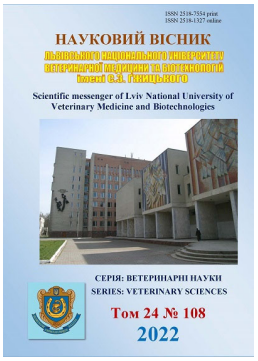

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The effect of antioxidants on biochemical and morphological indicators of the piglet's blood

K. Y. Leskiv[1]✉, B. V. Gutyj[1], V. M. Hunchak[1], I. I. Khariv[1], R. O. Vasiv[1], M. S. Romanovych[1], V. Ya. Prysiazhniuk[1], O. V. Pavliv[2], S. S. Adamiv[2]

[1]*Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Ukraine*
[2]*Separated subdivision of National University of Life and Environmental Sciences of Ukraine "Berezhany Agrotechnical Institute", Berezhany, Ukraine*

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Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Pekarska Str., 50, Lviv, 79010, Ukraine.
Tel.: +38-067-996-92-07
E-mail: xpystik@ukr.net

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The work aimed to study the influence of methifen, fenarone, and methionine on the biochemical and morphological indicators of the piglet's blood. We researched three-month-old large white piglets. 10 groups were formed, each with 5 animals: a control group and nine experimental groups. We established that antioxidants promote an increase in the erythrocyte number and the Hb level to the upper limits of physiological indicators from the 10th day of the test. On the 60th day, the erythrocyte number in the blood of piglets fed methionine varied between 7.15 ± 0.12 – 7.30 ± 0.12 B/l. This indicator was 7.34 ± 0.10 – 7.64 ± 0.11 B/l ($P < 0.05$) in the group fed fenarone, and 7.64 ± 0.14 – 7.65 ± 0.11 B/l ($P < 0.05$) in animals fed methifen. On the 90th day, the erythrocyte number was the highest in animals that were fed methifen at a dose of 0.9 mg/kg body weight. Relative to piglets of the C group, the aforementioned indicator increased by 12.9 %. We also found a slight growth in the activity of aminotransferases in the blood serum of piglets of the experimental groups. In addition, it was found that the studied drugs do not affect the mononuclear system. It was indicated by the number of leukocytes, which was within the limits of physiological parameters during the experiment and ranged from 10.0 ± 0.12 to 11.8 ± 0.6 G/l. The highest indicators of enzyme activity, hemoglobin content, and the number of erythrocytes in the blood of animals of the experimental groups were established on the 60th and 90th days of the trial. It manifested the best effect under methionine feeding at a dose of 4 mg/kg body weight, fenarone – 1.20 mg/kg body weight. and metifen – 0.9 mg/kg body weight.

Key words: *methifen, fenarone, methionine, antioxidants, piglets, aminotransferases.*

Introduction

Solving the problem of providing the population of Ukraine with high-quality pork is the development of new scientifically based approaches to managing the pig industry. It includes a full-fledged, optimized animal feeding system (Kramarenko et al., 2018; 2019; Krempa et al., 2021; Kozenko et al., 2022). The most rational way to provide nutrient resources for animals is the production of complete feed of combined composition. The effect of the use of premises is possible only when there is absolute compliance with the content of the introduced ingredients of the formula (Khalak, 2020; Khalak et al., 2020; 2021; 2022).

The method of intensive breeding of pigs with the use of highly productive lines and industrial technology of keeping is significantly different from traditional methods of their breeding. Under these conditions, early weaning of piglets from sows, transportation, etc., are stressful factors that contribute to the reduction of the protective and adaptive reactions of their bodies. In such conditions, the balance between the activity of the antioxidant system and the peroxidation of lipids is disturbed, and a stressful state occurs (Martyshuk et al., 2019; 2020; 2021). A delay in growth, an increase in morbidity and mortality of pigs, a violation of their ability to reproduce, and a decrease in the quality of meat products accompany this (Vyslotska et

al., 2021; Leskiv et al., 2021). That is why it is advisable to add natural antioxidants to piglet feed.

The purpose of the research

To study the influence of methifen, fenarone, and methionine on the biochemical and morphological parameters of the blood of piglets.

Material and methods

We researched three-month-old large white piglets. 10 groups were formed, each with 5 animals: a control group and nine experimental groups. We fed the piglets of group C the usual forage for the farm, which was balanced according to the norms without specifying the above-mentioned antioxidants.

Group E_1 piglets were fed methionine at a dose of 2 mg/kg body weight once a day with feed.

