Effect of the addition of activated charcoal on the blood parameters of fattening pigs

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Abstract

A physiological experiment was conducted for determining the effect of the added product *Carbovet*, containing activated charcoal, on some hematological and biochemical parameters in the blood of fattening male castrated pigs with live weight 58.333–58.667 kg. The animals were divided into three groups, each having three pigs. The pigs were placed in specialized cages for a 6-day preoperational and a 5-day reporting period. Animals from the individual groups were fed the same compound feed, and *Carbovet* (activated charcoal) was added to the ration of the second and third groups at a dosage of 5 and 10 g/day per animal, respectively. The pigs were fed twice a day – 1.8 kg of feed/day per animal. Faeces were collected twice a day. The inclusion of *Carbovet* in compound feeds for fattening pigs at a dose of 5 and 10 mg/day per animal had a positive effect on some hematological and biochemical parameters of the blood, changing them within physiological norms.

Key words: pigs, active charcoal, hematological parameters of the blood, biochemical parameters of the blood

Introduction

Antibiotics have been widely used in animal feed formulas as growth stimulants to reduce the animal's susceptibility to infections and to improve productivity. It is now thought that the continued use of antibiotics may contribute to the emergence of a reservoir of drug-resistant bacteria, which can then transfer their resistance to pathogenic bacteria in both animals and humans. As a result, much research has focused on alternative feeding strategies that can maintain pig productivity and health.

Adsorbents such as clay and activated charcoal are potentially good alternatives to antibiotics. Absorbents prevent diarrhea by absorbing toxins and therefore reducing the absorption of toxins from the intestinal tract. Absorbents are characterized by a porous structure and an enlarged surface. Many plants can be prepared as activated charcoal. Activated charcoal can be used in the treatment of diarrhea as an absorbent to attract substances that allow them to be cleared from the body in symptomatic therapy in humans. In animal models, activated charcoal can slow gastrointestinal transit and help remove excess moisture. In addition to that, activated charcoal is considered an antibacterial and antiviral material.

It can also help lower cholesterol levels. This is because activated charcoal can bind to cholesterol and cholesterol-containing bile acids in the gut, preventing their absorption. According to one study, taking 24 grams of activated charcoal per day for four weeks lowers total cholesterol by 25% and bad LDL cholesterol by 25%, and HDL cholesterol levels increase by 8% (Kuusisto et al., 1986). In a study by Neuvonen et al., 1989, it was found that the intake of 4–32 grams of activated charcoal per day helps to reduce total and LDL by 29–41%.

Studies with bamboo activated carbon have been performed in fish, ducks and pigs for fattening. Other studies have used soot derived from cedar wood and pine wood to develop an activated charcoal product. To enhance its antimicrobial activity, an extract of Chinese herbs (*Pulsatilla chinensis*, *Portulaca oleracea L.*, *Artemisiae argyi Folium* and *Pteris multifida Poir*) was added. The effects of this mixture of activated charcoal and Chinese herb extract, which have the dual absorption and antimicrobial properties, were studied in weaned pigs by Liqi Wang et al. (2019).

Phytogenic feed additives are plant products used in animal nutrition in order to improve productivity. This class of feed has gained increasing interest recently, especially after the complete ban on antibiotic feed additives by the EU in 2006. Phytogenics is a relatively new class of feed additives that do not include organic acids and probiotics, which are already better studied in animal nutrition. Knowledge of phytogenic additives is limited, in terms of their action and aspects of their application, as well as in terms of botanical origin, processing and composition (Windisch et al., 2007). One such product that is used in pig farming is activated charcoal.

Another study with pigs was done in South Korea, using different concentrations of activated charcoal and Stevia mixed in a total combined mixture. Pigs from the experimental groups had higher daily gain, more efficient feed utilization and better immune responses, as well as significantly higher meat quality and storage (Hans-Peter Schmidt et al., 2019).

Activated carbon is also used to absorb toxins in various feed materials, although some studies report that it shows a limited effect on the absorption of toxins. In vitro laboratory tests indicate that the absorption rate of mycotoxins DON, ZEN, AFB1 and OTA is over 90%. Once absorbed, the complex of activated charcoal and toxins is stable in saline and is not easily excreted by desorption assays (Liqi Wang et al., 2019).

The addition of activated charcoal to the rations of fattening pigs in our country has not been sufficiently studied. In our studies (Nedeva and Yordanova, 2013) with growing pigs, we found that the addition of charcoal (3 kg/t of feed) for 14, 21, 35 and 49 days after weaning, increases the growth rate by 17.65–24.81% and reduces the cost of mixture and nutrients by 5.47–18.17%. In the conditions of our experiment, the addition of charcoal successfully reduced the incidence of digestive disorders.

