# INFLUENCE OF FEED ADDITIVE FROM PEAT ON MORPHOLOGICAL AND BIOCHEMICAL BLOOD PROFILE OF PIGLETS

*Valentyn Yefimov, Kostiantyn Kostiushkevych, Vitalii Rakytianskyi*

*Dnipropetrovsk State Agrarian and Economic University*
*S. Yefremova st. 25, Dnipro, Ukraine, 49600*

*Corresponding author: Valentyn Yefimov e-mail yefimov.v.h@dsau.dp.ua tel.+38067 7275575*

**Absract.** The aim of our study was to establish the influence of feed additive based on peat on physiological and biochemical parameters of blood of piglets in the suckling and early post-weaning period. For this purpose, the control and the experimental groups of piglets were formed. The experimental animals were additionally getting feed additive from the 3rd to the 42nd day of life. Blood was sampled at the end of the experimental period.

At the end of the study, the feed additive was found to decrease the signs typical to the stress syndrome. It increases the basophiles and eosinophiles count in blood, as well as decreases the neutrophils count in blood by 26.7 % ($P<0.05$). Biochemical changes indicate an increase in the level of albumin by 9.0 % ($P<0.05$) and a decrease in glucose concentration by 17.4 % ($P<0.05$).

Thus, the use of a feed additive for piglets leads to greater adaptive capacity of piglets to the stress associated with their weaning and the transition to combifodder consumption.

**Keywords:** piglets, weaning, peat additive, blood morphology and biochemistry.

**Introduction.** Piglets are functionally immature in the early postnatal ontogenesis. Their digestive system (Zabielski et al., 2008), mechanisms of thermoregulation (Lossec et al., 1998) and organs of immune defense (Potočnjak et al., 2012) are not developed sufficiently.

The high rate of piglets' growth requires adequate intake of micronutrients such as vitamins and microelements. For this reason, piglets often suffer from deficiency of these substances (Miller and Kornegay, 1983). Because of this, the mortality rate of piglets in the sucking period and after weaning can reach 10% or more (Edwards, 2002; Zabielski et al., 2008).

Deficiency of microelements is one of the factors limiting the growth of piglets and decreasing their viability. Perucchietti and Litjens (2010) indicate that the most necessary microelements for piglets are iron, selenium and copper. Consumption of these elements for piglets depends largely on the availability of micronutrients in the organism of sows. At the same time, our previous studies (Yefimov et al., 2010) indicate an insufficient content of copper, zinc and iron in the blood serum of lactating sows. On the other hand, these microelements, as well as manganese are essential for piglets in the post-weaning period (Untea et al., 2013).

At present, various technological methods and biologically active substances are used for enhancing the adaptive capacities and resistance of pigs (Bowman et al., 2006). Peat and feed additives, which contain it, are recommended to influence the behavior of piglets. Piglets that had an access to peat behave more actively than piglets that did not receive it. It was established that the influence of peat on piglets in post-weaning period was more pronounced than the results of the previous studies regarding piglets in enclosures (Vanheukelom et al., 2011).

The analysis of the literature gives different data regarding the influence of peat on the functional state of pigs. Trckova et al. (2006b) indicate that it does not cause any effect on the organism of the fattened pigs. At the same time, it was also established that under the influence of peat as an additive, an increase in pig's body weight at the end of the post-weaning period was observed (Trckova et al., 2006a). The similar results were obtained after feeding piglets with the treated peat enriched with microelements (Yefimov et al., 2016).

Taking into account, that there is no sufficient evidence in literary sources in terms of the influence of peat additives on the physiological state of piglets, the purpose our work was to establish the effect of the peat-based feed additive on the physiological and biochemical blood indicators of piglets in the sucking and post-weaning periods.

**Materials and methods**

The data of the present study are in full compliance with the requirements of the Committee on Ethics of the Faculty of Veterinary Medicine in Dnipropetrovsk State Agrarian and Economic University, Dnipro, Ukraine.

*Animal model and design of experiment.* The study was carried out on the farm, where the three-bred hybrids (Yorkshire, Landrace and Duroc) of pigs are grown (Dnipropetrovsk region, the central part of Ukraine). The research material at the beginning of the experiment included 109 piglets, aged 3 days, divided into two groups: experimental (n=55) and control (n=54). The experimental period lasted 42 days. During that time, the animals of the control group received standard fodder, while animals of the experimental group besides the standard fodder received additive "TorVet" in the following doses: 200 ml per one socket in suckling period and 250 ml per ten piglets in the after-weaning period.

