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Research Article

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

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Abstract:
The objective of our study was to establish the effect of commercial feed additive based on peat on immune status, antioxidant parameters and performance of piglets in the suckling and early post-weaning period. Control and experimental groups of piglets were formed. The experimental animals were additionally getting feed additive from day 3 after birth until day 42. Blood was sampled at the end of the experimental period. Feeding the processed peat, enriched with micro-elements, has led to an increase in total T-lymphocyte count by 20.3 % ($p<0.02$) and resulted in a significant decrease of 0-lymphocyte by 22.7 % ($p<0.05$). The total activity of catalase in the blood under the influence of additives was reduced by 11.2 % at the same time as the total peroxidase activity is increased by 34.8 %. The level of TBA-active products decreased in experimental animals, but these changes were not significant. Accordingly, the use of the feed additive based on peat in diets of piglets in the early postnatal period stimulates the development of the immune system and increases resistance to weaning stress. This leads to increase in performance and reduction of the piglet's mortality.

Key Words: piglets, peat, supplement, cellular immunity, antioxidant parameters, growth

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Introduction

Under present conditions of commercial breeding piglets are conventionally weaned between 3 to 4 weeks old, which results in change in their behavior and welfare (Cox&Cooper 2001). Early weaning adversely affects the function of the digestive system (Berkeveld et al 2009) and as a result we can observe reduced activity of cellular immunity in general (Blecha et al 1983; Kick et al 2012) and in the small intestines as well (Stokes et al 2004).

In order to stimulate the development of the piglets' immune system in the early postnatal period it is recommended to use Glutamine (Johnson et al 2006), β-glucan extracted from Saccharomyces cerevisiae (Li et al 2005) and other substances (vitamins, lipopolysaccharide of bacterial origin, essentially trace elements etc).

Literature states that piglets given peat showed significantly calmer behavior than piglets who didn't receive any. The authors observed lower aggression levels and weight gain after weaning (Vanheukelom et al 2011).

There were reported various data regarding the influence of peat on the functional state of pigs. Trckova et al (2006a) indicate that peat does not have any effect on the growing-fattening pigs. At the same time they indicate that peat supplement might influence the increase of pig's body weight in the end of the post-weaning period (Trckova et al 2006b). At the same time, there was described the increasing of the piglets resistance level to the pathogen Actinobacillus pleuropneumiae and PRRSV infection after environmental peat enrichment in early life (Dixhoorn et al 2015).

Although, as there is no data regarding the influence of supplements made of processed peat on the piglets' immune system and because of the paucity of publications about the influence of the additive on piglets performance it will be discussed in our article.

Materials and methods

Animal model and design of experiment. The study was carried out on the farm that rears three-breed hybrid pigs (Yorkshire, Landrace and Duroc) (Dnipropetrovs'k region, the central part of Ukraine). The research material in the beginning of the experiment consisted of 109 piglets, 3 days old, divided into two groups: experimental (n=55) and control (n=54). Until day 28 they stayed with the breeding pig, in clusters of 10-11 piglets. The experimental period lasted 42 days. During that time, the

animals of the control group received standard fodder while animals of the experimental group received commercial supplements from heat treated peat with trace elements. It was given in addition to the standard diet in the following doses: 200 ml per one litter in suckling period and 250 ml per ten piglets after weaning.

Active biological feed additive for pigs, prepared using heat treatment of the peat and the subsequent addition of iron sulfate, copper sulfate, zinc sulfate, manganese sulfate and cobalt sulfate. Animals from both groups were kept under the same optimal zoo hygienic conditions and under standard veterinary medical care before and during the experiment.

Clinical examination and sampling procedures. The assessment of survival and weighing piglets were held twice: after their weaning (on the 28-th day after birth) and on the last day of the experiment. Blood samples were collected from five piglets from each group in the morning (prior to feeding) from the orbital sinus on the 42-nd after birth. The samples for determination of immunological parameters were placed in test tubes with potassium salt of EDTA; and samples for determination indices of antioxidant protection were placed in test tubes with a coagulant and were centrifuged.

The separated serum was frozen at -20°C and stored until analysis. The laboratory stage studies were conducted in the Scientific-research Centre for biosafety and environmental control of agro-industrial complex DSAEU.

Immunological exploration. The relative amount of T-lymphocytes and their subpopulations was determined by sheep erythrocyte rosette formation and the number of B- lymphocytes and natural killers (NK-lymphocytes) was determined using diagnostic erythrocytes that have antibodies to the receptor CD 22 and CD 16 respectively; null lymphocytes amount was determined by the calculation. The differential leukocyte count was performed in blood smears stained by Wright-Giemsa under a light microscope (Olympus CH 20). The lymphocytes with low, medium and high density of receptors, which are attached 3-5, 6-10 and more than 10 erythrocyte were identified by the counting the number of T- and B-lymphocytes and their subpopulations.

