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The Influence of Humic Acids on Metabolic and Immunological Parameters of Pigs

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Abstract: *The purpose of the work was to monitor the effects of humic acids on the biochemical parameters and growth of piglets. In our work, we monitored three litters of piglets. In group A, both sows and piglets received humic acids. In group B, only piglets received humic acids. The last group (C group) served as a control; it did not receive humic acids.*

We carried out blood sampling from piglets and sows to determine biochemical indicators and weighing all piglets on the 0th, 7th, 14th, 21st, 28th, 35th and 42nd day of life. The results of biochemical examinations of the piglets´ blood did not show any significant differences between the groups, although there were slightly lower values of blood serum total proteins and albumin levels in the control group. Biochemical examination of the sows´ blood showed different levels of cholesterol and urea between the experimental sow and the control sows, the results were better in sow fed with humic acids. Piglets of group A showed the best growth ability of all groups, they achieved the highest weight among all groups at the end of the experiment.

In the piglets, on the 5th day, we recorded a significantly higher total phagocytic activity (PhA) and the mean fluorescence intensity(MFI) ($p < 0.001$) in experimental groups compared to the control. Also between days 14 and day 28, we observed a significantly increased mean fluorescence value ($p<0.001$) in the humic acid groups compared to the control. On the 21st day, we measured significantly higher ($p < 0.001$) values of the metabolic activity index (MAI) of phagocytes in both experimental groups compared to the control.

I. Introduction

Humic substances are organic compounds that are formed naturally by the decomposition of organic matter by physical processes and the activity of microorganisms. Mainly caustobioliths are used - oxidized brown coal (oxyhumolite), peat, lignite. Oxyhumolite is the best source of humic acids (85%). There are several types of humic substances, but their properties are very similar. Humic substances are composed of fulvoacids, humic and humic acids [1].

Humic acids have an excellent ability to bind metals, forming insoluble compounds. As the molecular weight increases, this ability also increases, allowing them to be used for detoxification in heavy metal poisonings such as lead, copper, mercury, cadmium or barium. In addition to metals, they are able to bind other toxic compounds of endogenous and exogenous origin, e.g. microbial toxins, mycotoxins, phytotoxins, ammonia, PCBs, dioxins, benzpyrenes and others, thus becoming non-toxic.

It can be assumed that humic acids has an effect on accelerating the overall metabolism of the cell, because they increase the absorption of oxygen by the mitochondria, thereby supporting cellular respiration and the process of oxidative phosphorylation, and thus the production of ATP, which results in stimulation of the body to increased nutrient intake. The most important property of humic acids for human medicine is the prevention of resorption and accumulation of heavy metals and pesticides in organs and antioxidant ability [2].

A very important property of humic acids is their ability to bind insoluble ions and later slowly release them if required by the body. It has an important ability to bind dangerous substances for the body, such as heavy metals (Pb, Hg, Cd), which are found in the form of chelates, and free radicals, these substances are harmlessly excreted from the body. They also have the ability to directly neutralize free radicals, thus protecting cells from damage and thus preventing disruption of genetic information. Also, this mechanism prevents the development of infections and the development of civilization oncological diseases and endocrine diseases. Humic substances significantly help the utilization of nutrients from food. They support an increase in cell membrane permeability and thus facilitate the movement of minerals from the blood to the intracellular space [3].

Humic acids were primarily used to stimulate growth in agriculture [4]. Later, they began to be used in human and veterinary medicine due to their antiviral, antibacterial, detoxifying and immunostimulating properties [5]. Their metal-binding properties have also been used in the decontamination of polluted water [6].

Humic acids have proven effective in treating multifactorial gastrointestinal diseases. They have an astringent effect and have an antibacterial anti-inflammatory and viricidal effect. Humic acid supplements are suitable for treating the digestive tract and metabolic disorders. Another positive is that they have properties similar to antibiotic growth stimulants without undesirable effects and, accordingly, a beneficial effect on the growth of animals. Humic acids support the health of the intestinal epithelium, stabilization of the intestinal flora and effective feed conversion. They have the potential to replace antibiotics in feed supplements and in the treatment of gastrointestinal diseases [7].

