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ORIGINAL ARTICLE

Immunity of suckling pigs after administration of sow drug Glutam 1M and Nanoakvahelates

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The article investigates the effect of Nanoakvahelat Germanium and neurotropic metabolic drugs such as Glutam 1M and Kvatronan-Se on the immunological parameters of the blood of suckling piglets after feeding sows with these drugs. In previous exploratory studies, the doses of drugs were established: for the drug Glutam 1M – 18 mg/kg, for Kvatronan-Se – 0.02 ml/kg, for Nanoakvahelat Germanium – 5 μg/kg. The drugs were fed to sows four days before farrowing and for ten days after farrowing. Studies show that their feeding to sows does not significantly affect the quantitative content of leukocytes in the blood of newborn piglets. We found an increased lymphocyte content (63.21–82.08%) in all experimental animals. We found that on the 4th day of the suckling period in the blood of piglets of the experimental groups increased the number of erythrocytes by 10.1–26.3%, hemoglobin level – by 3.1–11.5%, hematocrit content – by 3.2–11.6% (the difference with the data of the control group). On the 11th day of the suckling period, the number of leukocytes in the experimental groups increased by 0.67–7.51%; in control, this indicator decreased by 20.43%. The concentration of hemoglobin in the experimental groups increased by 11.5, 26.3, 3.1, and 7.9%; the number of erythrocytes increased by 11.2, 26.3, 10.1, and 10.9%, with regards to control. The tested drugs can be safely fed to sows in the suggested doses. Using Nanoakvahelat Germanium, Glutam 1M, Kvatronan-Se drugs enhance newborn piglets' immunity within the first 11 days of their lives.
Keywords: suckling pigs, sow, Kvatronan-Se, Glutam 1M, Nanoakvahelat Germanium, blood, leukocytes, erythrocytes, hemoglobin.

Introduction

Improving the viability and resistance of animals to preserve their productivity potential is considered one of the current central tasks at the present stage of selection work in pig breeding (Quesnel, 2011). Currently, much attention is paid to studying the mechanisms of natural resistance and immune protection in animals and the study of their role in the adaptation of piglets to environmental conditions, including on the level of interaction "mother-fetus – newborn animals" (Kenney & Gantt, 2014).
Protein metabolism and the development of the immune system in piglets are formed depending on the mother's body. It is established that early and intensive consumption of colostrum is crucial for the survival and health of piglets in the postnatal period (Gajewski & Schreiber, 2013).
The higher the immunobiological reactivity of sows, the higher they are in fetuses and piglets (Kielland et al., 2015). That is why it is essential to provide an additional intake of microelements that have antioxidant properties and regulate the body's physiological processes (Lebedev, 1980; Moroz & Leskov, 1995; Biswas et al., 2006; Pilipchuk & Sheremeta, 2016; Del Vesco et al., 2017; Hassan et al., 2019). It is known that the use of nanoaquachelates supports the activity of the body's immune system (Goodman, 1988; Kaplunenko & Kosinov, 2009; Mamchenko, 2014; Seba & Khomenko, 2017; Jemelʾjanenko et al., 2020).
The organism of animals, its hematopoietic organs are susceptible to various pathological impacts, and the picture of blood reflects these influences. In this aspect, hematological studies play an essential role as, along with the clinical examination, they enable the possibility to diagnose hidden or subclinical forms of the disease. The primary importance of the hemogram belongs to the ratio and balance of its components (Quesnel, 2011). Particularly relevant is the analysis of quantitative and qualitative changes in blood serum, which allows without special studies to roughly evaluate the state of various chains of the immune system.
The purpose of conducting our research was to study the influence of Nanoakvahelat Germanium and drugs of neurotrophic metabolic action such as Glutam 1M and Kvatronan-Se on the immunological indices of the blood of suckling piglets after feeding sows with these drugs.