Biologically active fodder additive for pigs "Torvet" is a commercial additive, which is obtained by treatment with peat and further adding to its composition in the form of sulphates such microelements (mg/1 kg) as iron –

2850, zinc – 1175, copper – 590, manganese – 270, and cobalt – 28. Transitional peat, obtained in Ukraine, is used in the production.

Animals from both groups were kept under the same optimal zoohygienic conditions and under standard veterinary medical care before and during the experiment.

*Sampling procedures.* Blood samples were collected from five piglets from each group in the morning (prior to feeding) from the orbital sinus on the 42nd day of birth. The samples for determining hematological parameters were placed in test tubes with potassium salt of EDTA, and samples for determining biochemical profile were placed in test tubes with a coagulant and centrifuged. The separated serum was frozen at -20° C and stored until it was analyzed. The research at the laboratory stage was conducted at the Scientific-research centre of biosafety and environmental control of agro-industrial complex of DSAEU.

*Hematological and biochemical examination.* In blood samples, the number of leukocytes, erythrocytes and thrombocytes, the values of erythrocyte indices and haematocrit, the hemoglobin concentration were measured using the automated haematology analyser PCE 90-Vet, (High Technology, USA). The differential leukocyte count was performed in blood smears stained by Wright-Giemsa under a light microscope (Olympus CH 20). Serum contents of total protein, albumin, globulin, glucose, cholesterol, urea, creatinine, bilirubin, uric acid, and some minerals (such as calcium, phosphorus, magnesium, iron) as well as activity of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were determined. The tests were performed using the automated biochemical analyser BioChem FC-200 (High Technology, USA) with the reagents of High Technology.

*Statistical analysis.* The results were analyzed statistically with the Student's *t*-test using the MS Excel. The values of $P<0.05$ and lower were considered significant. The results are expressed in mean values ± standard deviations.

**Results**

*Hematological investigation.* Mean values of hematological parameters are presented in Table 1. The difference between the indices of erythropoiesis in the blood of animal from the experimental and the control groups was not found, the platelet count did not differ either.

Table 1. **The indices of haemocytopoesis under the influence of "TorVet" (mean ± SD)**

| Indices | Animal group | |
|---|---|---|
| | Control | Experimental |
| Packed cell volume, % | 28.58±1.20 | 30.62±1.43 |
| Hemoglobin, g/L | 69.2±3.03 | 75.0±4.34 |
| Red blood cells, $10^{12}$/L | 5.38±0.05 | 5.38±0.14 |
| MCV, fl ($10^{-15}$ L) | 53.08±1.98 | 56.91±2.03 |
| MCH, pg ($10^{-12}$ g) | 12.85±0.53 | 13.93±0.61 |
| MCHC, % | 24.24±0.70 | 24.47±0.52 |
| Platelets, $10^9$/L | 369.0±23.86 | 395.8±8.41 |
| White blood cells, $10^9$/L | 26.54±1.12 | 23.16±1.52 |

Table 2. **The differential leukocyte count in the blood of pigs in the early post-weaning period under the influence of additive "Torvet" (mean ± SD)**

| Indices | | Animal group | |
|---|---|---|---|
| | | Control | Experimental |
| Basophiles, % | % | 0.00±0.00 | 0.40±0.11** |
| | $10^9$/l | 0.00±0.00 | 0.09±0.03** |
| Eosinophiles, % | % | 0.70±0.22 | 2.00±0.73 |
| | $10^9$/l | 0.18±0.06 | 0.48±0.20 |
| Neutrophiles banded, % | % | 4.50±0.50 | 4.10±0.37 |
| | $10^9$/l | 1.20±0.15 | 0.95±0.12 |
| Neutrophiles segmented, % | % | 39.20±0.88 | 32.60±1.35** |
| | $10^9$/l | 10.43±0.63 | 7.58±0.69* |
| Lymphocytes, % | % | 52.30±1.23 | 58.30±1.26** |
| | $10^9$/l | 13.85±0.45 | 13.46±0.72 |
| Monocytes, % | % | 3.30±0.28 | 2.60±0.21 |
| | $10^9$/l | 0.88±0.10 | 0.61±0.07 |
| L:N ratio | | 1.20±0.06 | 1.60±0.10* |
| *- P<0.05; **- P<0.01 | | | |