II. Material and methods

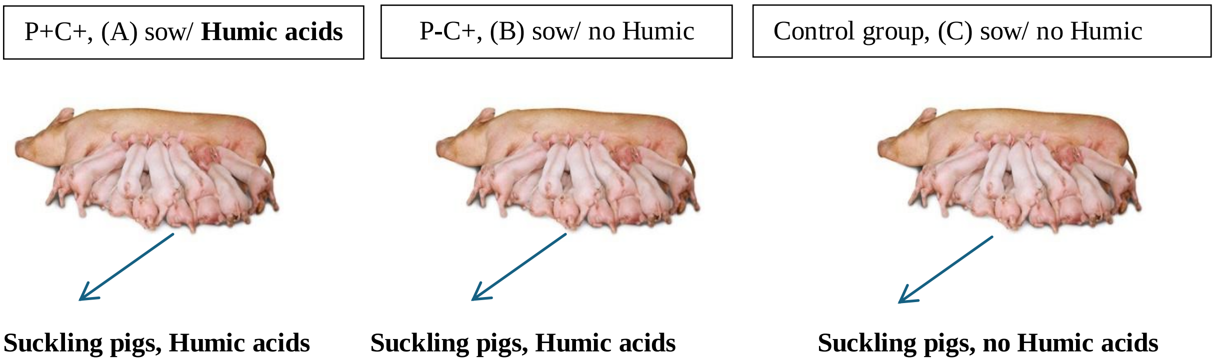
In the experiment, three sows and their piglets were used, which were housed at the UVMPh, Clinic of Swine in Košice, Slovakia. The animals were divided into groups (Table 1).

1. The experimental sow (group A) was fed with mixed feed supplemented with humic acids at 0.5% concentration throughout the gestation period until farrowing and the other sows (B, C) were fed only mixed feed.
2. Experimental piglets (A, B group) from the first day after birth were applied daily for 6 days of life, individually *per os* 2 ml of aqueous solution HUMALAC Natur AFM Liquid, which obtained humic acid in liquid form. From day 9 until weaning (day 31), we fed them powdered humic acids in preparation HUMALAC Natur AFM supplemented in the appropriate mixed feed in 0.5% concentration (0.5 kg/100 kg mixed feed).
3. We took blood from sows before farrowing and then at weekly intervals after farrowing. We carried out blood sampling from piglets to determine biochemical indicators and weighing all piglets on the 0th, 7th, 14th, 21st, 28th, 35th and 42nd day of life. To determine the indicators of the immunological profile of animals, blood from piglets was taken from *the sinus ophtalmicus* on the following days: 5, 14, 21, 28 and on the 42nd day after birth. We took blood from sows from *vena jugularis*. Biochemical parameters were determined from blood serum on the multifunctional analyzer Lisabio (France) at the Clinic of Ruminants UVMPh Košice. Total phagocytic activity, phagocyte metabolic activity index were determined from

heparinized blood (heparin 10-20 IU.ml^{-1} in PBS, Zentiva, Czech Republic). We determined phagocytic activity and average fluorescence intensity using the commercial test PHAGOTEST® (Celonic, Germany). The metabolic activity index of leukocytes was determined by the commercial test Bursttest® (Celonic, Germany).

Table 1: Distribution of animals into groups with and without addition of humic acids

Group name	Sow (administration of humic acids)	Piglets (administration of humic acids)
P+C+, (A)	Yes	Yes
P-C+, (B)	No	Yes
Control, (C)	No	No



Statistical analysis was done in GraphPad Prism version 9. The data were tested using a one-way ANOVA followed by a Tukey test to determine the differences between individual groups. We determined significant differences at significance levels $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