Materials and Methods

The study was conducted in the State Enterprise "Experimental Farm "Stepne" of Institute of Pig Breeding and Agro-industrial production of the National Academy of Agrarian Sciences of Ukraine" Poltava region.
For the planned research on the farm, 25 sows of the large white breed were selected. Experimental groups of sows were formed on analogs' principles, taking into account age, live weight, and farrowing. The sows selected for the experiment were divided into

five groups – four experimental and one control (5 heads in each). For insemination of sows were used the semen of boars of the large white breed. After artificial insemination, the sows were kept individually. Five days before the expected date of farrowing, the animals were transferred to a farrowing room, where they were kept with the piglets throughout the suckling period until weaning.
In previous exploratory studies, doses of drug administration were established. The obtained results show that the following doses are optimal for use: for the drug Glutam 1M – 18 mg/kg, for Kvatronan-Se – 0.02 ml/kg, for Nanoakvahelat Germanium – 5 μg/kg. Before and after farrowing, sows were administered the study drugs according to the scheme shown in Table 1.

Table 1. The scheme of administration of the studied drugs to sows.

Group	Number of feeding days		Drug and dose
	before farrowing	after farrowing	
Control	4	10	Saline solution – 20 ml
I experimental	-	3	Glutam 1M – 18 mg/kg
II experimental	4	10	Nanoakvahelat Germanium – 5 μg/kg
	-	3	Glutam 1M – 18 mg/kg
III experimental	4	10	Nanoakvahelat Germanium – 5 μg/kg
	-	3	Glutam 1M – 9 mg/kg
IV experimental	4	10	Kvatronan-Se – 0.02 ml/kg (Cu, Se, Cr, Ge, Mn)

Sows were administered the drugs individually, once a day (in the morning). Drugs were in liquid form in vials. The vial contents were added to 100 g of feed, and sows were fed before the main feed. Before farrowing, the amount of dry feed in the diet of sows was 3.3 kg per day, after farrowing – 6.3 kg per day.
Blood collection from piglets was performed in the morning from the jugular vein on the 1st day of life, on the 4th and 11th days of the suckling period (Table 2).

Table 2. Scheme of blood sampling in piglets.

Group	Blood collection days	Indicator
Control I experimental II experimental III experimental IV experimental	newborns on the 4th day of life on the 11th day of life	leukocyte profile of blood of piglets, number of erythrocytes, thrombocytes, hemoglobin concentration, hematocrit, erythrocyte sedimentation rate (ESR), anisocytosis

Blood was taken from the jugular vein in special tubes with a coagulant. The leukocyte profile of the blood of piglets, the number of erythrocytes, platelets, hemoglobin concentration, hematocrit, erythrocyte sedimentation rate, anisocytosis were determined.
Immunogenetic analysis was performed at the veterinary clinic "Vetline" (Poltava city) on a hematology analyzer NIHON KOHDEN (Japan). Statistical processing of the obtained results was performed using Microsoft Excel software.

Results and Discussion

The highest content of leukocytes (Figure 1) was found in the blood of newborn piglets of the control group – 11.16 x10^9/l, 21.2, 20.8, 18.7 and 19.4% higher than the corresponding indicator of animals of I, II, III, and IV experimental groups.

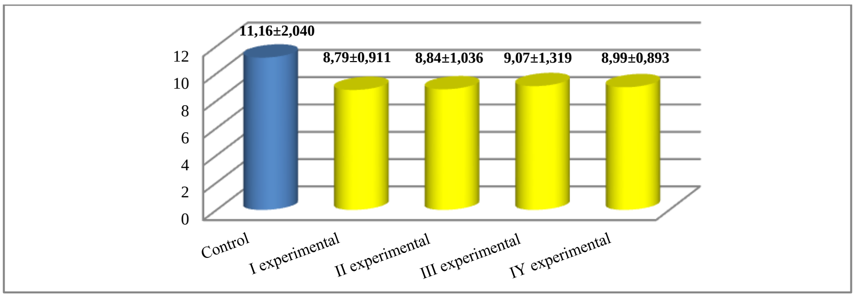

Fig. 1. The number of leukocytes (x10^9 /l) in the blood of newborn piglets.