The total white blood cells concentration under the influence of additives also appeared unchanged. However, some changes in the concentration of specific leukocyte types were established (Table 2). The tendency to an increase in the eosinophiles count was detected. The eosinophiles count in the blood of animals of the experimental group was 2.0% (0.48 x $10^9$/L), and of the control group – 0.70% (0.18 x $10^9$/L). The number of segmented neutrophiles appeared less in the blood of piglets of the experimental group compared to the control group and accounted for 32.6% (7.58 x $10^9$/L) and 39.2% (10.43 x $10^9$/L), respectively (P<0.05; P<0.01). We observed statistically significant differences (P<0.01) between the number of lymphocytes, which increased from 52.3% to 58.3% under the influence of feed additive. L:N ratio was probably 33.3% (P<0.05) higher in animals, receiving the additive, than in animals of the control group.

*Biochemical examination.* The difference between the total protein content in the serum of piglets from the experimental and control groups was not observed (Table 3).

Table 3. **The biochemical indices in blood serum of piglets in the early post-weaning period under the influence of additive "TorVet" (mean ± SD)**

| Indices | Animal group | |
|---|---|---|
| | Control | Experimental |
| Total bilirubin, µmol/L | 3.02±0.90 | 2.60±0.48 |
| Glucose, mmol/L | 5.41±0.30 | 4.47±0.22* |
| Cholesterol, mmol/L | 1.60±0.25 | 1.51±0.26 |
| Uric acid, µmol/L | 25.0±6.74 | 27.8±8.00 |
| *The indices of protein metabolism* | | |
| Total protein, g/L | 48.2±1.88 | 50.6±1.56 |
| Albumin, g/L | 28.8±0.55 | 31.4±0.97* |
| Globulin, g/L | 19.2±1.43 | 19.4±0.91 |
| Urea, mmol/L | 5.72±0.27 | 5.82±0.56 |
| Creatinine, µmol/L | 64.6±8.03 | 55.8±6.80 |
| Aspartate aminotransferase, U/L | 121.4±25.26 | 164.8±32.86 |
| Alanine aminotransferase, U/L | 87.8±7.43 | 86.0±6.49 |
| *The indices of mineral metabolism* | | |
| Alkaline phosphatase, U/L | 411.2±32.67 | 305.4±24.78* |
| Total calcium, mmol/L | 3.13±0.07 | 3.10±0.05 |
| Inorganic phosphorus, mmol/L | 2.46±0.18 | 2.40±0.15 |
| Magnesium, mg/100 mL | 3.92±0.30 | 3.06±0.42 |
| Iron, µg/100 mL | 96.84±20.57 | 113.26±10.90 |
| *- P<0.05 | | |

At the same time, there is a statistically significant increase in the concentration of albumin from 28.8 g/L to 31.4 g/L (9.0%, P<0.05). The total globulin concentration in pigs of the control and experimental groups had no significant difference. The content of the finished products of protein metabolism (urea and creatinine) did not change significantly, although there was a trend to lower creatinine levels (64.6 µmol/L in the control group and 55.8 µmol/L in the experimental group) under the influence of the additive.

The enzyme activity of transaminases was characterized by various dynamics. There was an increase in the AST activity in the blood of experimental pigs in comparison with the activity of the enzyme in the blood of the control pigs (121.4 U/L and 164.8 U/L respectively), which, however, was not statistically reliable. At the same time, there were no changes in the ALT activity. The level of glucose in blood serum decreased by 17.4 % (P<0.05) under the influence of the additive: the glucose content in blood serum of the control and experimental animals was 5.41 mmol/L and 4.47 mmol/L respectively.

The activity of alkaline phosphatase significantly decreased from 411.2 U/L to 305.4 U/L by 25.7% (P<0.05). Among other indices, it should be noted, that the level of iron was higher in blood serum of piglets of the experimental group (113.3 µg/100 mL vs 96.8 µg/100 mL in serum of piglets of the control group). However, due to considerable individual variability of this index, the difference was not statistically reliable.

**Discussion**

Feeding the peat-based additive does not significantly influence the indices of haemopoiesis. At the same time, there is a tendency to an increase in the hemoglobin concentration in blood. The synthesis of hemoglobin depends on existence of iron, zinc and copper, which are the components of additive "TorVet". These elements are known to be involved in the synthesis of heme and hemoglobin (Puy and Deybach, 2007).

