Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Morpho-Functional State of the Liver of the Rats Fed the Rations with Meat of the Pigs Grown with Antimicrobials

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ARTICLE HISTORY (14-501)

Received: September 26, 2014
Revised: November 08, 2014
Accepted: January 19, 2015

Key words:
Antimicrobial agents
Lard
Meat
Steatosis
The functional activity of the liver

ABSTRACT

The objective of the research is to study the effects of the diets containing meat and fat of the pigs fed with antibiotics on the morpho-functional activity of the liver by the biochemical and morphological indexes of the rats. Sixty albino rats were randomized into five groups that were feeding the same ration within 30 days; the difference was only in protein or fat component. Groups I and III received meat and fat of the pigs fed with antimicrobials, Groups II and IV received meat and fat of the pigs grown on conventional diet, the control Group V was fed with egg protein and vegetable oil. The biochemical investigations included the determination of total protein, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), urea, creatinine, total lipids, cholesterol, bilirubin, and glucose in serum by the standard methods with standard reagent kits. The morphological study included a light-optical analysis of the histological section of the liver tissue. It has been found that the application of pork in the diets of rats leads to the increased functional activity of the liver mainly due to the activation of the protein metabolism (the increase of total protein and albumin in serum). The application of lard in the diet of rats causes intensification of the detoxicative function of the liver that results in the increased transaminase activity in the blood and the liver tissue, the change of the lipid metabolism, and the appearance of the fat modified hepatocytes.

To Cite This Article: Belik SN, IF Gorlov, MI Slozhenkina, EY Zlobina and AS Pavlenko, 2015. Morpho-functional state of the liver of the rats fed the rations with meat of the pigs grown with antimicrobials. Pak Vet J, 35(3): 325-328.

INTRODUCTION

One of the most important sources of native protein, fats, minerals, vitamins, enzymes, and other nutrients necessary for life of the human body is meat. Currently, the major share of the total Russian imports of meat and edible meat by-products (34%) is accounted for pork, 10% of the imports is accounted for poultry, which exceeds the threshold of food security by 10-15%. In this regard, a critical factor for the long-term sustainable development of the pig industry is its intensification. Currently, one of the most widely used ways of intensification is the application of the animal feed supplements containing antimicrobial agents in breeding (Jacela *et al.*, 2009; Pluske, 2013; Chattopadhyay, 2014).

Good results in the pork production were obtained on the farms that apply forage protein-vitamin and mineral supplements (PVMS) containing all the necessary vitamins, minerals, trace elements, antioxidants, synthetic amino acids, and antimicrobial agents – tylosin, olaquindox, and zinc bacitracin in the amount of 50, 250, and 200 parts per million, respectively. According to our references, the intervention of this supplement to the pig diet can improve the ability to have a big fetus, the milking capacity of the sows, the livability of their piglets at weaning, stimulates the growth rate of young animals (Kulmakova, 2011). At the same time there is evidence of a negative impact of PVMS on the metabolic enzymatic redox processes in the liver of the pigs at the age of 2.5 months, that is the increase in the level of transaminases and alkaline phosphatase in the serum and the liver histological structure violation in the form of microvesicular fat transformation (Prokopeva and Grigorieva, 2008). These facts and the results of the analysis of scientific sources (Piatkowska *et al.*, 2012; Adesokan *et al.*, 2013; Er *et al.*, 2013; Rasheed *et al.*, 2014) suggest the possibility of contamination of the killing products (directly entering the retail network for

sale to consumers) with residual amounts and metabolites of the biologically active agents PVMS.

The liver is the central organ of the chemical homeostasis in the body, whose main function is the metabolism of proteins, fats, carbohydrates, vitamins, pigments, and occupies the central place in the process of the detoxification of exogenous and endogenous toxic compounds. Active admission of xenobiotics in the body causes a violation of detoxification capacity, which may lead to the violation of other metabolic functions of the liver and its organic damage (Bigoniya *et al.,* 2009; Esaulenko *et al.*, 2011).

In this regard, the main objective of our study was to investigate the influence of the diets containing meat and lard of the pigs, fed with PVMS on the morpho-functional activity of the liver by biochemical and morphological indicators in a biological experiment.