The adsorption of activated charcoal chemicals depends on several factors: pore size, surface area, chemical nature of the xenobiotic and charcoal dose, pH and gastrointestinal content are also very important. The specific surface area measured for different types of activated carbon can range from 500 m2/g (with lignin as starting material) to over 2000 m2/g (with oil as starting material) (Diamadopoulos et al., 1992). The development of ”super active” coal (with surfaces up to 3500 m2/g) increased the effectiveness of this antidote. By controlling the distribution and size of the pores, an adsorption rate of 10 to 100 times higher than that of granular carbon is achieved (Basta et al., 1994).

The aim of the study was to establish the effects of the added activated charcoal on some hematological and biochemical parameters in the blood of fattening pigs.

Material and methods

A physiological experiment was conducted for determining the effect of activated charcoal – *Carbovet* on the blood parameters in three groups with three male castrated pigs in each with live weight 58.333–58.667 kg from the ♀ Danube White x ♂ Duroc cross. The pigs were placed in specialized cages for a 6-day preoperational and a 5-day reporting period.

Experiment scheme

The experiments' scheme is presented in Table 1. The animals of the individual groups were fed the same compound feed presented in Table 2, and *Carbovet* was added to the ration of the second and third groups at a dose of 5 and 10 g/day per animal, respectively.

Natural plants-based charcoal – *Carbovet* is a feed additive obtained from specially selected French oak by carbonization. The product has a very good absorption capacity and captures enterotoxins produced by harmful bacteria and mycotoxins from feed, as well as some pathogenic bacteria such as *clostridia*, *salmonella* and *E. coli*. *Carbovet* is a feed additive designed to be added to poultry and pig feeds. Active ingredient: 80% consists of charcoal obtained by carbonization of French oak wood. It acts in the digestive tract. Due to its large adsorbing surface, it quickly binds myco- and enterotoxins, which prevents them from penetrating the blood, liver and other organs.

Table 1. Experiment scheme

Indicators	Groups		
	1	2	3
Control	0	0	0
Carbovet, 5g/day per animal	0	+	0
Carbovet, 10 g/day per animal	0	0	+

Feeding and water consumption

The pigs were fed twice a day with 1.8 kg feed per day per animal (Table 2). The rations of the compound feed for the preoperational and reporting period were set aside in advance and stored in buckets with lids that close tightly.

Blood samples and analyses

Blood samples were taken from each pig from the orbital venous sinus using a closed system method. All samples were collected in plastic blood tubes (Vacusera, Izmir, Turkey) and immediately inverted 10 times. Serum biochemistry samples were collected in serum tubes and allowed to clot at room temperature for 2–3 hours before centrifugation (2000×g for 15 minutes). Serum was collected and stored at -20 °C for subsequent biochemical analysis. Whole blood samples were collected in EDTA tubes and stored at room temperature for hematological analysis within 6 hours of sampling. Analytical procedures for blood counting were performed with an automatic hematology analyzer with 5-type differential count SYSMEX XS 500i (Sysmex Europe GmbH, Norderstedt, Germany) and an automatic biochemical analyzer Selectra Pro XL (ELITech Group, Puteaux, France) in accordance with the instructions of manufacturer. These include leukocyte (WBC) determination by conductometric and visual optical method, erythrocytes (RBC) by conductometric method, hemoglobin (HGL) by cyanomethaemoglobin method, hematocrit (HCT) by indirect method based on conductometric analysis, cells (MCV) by conductometric method, mean hemoglobin content in erythrocyte (MCH), mean hemoglobin concentration in erythrocytes (MCHC).

Table 2. Component composition and nutrient content in kg of compound feed

Components, %	Groups – 1, 2, 3
Maize	18.88
Barley	20.00
Wheat	25.00
Wheat bran	10.00
Bioconcentrate mixture – 14	26.00
Synthetic lysine, 98	0.12
Total:	100.00
1 kg of feed contains:	
Metabolizable energy, kcal	3015
Crude protein	18.01
Lysine	0.95
Methionine + cystine	0.64
Threonine	0.64
Tryptophan	0.1
Crude fats	1.93
Crude fibers	6.74
Calcium	0.85
Phosphorus	0.78

Statistical analyses

Results were calculated using the variation statistics method. Established were the following: regression coefficients, correlation and determination to establish the strength and direction of influence of the tested variable, as well as the level of its significance accepted for $p \leq 0.05$.

