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PLASMA METABOLITES IN FATTENING PIGS

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Abstract

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The experiments are carried out with castrated male pigs fed individually from 20 kg to 100 kg live weight. The animals from the control group (12 pigs) are fed with standard soybean-corn feedstuff and these from the experimental group (12 pigs) – with soybean-barley mixture. The pigs are fed with rations equal in content of protein and energy. The objective of the investigation is to establish the level of the main plasma metabolites, participating in the cholesterol transport and the possibility to affect them in fattening pigs by the effect of barley included in the feed content. The blood plasma is analyzed for content of total protein, cholesterol, phospholipids, triacylglycerols and lipoprotein classes: chylomicrons, low density lipoproteins, very low density lipoproteins and high density lipoproteins.

The obtained concentrations of the main plasma metabolites in the control and experimental group according to the biological norms and show a presence of a full value synthesis and secretion of liver proteins, phospholipids, cholesterol, triacylglycerols and lipoproteins. The main lipoprotein classes in the blood plasma of both animal groups are in the physiological optimum range and don't show differences obligated to the feeding factor. The ratio high density lipoproteins/ low density lipoproteins for the two groups increases from 0.81 for 20 kg up to 1.04 and 1.18 for 50 kg and reaches values from 1.45 and 1.57 for 100 kg animals, for the control and experimental group respectively.

Key words: proteins, lipids, cylomicrons, low density lipoproteins, very low density lipoproteins, high density lipoproteins

Abbreviations: HMG-CoA reductase - hydroxyl–methyl-glutaryl–CoA reductase, CM - cylomicrons, LDL - low density lipoproteins, VLDL - very low density lipoproteins, HDL - high density lipoproteins, TG - triacylglycerols, LCAT - lecithin-cholesterol-acyltransferase

Introduction

The fibers in the feedstuff, along with the lipids and proteins, are an important factor influencing the cholesterol metabolism in the organism. The results from a considerable number of studies give the idea that the adding of fibres to the diets of experimental and productive animals lead to a decrease of its plasma and tissue concentrations. The effects of the fibres on the cholesterol in the organism of animals and man, however, are not unidirectional. It is assumed that the main fractions of plant fibres, rich of water soluble polysaccharides have an expressed hypocholesterolemic effect (Chapman**,** 2009). On the contrary, the intakes of insoluble fibers - cellulose, lignin, wheat bran, don't affect the cholesterol levels in the main plasma lipoproteins. The introduction in the feedstuff of water soluble fibers leads to a reduction of the plasma concentrations of the LDL-cholesterol for the normal and hypercholesterolemic individuals.

Barley, which is rich of soluble fibres (including considerable quantities of β-glucans) is with a proved hypocholesterolemic effect on chickens, experimental animals and people (Chapman et al., 2007; Babin and Gibbons, 2009). Most of the barley varieties are rich in fibers as they contain as well another hypocholesterolemic component - fat-soluble tocopherols which have an inhibiting effect on the cholesterol synthesis. Chickens fed with barley, rye and oat and pigs – with barley rations (Chapman and Goldstein, 2006; Chapman et al., 2007) demonstrate a decreased activity of the hepatic HMG-CoA reductase from 34 to 49% compared to control ones, fed with corn mixtures. The total plasma and LDL-cholesterol are with decreased concentrations in the experimental groups. This may be due to a presence of

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soluble fibers and α-tocopherols in the experimental diets. Investigators relate it to the higher decomposition speed of the hepatic cholesterol to bile acids, their increased excretion and from there a lower concentration in the enterohepatic circulation.

The high sick rate of cardio vascular diseases and their proved relation with some plasma metabolites as total cholesterol, its content in main lipoprotein fractions, is a challenge for carrying out of thorough scientific investigations. The present study is related to the follow up of plasma metabolites, necessary for the synthesis of main lipoprotein fractions and the possibilities for changes of their correlations under the effect of age and the feeding factor in the case of fattening pigs.

Materials and Methods

The experiment is carried out with male castrated pigs of the double-line hybrid fed and bred in individual boxes from 20 to 100 kg live weight. The blood samples are taken from the eye vein sinus, 14 hours after the last feeding at 20, 50 and 100 kg live weight of the pigs. The blood is immediately centrifuged at 700 x g for 30min at 4^0C, and as an anticoagulant was used a12% EDTA solution. A blood plasma aliquot part is analyzed for content of main metabolites: total protein (Lowry et al., 1951), cholesterol (Sperry and Webb, 1950), phospholipids (Touchstone et al., 1980) and lipoprotein fractions. Lipoproteins are separated by sequential ultracentrifugation (Hatch and Lee, 1988) by using a Beckman ultracentrifuge. Plasma chylomicra are removed by rapid flotation into saline and VLDL, LDL and HDL are centrifuged for 1.09 x 10^8, 1.37 x 10^8, and 2.74 x 10^8 g-minutes, respectively. For each fraction, lipoproteins are aspirated from the top 1.5 cm of the tube. The statistical analysis of the obtained results is carried out by Snedecor and Cochran (1971).