No specific antibodies were detected in the study of placental tissues and fetus, amniotic fluid, and fetal blood serum (Masiuk et al., 2018). Therefore, Kvatronan-Se and Nanoakvahelat Germanium administration to sow before farrowing could not affect newborn piglets' white blood cell count.

It worth mentioning that if at birth the piglets of the experimental groups did not differ significantly in the content of leukocytes in the blood, then on the 4th and 11th day of their lives, the number of leukocytes in the blood changed differently.
Thus, on day 4 of the suckling period, the lowest content of leukocytes (Figure 2) we found in the blood of piglets IV of the experimental group – 7.54 x 10^9/l, which was 17.8, 11.2, 2.3, and 19.3%, respectively, less compared to this indicator of animals of the control, I, II and III groups. At the same time, the content of leukocytes in the blood of piglets of experimental group III was 1.9% higher compared to animals of the control group.

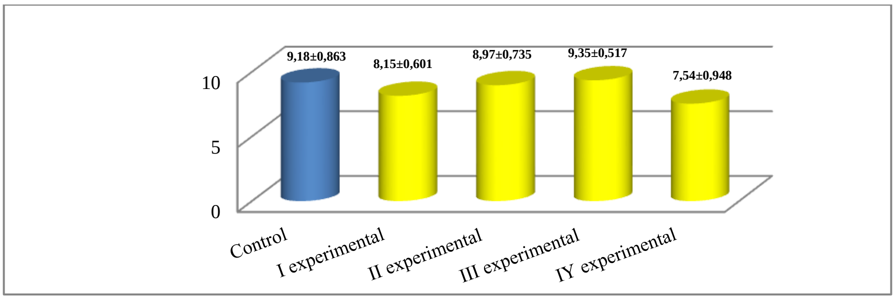

Fig. 2. The number of leukocytes (x10^9/l) in the blood of piglets on the 4th day of the suckling period.

Therefore, an increase in the number of leukocytes in the blood of animals of experimental group III may indicate a stimulating effect on the immune protection of suckling piglets of such drugs as Glutam 1M and Nanoakvahelat Germanium, which we administered to sows.
On day 11 of the suckling period, the highest content of leukocytes was found in the blood of piglets I, II, and IV experimental groups, which was 2.2, 2.4 and 1.8% more than control animals (Figure 3).
The analysis results show that the number of leukocytes in the blood of animals of all experimental groups for 11 days of the suckling period increased by 0.67–7.51%. Whereas in control animals, this figure decreased by 20.43% during this period.

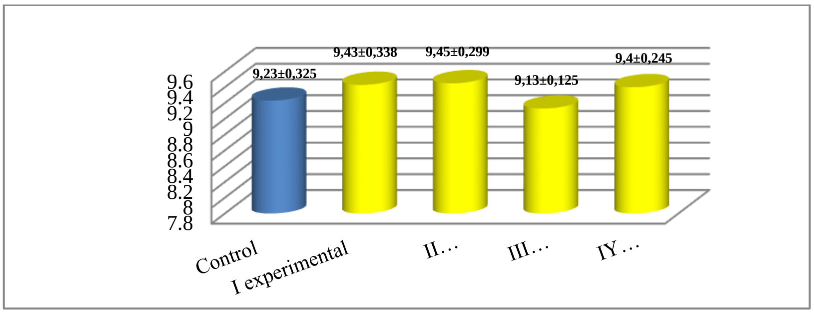

Fig. 3. The number of leukocytes (x10^9/l) in the blood of piglets on the 11th day of the suckling period.