Results

The results from the blood analyses are presented in Tables 3 and 4. The blood sample analyses indicate that the added *Carbovet* in the feed rations of pigs had a positive effect on the hematological and biochemical parameters of the blood, changing them within physiological norms. Characterizing the results of the values of AST and ALT in the blood serum, we observe a tendency to a gradual increase of these indicators in the experimental groups. The effects of AST for the second and third groups were 3.65 (12.76%) and 7.63 (26.21%), respectively, and in ALT – 11.30 (23.99%) and 2.03 (4.25%) which indicates the increase in the experimental groups compared to the control. The correlation and determination coefficients ($R = 0.425$, $R^2 = 180$ and $R = 0.472$, R2 = 0.223) determined a moderate connection between *Carbovet* and the traits (Table 5). Increases in values were also observed in ALP (-5.08 – 18.45%), WBC (-1.45 – 14.53%), MCV (4.24 – 3.90%) and MCH (2.20 – 1.10%).

For the other indicators, we have reported a negative effect of activated charcoal in pigs from the experimental groups compared to the control – HGB (-2.54 – -3.39%), RBC (-4.64 – -4.48%), HCT (-0.26 – -0.79%), MCHC (-2.27 – -2.91%) and PLT (-18.28 – -18.06%). All results obtained from the blood analyses were within the reference values. The differences between the groups were not statistically significant, but there were close to significant – RBC $p = 0.091$ and MCHC $p = 0.087$. With the same traits we have registered higher coefficients of correlation ($R = 0.725$ and $R = 0.704$) and determination ($R^2 = 0.526$ and $R^2 = 0.496$). These correlation coefficients indicated a strong relationship between *Carbovet* and red blood cell count, as well as the mean hemoglobin concentration in erythrocytes. And from the determination coefficients we established that the statistical model as a whole explains about 50% of the variability of the variables, which is a good result.

Discussion

The liver is the most important organ for the disposal of toxic substances formed in the body and from outside. The preparation used *Carbovet* is characterized by a high rate of bind-

Table 3. Blood analyses results

Traits	Group	AST	SD	ALT	SD	ALP	SD	WBC	SD	HGB	SD	RBC	SD
	1	29.0	11.2	47.1	15.6	374	118	13.4	5.22	118	1.15	6.47	0.198
Mean	2	32.7	1.48	58.4	8.06	355	102	13.2	0.502	115	2.12	6.17	0.0283
	3	36.6	8.45	49.1	3.40	443	47.1	15.4	6.04	114	4.73	6.18	0.182

Table 4. Blood analyses results

Traits	Group	HCT	SD	MCV	SD	MCH	SD	MCHC	SD	PLT	SD
	1	0.381	0.00321	59.0	1.40	18.2	0.700	309	4.51	443	68.5
Mean	2	0.380	0.0163	61.5	2.90	18.6	0.424	302	7.07	362	42.4
	3	0.378	0.0121	61.3	1.25	18.4	0.379	300	3.51	363	130

Table 5. Correlation, determination and regression coefficients

Predictor	Estimate	SE	t	p	R	R^2
Model Coefficients - AST						
Intercept [a]	29.00	5.15	5.636	0.002	0.425	0.180
2 – 1	3.65	8.14	0.449	0.672		
3 – 1	7.63	7.28	1.049	0.342		
Model Coefficients - ALT						
Intercept [a]	47.10	6.19	7.611	< 0.001	0.472	0.223
2 – 1	11.30	9.78	1.155	0.300		
3 – 1	2.03	8.75	0.232	0.825		
Model Coefficients - ALP						
Intercept [a]	374.0	53.3	7.013	< 0.001	0.460	0.212
2 – 1	-18.6	84.3	-0.221	0.834		
3 – 1	69.3	75.4	0.918	0.401		
Model Coefficients - WBC						
Intercept [a]	13.410	2.92	4.5992	0.006	0.248	0.0614
2 – 1	-0.255	4.61	-0.0553	0.958		
3 – 1	1.997	4.12	0.4842	0.649		
Model Coefficients - HGB						
Intercept [a]	117.67	1.86	63.30	< 0.001	0.578	0.334
2 – 1	-3.17	2.94	-1.08	0.331		
3 – 1	-4.00	2.63	-1.52	0.189		
Model Coefficients - RBC						
Intercept [a]	6.467	0.0983	65.79	< 0.001	0.725	0.526
2 – 1	-0.297	0.1554	-1.91	0.115		
3 – 1	-0.290	0.1390	-2.09	0.091		
Model Coefficients - HCT						
Intercept [a]	0.38133	0.00620	61.546	< 0.001	0.152	0.0232
2 – 1	-0.00183	0.00980	-0.187	0.859		
3 – 1	-0.00300	0.00876	-0.342	0.746		
Model Coefficients - MCV						
Intercept [a]	58.97	1.02	58.09	< 0.001	0.645	0.416
2 – 1	2.58	1.61	1.61	0.168		
3 – 1	2.30	1.44	1.60	0.170		
Model Coefficients - MCH						
Intercept [a]	18.200	0.311	58.605	< 0.001	0.350	0.123
2 – 1	0.400	0.491	0.815	0.452		
3 – 1	0.233	0.439	0.531	0.618		
Model Coefficients - MCHC						
Intercept [a]	308.67	2.77	111.32	< 0.001	0.704	0.496
2 – 1	-6.67	4.38	-1.52	0.189		
3 – 1	-8.33	3.92	-2.13	0.087		
Model Coefficients - PLT						
Intercept [a]	443.0	54.8	8.087	< 0.001	0.461	0.212
2 – 1	-81.0	86.6	-0.935	0.393		
3 – 1	-80.0	77.5	-1.033	0.349		