MATERIALS AND METHODS

The experiments were carried out on 60 albino weaned rats with an average weight of 53 g. The animals were kept under identical conditions with free access to food and water. Under the analogy principle, these rats were divided in five equal groups. All group members fed their basic diet (Table 1) compiled in accordance with the recommended daily feed standards, characterized by protein and fat components within 30 days. The quantitative composition of the daily diet is presented in Table 2.

After the experiment, the animals were sacrificed by decapitation, the blood was centrifuged, the serum obtained was studied in terms of the following indicators: the concentration of the total protein (TP), urea, creatinine, total lipids (TL), triglycerides (TG), cholesterol (TC) was determined spectrophotometrically with standardized techniques using standard chemical sets by the firm "Lakhema" (Czech Republic). The activity of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined colorimetrically using the Reitman-Fraenkel method by the set of standard diagnostic reagents. The activity of ALT, AST in 10% homogenate of the liver tissue was determined by the same method. The protein fractions were determined by electrophoresis.

For the histological examination, the liver slices from each animal of the corresponding experimental group were inserted into 10% neutral formalin solution, and further, optionally, - into Carnoy's fluid. After the standard preparation, the paraffin sections were stained with hematoxylin-eosin and Schiff's reagent with an amylase test. 60 bodies were studied light-optically. All manipulations with animals were carried out in accordance with the international principles of the Declaration of Helsinki 2000 of humane treatment of animals.

Statistical processing was performed using the software package Statsoft Statistica 6.0 and conventional methods of the parametric statistics. To assess the inter-group differences, the Student's t-test was used. These differences were considered to be significant when the error probability is $P<0.05$.

Table 1: Scheme of the feeding the laboratory animals

Groups	Characteristics of feeding[4]
Group I	BD + PC – meat AB, FC – oil
Group II	BD + PC – meat NR, FC – oil
Group III	BD + PC – lowfat quark, FC – lard AB
Group IV	BD + PC – lowfat quark, FC – lard NR
Group V	BD + PC – white of the egg, FC – oil

[4]BD - basic diet; PC - a protein component; FC - a fat component; meat AB and lard AB - meat and lard of the pigs fed with antimicrobials; meat NR and fat NR - meat and lad of the pigs fed a normal agricultural ration.

Table 2: The composition of the daily diet

Feed item (g)	Groups				
	I	II	III	IV	V
Wheat bread	15.0	15.0	15.0	15.0	15.0
Maize groats	5.0	5.0	5.0	5.0	5.0
Oat groats	10.0	10.0	10.0	10.0	10.0
Potato starch	10.5	10.5	10.5	10.5	10.5
Pork (experimental)	5.0	-	-	-	-
Pork (control)	-	5.0	-	-	-
Lowfat quark	-	-	7.0	7.0	-
Whole egg	-	-	-	-	9.5
Lard (experimental)	-	-	1.2	-	-
Lard (control)	-	-	-	1.2	-
Oil	1.0	1.0	-	-	0.1

RESULTS AND DISCUSSION

The most revealing markers characterizing protein-synthetic function of the liver is the content of protein and of its fractions in the blood serum (Bigoniya *et al.,* 2009). The highest activation of the protein metabolism was observed in animals fed with meat of the pigs grown with antibiotics in their diets. This was reflected in the increase of total protein content by 44.36% ($P<0.05$) and of albumin by 12.17% ($P<0.05$) with respect to the control group indexes. In view of mainly transport function of albumin (transfer of water, ions, magnesium, calcium, fatty acids, certain hormones, etc.), we can assume that a diet containing meat AB has an anabolic effect, i.e. reinforces for all metabolic processes in the body. This probably reflected the higher biological value of protein in the diet that had been found earlier (Belik *et al.*, 2006).

The rats in Groups II, III, and IV had a statistically significant increase in TP by 10.12, 16.36 and 8.00%, respectively, with the albumin content in these animals to remain at the target level. Concentrations of AST and ALT in serum are often used as biochemical markers of the hepatocellular insufficiency. The activity of these enzymes increases significantly as a result of the cell membranes damage. ALT is specific for the liver tissue and has a higher diagnostic value in assessing the degree of the liver cell damage, while the AST level may rise when the cells of other organs and tissues (heart, skeletal muscle, red blood cells) are damaged (Kanda *et al.*, 2014). The greatest increase in activity of these enzymes is observed in the xenobiotic induced hepatitis (Krier and Ahmed, 2009).