Piglets of the E_2 group were fed methionine at a dose of 3 mg/kg body weight once a day with feed.

Group E_3 piglets were fed methionine at a dose of 4 mg/kg body weight once a day with feed.

The E_4 group was fed fenarone at a dose of 1.10 mg/kg body weight once a day with feed.

The E_5 group was fed fenarone at a dose of 1.15 mg/kg body weight once a day with food.

Piglets of the E6 group were fed fenarone at a dose of 1.20 mg/kg body weight once a day with feed.

Group E_7 was fed methifen at a dose of 0.85 mg/kg body weight once with food.

Piglets of the E_8 group were fed methifen at a dose of 0.9 mg/kg body weight once a day with feed.

Piglets of the E_9 group were fed methifen at a dose of 0.95 mg/kg body weight once a day with feed.

Methionin – Methioninum, is a white crystalline powder, with an active substance content of at least 98.5 %. Well soluble in diluted mineral acids, caustic alkalis, and ammonia, hardly soluble in water (Gutyj et al., 2017; Martyshchuk & Gutyj, 2019).

Fenaronum is a complex compound containing 70 % phenazanic acid and 30 % zeolite. It is thermostable, and dissolves poorly in cold water, but better in hot water (Gutyj et al., 2016).

Metiphen – Metiphenum is a white crystalline powder, sweet to the taste, with the smell of sulfur. It dissolves poorly in cold water, better in hot water (1:20). Thermostable. The drug contains fenarone and methionine.

Blood for research was taken from the cranial vena cava at the beginning of the experiment and 10, 30, 60, and 90 days after feeding antioxidants.

We carried statistical processing of research results out according to the method described by V. A. Oivin. The degree of probability compared to the data of the control group was: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

When conducting the research, the rules mandatory for performing zootechnical experiments regarding the selection and maintenance of analogous animals in groups were followed. The diet of the piglets was balanced in terms of nutrients and minerals. During the research, the principles of bioethics were followed, following the requirements of the European Convention on the Protection of Experimental Animals (86/609 EEC).

Results and discussion

Physiological processes occurring in the body largely affect the quality of blood. Hematological studies make it possible to examine more deeply the effect of methionine, fenarone, and methifen, based on which it is viable to evaluate the drugs and choose the optimal dose of the drug for this type of animal.

According to the data in Table 1, the erythrocyte number, both in experimental and control groups, was within the limits of physiological parameters, within 6.56 ± 0.28 – 6.65 ± 0.12 T/l. In animals of the C group, the number of erythrocytes on the 90th day raised by almost 2 % relative to the initial values. The feeding of antioxidants with feed contributed to an increase in the number of erythrocytes in the blood of piglets starting from the 10th day of the experiment. The largest number was in groups E_6, E_7, and E_8. On the 30th day, the number of erythrocytes in the blood of experimental piglets continued to increase. Thus, during the indicated period, the number of erythrocytes in the blood of piglets, which were fed fenarone increased by 4.7, 5.9, and 6.9 %, relative to the values of animals in the C group. In animals that were fed metifen, this indicator increased by 7.7, 10.6 and 8.4 %.

Table 1

The effect of methifen, fenarone, and methionine on the number of erythrocytes in the blood of piglets, T/l (M ± m, n = 5)

Groups	Study periods				
	At the beginning of the experiment	10-та доба	30th day	60th day	90th day
C	6.56 ± 0.28	6.62 ± 0.11	6.78 ± 0.11	7.10 ± 0.12	7.05 ± 0.12
E_1	6.58 ± 0.25	6.70 ± 0.12	7.01 ± 0.11	7.15 ± 0.12	7.14 ± 0.12
E_2	6.60 ± 0.21	6.74 ± 0.22	7.04 ± 0.13	7.28 ± 0.11	7.26 ± 0.13
E_3	6.59 ± 0.23	6.76 ± 0.21	7.04 ± 0.11	7.30 ± 0,12	7.31 ± 0.13
E_4	6.62 ± 0.12	6.74 ± 0.11	7.10 ± 0.12	7.34 ± 0.10	7.34 ± 0.12
E_5	6.58 ± 0.12	6.80 ± 0.11	7.18 ± 0.10	7.42 ± 0.12	7.40 ± 0.11*
E_6	6.62 ± 0.13	6.85 ± 0.12	7.25 ± 0.10*	7.64 ± 0.11*	7.60 ± 0.13*
E_7	6.65 ± 0.12	6.81 ± 0,12	7.30 ± 0.12*	7.65 ± 0.11*	7.80 ± 0.14**
E_8	6.63 ± 0.12	6.92 ± 0.37	7.50 ± 0.13**	7.95 ± 0.14**	7.96 ± 0.15**
E_9	6.59 ± 0.15	6.80 ± 0.26	7.35 ± 0.14*	7.64 ± 0.14*	7.6 ± 0.13*