Lymphocyte and monocyte cells provide a clearer picture of the leukocyte profile of the blood of suckling piglets.
Thus, on the day of farrowing, the lowest number of monocytes observed in the blood of piglets of the control group – 1.98%, which was respectively 47.5, 28.3, 48.9, and 18.2% less compared to the analogs of I, II, III, and IV experimental groups (Table 3).
We have not found a significant difference between the animals of the control and experimental groups in terms of eosinophils and basophils. It worth noting that the above indicators were within the physiological norm. The highest number of neutrophils observed in the blood of animals of experimental group I – 31.6%, which was respectively 59.2, 24.5, 30.9, and 52.7% higher compared to the newborn piglets of the control ($p<0.01$), II, III ($p<0.05$) and IV ($p<0.01$) experimental groups. The difference between animals of I and II experimental groups was statistically nonsignificant. The number of neutrophils in the blood of piglets of experimental group IV was 14.95%, which is significantly less (24.7%) compared to this indicator in control, but at the same time, the above indicator was within the limits of experimental accuracy.
The most significant number of lymphocytes found in the blood of piglets of the IV experimental group – 82.08%, which was 4.4, 29.9, 12.7, and 10.1% more compared to the same indicator in the blood of control piglets, I ($p<0.01$), II and III experimental groups. Besides, animals of the I experimental group had a lower number of lymphocytes by 19.6, 15.2, and 13.2%, respectively, compared to animals of the control ($p<0.01$), III ($p<0.05$), and II experimental groups. Increased levels of lymphocytes in experimental suckling piglets may indicate the influence of stress factors related to their birth.
The results of the analysis show that the administration of Nanoakvahelat Germanium and Kvatronan-Se in sows in the last decade of the prenatal period, four days before farrowing, did not significantly affect the quantitative content of leukocytes in the blood of newborn piglets, as this figure was within the limits of experimental accuracy. On the day of farrowing in the blood serum of all experimental animals, we observed an increased percentage of lymphocytes.
Analysis of the leukocyte formula showed that on the 4th day of the suckling period in piglets of I, II, and IV experimental groups, the number of neutrophils in the blood exceeded the control analogs by 7.1, 12.6, and 15.7%. At the same time, according to this indicator, piglets of experimental group III conceded by 6.8% before animals of the control group (Table 3).
At this age, the lowest lymphocytes we observed in the blood of piglets of experimental group IV, where it is 5.04, 1.75, 2.42, and 4.85% less compared to the same indicator of the animals of the control, II, and III experimental groups. The level of eosinophils in the blood of piglets of II, III, and IV experimental groups increased by 16.7–83.3% compared with the control.
We found that the content of basophils and monocytes in the blood of animals of I, II, III, and IV experimental groups decreased compared to control analogs by 7.1–28.6 and 36.1–54.9%, respectively. On the 11th day of life (Table 3), the highest level of neutrophils in the blood we found in piglets of II and IV experimental groups. It was 8.4 and 3.8% higher than the same indicator

of piglets in the control group. We observed the lowest level of neutrophils in the blood of piglets of I and III experimental groups, which was 10.6 and 22.37% less compared with animals of the control group. At the same time, the level of neutrophils in piglets of the III experimental group was 13.1% lower ($p<0.01$) compared to this indicator in the II experimental group and analogs of the IV experimental group – 33.7% higher ($p<0.001$) than it was in animals of experimental group III.

Table 3. Leukocyte profile of piglet blood on different days of the suckling period, %.

Indicator	Group, M±m				
	Control	I experimental	II experimental	III experimental	IV experimental
Newborns, n=10					
Neutrophils	19.85±2.376	31.61±2.395**	23.87±3.872	21.83±2.879[1]	14.95±2.913[2]
Lymphocytes	78.65±2.947	63.21±2.698**	72.84±3.812	74.55±2.744[1]	82.08±2.715[2]
Monocytes	1.98±0.403	2.92±0.394	2.54±0.377	2.95±0.338	2.34±0.319
Eosinophils	0.55±0.093	0.47±0.068	0.65±0.163	0.57±0.068	0.48±0.117
Basophils	0.13±0.021	0.11±0.014	0.14±0.020	0.14±0.020	0.18±0.025
On the 4th day of life, n=10					
Neutrophils	16.63±2.557	17.81±2.377	18.72±2.785	15.49±2.123	19.24±3.249
Lymphocytes	80.55±2.403	77.85±2.768	78.39±2.462	80.19±2.462	76.49±3.214
Monocytes	2.44±0.377	3.98±0.720	2.41±0.689	3.68±0.803	3.69±0.576
Eosinophils	0.30±0.027	0.29±0.041	0.35±0.082	0.54±0.121	0.55±0.128
Basophils	0.14±0.024	0.12±0.017	0.13±0.021	0.11±0.010	0.1
On the 11th day of life, n=4					
Neutrophils	29.15±4.279	26.05±3.613	31.6±1.332	22.63±1.331[b]	30.25±0,463[f]
Lymphocytes	68.83±4.101	71.65±3.507	66.88±1.440	74.95±1.167[b]	68.03±0.413[f]
Monocytes	1.30±0.252	1.10±0.238	1.23±0.184	1.10±0.196	1.13±0.206
Eosinophils	0.68±0.307	1.13±0.312	0.25±0.087[1]	1.33±0.075[c]	0.43±0.149[f]
Basophils	0.10	0.10	0.10	-	0.10