a - Regression constant

ing of a wide range of myco- and enterotoxins, which completely normally leads to optimization of liver function, which, respectively, is reflected in slightly elevated values of AST and ALT, such as the coefficient of De Rytis remains virtually the same. AST and ALT levels have a parallel change and the observed increased serum transaminase activity indicates regenerative / reparative liver activity, i.e. the adaptation of the organism to the intake of the preparation. Despite the non-specificity of the changes in AST and ALT, their determination in the blood serum is a valuable and important diagnostic method.

PLTs are involved in hemostasis and, in addition, in inflammation, by secreting cytokines and chemokines. Lower platelet counts in pigs receiving activated charcoal may be associated with reduced levels of infection in their bodies. Chu et al. (2013) found that the inclusion of bamboo charcoal in the rations of fattened pigs increased productivity and feeding efficiency by reducing gaseous emissions and harmful microflora in the faeces, leading to reduced stress (lower cortisol levels) and increased IgG levels in the serum. The authors point out that bamboo charcoal can protect pigs from infections and reduce stress due to reduced cortisol levels and increased serum IgG levels in fattened pigs, but does not affect the concentration of leukocytes, erythrocytes, hemoglobin and hematocrit in the blood.

The amount of PLT in the peripheral blood of the animals in the experimental groups was lower, but within normal physiological limits. The synthesis of prothrombin of factors VII and IX requires vitamin K. In previous studies on the effect of *Carbovet* included in the feed of pigs, it was found that the preparation has absorbed some of the useful nutrients, to which we can attribute a possible deficiency of vitamin K. Decreased values may also be the result of improper storage of blood samples, hemolysis, hydrolysis, self-degradation. Therefore, reduced PLT values are not a definite indicator in relation to the test preparation. Decreased PLT values in the experimental groups cannot be considered as a sign of thrombocytopenia because they are within the norm, but can be interpreted as a pronounced suppression of liver function in relation to the formation of coagulation factors.

WBCs were within normal levels, despite the observed increase in group 3 pigs. The mild form of leukocytosis compared to the control group was probably due to the stimulating effect of activated charcoal, which supports the function of the immune system and improves its ability to respond to attacks by pathogens such as viruses and bacteria.

Adsorption therapy with activated charcoal as an indigestible carrier is one of the important methods for neutralizing the absorbed toxic substances or harmful substances formed in the gastrointestinal tract (McLennan and Amos, 1989; McKenzie, 1991; Jindal et al., 1994). Activated charcoal works by trapping toxins and chemicals in the gut, preventing their absorption. Its porous structure has a negative electrical charge and attracts positively charged molecules, such as toxins and gases. It has the ability to bind to urea and other toxins, helping the body eliminate them. Urea and other waste products can pass from the blood into the intestines through a process known as diffusion. In the gut, they bind to activated charcoal and because it is not absorbed by the body, it carries toxins associated with its surface outside the body and they are excreted in the feces. It has been studied that in people suffering from chronic kidney disease, activated charcoal helps to improve kidney function.

In a study by Petre (2017), it was shown that activated charcoal supplements can help lower levels of urea and other waste products in the blood and can help improve kidney function by promoting the elimination of toxic waste products and reducing their number and quantity. Struhsaker et al. (1997) state that activated carbon adsorbs a wide range of compounds. The slight decrease in RBC and MCHC in the peripheral blood of experimental pigs correlates with the absorption properties of activated carbon, which can be associated not only with toxic but also with beneficial substances, thus causing nutritional deficiency of vitamin B6, B12 and folic acid and affect hematopoiesis.

Conclusion

The addition of *Carbovet* had a positive effect on some hematological and biochemical parameters of the blood, as well as changing them within the physiological norms.

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