Antioxidant protection examination. Peroxidase activity in blood was determined in a reaction mixture containing: 0,2 M acetic buffer (pH 5.0), 2,5 mmol benzidine (2 ml); 0.6 % H_2O_2 (0.5 ml) and 0.1 ml blood dilution with distillated water (1:200). Activity was measured by following the decrease in absorbance at 520 nm during one minute at 37°C using Humalazer 3000 (Human, Germany).

Plasma thiobarbituric-acid-reactive substances (TBARS) were determined by assaying malondialdehyde (MDA) formation according to the method of Sinnhuber et al (1958) in modification. Blood and 20 % trichloroacetic acid (TCA) were mixed evenly in an amount of 2,5 ml in centrifuge tube. After centrifugation at 3 000 rpm for 5 min 3 ml of the supernatant was collected and added to 1,5 ml of 0.8% thiobarbituric acid, then the mixture was shaken and warmed for 15 min in a boiling water bath followed by rapid cooling. The absorbance of the supernatant was read at 540 nm at room temperature against blank. The optical density of the obtained pink chromogen was read at 540 nm in a semi-automatic analyzer HumaLyzer 3000 (Human, Germany). Blood catalase activity was determined according to the method of Goth (1991).

Table 1. Percentage of various lymphocyte populations in the blood of piglets under the influence of peat additive (mean ± SD)

Parameters	Animal group	
	Control	Experimental
T-lymphocytes, %		
Total	28.83±1.43	34.67±0.54**
Low density of receptors	21.5±1.54	28.67±0.41**
Medium density of receptors	6.33±0.41	4.83±0.20*
High density of receptors	1.00±0.00	1.17±0.20
Theophylline resistant T-lymphocytes. %		
Total	16.50±1.62	20.83±0.41
Low density of receptors	14.33±1.47	18.17±0.41
Medium density of receptors	2.17±0.20	2.50±0.35
High density of receptors	0.00±0.00	0.17±0.20
Theophylline sensitive T-lymphocytes. %		
Total	12.33±0.54	13.83±0.20
Low density of receptors	7.17±1.02	10.50±0.00*
Medium density of receptors	4.17±0.54	2.33±0.20*
High density of receptors	1.00±0.00	1.00±0.00
B-lymphocytes. %		
Total	16.83±1.08	18.67±0.89
Low density of receptors	16.00±0.71	16.50±0.61
Medium density of receptors	0.83±0.41	2.17±0.54
High density of receptors	0.00±0.00	0.00±0.00
NK-lymphocytes. %	12.67±1.34	14.50±0.00
Null lymphocytes. %	41.67±2.88	32.17±1.34*

* – $p \leq 0.05$; ** – $p \leq 0.02$

Statistical analysis. Results were analyzed statistically by the Student's t-test using the Microsoft Excel. The values $P<0.05$ and lower were considered as accurate. Results are expressed in average values ± standard deviations.

Results

Immunological investigation. Table 1 shows the results of immunological studies. The number of T-lymphocytes increased by 16.4 % (from 28.83 % to 34.67 %, $p<0.02$) for the actions of fodder additives from peat. This increase in the number of T -lymphocytes was due to the greater number of cells with low density of receptors. Statistically significant difference between the number of these cells in experimental and control group amounted 24.4 % ($p<0.02$).

Some subpopulations revealed probable increase of theophylline-sensitive cells with low receptor density by 30.2 % (from 7.17% to 10.50 %, $p < 0.05$). Also, there is a tendency of increase in theophylline -resistant T-lymphocytes by 20.8% with a simultaneous increase in the density of membrane receptors. There was a tendency of increase in B-lymphocytes and NK-lymphocytes under the influence of the additive by 9.9 % and 12.6 % respectively.

At the same time, the blood of piglets in experimental group showed significant decrease in the number of null-lymphocytes from 41.7 % to 32.2 % ($p<0.05$).

Table 2. The parameters of antioxidant protection system of piglets in the early post-weaning period under the influence of the peat feed additive (mean ± SD)

Parameters	Animal group	
	Control	Experimental
Catalase activity. mmol H_2O_2/L • min^{-1}	25.53±0.35	22.66±0.85*
Peroxidase activity. OD/L • s^{-1}	0.515±0.023	0.694±0.054*
TBARS. μmol/L	1.80±0.15	1.55±0.22

*- P≤0,05

Antioxidant protection examination. Table 2 shows the indicators of the antioxidant protection system. There was found significantly higher level of peroxidase activity by 34.8% ($p<0.05$) and lower - catalase activity by 11.2% ($p<0.05$) under the influence of supplement.

The concentration of TBARS tended to decrease in the blood of piglets receiving supplement (1.80 μmol/L and 1.55 μmol/ L in the control and experimental group, respectively).