*The degree of probability, compared to indicators in piglets of the control group: * – $P \le 0.05$; ** – $P \le 0.01$; *** – $P \le 0.001$*

On the 60th day, the erythrocyte number in the blood of piglets fed methionine varied between 7.15 ± 0.12, 7.30 ± 0.12 B/l, and 7.34 ± 0.10 7, fed fenarone 64 ± 0.11 B/l, and fed metifen, their amount was within the range of 7.64 ± 0.14 – 7.65 ± 0.11 B/l. On the 90th day, this indicator was the highest in the animals of the experimental groups, which were fed with methifen. Thus, the erythrocyte number raised by 10.6 % in piglets fed metifen at a dose of 0.85 mg/kg body weight, and by 12.9% at a dose of 0.9 mg/kg, respectively. The number of erythrocytes in the blood of piglets fed methifen at a dose of 1.0 mg/kg body weight, was lower than the indicators of groups E_7 and E_8 in this period.

We consider that the increase in the erythrocyte number in the blood of piglets after feeding them with methionine, fenarone, and methifen is caused by a growth in the erythropoietic function of the bone marrow. Confirmation of this hypothesis is an examination of chickens and cattle.

A change in the erythrocyte number in the blood of experimental piglets also contributes to an increase in the hemoglobin content, as indicated by the research results shown in Table 2.

When feeding methionine, the hemoglobin content on the 10th day increased by 2 % in animals of all three groups. On the 30th day, the hemoglobin content in the blood of animals of the group, which were fed methionine at a dose of 4 mg/kg body weight. increased by 4% relative to the size of animals in the C group.

On the 90th day, the hemoglobin content in animals of group E_1 raised to 102.3 ± 1.13 g/l, in animals of group E_2 to 102.2 ± 1.19 g/l, and in group E_3 to 103.1 ± 1,11 g/l.

When analyzing the results of studies on the effect of fenaron on the hemoglobin content in the blood of intact piglets, it was established that on the 10th day, the hemoglobin content relative to the indicator in the piglets of the control group increased by 2.2, 2.6 and 3.0 %, respectively, in the animals of the groups E_4, E_5 and E_6. On the 30th day of the experiment, this index in the animals of the experimental groups increased by 4 and 4.3 %, respectively, relative to the values of the C group of animals. On the 60th day of the trial, the hemoglobin content in the animals of the experimental groups continued to increase and on the 90th day, it reached 103.0 ± 1.17 g/l in group E_4 and 103.5 ± 1.18 g/l in group E_5 and group E_6 104.1 ± 1.16 g/l.

Slightly higher values of the studied indicator were established in piglets fed metifen.

Table 2

The influence of methifen, fenarone, and methionine on the hemoglobin level in the blood of intact piglets, g/l (M ± m, n = 5)

Groups	Study periods				
	At the beginning of the experiment	10th day	30th day	60th day	90th day
C	96.4 ± 1.32	97.0 ± 1.36	97.6 ± 1.17	100.8 ± 1.17	101.0 ± 1.16
E_1	95.6 ± 1.36	98.9 ± 1.12	100,0 ± 1.32	102.4 ± 1.20	102.3 ± 1.13
E_2	96.6 ± 1.02	99.3 ± 1.02	100.7 ± 1.12	102.8 ± 1.26	102.2 ± 1.19
E_3	96.4 ± 0.50	98.9 ± 1.50	101.4 ± 1.54	103.3 ± 1.54	103.1 ± 1.11
E_4	96.1 ± 0.14	99.1 ± 1.16	101.5 ± 1.17	103.3 ± 1.16	103.0 ± 1.17
E_5	96.6 ± 1.02	99.5 ± 1.11	101.8 ± 1.12	103.8 ± 1.18	103.5 ± 1.18
E_6	97.0 ± 1.14	99.9 ± 1.15	101.8 ± 1.15	104.1 ± 1.15	104.1 ± 1.16
E_7	96.4 ± 1.18	99.8 ± 1.18	101.7 ± 1.20	104.3 ± 1.59	104.1 ± 1.14
E_8	96.2 ± 1.17	100.8 ± 1.52	102.8 ± 1.15	105.8 ± 1.13*	105.7 ± 1.17*
E_9	96.8 ± 1.18	100.4 ± 1.67	102.0 ± 1.13	104.6 ± 1.16	104.5 ± 1.18