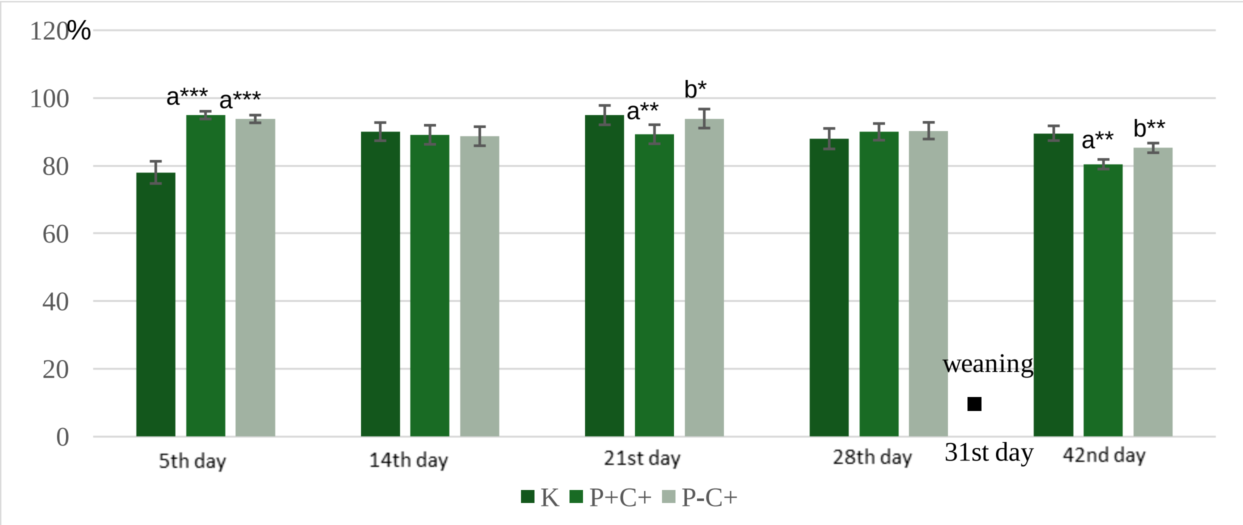
III. Results

In the experiment, we observed significantly higher total phagocytic activity (PhA) as well as mean fluorescence (MFI) ($p < 0.001$) on day 5 in both humic acid supplemented groups compared to the control group. There were no significant differences in phagocytic activity among groups on days 14 and 28. However, between days 14 and day 28, we observed a significantly increased mean fluorescence value ($p<0.001$) in the humic acid groups compared to the control. On the last (42nd) day, we observed significantly lower ($p < 0.01$; $p < 0.001$) overall phagocytic activity as well as mean fluorescence intensity in the P+C+ group compared to the P-C+ group (Graph 1, 2).