In the blood of animals which were fed with the peat-based additive, the greater number of basophils and eosinophils was found. Eosinopenia is the result of stress of various geneses and is associated with the increased synthesis of corticotropin-releasing hormone, and the

increased levels of ACTH and glucocorticoids (Martin and Pattee, 1953; O'Connor et al., 2000). The number of basophils is reduced at higher concentrations of glucocorticoids, and in case when the IgE-induced release of histamine and leukotrienes is impaired (Schleimer et al., 1981).

Glucocorticoids are involved in the mobilization of neutrophiles from the places of their formation in an organism to the pheripheral circulation. Lymphopenia, neutrophilia and a decrease in L:N ratio may serve as good indicators of the transportation stress and the weaning stress in pigs (Niekamp et al., 2007; Adenkola et al., 2011).

We observed a significantly greater number of neutrophiles in the blood of animals in the control group, whereas in the blood of the experimental animals, the number of lymphocytes and L:N ratio were significantly higher. Therefore, changes in the differential leukocyte count in pigs, which were fed with the peat additive, indicate a decrease in correspondent response to stress and testify to a better adaptation to the new conditions of existence.

The use of "TorVet" does not affect the total protein content in the serum of piglets. At the same time, there is a statistically significant increase in the albumin concentration. Considering that these proteins are completely synthesized in the liver (Kaneko et al., 1997), it is possible to state about intensification of its protein synthetic activity. In addition, the cause of an increase in the index may be the increased absorption of protein with pre-starter as a result of its greater consumption.

Glucose is the main source of energy for most tissues of piglets. Its level was 17.4% ($P<0.05$) lower in the experimental animals, but it was within the limits of reference magnitudes (Kaneko et al., 1997). This can be explained by two factors. The first factor is a greater glucose usage by tissues as a result of adaptation to new conditions in the post-weaning period. The second factor is a higher intensity of gluconeogenesis in control animals under the influence of glucocorticoids (Mota-Rojas et al., 2011).

The state of calcium-phosphorus metabolism can be assessed by the content of total calcium and inorganic phosphorus in blood serum, as well as by alkaline phosphatase activity (Kaneko et al., 1997). We have established rather high levels of total calcium and alkaline phosphatase activity, which can be explained by the high intensity of piglets' growth within the research period. At the same time, the alkaline phosphatase activity significantly decreased by 25.7% ($P<0.05$) under the influence of the additive. These changes may indicate the better process of mineralization of bone tissue under the influence of peat additives. In the study of Tymczyna et al. (2012), the highest prognostic value of determining alkaline phosphatase in blood serum in relation to assessment of morphological, densitometric and biomechanical properties of the mandible in 6-month-old pigs.

In the blood serum of animals, which were fed with the additive, a higher level of iron was defined, which may be due to its additional consumption from feeds. This indicates a better supply of the organism of piglets with iron, which is especially important at their early age (Victor and Mary, 2012).

**Conclusion**

Assessing the obtained data, we believe that positive changes in physiological state of piglets under the influence of the additive are caused by several factors. Firstly, it is the change in the count of particular leukocytes, which indicates the less expressed state of stress in piglets. Secondly, the positive biochemical changes, associated with more pronounced anabolic processes, are noticed in the organisms of animals.

**References**

1. Adenkola A.Y., Ayo J.O., Sackey A.K.B., Adelaiye A.B. Eight hours road transportation and ascorbic acid administration effects on haematological parameters of pigs during the harmattan season. Agric. Biol. J. N. Am. 2011. Vol. 2. P. 1143-1150.

2. Bowman G.L., Ott S.L., Bush E.J. Management effects on preweaning mortality: A report of the NAHMS National Swine Survey. Swine Health Prod. 1996. Vol. 4. P. 25-32.

3. Edwards S.A. (2002): Perinatal mortality in the pig: environmental or physiological solutions? Livest. Prod. Sci. Vol. 78. P. 3-12.

4. Kaneko J.J., Harvey J.W., Bruss M.L. Clinical Biochemistry of Domestic Animals. 5th ed. Oxford, Gulf Professional Publishing, 1997. 932 p.

5. Lossec G., Herpin P., Dividich J.L. Thermoregulatory responses of newborn pig during experimentally induced hypotermia and rewarming. Exp. Physiol. 1998. Vol. 83. P. 667-678.