Results and Discussion

The animals of the control group are fed with standard mixture according by Bulgarian State Standard and the experimental group – with barley as a main grain component in the diet. The nutrient composition of the diets is showed in Table 1.

The pigs are fed with ratio equal in content of protein and energy – 11.80 – 11.85 at 20kg – 50 kg live weight and 9.93-9.98 at 50-100 kg live weight, respectively. The results from the analysis of the plasma protein (Table 2) show concentrations in all the three weight periods in the range of the physiological optimum from 8.71 to 9.61 g/100ml. This is an evidence for an absence of protein deficit in the feedstuff and

Table 1
Nutrient composition of the diets

Ingradients, %	20 - 50 kg		50 - 100 kg	
	control group	experimental group	control group	experimental group
Corn	73.53	-	79.51	-
Barley	-	84.51	-	91.36
Soybean isolate	21.21	10.32	15.49	3.61
Alfalfa meal	22.01	2.01	2.01	2.01
Vitamin premix*	0.62	.0.62	0.51	0.51
Mineral premix**	0.15	0.15	0.15	0.15
Salt	0.35	0.25	0.41	0.41
L-lysine, 98%	0.07	0.21	-	0.14
Components:				
Protein, %	15.51	15.54	13.57	13.55
Fat,%	2.97	1.71	2.62	2.38
Fibers,%	3.47	5.22	3.28	5.14
Protein/Energy	11.81	11.85	9.93	9.98

* Vitamin premix: 725 000UI Vit A; 720 000UI Vit D_3; 3.25g Vit E; 0.1g thiamin; 0.5g riboflavin; 3.5g cyancobalamin; 1.5g dicalcium pantothenate; 2.25g Vit B12; 35g cholin chloride.

** Mineral premix: 30% $FeSO_4$.$7H_2O$; 8% $MnSO_4$.$5H_2O$; 2% $CuSO_4$.$5H_2O$; 20% $ZnSO_4$.$7H_2O$; 0.04% KIO_3; 39.76% $CaSO_4$.$2H_2O$

presence of full value synthesis and secretion of hepar proteins in blood.

A reliable higher content of proteins ($p \leq 0.05$) for 100 kg animals compared to 20 kg animals, the control and experimental group has been established. The quantity of the plasma total phospholipids doesn't show significant changes with the age and live weight of the pigs. The correlation of their quantities with those of TG and cholesterol give a notion of the forming and secretion of lipoproteins with spectrum specific to this hybrid. The cholesterol content from 92 to 106 mg/100 ml, similar to that of triacylglycerols from 34 to 40 nM/100 ml for both groups is comparatively low which is probably due to a hybrid feature and it can be also a result of the presence of considerable quantities soybean protein in the diet. A cholesterol lowering effect of the barley plant fibers, proved in investigations with chickens (Babin et al., 2007), has not been established for the experimental animals.

The chylomicrons (Figure 1), synthesized in the intestinal wall and participating in the tissue lipid (Mansbach and Siddi, 2010) exchange by the lymph and the blood flow are with low concentrations in the plasma of the control and experimental animals.

Reliable differences related to age as well as a result of varied feeding rations have not been established. This is probably connected to the fact that the pigs are fed with mixtures with relatively low lipid content. The limited quantities of available lipids for chylomicron synthesis in the intestinal mucosa lead to a relatively low level of chylomicrons forming. The lipoproteins with very low density, participating together with the chylomicrons in the transport of fatty acids as TG to the peripheral tissues, are in the limits of the norm and correspond to the data obtained by Hevonoja et al. (2000), Jonas and Phillips (2008), and Weber (2009) from experiments with pigs fed with standard corn-soybean rations. The observed changes in the VLDL plasma levels with the pig age and live weight growing are insignificantly. The absence of significant differences in the correlation between the animals of the three weight classes show that the established levels of the ration carbohydrates and proteins create conditions both for the normal flow of the lipids and apoprotein CII and E synthesis in the liver and for the VLDL secretion in the pigs blood plasma during the whole fattening period. Probably of importance for the not high TG and VLDL concentrations is the presence of considerable starch quantities in the feeding rations of the control and experimental groups (Jenkins et al., 2003; Fielding and Fielding (2008)).

The substitution of corn as main grain components for the control group rations with barley for the experimental group doesn't lead to changes in plasma VLDL concentrations. These results correspond to the established changed levels of the plasma TG and VLDL-triacylglycerols for the experimental animals fed with low-lipid, low-cholesterol diets with added fibres to the feedstuff (Chapman and Goldstein, 2006). The adding of wheat bran to high-lipid, cholesterol-containing rations lead to a considerable decrease of the VLDL-tryaglycerols plasma concentrations. The main plasma choles-