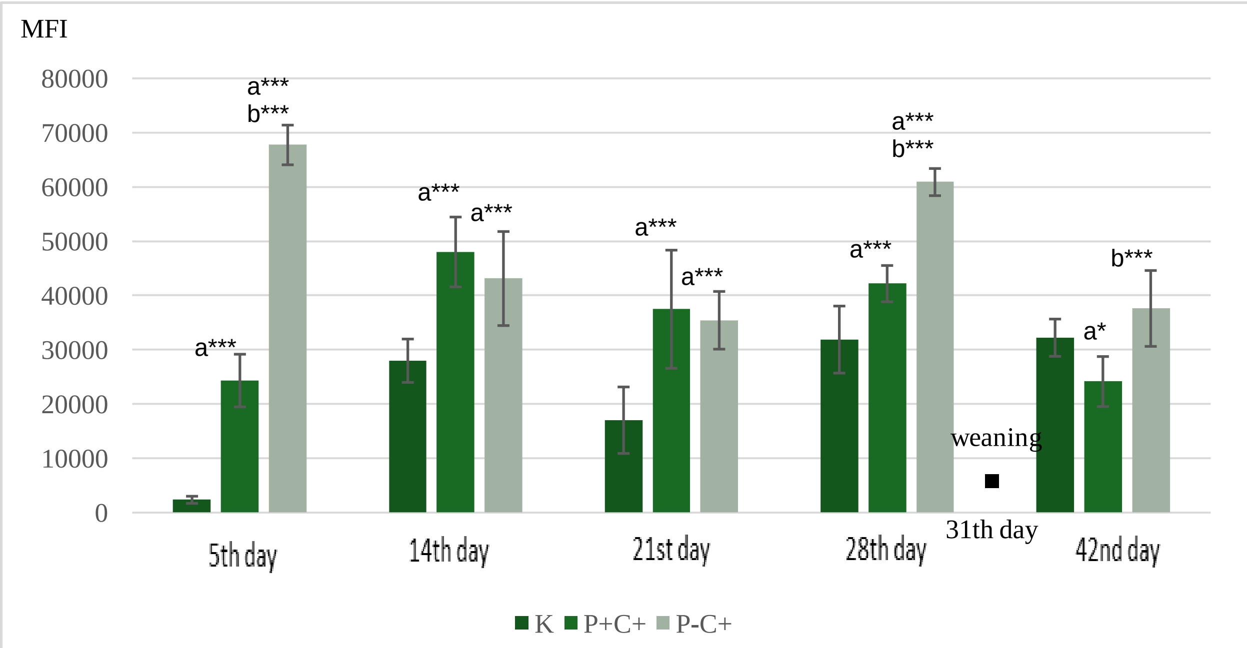
The phagocyte metabolic activity index (MAI) representing the oxidative flash level is shown in Graph 3. On day 5 of piglet life, we observed significantly lower levels of oxidative flare in the P+C+ group compared to the control group ($p < 0.001$), while in the P-C+ group the level was significantly higher than in the control group ($p < 0.01$) and at the same time compared to the P+C+ group ($p < 0.001$). On day 14, we did not notice any significant difference among the groups. On day 21, we measured significantly higher ($p < 0.001$) metabolic activity index values in both experimental groups compared to the control group. In the P-C+ group, we observed significantly higher MAI values on day 28 compared to both the control ($p < 0.01$) and the P+C+ group ($p < 0.05$). On the last (42nd) day, in the group where only piglets received humic acid, we experienced the highest levels of oxidative flare-up.

Throughout the trial, the best results in terms of immune parameters were shown in the group in which humic acids were ingested only by piglets.

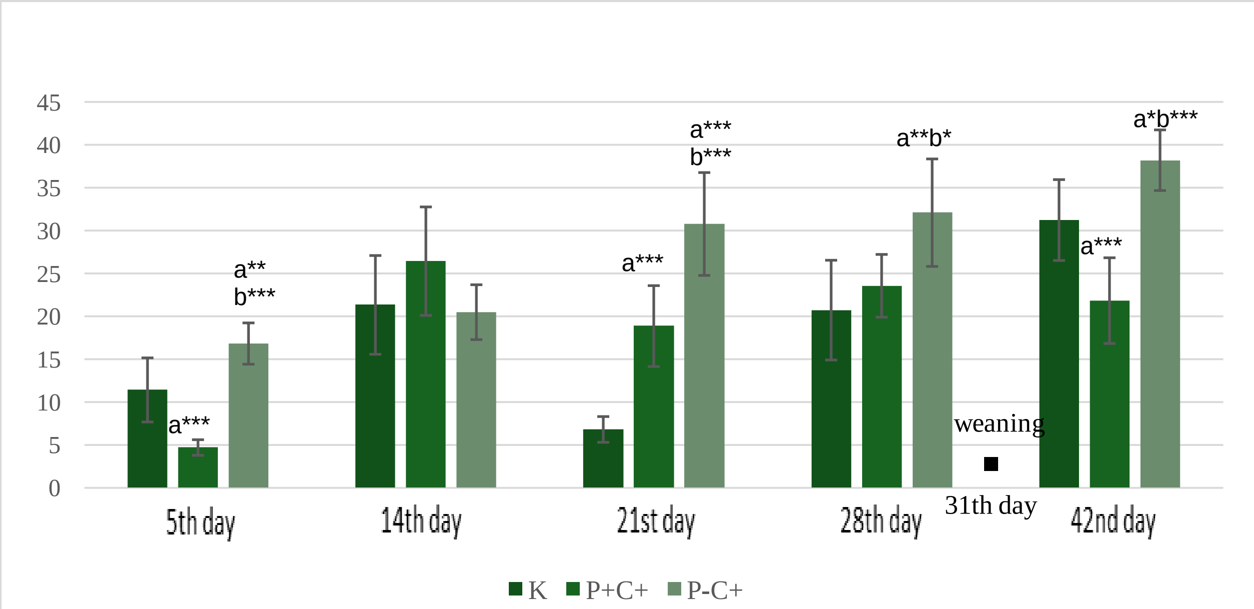
Graph 1: Total phagocytic activity (PhA)