The biochemical studies of the serum showed (Table 3) that the ALT blood content in the experimental Groups I and II were significantly higher than in the control animals by 41.32% and 26.93% respectively, while Group IV showed a decrease in the activity of the enzyme under study by 23.54% ($P<0.05$).

The level of ALT activity in the liver tissue in all groups was within the control value ($P>0.05$). This allows to assume that the increase in the activity of the studied

Table 3: Biochemical indexes of the functional activity of the rat liver/blood/serum

Indices	Units	Groups				
		I	II	III	IV	V
Total protein	g/l	12.27±0.21	9.36±0.22	9.89±0.36	9.18±0.21	8.50±0.19
Albumins	g/l	6.64±0.35	4.56±0.32	4.57±0.23	4.43±0.16	4.10±0.27
ALT blood	nmol/g	27.19±0.49	24.42±0.47	28.4±0.77*	14.71±0.51*	19.24±0.46
ALT liver	nmol/g	2.37±0.04	2.33±0.03	2.32±0.10	2.10±0.08*	2.34±0.03
AST blood	nmol/g	18.52±0.36	16.10±0.31	22.97±0.57*	16.91±0.6	17.11±0.27
AST liver	nmol/g	0.95±0.02	0.93±0.01	1.46±0.01*	1.09±0.03	0.91±0.01
Urea	mmol/l	4.87±0.12*	4.19±0.07*	3.27±0.06	3.46±0.12	3.42±0.09
Creatinine	mmol/l	79.22±1.72*	80.7±1.46*	97.1±0.63*	99.7±0.52*	72.3±0.75
Total bilirubin	mcmol/l	3.3±0.54	3.6±0.23	3.9±0.26	4.1±0.35	3.8±0.33
Glucose	mmol/l	5.91±0.46	4.35±0.72	5.83±0.30	5.70±0.33	4.83±0.57
Total lipids	g/l	11.62 ±0.31*	10.25±0.37	10.60±0.28*	9.69±0.18	9.70±0.16
Cholesterol	mmol/l	1.74±0.03	1.98±0.02	2.15±0.07	1.85±0.03	1.66±0.01
Triglycerides	mmol/l	1.37±0.03	1.11±0.01	1.47±0.02	1.30±0.02	1.51±0.01

Values (mean±SE) asterisk in a row differ significantly (P<0.05) than group-V (control).

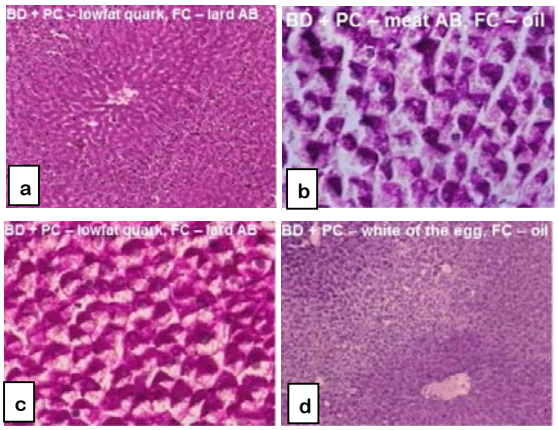

Fig. 1: Hepatocytes of the peripheral zone of the hepatic lobules with signs of microvesicular transformation of fat in the rats of group III (a), high glycogen content in hepatocytes of the rats in group I (b) & group III (c) and prevalence of glycogen inclusions in pericentral parts of the hepatic lobules in group V (control) (d). Stain: a & c: H and E; d & d: PAS-reaction. Magnification: a & d: 100×; b & c: 400×.

enzymes in the blood is not caused by injury of hepatocytes, but rather, is adaptive to the diets with anabolic effect and confirms the increased protein homeostasis with maintaining its stability.

Another indication of the synthetic liver activity is the urea content in the blood serum. The most significant increase in the urea level was observed in the animals of Groups I and II by 42.4% and 22.52%. This fact confirms the activation of the protein metabolism in dynamic equilibrium processes of synthesis and degradation, as evidenced by the creatinine preservation in these groups within the target values.

