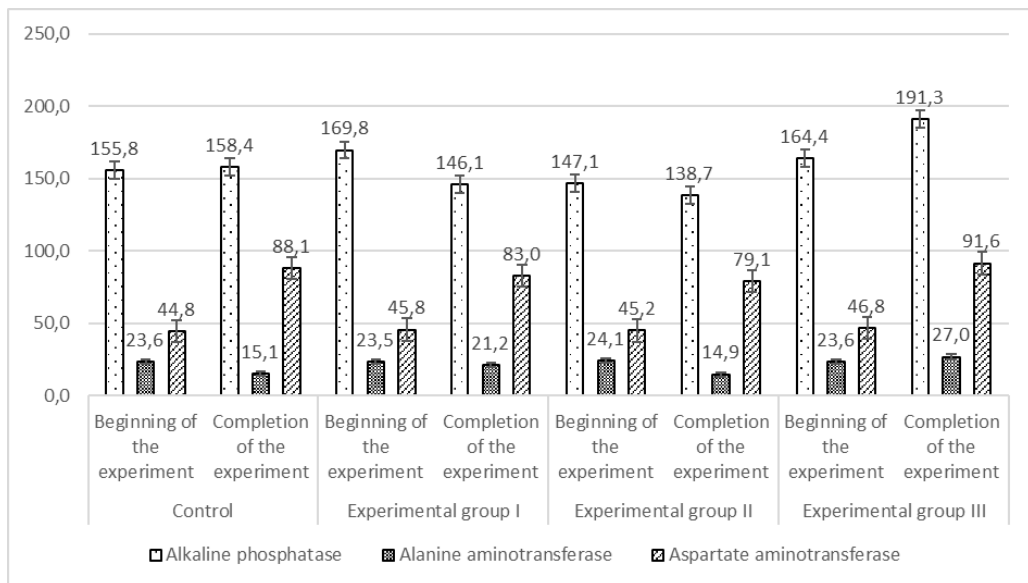
Biochemical parameters of blood serum indicated that metabolism direction in calves of the experimental and control groups corresponds to age characteristics, and the most optimal dose of cranberry meal extract is 250.0 mg per animal per day (Figure 2, 3, 4).



* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ — the difference is statistically significant in comparison with the control group.

Fig. 2. Indicators of protein metabolism in calves of Holstein black-and-white breed, g/l. *Source: "Compiled by the authors"*

This was reflected in a higher level of total protein and albumins by 0.4 and 4.5%, and a decrease in the content of ALT, AST, and alkaline phosphatase by 11.4; 1.3, and 14.2%, respectively (Figure 2 and 3), compared with the control group of animals.