*The degree of probability, compared to indicators in piglets of the control group: * – $P \le 0.05$; ** – $P \le 0.01$; *** –$P \le 0.001$*

So, on the 10th day, the hemoglobin content in the animals of the experimental group, which were fed metifen at a dose of 0.85 mg/kg body weight, increased by 2.9 %, and at a dose of 0.9 mg/kg body weight, respectively by 3.9 %. When feeding methifen at a dose of 1.0 mg/kg body weight, the hemoglobin content in the blood of piglets increased by 3.5 % relative to the values in animals of the control group. On the 30th day, the hemoglobin content in the blood of the experimental animals increased by 4.2, 5.3, and 4.5 %, respectively, compared to the C group. On the 60th and 90th days, the hemoglobin level in the blood of piglets, which were fed metifen at a dose of 0.85 mg/kg body weight was within the range of 104.3 ± 1.59 – 104.1 ± 1.14 g/l. In pigs that were fed metifen at a dose of 0.9 mg/kg body weight, the hemoglobin level was within the range of 105.8 ± 1.13 – 105.7 ± 1.17 g/l. In animals that were fed metifen at a dose of 1.0 mg/kg body weight, this indicator was within 104.6 ± 1.16 – 104.5 ± 1.18 g/l.

Therefore, the increase in the hemoglobin level in the blood of experimental animals that were fed antioxidants is because the level of hemoglobin in the blood is directly dependent on the number of erythrocytes, and these drugs enhanced the erythropoietic function of the bone marrow of piglets.

From the data presented in Table 3, the number of leukocytes in the blood of animals during the experiment fluctuated within the physiological parameters.

Therefore, methionine, fenarone, and metiphen do not contain irritating substances, and do not affect the mononuclear system, which is indicated by the number of leukocytes during the 90 days of the experiment.

Determining the activity of aminotransferases plays an important role in the study of the therapeutic effectiveness of antioxidant drugs since the degree of liver damage can

be judged by the activity of these enzymes. Aminotransferases are enzymes that catalyze the reaction of transferring an amino group (NH2) together with a proton (hydrogen ion) and a pair of electrons from amino acids or amines to keto acids. The biological role of aminotransferases is extremely large because they take part in transamination, a process that is most important for energy metabolism.

The activity of aminotransferases in the blood is an important diagnostic feature of many diseases. Aspartate aminotransferase (AsAT) and alanine aminotransferase (AlAT) have the greatest clinical and diagnostic value. An increase in the activity of these enzymes in the blood makes it possible to recognize pathological conditions accompanied by tissue necrosis.

Table 3

The effect of methifen, fenarone, and methionine on the number of leukocytes in the blood of intact piglets, G/l (M ± m, n = 5)

Groups	Study periods				
	At the beginning of the experiment	10th day	30th day	60th day	90th day
C	10.4 ± 0.52	10.5 ± 0.48	11.6 ± 0.50	11.4 ± 0.16	11.6 ± 0.50
E_1	10.0 ± 0.12	10.6 ± 0.14	11.7 ± 0.16	11.4 ± 0.17	11.5 ± 0.15
E_2	10.4 ± 0.17	11.0 ± 0.11	11.0 ± 0.11	11.5 ± 0.14	11.4 ± 0.14
E_3	10.8 ± 0.12	10.8 ± 0.18	11.4 ± 0.17	11.4 ± 0.17	11.6 ± 0.14
E_4	10.1 ± 0.58	10.6 ± 0.50	11.0 ± 0.53	11.4 ± 0.45	11.7 ± 0.40
E_5	10.2 ± 0.67	10.4 ± 0.50	10.4 ± 0.50	10.4 ± 0.50	11.8 ± 0.6
E_6	10.4 ± 0.74	11.0 ± 0.56	11.2 ± 0.66	11.5 ± 0.37	11.5 ± 0.37
E_7	10.0 ± 0.67	10.2 ± 0.37	11.5 ± 0.67	11.6 ± 0.67	11.6 ± 0.45
E_8	10.6 ± 0.6	10.2 ± 0.37	11.6 ± 0.68	11.5 ± 0.44	11.4 ± 0.48
E_9	10.3 ± 0.6	10.6 ± 0.50	11.6 ± 0.65	11.6 ± 0.62	11.5 ± 0.56

*The degree of probability, compared to indicators in piglets of the control group: * – $P \le 0.05$; ** – $P \le 0.01$; *** –$P \le 0.001$*

We established that in experimental piglets, the activity of AsAT at the beginning of the experiment was within the range of 0.350 ± 0.016 – 0.360 ± 0.017 mmol/l/h (Table 4).