The application of the lard of the pigs fed with antimicrobials in the diets of the rats has resulted in a statistically significant increase in activity of both AST and ALT in blood by 34.25% and 47.61%, respectively, and of AST of the liver tissue by 60.44%, whereas in the other groups, the statistically significant difference from the respective values in the control group was not found. We suggest that the increased activity of transamination enzymes in rats fed diets with lard AB can be indicative of destabilization of the hepatocyte membranes and is an indicator of the intensification of the detoxicative function of the liver (Prutkina and Tsybikov, 2007). Besides, the increased activity of AST in the liver may be an indicator of increasing of the primary energy metabolism, which is connected with the synthesis of aspartic acid in the liver. The proof of that is the highest creatinine level in the blood and high glycogen content (Fig. 1) in the liver tissue of animals in this group.

The liver plays a key role in the lipid metabolism. The synthesis of such important lipids as cholesterol, which is a component of the cell membranes, the reproductive hormones, and the nerve tissue, occurs in the liver, as well as the synthesis of triglycerides, which constitute about 90% of the fats in the body, takes place there (Kamyshnikov, 2013). In our experiment we observed the preservation of the total lipid, triacylglycerol, and cholesterol content within the normal range of the physiological responses in the animals consumed a diet containing meat AB, which confirms the absence of its stress effect on the body.

At the same time, we observed an increase in the cholesterol level in the blood serum of the animals in Group III by 16.2% compared with the animals fed lard NR and by 29.52% compared with the control animals that may be an additional indicator of the stress detoxicative function of the liver.

One of the most important tests characterizing the functional activity of the liver is the bilirubin level in the blood serum. Our data obtained show maintaining a balance between saturation, conjugation, and excretion of the pigment by hepatocytes. In all groups studied, the value of this index was within the physiological range. This confirms the stability of the synthetic and secretory function of the liver in relation to the pigment metabolism.

Glycogen is found in all tissues of the body, but as a primary energy repository it is stored only in the liver and the muscles. The glycogen in the muscle tissue can be used only by its own cells, whereas the liver glycogen is a source of glucose for almost all types of tissues, as enzyme glucose-6-phosphatase catalyzing the cleavage of the phosphate group with the formation of free glucose, which enters the general circulation, is found in hepatocytes.

The results of the morphological analysis of the liver preparations showed that liver slices of the rats, which had fed rations both with the meat from the pigs in the experimental group and with the meat and the lard of the pigs on a normal diet, as well as the rats in the control group, have a similar characteristic structure plan, the cell structure is not modified, pathological changes were not observed. All animals are characterized by a high content and a uniform distribution of glycogen in the liver cells (Fig. 2 to 4) that indicates a high functional activity of the organ without any destabilization elements.

The application of the lard of the pigs grown with the feed supplement in the diets of rats led to a higher content of the fat inclusions with respect to the norm, with hepatocytes with an increased content of fat inclusions to be always located on the edge of the liver lobules, and to take no more than one quarter of their parts. It is important to emphasize that the glycogen content remained high especially in the pericentral area that is a sign of correct functioning of the energy homeostasis and is confirmed by the preservation of blood glucose at the level of control values.

The distribution nature and the paucity of the fat modified hepatocytes (less than 5% of cells) allow to consider the changes identified to be occasional and not dangerous to the health of consumers at the given shelf-life period of the studied products (Sen *et al.*, 2007; Lelli *et al.*, 2014). At the same time, the activity nature of the transaminases in liver and blood, as well as the indexes of the lipid metabolism suggest the presence of residual amounts and metabolites of biologically active agents of the feed supplement in the lard. When ingested, the agents cause the intensification of the detoxicative function of the liver and can cause steatosis (Yagmur *et al.*, 2010).

Conclusion: The application of the meat of the pigs grown with antimicrobials in the diets of rats leads to the increased functional activity of the liver due to the activation of the protein metabolism while retaining the lipid, pigment, and carbohydrate metabolism within the normal physiological response. The application of the lard of the pigs grown with antimicrobials in the diets of rats increases intensity of the detoxicative function of the liver, as evidenced by the indicators of the transaminase activity, of the lipid metabolism, and the results of the morphological estimation of the liver tissue. The established fact of the negative impact of the lard AB on the functional activity of the liver allows to consider the application of the lard to be unacceptable for nutrition of high risk population (children, pregnant and lactating women, senior citizens).

Acknowledgement: The work has been performed under the grant of the President of the Russian Federation for support of leading scientific schools SS-2602.2014.4 "New approaches to quality assurance and environmental safety of food products on the basis of the management of living systems throughout the biotech chain".

Author's contribution: All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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