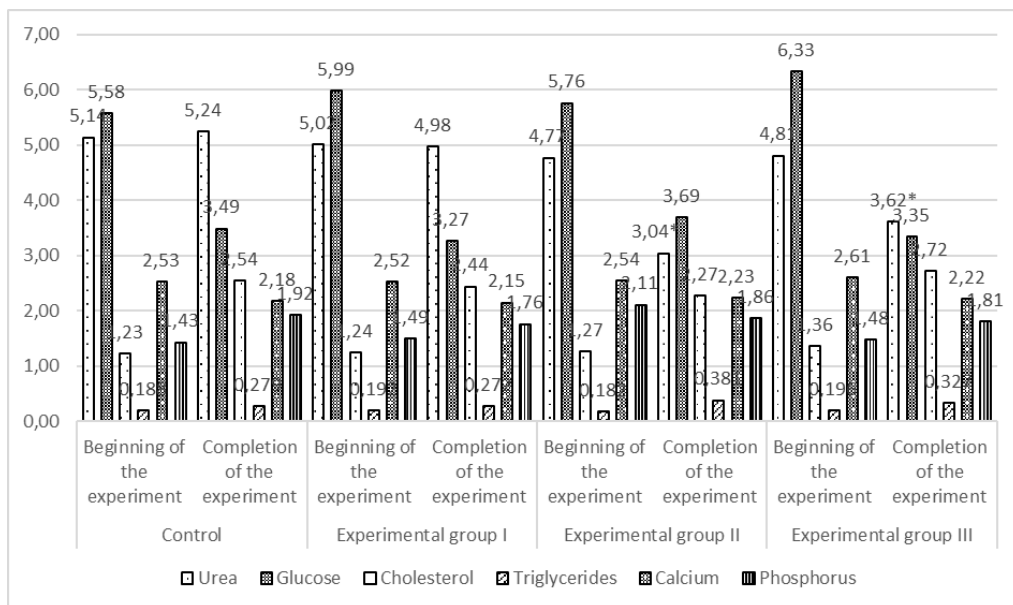


*P ≤0.05; **P ≤0.01; ***P≤0.001 — the difference is statistically significant in comparison with the control group.

Fig. 3. Activity indicators of liver enzymes in calves of Holstein black-and-white breed, units/l *Source: "Compiled by the authors"*

It should be noted that the indicators of protein metabolism in calves that received cranberry meal extract in addition to the main diet had some peculiarities depending on the dose of the drug. Thus, when feeding cranberry meal extract at a dose of 250.0 mg (experimental group 2), in comparison with the control, experimental groups 1 and 3, the level of total protein and albumins is higher by 2.8; 12.1 and 5.4; 9.2%, respectively, in comparison with experimental groups 1 and 3.

The level of urea in the blood of calves of the experimental group 2 was 41.9 (P <0.05); 38.9; 16.2%, respectively, in comparison with the control and experimental groups 1 and 3 (Figure 4).



*P < 0.05; **P < 0.01; ***P < 0.001 — the difference is statistically significant in comparison with the control group.

Fig. 4. Dynamics of biochemical parameters of calves' blood of Holstein black-and-white breed, mmol/l. Source: "Compiled by the authors"

The extract dosage of 100.0 mg per day per animal (experimental group 1) does not have significant metabolic effects.

It was found that the introduction of cranberry meal extract into the diet contributes to the normalization of carbohydrate and lipid metabolism. So, when cranberry meal extract is introduced into the diet at a dose of 250.0 mg per animal per day, a higher glucose level by 5.7; 12.8; 10.1%, and the cholesterol concentration lower by 10.6; 7.0; 16.5% compared with the control and experimental groups 1 and 3, were observed.

It should also be noted that in the second experimental group there is an increase in the concentration of triglycerides by 26.7; 28.6 and 14.2% amid a decrease in cholesterol by 11.9; 7.5 and 19.8% compared with the control, first and third experimental groups, which may be due to the most optimal ratio of thyroid hormones (Table 2), which enhance it breaks down and activates the utilization of lipids, which leads to hypercholesterolemia and an increase in triglycerides in the blood [17].

To assess the effect of cranberry meal extract on the level of adaptation of calves based on the method of correlation adaptometry [14], the coefficient of organism adaptation was calculated.

According to the methodology, the highest adaptability level corresponds to a coefficient value below 2.60 conditional points (satisfactory adaptation) and, vice versa, values above 3.49 conditional points are characteristic of adaptation failure. The results are shown in Figure 5.