Graph 2: Mean fluorescence intensity (MFI)



Graph 3: Metabolic activity index



Legend: **K** – control group,
P+C+ – humic acids were added to both sows and piglets, **P-C+** – humic acids were added only to piglets
a –significant difference from the control, **b** – significant difference from the P +C+ group
***** – p < 0.05, ****** – p < 0.01, ******* – p < 0.001

In our results, we did not see any significant changes in blood serum total proteins and albumin levels between the piglet groups, although slightly lower values were at the control (Table 2).

In our experiment, there was no significant difference between groups of piglets in the measured levels of cholesterol, urea, AST and ALT.

Table 2: Level of total protein (TP) and albumin (Alb) in the blood of piglets of groups A, B, C

	Group A TP g/l	Group B TP g/l	Group C TP g/l	Group A Alb g/l	Group B Alb g/l	Group C Alb g/l
Sampling 0	26,5±1,9	24,1±1,5	23,1±2,2	9,4±0,9	8,5±1,4	7,3±1,1
Sampling 1	61,4±2,9	57,1±3,2	64,0±8,3	25,3±1,8	22,0±1,8	19,9±1,3
Sampling 2	58,4±5,9	61,8±7,7	55,0±3,6	34,9±2,7	31,6±2,8	27,6±1,7
Sampling 3	51,9±9,7	58,4±2,3	49,9±2,8	39,2±1,3	35,6±1,5	30,5±2,7
Sampling 4	54,6±4,4	58,0±6,2	53,1±3,8	40,9±2,7	36,9±1,8	32,9±2,5
Sampling 5	49,5±3,6	53,7±3,9	51,1±3,7	41,1±2,3	37,4±0,9	34,3±2,3
Sampling 6	47,2±4,1	49,4±2,9	48,7±2,1	38,7±3,5	33,7±2,1	35,5±1,8

Legend: TP - total proteins, norm 70-90 g/l, ALB – albumin, norm 35-45 g/l

In the Table 3, we can see that the best growing groups of animals were the A group, in which both sow and piglets received humic acid. It achieved the highest weight among all groups (Table 3).

Cholesterol levels in sow A, supplemented with humic acids, were slightly higher than others. The level of urea in the control is slightly higher after farrowing than in sow A. An increase was not above the reference value, but this level in combination with cholesterol indicates that the overall metabolism of experimental sow A was in better condition than control animals B, C (Table 4).

Table 3: Piglets´ weight (kg) and average weight gains in % (AWG) of piglets in groups A, B, C

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group A, average weight of pig	1,3±0,2	2,8±0,6	4,4±0,7	6,3±1,2	8,9±1,1	10,1±1,7	13,9±2,4
AWG, %		134%	55%	43%	41%	13%	38%
Group B, average weight of pig	1,6±0,3	2,8±0,5	4,3±0,8	6,5±1,0	8,5±1,4	10,6±1,8	13,5±3,6
AWG, %		74%	56%	49%	32%	23%	27%
Group C, average weight of pig	1,4±0,2	3,3±0,6	4,9±0,9	6,7±1,3	9,2±1,4	11,6±1,7	13,6±1,8
AWG, %		127%	51%	37%	36%	27%	18%

Legend: Group A – humic acids were added to both sows and piglets
Group B – humic acids were added only to piglet, Group C – control group