***p<0.01 – compared to control, [1]p<0.05, [2]p<0.01 – compared to I experimental, [b]p<0.01, [c]p<0.001 – compared to II experimental, [f]p<0.001 – compared to III experimental.*

We found that the content of eosinophils in the blood of piglets of group II compared with analogs of I and III experimental groups were lower by 77.9 ($p<0.05$) and 81.2% ($p<0.001$), respectively. Besides, in animals of experimental group III, it was 67.6% higher ($p<0.001$) compared with that one of the piglets in experimental group IV. In animals of the experimental groups, the content of basophils was almost at the same level.

On the 11th day of the suckling period, the content of lymphocytes in the blood of piglets of the control and IV experimental groups was almost at the same level and was within the physiological norm. In piglets of experimental group III, the above indicator was 4.6 ($p<0.01$) and 10.2% ($p<0.001$) higher compared to that of analogs of experimental groups I and IV.

The data in Table 4 show that the highest number of erythrocytes observed in the blood of newborn piglets of I, II, and III experimental groups, which was 19.6, 21.3, and 3.5% higher compared to the same indicator of animals in the control group. Additionally, in animals of the control group, the content of erythrocytes was 2.97% higher compared with the same indicator of piglets of the IV experimental group. The concentration of hemoglobin in the blood of piglets of I, II, III, and IV experimental groups was respectively lower by 11.3, 7.2, 9.7, and 2.3% compared with the control group. In the specified age period, there was a higher ($p<0.05$) hematocrit content in the serum of animals of I and II experimental groups, while piglets of III and IV experimental groups on the above indicator were inferior to the control analogs, respectively, by 10.5 and 16.7% and 4.2 and 6.3%. We found that in terms of anisocytosis, newborn piglets of the experimental group exceeded the control analogs by 3.5%. There is no significant difference in the amount of anisocytosis in the blood of animals of the control, II, III, and IV experimental groups.

The content of platelets in the blood of animals of I and III experimental groups in the specified age period was 14.1 and 6.6% lower than in control. The above figure was higher in piglets of II and IV experimental groups – respectively, by 0.8 and 20.5%. The lowest erythrocyte sedimentation rate we observed in the blood of piglets of experimental group III – 2.5 mm/h, which was 4.9% less than in the control group. On this indicator, animals of I, II, and IV experimental groups predominated piglets of the control group, respectively, by 21.7, 6.5, and 36.9%. The analysis results indicate a slight effect of the studied drugs on the blood composition of piglets in the prenatal period, as all studied indicators after farrowing in the experimental groups had slight fluctuations. On the 4th day of the suckling period, the highest content of erythrocytes and hemoglobin we found in the blood of piglets of the experimental groups (Table 4). The concentration of hemoglobin in the blood of animals of I, II, III, and IV experimental groups compared with the control was higher by 11.5, 5.7, 3.1, and 7.9%, the number of erythrocytes was 11.2 26.3, 10.1, and 10.9%, respectively. At this age, the hematocrit indicator of the piglets of the experimental groups outperformed the control analogs by 3.2–11.6%.

Table 4. Hematological analysis of the piglets' blood on different days of the suckling period.