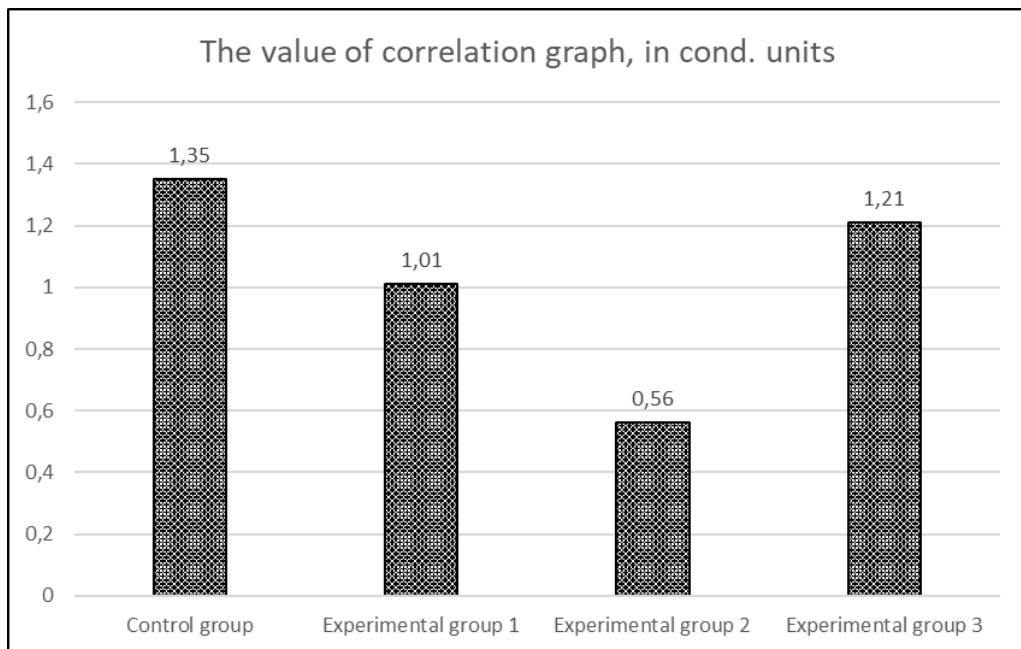


Fig. 5. Assessment of adaptation level in calves. Source: “Compiled by the authors”

It was found that the lowest value of the correlation graph — 0.56 was observed in calves of the second experimental group, who received 250.0 mg of cranberry meal extract in addition to the diet, which indicates the optimal function of the calves’ body during the period of technological stress. In calves of the control group, the value of the correlation graph 1.35 indicates the maximum degree of functional stress of the body.

5 Discussion

Ursolic acid is a pentacyclic triterpenoid capable of influencing the transmission of insulin signals in adipose tissue, the expression of heart damage markers, the process of inflammation and the level of antioxidants in the brain, the intensity of metabolic signals, and the level of oxidants in the liver, as well as atrophy signals in muscles. In addition, this phytonutrient is non-toxic, and its safety and ability to affect various key targets make it very promising as a platform for the development of dietary supplements and medicines [18].

It is important to note that cranberry meal extract containing ursolic acid (at least 40%) is produced in an environmentally friendly way at the experimental chemical production of Novosibirsk Institute of Organic Chemistry named after N.N. Vorozhtsov (NIOC SB of RAS).

Due to the vast number of positive effects that drugs containing ursolic acid have, it is of great scientific and practical interest to study the possibility of using medicinal plants containing it to increase the adaptive capacity of young farm animals

The established changes in the concentration of hormones indicate that the best adaptive potential was possessed by calves of the experimental group 2, who received 250.0 mg of cranberry meal extract as part of the base diet. It is manifested in the fact that with an increase in the functional activity of the adrenal cortex, a higher level of thyroid hormone metabolism was noted, which is reflected in a higher concentration of thyroxine and triiodothyronine, as well as a higher ratio of T3/T4, respectively, in comparison with the control group of animals.

At the same time, the lowest T3/T4 ratio, amid the increase in cortisol levels, was recorded in the experimental group 3, which indicates the functional tension of the endocrine system and adaptation processes. No significant differences in hormonal status between the experimental group 1 and control group were observed.