Table 4: Biochemical examination of sows, mothers of groups A, B, C

Day	**CH(A)**	**CH(B)**	**CH(C)**	**Alb(A)**	**Alb(B)**	**Alb(C)**	**U (A)**	**U (B)**	**U (C)**
0.	1,68	1,53	1,98	44,1	47,9	45,2	7,13	7,3	10,6
7.	2,71	1,77	1,72	45,2	43,10	47,5	6,36	7,22	9,28
14.	2,97	2,01	1,41	42,8	47,1	45,2	5,9	10,3	7,65
21.	2,22	1,8	2,46	45,1	47,8	43,4	6,56	7,43	5,65
28.	2,08	2,04	1,71	46	43,4	41,7	6,47	6,42	4,99
35.	2,01	1,96	1,53	43,1	45,8	39,8	5,98	6,5	5,92
42.	3	2,16	1,63	46,3	46,7	41,8	6,2	5,29	6,89

Norm: CH (cholesterol) 2,60-3,90 mmol/l, Alb (albumin) 35-45 g/l, U (urea) 2,5-10,7 mmol/l
Group A – humic acids were added to both sows and piglets
Group B – humic acids were added only to piglets
Group C – control group

IV. Discussion

In our experiment, we found that feeding humic acids favourably affects the ability of leukocytes to phagocyte and also the average fluorescence value. A similar finding emerges from a study investigating the effect of humic acids on the phagocytic activity of mononuclear cells in rats [8].

The greatest improvement was observed during the first week, and the ability of phagocytes to absorb as many potential pathogens as possible was significantly better in the group where humic acids were fed only to piglets, compared to the group where both piglets and sows received humic acid. During the further course of the experiment, the differences between the groups had a downward trend. It follows that, unlike the first week, long-term use of humic acids does not have a significant effect on phagocytic activity. This claim is also supported by other authors [9], from whose studies show that the effect on phagocytes is time-dependent. In their experiment, they fed humic substances in poultry at concentrations of 0.1% and 0.2% for 60 days, where phagocytic activity was higher during days 8 and 30 after application, but during day 60 the values of phagocytic activity also dropped significantly compared to the control group. Thus, we assume that short-term use of humic acids as an immunostimulant is better for inducing better results of phagocytic activity.

The results of this work prove that humic acids, as an immunostimulating agent, favourably affect the immune profile of animals and thereby reduce the risk of developing infectious diseases.

Oxidative flash values were higher in the humic acid-added experimental groups compared to control for almost the entire duration of the experiment. Several authors have confirmed the stimulating effect of humic acids on oxidative flare in human neutrophils [10], [11].

Sows B, C who did not receive humic acids had lower cholesterol levels than the experimental sow. These measured values were below the reference standard. At the same time, the level of urea was higher in the control than in a sow fed with humic acids. Similar results have been reported by other authors [12].In their results, pigs which received humic substances also showed reduced urea levels and increased cholesterol.

The weight gains showed that piglets from group A, in which both the sow and piglets received humic acids for the entire duration of the trial, showed the best growth gains, its weight at the end of our trial was the highest. Other authors referred, that fattening pigs fed humic acids had increased daily gain compared to the control group, increased lymphocyte counts, less back fat thickness, and better fat overgrowth through meat, indicating better meat quality [13].

The dosage of humic acids is very important. If we add them to feed in less than 0.5%, it is possible that no parameter will be affected. Conversely, if more than 1% is added, there may be problems with the digestibility of other nutrients [10].

V. Conclusion

We revealed that the best growing groups of animals were the A group, in which both sow and piglets received humic acid. It achieved the highest weight among all groups.

The results of this work prove that humic acids, as an immunostimulating agent, favourably affect the immune profile of animals. In the piglets, on the 5th day, we recorded a significantly higher total phagocytic activity (PhA) and the mean fluorescence intensity(MFI) ($p < 0.001$) in experimental groups compared to the control. Also between days 14 and day 28, we observed a significantly increased mean fluorescence value ($p<0.001$) in the humic acid groups compared to the control. On the 21st day, we measured significantly higher ($p < 0.001$) values of the metabolic activity index (MAI) of phagocytes in both experimental groups compared to the control.

Sows B, C who did not receive humic acids had lower cholesterol levels than the experimental sow. These measured values were below the reference standard. At the same time, the level of urea was higher in the control than in a sow fed with humic acids.

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