BIOCHEMICAL PARAMETERS OF BLOOD PLASMA AND FEED CONVERSION RATE DEPENDING ON THE DIET IN THE MODEL ORGANISM

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Abstract: The aim of the present study was to determine the effect of diet enriched with 2.5% fish oil (polyunsaturated fatty acids source) resp. 2.5% palm oil (saturated fatty acids source) during the feeding experiment to the overall health status of the model organism (*Sus scrofa f. domestica*). Biochemical indicators of blood (alanineaminotransferase, aspartateaminotransferase, alkaline phosphatase, urea, total cholesterol, HDL-fraction and LDL-fraction) and feed conversion rate were analyzed to determine the overall health status of the experimental animal. Diet enriched with 2.5% fish oil significantly decreased ($P<0.05$) aspartateaminotransferase and alkaline phosphatase, but there was no change ($P>0.05$) in alanineaminotransferase. Diet enriched with 2.5% palm oil significantly decreased ($P<0.05$) aspartateaminotransferase, alkaline phosphatase and also alanineaminotransferase. Both diets decreased ($P<0.05$) level of total cholesterol, although we expected a reduction only in the diet enriched with fish oil and increasing in the diet enriched with palm oil. The level of HDL-fraction was increased ($P<0.05$) in the diet enriched with palm oil, but not in the diet enriched with fish oil ($P>0.05$). The level of LDL-fraction was decreased ($P<0.05$) in both diets, which was expected in the diet enriched with fish oil, but not with palm oil. The level of urea was decreased ($P<0.05$) in both diets. The effect of diet enriched with 2.5% fish resp. palm oil to the feed consumption, body weight gains and feed conversion rate was tested - there were not significant differences ($P>0.05$) between two experimental diets. These are only preliminary results obtained during the experiment, which are so far unclear and ambiguous, therefore further research is needed in this area.

Key Words: polyunsaturated fatty acids, feed conversion rate, HDL-cholesterol, LDL-cholesterol, alaninaminotransferase, aspartateamino transferase, alkaline phosphatase, urea, fish oil, palm oil

INTRODUCTION

Study of the positive impact of functional foods on the human body is in the interest of scientists from around the world. Fish oil due to its high content of polyunsaturated fatty acids n-3 (PUFA), especially eicosapentaenoic and docosahexaenoic acid, can act just as a functional food. PUFAs are important components of cell membranes, are involved in the regulation of many functions in the body - for example regulation of blood pressure, proper development of the central and peripheral nervous system, inflammatory response of the organism and cholesterol homeostasis.

Therefore, the aim of this study was to compare the effects of diet enriched with 2.5% fish oil and diet enriched with 2.5% palm oil to the overall health status in the model organism. In the experimental group fed with diet enriched with 2.5% fish oil we expected overall improvement in biochemical markers, especially reducing total cholesterol, increasing HDL and reducing LDL-fraction. On the other hand, palm oil, high in saturated fatty acids was used as a negative control in

this experiment. In this group we do not expected overall improvement in biochemical markers, especially we expected increased total cholesterol, reduced HDL-fraction and increased LDL.

To determine the overall health status of the model organism, biochemical indicators of blood (alanineaminotransferase (ALT), aspartateaminotransferase (AST), alkaline phosphatase (ALP), HDL-cholesterol and LDL-cholesterol) and feed conversion rate were analyzed.

Biochemical values in animals are important to assess the clinical condition of the animal. In this study we were focused on three enzymes (ALT, AST and ALP), which are biomarkers for liver health (Ghouri et al. 2010, Hirotsu et al. 2005). Also level of HDL-cholesterol and LDL-cholesterol were measured, to determine whether also cholesterol metabolism is affected.

Urea is the final degradation product of proteins (specifically nitrogen from the amino acids) in the body. Urea is excreted from the body via the kidney and determination of the concentration of urea is mainly used to assess kidney function (Kato 2015).

Feed conversion rate is an important indicator of fattening pigs and shows us how many kilograms of feed animal need to consume to gain 1 kg bodyweight. Conversion of nutrients was not the main point of interest in our study, but it served us as a fast and approximate indicator of the overall health status of the organism.