The effect of introducing dry cranberry meal extract into the calves' diet on the morphological blood composition also depends on the drug dose. It was found that the introduction of an additional 250.0 mg of cranberry meal extract into the calves' diet, during the period of exposure to technological stress factors, positively affects the processes of hematopoiesis, improves oxygen supply of the body and promotes a more intensive course of redox processes, which may be associated with an increase in the activity of neuroendocrine regulation of hematopoietic organs under the action of biologically active substances included in the composition of cranberry meal extract and leads to an increase of red blood cells in the blood of calves of experimental group 2.

Analysis of blood biochemical parameters also confirms that the most optimal dose of cranberry meal extract is 250.0 mg per animal per day. Thus, calves of the experimental group 2 have a higher level of total protein and albumins and a low content of ALT, AST, and alkaline phosphatase, indicating the predominance of anabolic processes over catabolic, that is, the intensity of growth and formation of young animals. This may be due to the optimal concentration of ursolic acid and its ability to increase the activity of mTORC1 (the target of mammalian rapamycin), one of the most important regulators of neurophysiological processes of protein synthesis [19, 20; 21] and reduce protein breakdown [19], as indirectly evidenced by the low level of urea.

Blood albumins are involved in the transport of low molecular weight compounds and are a plastic material for the synthesis of tissue structures. Therefore, calves of the experimental group 2, with a high concentration of albumins in the blood, showed more active growth and high rates of live weight gain, which was also established by us in previous studies. In calves receiving 250.0 mg of cranberry meal extract in addition to the diet, a statistically significant ($p < 0.05$) difference in live weight was noted, compared with the control group by 5.1 kg (5.7%). The average daily increase in live weight during the study period was also 15.7% higher ($p < 0.05$), and the growth rate was 8.4% [22].

It should be noted that the studies of Ou X. et al. [23] confirm the fact that a high concentration of ursolic acid can inhibit the leucine-stimulated activity of mTORC1, disrupting the lysosomal localization of mTOR, thereby probably reducing the intensity of metabolic processes. It is confirmed by the results of our studies, which indicate that when the maximum dosage of cranberry meal is introduced into the diet, a decrease in the level of total protein and albumins by 4.7 and 4.1% is observed, compared with the control group.

When cranberry meal extract is introduced into the diet at a dose of 250.0 mg per animal per day, as noted above, it contributes to the normalization of carbohydrate and lipid indicators by increasing glucose levels and reducing cholesterol concentration.

A stable glucose level in the blood of ruminants is maintained due to the process of gluconeogenesis, in which glycogen acts as a substrate for glucose synthesis, and the absorption of carbohydrates from the digestive tract is insignificant. Therefore, a lower concentration of glucose in the blood of calves of the control, first and third experimental groups is adaptive in nature and may indicate a decrease in glycogen reserves in the liver and muscles [24], while the body of calves tries to compensate for the low concentration of glucose by splitting lipids, which is reflected in an increase in cholesterol levels, it is confirmed by our research and is consistent with the data of Dai S. et al. [25].

In addition, a higher glucose level in the second experimental group may be associated with greater thyroid activity and an optimal T3/T4 ratio (Table 2), which is one of the important

factors activating carbohydrate metabolism, enhancing glucose absorption in the intestine, and accelerates its utilization by cells [17, 26, 27].

An increase in the concentration of triglycerides in calves of the experimental group 2 amid a decrease in cholesterol levels may be associated with the most optimal ratio of thyroid hormones, which enhance the breakdown and activate the utilization of lipids, which leads to hypercholesterolemia and an increase in triglycerides in the blood [17].

6 Conclusion

Introduction of cranberry meal extract in a diet at a dose of 250.0 mg per day per 1 animal for 10 days before transfer to the main group and 10 days after contributes to moderate stimulation of the adaptive ability, normalization of metabolism and increase of adaptive potential.

Acknowledgments

The state task No. 122030100437-9 of the Ministry of Agriculture of the Russian Federation on the topic “The use of phytoadaptogens from regional raw materials of plant origin in the cultivation of replacing young cattle in the Altai Territory” was a job authorization for this paper.

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