After feeding methionine to piglets, the activity of AsAT in their blood serum increased slightly. On the 60th day, in the animals that were fed methionine at a dose of 3 mg/kg body weight, it increased by 2 %, and in animals that were fed methionine at a dose of 4 mg/kg body weight, the activity of the enzyme raised by 3 % relative to the values of animals in the C group.

Table 4

The effect of methifen, fenarone, and methionine on the activity of aspartate aminotransferase in the blood of intact piglets, mmol/l/h (M ± m, n = 5)

Groups	Study periods				
	At the beginning of the experiment	10th day	30th day	60th day	90th day
C	0.360 ± 0.017	0.358 ± 0.010	0.355 ± 0.012	0.350 ± 0.011	0.353 ± 0.015
E_1	0.355 ± 0.014	0.356 ± 0.014	0.357 ± 0.012	0.356 ± 0.014	0.354 ± 0.014
E_2	0.350 ± 0.016	0.354 ± 0.012	0.356 ± 0.011	0.357 ± 0.013	0.354 ± 0.012
E_3	0.356 ± 0.015	0.357 ± 0.013	0.360 ± 0.011	0.360 ± 0.012	0.362 ± 0.013
E_4	0.359 ± 0.010	0.359 ± 0.013	0.362 ± 0.011	0.365 ± 0.014	0.363 ± 0.015
E_5	0.350 ± 0.016	0.358 ± 0.016	0.364 ± 0.015	0.367 ± 0.010	0.366 ± 0.012
E_6	0.357 ± 0.015	0.361 ± 0.013	0.365 ± 0.012	0.369 ± 0.013*	0.369 ± 0.014
E_7	0.355 ± 0.016	0.360 ± 0.012	0.364 ± 0.014	0.370 ± 0.015*	0,369 ± 0.013
E_8	0.360 ± 0.014	0.365 ± 0.011	0.371 ± 0.012	0.386 ± 0.014*	0.374 ± 0.014*
E_9	0.355 ± 0.012	0.360 ± 0.014	0.365 ± 0.013	0.374 ± 0.017*	0.370 ± 0.15*

*The degree of probability, compared to indicators in piglets of the control group: * – $P \le 0.05$; ** – $P \le 0.01$; *** –$P \le 0.001$*

Feeding fenarone raised the AST activity in blood serum. Thus, on the 30th day, the activity of the researched enzyme increased by 2 % in group E_4, by 2.5 % in group E_5, and by 2.8 % in group E_6 relative to the indicator of animals of the C group. On the 60th day, the activity of the enzyme was the highest and relative to the animals of the C group, it raised by 4.3, 4.9, and 5.4 %, in groups of animals that were fed fenarone. On the 90th day, the AsAT activity fluctuated within the same range of values as on the 60th day of the experiment.

We found similar changes in the activity of this enzyme in piglets fed metifen. Thus, on the 30th day, the activity of AsAT in the blood serum of the animals increased by 2.5, 4.5, and 2.8 %, relative to the values of the C group. On the 60th day, the activity of the enzyme in the blood serum of the animals of groups E_7, E_8 and E_9 continued to rise and fluctuated, within the range of 0.370 ± 0.015 – 0.386 ± 0.014 mmol/l/h. In animals of the C group, this indicator was 0.350 ± 0.011 mmol/l/h. Feeding metifen at a dose of 0.9 mg/kg body weight, contributed to an extent to the activity of AsAT by 10 %

relative to animals of the C group. On the 90th day, the activity of the enzyme in the blood serum of the animals of the experimental groups remained at a high level.

The activity of alanine aminotransferase at the beginning of the test in the blood serum of piglets of all experimental groups ranged from 0.215 ± 0.011 to 0.224 ± 0.011 mmol/l/h (Table 5). Feeding methionine to piglets increased the activity of the enzyme in blood serum on the 30th day by 7.7, 9, and 11 %, respectively, in animals of experimental groups E_1, E_2, and E_3. On the 60th day of the experiment, the activity of AlAT in the blood of these animals was the highest and, accordingly, ranged from 0.250 ± 0.013 to 0.257 ± 0.013 mmol/l/h.