MATERIAL AND METHODS

The experiment was carried out on 20 piglets (Bioprodukt Knapovec a.s., Czech Republic), both male and female, with the initial mean live body weight of 25.98±3.67 kg divided to two experimental groups (n=10) with different composition of diet. Experimental groups were fed with standard feed mixture for pigs with addition of 2.5% fish oil (comercial oleum jecoris asseli, Czech Republic) and 2.5% palm oil (VOG s.r.o., Strančice, Czech Republic), respectivelly. The animals were earmarked by tattooing and housed in pens with 5 pigs to each, under good hygienic conditions of accredited animal facilities in the Veterinary Research Institute (Brno, Czech Republic). Before the beginning of the experiment, the animals were dewormed (Ivomec, inj., Agvet, USA) and allocated into two groups based on individual live body weight and sex. During the course of the experiment (29 days) the pigs were fed partly *ad libitum* twice a day at 7.00 and 16.00 h, drinking water was available *ad libitum*. Thirty minutes after the beginning of feeding, the refusals were removed, weighed and taken into account in the calculations of feed consumption. Live body weight of pigs was taken at day 1, 15, 29 and 43 (each time 2 h post feeding). Individual and group body weight gains (BWG) were calculated. Feed conversion rate was calculated as the ratio of feed consumption (kg) and BWG (kg) of respective groups. At the day 1 and 29 of the trial, blood samples were drawn from *v. cava cranialis* for biochemical analysis 3 h post feeding. Blood was collected into tubes with Heparinum natricum (25 IU/ml of blood; Zentiva, Praha, Czech Republic) to prevent blood clotting and then centrifuged at 1000 g for 15 minutes. Blood plasma was used for biochemical analysis. Biochemical analysis was performed on automatic Chemistry analyser BS-200 (Mindray, China) according to the manufacturer's protocol. All data were statistically analyzed using Statistica and MS Excel (2010). For statistical evaluation t-test for paired samples was used.

RESULTS AND DISCUSSION

Biochemical analysis

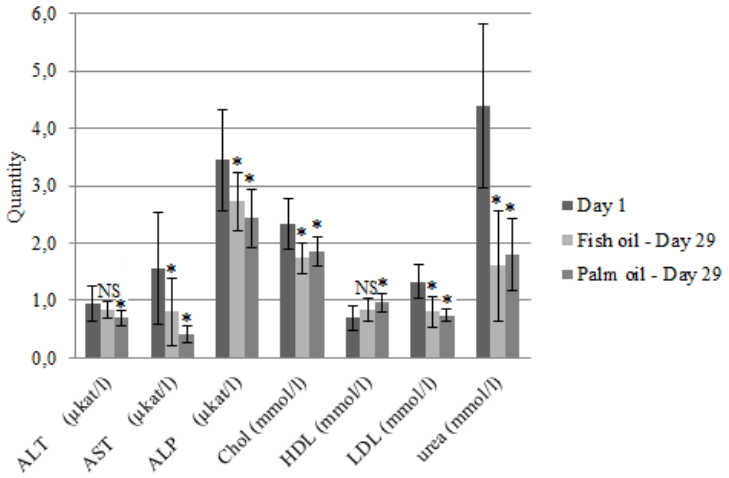
The reference values for ALT, AST, resp. ALP are 0.5-1.0 µkat/l, 0.5-1.5 µkat/l, resp. 2.0-6.6 µkat/l. The maximum reference value for urea is 7.992 mmol/l (Doubek 2003). Reference values for total cholesterol, HDL- and LDL-fractions are unknown. All other biochemical values, we have measured are within the reference range, that gave evidence of the animals being in a good state of health.

The initial levels of biochemical markers and levels at day 29 of the feeding experiment are shown in the Figure 1 A). Diet enriched with 2.5% fish oil significantly decreased ($P<0.05$) AST and ALP enzymes, but there were no change ($P>0.05$) in ALT enzyme. Diet enriched with 2.5% palm oil significantly decreased ($P<0.05$) AST, ALP and also ALT enzymes. Both diets decreased ($P<0.05$) level of total cholesterol, although we expected a reduction only in the diet enriched with fish oil and increasing in the diet enriched with palm oil. The level of HDL-fraction was increased ($P<0.05$) in the

diet enriched with palm oil, but not in the diet enriched with fish oil (P>0.05). This result is exactly the opposite than we expected, and yet we are not able to explain why this occurred. The level of LDL-fraction was decreased (P<0.05) in both diets, which was expected in the diet enriched with fish oil, but not with palm oil. The level of urea was decreased (P<0.05) in both diets. Increased level of urea at the beginning of the experiment can be explained with a heavy load of stress on experimental animals during transport and movement at the beginning of the experiment.

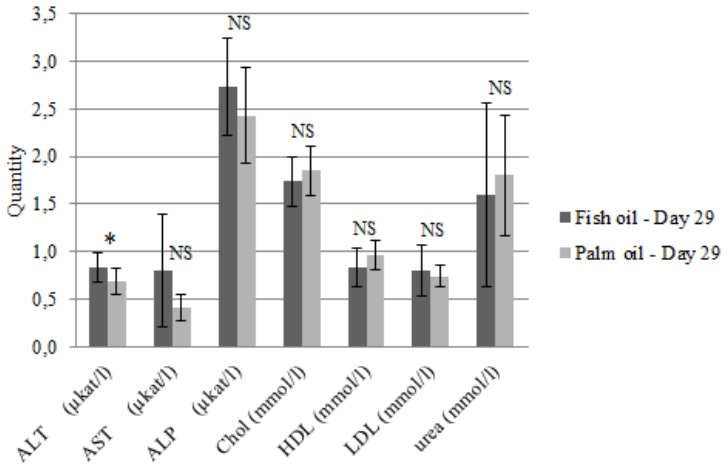
Figure 1 The effect of diet enriched with 2.5% fish resp. palm oil to the biochemical markers