Pigs body weight and health state. Table 3 shows data on mortality, morbidity and body weight of piglets. While we can indicate low levels of mortality in both groups, it is worth to point out that the experimental group survival reached 100% in both sucking and in the early post-weaning period.

The use of supplements has resulted in insignificant decrease in morbidity of piglets (by 2.1% and 4.2% in the sucking and early post-weaning period respectively). All diseased pigs were diagnosed with gastroenteritis. Increases in piglets' body weight in sucking period in the control group were 209.3 grams per day and – 234.3 grams per day in the experimental group, which is 11.9 % more. Similar changes were observed in the early period after weaning: average daily gain in the control group was 110.6 g per day, and in the experimental group – 144.6 grams per day, which is 30.7 % higher.

Table 3. Influence of the feed additive from peat on piglets performance and health

Parameters	Animal group	
	Control (n=54)	Experimental (n=55)
Suckling period		
Mortality. %	1.9	0
Average weight at the weaning. kg	7.56	8.26
Daily gain. g	209.3	234.3
Morbidity. %	9.4	7.3
Early post-weaning period		
Mortality. %	1.9	0
Average weight in two weeks after weaning. kg	9.11	10.28
Daily gain. g	110.6	144.6
Morbidity. %	11.5	7.3

Discussion

T-lymphocytes may be identified by their capacity to bind sheep red blood cells spontaneously in a characteristic pattern, termed E-rosettes. Lability of T lymphocyte sheep erythrocyte receptors to theophylline exposure permits the division of T lymphocytes into theophylline resistant, TR, and theophylline sensitive, Ts, subsets (Limatibul et al 1978). TR lymphocytes, comprising 80% of the T cells, are RFcμ-enriched, RFcγ-depleted and function as inducers of B lymphocyte differentiation. In contrast Ts cells, comprising 20% T cells, are RFcy-enriched, RFcp-depleted and suppress B lymphocyte differentiation (Shore et al 1978). Recent advances in the study of CD22 indicate a complex role of this transmembrane glycoprotein member of the immunoglobulin superfamily in the regulation of B lymphocyte survival and proliferation (Tedder et al 2005). CD16 (Fcγ receptor III) has been described as a receptor expressed on NK cells that facilitates antibody-dependent cellular cytotoxicity by binding to the Fc portion of various antibodies. However, CD16 has a broader function and is directly involved in the lysis of some virus-infected cells and tumor cells, independent of antibody binding (Mandelboim et al 1999).

Our studies observed increased number of T lymphocytes along with increased proportion of Ts cells by 30.2% $p<0.05$ at the expense of cells that have a small number of membrane receptors. The number of TR had a tendency to increase with a simultaneous increase in the density of membrane receptors. Similar results of increasing the number of immune cells with low density of membrane receptor were obtained by Popyk&Vischur (2013) using vitamin A.

Also under the influence of additive we observed an increase in the number of B-lymphocytes and NK-lymphocyte by 9.9 % and 12.6 % respectively. At the same time, the blood of experimental piglets showed probable decrease in the number of null lymphocytes by 22.8% ($p<0.05$).

The data obtained indicates that the use of feed peat additive in sucking and early post-weaning period stimulates the differentiation of immune cells mainly due to the T cells. In our view this is consistent with the literature data available, according to which the cytotoxic T-cells have the leading role in providing response to antigenic load in piglets' before weaning period (Brown et al 2006).

We found that peat feeding supplements lead to significantly higher levels of peroxidase activity by 34.8% (P≤0,05) and conversely decreased catalase activity by 11.2% (P ≤ 0,05). We consider that these changes in parameters of antioxidant protection are the result of a better compensatory response to intensification of lipid peroxidation process, which is typical for the stress (Halliwell&Chirico 1993). The TBA-active products are endogenous genotoxic products of enzymatic and oxygen radical-induced lipid peroxidation. Their concentration in the blood of piglets had a tendency to decrease under action of supplements. These changes in antioxidant status should be evaluated from the standpoint of the micronutrient action because supplement contains iron, copper, zinc and manganese, which are part of the active centers of antioxidant enzymes (Kaneko et al 1997). In addition, some researchers (Johnson et al 2005) suggest that catalase shows its antioxidant properties only at high concentrations of hydrogen peroxide.

These changes in the course of physiological and biochemical processes were displayed on indicators of morbidity and weight gain. The increase of the same parameter was also discovered by Trckova et al (2006b) under the influence of supplements containing peat.

Based on data obtained, we believe that positive changes in physical state and performance of piglets are due to several factors. First of all it is change in the animal behavior and their early accustoming to self-feeding that can be used as part of herd management technology reducing the effects of stress caused by weaning. The second factor is a positive effect of microelements

included in additive. Thirdly, these changes are connected to immune structure stimulation. Further studies will be held to research and support these hypotheses.

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