Indicator	Group, M±m				
	Control	I experimental	II experimental	III experimental	IV experimental
Newborns, n=10					
Erythrocytes, $x10^{12}/l$	4.04±0.37	4.83±0.41	4.90±0.46	4.18±0.23	3.92±0.19
Hemoglobin, g/l	76.19±2.55	67.59±3.76	70.71±5.21	68.81±4.43	74.45±2.67
Hematocrit,%	24.56±1.07	27.15±2.36	28.67±1.07*	23.54±0.72^{b}	23.01±1.09^{b}
The average concentration of hemoglobin in the erythrocyte, g/l	332±7.64	334.4±9.65	321.6±9.44	335±9.62	343.3±12.8
Anisocytosis,%	15.95±0.46	16.51±0.35	15.74±0.44	15.66±0.57	15.47±0.49
Platelets, $x10^{9}/l$	278.13±63.77	238.8±55.38	280.3±42.19	259.8±55.1	335.1±36.55
ESR, mm/h	2.63±0.46	3.2±0.33	2.8±0.25	2.5±0.27	3.60±0.65
On the 4th day of life, n=10					
Erythrocytes, $x10^{12}/l$	3.76±0.35	4.18±0.31	4.75±0.47	4.14±0.58	4.17±0.27
Hemoglobin, g/l	75.25±2.85	83.92±4.48	79.53±4.60	77.60±6.72	81.22±3.25
Hematocrit,%	21.85±1.31	23.99±1.63	24.39±0.86	22.56±1.41	23.35±0.72
The average concentration of hemoglobin in the erythrocyte, g/l	337.75±10.80	354.32±9.67	346.20±8.97	351.63±8.17	345.68±5.52
Anisocytosis,%	15.79±0.49	16.42±0.44	16.71±0.46	17.20±0.41	16.28±0.43
Platelets, $x10^{9}/l$	405.25±60.03	379.40±42.07	331.32±68.61	443.70±26.56	381.73±16.01
ESR, mm/h	3.75±0.77	3.30±0.73	3.50±0.45	3.90±0.72	2.90±0.28
On the 11th day of life, n=4					
Erythrocytes, $x10^{12}/l$	3.21±0.35	2.76±0.34	3.0±0.31	3.23±0.40	3.65±0.12^{1}
Hemoglobin, g/l	55.93±1.71	53.08±1.94	59.25±2.54	57.25±3.01	58.65±2.62
Hematocrit,%	15.35±1.14	14.33±0.85	17.40±1.72	13.85±0.31	19.20±0.98^{*2f}
The average concentration of hemoglobin in the erythrocyte, g/l	362.75±22.05	369.28±14.11	343.0±15.59	393.30±5.90	326.75±4.15^{f}
Anisocytosis,%	19.03±2.13	18.30±2.0	18.10±1.19	16.65±0.10	19.83±2.34
Platelets, $x10^{9}/l$	903±39.91	904.05±20.37	743.25±111.55	741.50±98.66	778±139.22
ESR, mm/h	7.0±1.08	6.0±0.82	7.0±0.71	5.75±0.25	6.75±0.75

$^{*}p<0.05$ – compared to control, $^{1}p<0.05$, $^{2}p<0.01$ – compared to I experimental, $^{a}p<0.05$, $^{b}p<0.01$ – compared to II experimental, $^{f}p<0.001$ – compared to III experimental.

The increased content of hemoglobin, erythrocytes, and hematocrit indicators in the blood of piglets of the experimental groups on the 4th day of life indicates a positive effect of the studied drugs on respiratory function, hemoglobin synthesis, and metabolic levels in the body. In terms of anisocytosis, piglets of the experimental groups outperformed the control analogs by 3.1–8.9%. We found that the content of platelets in the blood of piglets of I, II, and IV experimental groups at the above age was 6.4, 18.2, and 5.8% lower than in control, and in the III experimental group, it was respectively higher – by 9.5%.