A) Comparison of the initial levels of biochemical markers and levels at day 29



*Legend: NS – non significant difference, * - significant difference; t-test*

B) Comparison of the effect of diet enriched with 2.5% fish resp. palm oil to the biochemical markers at day 29



*Legend: NS – non significant difference, * - significant difference; t-test*

The comparison of diet enriched with 2.5% fish and palm oil, respectively at day 29 of the feeding experiment is shown in the Figure 1 B). There is significant difference between diet enriched with 2.5% fish resp. palm oil at day 29 of feeding experiment for the enzyme alanine aminotransaminase (ALT). Diet enriched with 2.5% palm oil decreased (P<0.05) level of ALT at day 29 of the feeding experiment. ALT is a transaminase, liver enzyme that is an indicator of good state of health of the liver. Elevated levels of this enzyme indicate increased burden of liver or liver disease.

There were no significant differences (P>0.05) between diet enriched with 2.5% fish and palm oil, respectively for other biochemical markers.

Our results are comparable with the results of the study Merritt et al., 2003, which dealt with the safety evaluation of sources of docosahexaenoic acid and arachidonic acid for use in infant formulas in newborn piglets. They found, that administration of ARA, DHA or ARA+DHA to neonatal piglets, under the conditions of this study, did not result in adverse health effects at the highest doses tested.

Tyburczy et al. (2012) studied growth, clinical chemistry and immune function in domestic piglets fed varying ratios of arachidonic acid and DHA and also had similar result as we had. They found, that milk replacer formulas supplemented with physiologically high levels of ARA and DHA supported normal growth, development and immune function in rapidly growing domestic piglets up to 28 day of age and there were no adverse effects in any of the clinical chemistry, haematology or immune function parameters that were measured.

On the other hand, Langerhuus et al. (2012) has shown that, preoperative treatment with diet rich in EPA and DHA (enriched with fish oil) significantly improved clinical outcome in pigs with aortic vascular prosthetic graft infection (*Staphylococcus aureus*) by improving feed intake and body-weight gain post-operatively.

Feed consumption, body weight gains and feed conversion rate

The effect of diet enriched with 2.5% fish resp. palm oil to the feed consumption, body weight gains and feed conversion rate we can see in the Table 2. There is not significant difference (P>0.05) between two experimental diets.

Table 2 The effect of diet enriched with 2.5% fish resp. palm oil to the feed consumption, body weight gains and feed conversion rate

	Diet enriched with Fish oil	Diet enriched with Palm oil
Feed consumption Day 1-15 (kg)	132.8	130.6
Feed consumption Day 16-29 (kg)	197.6	205.8
Feed consumption Day 30-43 (kg)	243.2	247,1
Average (kg)	**573.6**	**583.5**
Body weight gains Day 1-15 (kg)	102.5	99
Body weight gains Day 16-29 (kg)	62.0	60.5
Body weight gains Day 30-43 (kg)	108.0	118.5
Average (kg)	**272.5**	**288**
Feed conversion rate Day 1-15 (kg/kg)	1.3	1.3
Feed conversion rate Day 16-29 (kg/kg)	3.2	3.4
Feed conversion rate Day 30-43 (kg/kg)	2.3	2.1
Average (kg/kg)	**2.1**	**2.0**

CONCLUSION

The aim of the present study was to determine the influence of diet enriched with polyunsaturated resp. saturated fatty acids to the overall health status of the model organism (*Sus scrofa f. domestica*) Biochemical indicators of blood (alanineaminotransferase (ALT), aspartateaminotransferase (AST), alkaline phosphatase (ALP), urea, total cholesterol, HDL-fraction and LDL-fraction) and conversion of nutrients were analysed to determine the overall health status of the experimental animals.

Diet enriched with 2.5% fish oil decreased AST and ALP enzymes, but there were no change in ALT enzyme. Diet enriched with 2.5% palm oil decreased AST, ALP and also ALT enzymes. Both diets decreased level of total cholesterol, although we expected a reduction only in the diet enriched with fish oil and increasing in the diet enriched with palm oil. The level of HDL-fraction was increased in the diet enriched with palm oil, but not in the diet enriched with fish oil. The level of LDL-fraction and urea was decreased in both diets. The effect of diet enriched with 2.5% fish resp. palm oil to the feed consumption, body weight gains and feed conversion rate was tested - there were not significant differences between two experimental diets. All values, we have measured are within the reference range, that gave evidence of the animals being in a good state of health and there is no effect of diet.

Our results are so far unclear and ambiguous, therefore further research is needed in this area. These are only preliminary results obtained during the experiment. Now the experiment continues and immediately before termination of the experiment, acute inflammation reversal by injection of lipopolysaccharide (LPS) will be caused in the organism. Then further samples of blood, liver, adipose and muscle tissue will be taken for subsequent biochemical, hematological, immunological, and genetic analysis.

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