6. Martin J.R., Pattee C.J. Eosinophil Count and Cortisone and ACTH. Can. Med. Assoc. J. 1953. Vol. 68. P. 565-569.

7. Miller E.R., Kornegay E.T. Mineral and Vitamin Nutrition of Swine. J. Anim. Sci. 1983. Vol. 57. P. 315-329.

8. Mota-Rojas D., Orozco-Gregorio H., Villanueva-Garcia D., Bonilla-Jaime H., Suarez-Bonilla X., Hernandez-Gonzalez R., Roldan-Santiago P., Trujillo-Ortega M.E. Foetal and neonatal energy metabolism in pigs and humans: a review. Vet. Med-Czech. 2011. Vol. 56. P. 215-225.

9. Niekamp S.R., Sutherland M.A., Dahl G.E., Salak-Johnson J.L. Immune responses of piglets to weaning stress: Impacts of photoperiod. J. Anim. Sci. 2007. Vol. 85. P. 93-100.

10. O'Connor T.M., O'Halloran D.J., Shanahan F. The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. Q. J. Med. 2000. Vol. 93. P. 323-333.

11. Perucchietti P., Litjens W. Balanced trace element feed improves sow performance. Pig Progress. 2010. Vol. 26. P. 2-3.

12. Potočnjak D., Kezić D., Popović M., Zdolec N., Valpotić H., Benković V., Mršić G., Janjatović A.K., Lacković G., Valpotić I. Age-related changes in porcine humoral and cellular immune parameters. 2012. Vet. Arhiv. Vol. 82. P. 167-181.

13. Puy H., Deybach J.-C. Haem biosynthesis and excretion of porphyrins. In: The Textbook of Hepatology: From Basic Science to Clinical Practice. (Ed. J. Rodés, J.-P. Benhamou, A. Blei, J. Reichen, M. Rizzetto). Wiley-Blackwell. 2007. P. 207-214.

14. Schleimer R.P., Lichtenstein L.M., Gillespie E. Inhibition of basophil histamine release by anti-inflammatory steroids. Nature. 1981. Vol. 292. P. 454-455.

15. Trckova M., Zraly Z., Bejcek P., Matlova L., Beran V., Horvathova A., Faldyna M., Moravkova M., Shitaye J.E., Svobodova J., Pavlik I. Effect of feeding treated peat as supplement to newborn piglets on the growth, health status and occurrence of conditionally pathogenic mycobacteria. Vet. Med-Czech. 2006a. Vol. 51. P. 544-554.

16. Trckova M., Zraly Z., Matlova L., Beran V., Moravkova M., Svobodova J., Pavlik I. Effects of peat feeding on the performance and health status of fattening pigs and environmentally derived mycobacteria. Vet. Med-Czech. 2006b. Vol. 51. P. 533-543.

17. Tymczyna B., Tatara M.R., Krupski W., Tymczyna-Sobotka M., Luszczewska-Sierakowska I., Bachanek T. Relationships between biochemical bone metabolism indices and morphometric, densitometric and mechanical properties of mandible in 6-month-old pigs. Ann. Agric. Environ. Med. 2012. Vol. 19. P. 535-539.

18. Untea A., Criste R.D., Taranu I., Souffrant W.-B., Janczyk P., Vladescu L., Dragomir C. Availability of microelements in recently weaned piglets fed diet supplemented with inulin. Czech J. Anim. Sci. 2013. Vol. 58. P. 369-374.

19. Vanheukelom V., Driessen B., Maenhout D., Geers R. Peat as environmental enrichment for piglets: The effect on behavior, skin lesions and production results. Appl. Anim. Behav. Sci. 2011. Vol. 134. P. 42-47.

20. Victor I., Mary I. Iron Nutrition and Anaemia in Piglets: a review. J. Vet. Adv. 2012. Vol. 2. P. 261-265.

21. Yefimov V., Kostiushkevych K., Loseva Ye. Condition of a mineral metabolism at pigs an industrial complex. Nauk. visnyk vet. med. 2010. Vol. 5. P. 68-71 (in Ukranian).

22. Yefimov V., Kostiushkevych K., Rakytianskyi V. Effect of feeding treated peat as a supplement on the parameters of cellular immunity, antioxidant status and performance of piglets in early post-weaning period. HVM Bioflux. 2016. Vol. 8 (3). P. 133- 136.

23. Zabielski R., Goldlewski M.M., Guilloteau P. Control of development of gastrointestinal system in neonates. J. Physiol. Pharmacol. 2008. Vol. 59. P. 35-54.

Received 19 December 2016
Accepted 7 April 2017