When piglets were fed fenarone, similar changes in the activity of AlAT were established, as when feeding methionine, but the activity of the enzyme was slightly higher. Thus, on the 30th day, the activity of AlAT in the blood serum of animals of group E_5 increased by 13 %, in animals of groups E_6 and E_7 – by 14 %, respectively, relative to the indicator of animals of the C group.

Table 5

The influence of methionine, fenarone, and methifen on the activity of alanine aminotransferase in the blood of intact piglets, mmol/l/h (M±m, n = 5)

Groups	Study periods				
	At the beginning of the experiment	10th day	30th day	60th day	90th day
C	0.220 ± 0.011	0.215 ± 0.013	0.220 ± 0.011	0.225 ± 0.011	0.225 ± 0.012
E_1	0.218 ± 0.011	0.225 ± 0.010	0.237 ± 0.011	0.250 ± 0.013*	0.244 ± 0.011*
E_2	0.222 ± 0.012	0.228 ± 0.012	0.240 ± 0.012	0.250 ± 0.010*	0.247 ± 0.012*
E_3	0.215 ± 0.011	0.230 ± 0.011*	0.244 ± 0.010*	0.257 ± 0.013*	0.251 ± 0.011*
E_4	0.216 ± 0.010	0.230 ± 0.012*	0.249 ± 0.010*	0.260 ± 0.013*	0.260 ± 0.010*
E_5	0.220 ± 0.010	0.233 ± 0.014*	0.251 ± 0.011*	0.260 ± 0.012**	0.261 ± 0.011**
E_6	0.219 ± 0.012	0.237 ± 0.013**	0.250 ± 0.012*	0.264 ± 0.011**	0.265 ± 0.013**
E_7	0.224 ± 0.011	0.240 ± 0.011**	0.258 ± 0.011**	0.270 ± 0.012**	0.270 ± 0.011**
E_8	0.223 ± 0.012	0.246 ± 0.012*	0.261 ± 0.010**	0.276 ± 0.011**	0.270 ± 0.012**
E_9	0.217 ± 0.011	0.242 ± 0.01*	0.257 ± 0.014**	0.272 ± 0.014**	0.271 ± 0.011**

*The degree of probability, compared to indicators in piglets of the control group: * – $P \le 0.05$; ** – $P \le 0.01$; *** –$P \le 0.001$*

On the 60th day, the activity of AlAT in the blood serum of groups, which were fed fenarone in doses of 1.10 and 1.15 mg/kg body weight was 0.260 ± 0.013 mmol/l/h, and animals that were fed fenarone at a dose of 1.20, was 0.264 ± 0.011 mmol/l/h. On the 90th day of the experiment, the activity of the enzyme continued to raise in the blood serum of group E_5 and E_6 animals, where it raised by 16 and 18 % relative to the level of animals in the C group.

When feeding with methifen, a change in the activity of AlAT was established starting from the 10th day of the experiment, where it increased by 11.6, 14.4, and 12.6 % relative to the values in the animals of the C group. Subsequently, the activity of the enzyme continued to process, and on the 30th day, it was 0.258 ± 0.011 mmol/l/h in piglets of group E_7, in group E_8 it was 0.261 ± 0.010 mmol/l/h, and in group E_9 0.257 ± 0.014 mmol/l/h. On the 60th day, it was the highest activity of AlAT in the blood serum of animals that were fed methifen at a dose of 0.9 mg/kg body weight, relative to the control, it raised by 22.7 %.

So, methionine, fenarone, and methifen contribute to a physiological increase in the activity of aminotransferases in blood serum due to the activation of metabolic processes in the piglets' bodies.

Conclusions

The use of methionine, fenarone, and metifen in piglets helps to increase the hemoglobin level, the erythrocyte number, and the activity of aminotransferases in their blood.

The best normalizing effect is manifested when feeding piglets with methionine at a dose of 4 mg/kg body weight, fenarone at a dose of 1.20 mg/kg body weight, and metifen at a dose of 0.9 mg/kg body weight.

The highest indicators of enzyme activity, hemoglobin content, and the number of erythrocytes in the blood of the animals of the experimental groups were established on the 60th and 90th days of the experiment.

Conflict of interest

The authors declare that there is no conflict of interest.

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