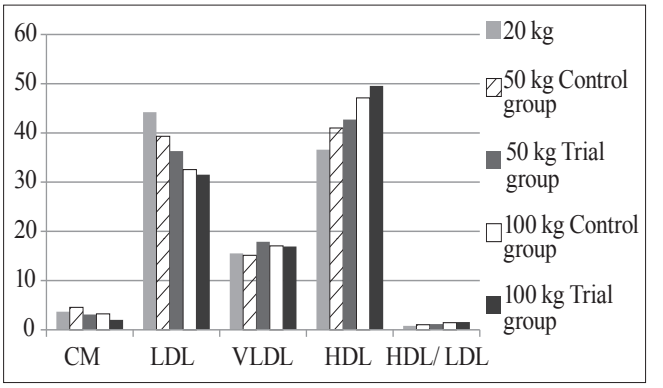

Fig. 1. Main plasma lipoprotein classes, %

Table 2
Plasma metabolites

Plasma metabolites	20 kg	50 kg		100 kg	
		Control group	Experimentall group	Control group	Experimental group
Total protein, g/100 ml	8.71± 0.11	9.47 ± 0.13	9.18 ± 0.21	9.61 ± 0.16[c]	9.15 ± 0.10[d]
Total phospholipids, mg/100 ml	69 ± 7	89 ± 7	73 ± 5	80 ± 3	98 ± 5[k]
Cholesterol, mg/100 ml	96 ± 3	95 ± 2	92 ± 7	105 ± 7	106 ± 2[j]
Triacylglicerols, nM/100ml	40 ± 8	39 ± 7	34 ± 5	34 ± 3	39 ± 3

*$p \leq 0{,}05$; a-20/50 kg control; b-20/50 kg trial; c-20/100 kg control;
d-20/100 kg trial; e- 50 control/50 kg trial;
f- 50 control/100 kg control; j– 50 trial/100 kg trial

terol carrier for pigs are LDL (Hevonoja et al., 2000; Weber et al., 2009), whose major role is to transfer the cholesterol to the tissues for membrane synthesis. Their levels are considerably higher in the plasma of the fattened animals compared to those of chylomicrons and VLDL. The decreased LDL content proved for 100 kg control and experimental groups compared to 20 kg animals is related to age characteristics of the pigs and is probably a hybrid feature. It is known (Brown, 2007) that in a number of cases with the growing of the animals age and live weight an increase of the total plasma and LDL-cholesterol is observed. For the animals of the bilinear hybrid the total cholesterol content doesn't change considerably and the LDL plasma concentration is decreased. Probably of importance here is the participation of soybean protein in the diets of both animal groups. The effect of the soybean protein is may be due to an increased ileal and faecal cholesterol secretion or it is possible that the plant proteins may lead to a greater transfer of cholesterol from the plasma to the tissues (Fielding and Fielding, 2008).This is connected with changed in the tissue receptor mechanism. However, in other experiments (Chapman, 2009) it is pointed that the nature of the protein source from the ration doesn't affect the plasma lipoprotein profile. The changes for the circulating lipids, caused by varied experimental rations, depend on the sensitivity of the separate animal species and it is not rare when in one and the same species considerable variations are observed.

The HDL fraction is with a high relative share for both animal groups. With the advance of their growth and fattening, the level of their plasma concentration increases, as they are proved highest for the 100 kg control and experimental group, compared to the 20 kg. The high HDL levels, secreted by the hepatocytes in the blood plasma, are connected with their participation in the transfer (reverse transport) of the cholesterol from the membrane structures to the liver for further decomposition (Jenkins et al., 2003; Jonas and Phillips, 2008). The absence of proved differences in the content of the main plasma lipids – TG, cholesterol, phospholipids for these animals is also corresponding to the obtained results. The considerable quantities soybean protein in both fed rations probably "masc" the cholesterol-lowering effect of the fibres in the mixtures for the experimental groups. It is considered that the main fractions plant fibres from the feedstuff, rich of water soluble polysaccharides cause a decrease of the total plasma and LDL cholesterol levels and increase of those of HDL cholesterol. The ratio HDL/LDL is with a tendency toward increase for the control and experimental animals with increased of live weight.

The results from the investigations of the plasma lipoprotein spectra give an idea of a normal course of their synthesis and secretion in the blood plasma for the animals fed with 100% corn as well as with 100% barley in the mixtures. Under the conditions of the carried out experiments the increasing of the ratio HDL/LDL with the age an live weight of the pigs from the experimental group favors the achievement of a muscle and fat tissue with good taste and dietetic qualities.

Conclusion

The obtained results for content of main plasma metabolites: total protein, phospholipids, cholesterol and triacylglycerols for the investigated animals from the control and the experimental group are according to the physiological norms.

The experiment shows that the applied feeding provides for the introduction in the organism of the necessary substrates for normal forming and secretion of the plasma lipoproteins.

The established concentrations for the plasma chylomicrons, VLDL, LDL and HDL in control and experimental animals don't show differences resulting from the complete substitution of corn with barley in the feeding mixtures.

A reliable decrease of the LDL plasma concentrations ($p \leq 0.01$) in 100 kg control and experimental group compared to 20 kg animals has been established, while the VLDL level remains relatively constant.

The HDL values increases considerably with the age and live weight ($p \leq 0.01$) for the animals from the experimental and control group. The ratio HDL/LDL, which is of importance for the atherogenic process, increases with the age and live weight. This creates conditions for obtaining of animal production with favorable qualities in terms of dietetics.

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