On the 4th day of life, animals of I, II, and IV experimental groups had an erythrocyte sedimentation rate of 22, 6.6, and 22.6% lower than in the control group. In addition, animals of experimental group III had this indicator of 4.0, 18.2, 11.4, and 34.4% higher than this indicator in and I, II, and IV experimental groups. Therefore, the administration of Glutam 1M, Kvatronan-Se, and Nanoakvahelat Germanium on the 4th day of the sucking period increases the content of erythrocytes, hemoglobin, and hematocrit, while reducing the platelet content and erythrocyte sedimentation rate.

We found that on the 11th day of the suckling period (Table 4), the lowest erythrocyte content in the blood animals of I and II experimental groups, which by this indicator were inferior to the analogs of the control group by 14.01 and 6.5%, respectively. The highest content of erythrocytes in the blood we found in piglets of III and IV experimental groups, which is 0.6 and 13.7% more than in control animals, respectively. In addition, at this age, piglets of the IV experimental group had the highest number of erythrocytes in the blood – 3.65 $x10^{12}/l$, which was 32.2% ($p<0.05$), 21.7, and 13%, higher compared to this indicator in animals of I, II and III experimental groups. On the 11th day of life, the content of platelets in the blood of piglets of the control and I experimental groups was almost at the same level. We also found that the animals of II, III, and IV experimental groups according to this indicator were inferior to the analogs of the control and I experimental groups, respectively, by 17.7, 17.9, and 13.8%. The levels of hemoglobin in the blood of animals of the I experimental group compared with the control, II, III, and IV experimental groups were lower, respectively, by 5.1, 10.4, 7.3, and 2.5%. The decrease in the content of erythrocytes and hemoglobin in the blood of piglets on the 11th day of their life, compared with the day of farrowing and the 4th day of the postnatal period, was because piglets synthesize their antibodies at a low level up to 4 weeks of age. The immune system reaches full development by one and a half to three months of age. Piglets of the IV experimental group, according to the hematocrit indicator, predominated animals of the control, I, II, and III experimental groups, respectively, by 25.1% ($p<0.05$), 33.9% ($p<0.01$), 10.3, and 38.6% ($p<0.001$). In the blood of the animals of II and IV experimental groups in the specified age period, we found a lower average concentration of hemoglobin in an erythrocyte. Animals of I and III experimental groups had this indicator higher than the control group, respectively by 5.4, 9.9%, and 1.8, 8.4%. On the 11th day of the suckling period, the highest level of anisocytosis we observed in piglets of the IV experimental group (19.83%), which was 4.2, 8.4, 9.6, and 19.1% higher than this indicator of the analogs of the control, I, II and III experimental groups. At this for animals of the control and II experimental groups, the erythrocyte sedimentation rate was at the same level – 7.0 mm/h, respectively 14.3, 17.9, and 3.6%, higher than piglets I, III, and IV experimental groups had. Therefore, the studied drugs can be safely administered to sows in the proposed doses. Apart from that, the use of Nanoakvahelat Germanium and Glutam 1M, and Kvatronan-Se strengthened the immunity of newborn piglets for the first 11 days of their lives. This is evidenced by an increase of hemoglobin by 11.5, 26.3, 3.1, and 7.9% in the blood of suckling piglets of the experimental groups and an increase in the number of erythrocytes by 11.2, 26.3, 10.1, and 10.9% compared with control.

Conclusion

Feeding sows (before and after farrowing) with Nanoakvahelat Germanium and drugs of neurotrophic metabolic action Glutam 1M and Kvatronan-Se positively affect the general state of immunity suckling piglets in the postnatal period.
On the 4th day of the suckling period, after administration of sows with the drugs mentioned above in the blood of piglets of experimental groups increases the number of erythrocytes by 10.1–26.3%, the level of hemoglobin – by 3.1–11.5%, the content of hematocrit – by 3.2–11.6% (the difference with the data of the control group).
On the 11th day of the suckling period, the number of leukocytes in the blood of piglets of experimental groups increases by 0.67–7.51%; in control animals, this figure decreases by 20.43% (the difference with data at birth).

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