EFFECT OF FISH OIL IN THE DIET OF THE MODEL ORGANISM ON HEMATOLOGICAL PARAMETERS AND CHEMILUMINISCENCE OF LEUKOCYTES

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Abstract: The purpose of the present study was to assess the effect of diet enriched with 2.5% fish oil (polyunsaturated fatty acids source) and the effect of diet enriched with 2.5% palm oil (saturated fatty acids source) to the overall health status of the model organism (*Sus scrofa f. domestica*). To determine the overall health status of the model organism, following hematological indicators of blood were analyzed: number of white blood cells, number of red blood cells, level of hemoglobin and hematocrit. There were non-significant differences in the investigated parameters of white and red blood cells, hemoglobine and hematocrit between groups of animals fed respective diets at day 29. No clinical signs of disease were observed during the entire experiment and hematological analysis gave results within the reference range, that gave evidence of the animals being in a good state of health. The level of oxidative stress of organism was measured via chemiluminiscence of leukocytes. There was no difference between fish oil diet and control group in the level of integral intensity of spontaneous CL as well as after stimulation by Zymozan. But the level of integral intensity of activated CL by PMA was increased by diet enriched with 2.5% fish oil compared with control group fed with diet enriched with 2.5% palm oil. Fish oil probably created oxidative stress in organism and antioxidants (in our case tocoferol) presented in feed were not able to avoid oxidative reaction of double bounds in the molecules of fish oil.

Key Words: polyunsaturated fatty acids, fish oil, palm oil, white blood cells, red blood cells, hemoglobin, hematocrit, chemiluminiscence

INTRODUCTION

The positive impact of nutriceutics (components of functional food) on the human body is at the present time in the worldwide interest of scientists and nutritionists. Fish oil due to its high content of PUFA n-3 (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. In the experimental group 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 increasing total cholesterol, reducing HDL and increasing LDL-fraction.

In this study we have focused on these hematological parameters: number of red blood cells (RBC), number of white blood cells (WBC), hemoglobin and hematocrit.

Red blood cells (erythrocytes) serve as a carrier of hemoglobin. It is this hemoglobin that reacts with oxygen carried in the blood to form oxyhemoglobin during respiration (NseAbasi et al. 2014). According to Isaac et al. (2013) red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Isaac et al. 2013).

The major functions of the white blood cell are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions (NseAbasi et al. 2014).

Hematocrit (HCT) which is also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the percentage (%) of red blood cells in blood (Purves et al. 2003). Hematocrit is involved in the transport of oxygen and absorbed nutrients. Increased hematocrit shows a better transportation and thus results in an increased primary and secondary polycythemia (Isaac et al. 2013).

Hemoglobin (HGB) is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates with the exception of the fish family, channichthyldae as well as tissues of invertebrates. Hemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (NseAbasi et al. 2014).

The aim of the present study was to compare the effect of diet enriched with 2.5% fish oil (polyunsaturated fatty acids source) and diet enriched with 2.5% palm oil (saturated fatty acids source) to the overall health status of the model organism (*Sus scrofa f. domestica*). To determine the overall health status of the model organism, following hematological indicators of blood were analyzed: number of white blood cells (WBC), number of red blood cells (RBC), level of hemoglobin (HGB) and hematocrit (HCT). In the second part of this study we have focused on chemiluminiscence of leukocytes.

Hematological analysis is very important, quick, easy and cheap method for screening of the physiological, nutritional and pathological status of the experimental animals. The examination of blood provides the opportunity to clinically investigate the presence of metabolites and other constituents in the body of animals. Blood constituents change in relation to the physiological status of an animal. These changes are important in assessing the response of farm animals to various physiological situations (NseAbasi et al. 2014)

MATERIAL AND METHODS

Experimental animals and feeding

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. First experimental group (F) was fed with standard feed mixture for pigs with addition of 2.5% fish oil (comercial oleum jecoris asseli, Fargon s r.o., Czech Republic), second experimental group (P) was fed with standard feed mixture for pigs with addition of 2.5% palm oil (VOG s.r.o., Strančice, Czech Republic). 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. Average ambient temperature and relative humidity were 19±3°C and 55±10%, respectively. 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 (each time 2 h post feeding). Individual and group body weight gains (BWG) were calculated. Feed conversion rate (FCR) was calculated from feed consumption and BWG of respective groups. The health status of animals was monitored

daily by observation at regular intervals. Occasional morbidity and mortality were recorded. At the day 1 and 29 of the trial, blood samples were drawn from v. cava cranialis for hematological analysis 3 h post feeding. Blood was collected into tubes with Heparinum natricum (25IU.ml^{-1} of blood; Zentiva, Praha, Czech Republic) to prevent blood clotting.

Hematological analysis

Hematological analysis was performed on automatic hematological analysator MINDRAY BC-2800 Vet (Mindray, China) according to the manufacturer's instructions. The following parameters were monitored: red and white blood cell count, hematocrit and hemoglobin.

Chemiluminiscence assay

First, leukocytes were isolated from the collected blood by use of the hypotonic lysis method. Whole blood was mixed with H_2O (USP Wfi, Lonza) (ratio 1:12). After lysis for 30 s the tonicity was increased by 10× DPBS (Dulbecco's phosphate-buffered saline; Lonza). Cells were washing two times in HBSS (Hanks' balanced salt solution; Lonza) and counted on analysator MINDRAY BC-2800 Vet (Mindray, China) according to the manufacturer's instructions.

Chemiluminescence (CL) assay was used for detection of respiratory burst of isolated leukocytes. Leukocytes were seeded in HBSS at a concentration 10^6 cells per well. To amplify the CL was added luminol-derivative L-012 (Wako Chemicals GmbH) which was diluted in HBSS to the final concentration 10nmol.L^{-1}.

Two types of measuring were performed: spontaneous and activated. For activation of leukocytes was used zymosan at the final concentration 0.05mg.mL^{-1} (Sigma-Aldrich) or phorbol myristate acetate (PMA) at the final concentration 0.5µg.mL^{-1} (Sigma-Aldrich). Chemiluminescence was measured at 37°C using a multidetection microplate reader Synergy H1 (BioTek) in kinetic mode for 2 h. The results are expressed as integrals of chemiluminescence intensity.

All data were statistically analyzed using Statistica and MS Excel (2010). For statistical evaluation t-test for paired samples was used.

RESULTS AND DISCUSSION

Hematological analysis

The effect of the diets enriched with 2.5% fish oil (polyunsaturated fatty acids source) resp. enriched with 2.5% palm oil (saturated fatty acids source) on hematological parameters of experimental aminals is presented in Table 1. In the Table 1 we can also compare measured values to reference values.

Table 1 The effect of different types of diet on hematological parameters

	Type of haematolog. parameters	Units	Feed with 2.5% Fish oil		Feed with 2.5% Palm oil		Reference values[#]
			Day 1	Day 29	Day 1	Day 29	
WBC	White blood cells	G.l^{-1}	19.12 ± 2.72	19.85 ± 3.77	19.12 ± 2.72	18.86 ± 5.68	11–22
RBC	Red blood cells	T.l^{-1}	7.18 ± 0.3	6.3 ± 0.83	7.18 ± 0.3	6.71 ± 0.4	5–8
HGB	Hemoglobine	g.l^{-1}	102.7 ± 5.04	109.8 ± 10.95	102.7 ± 5.04	112.3 ± 4.9	100–160
HCT	Hematocrit	%	38.58 ± 1.99	32.98 ± 4.48	38.58 ± 1.99	34.77 ± 2.1	32–50

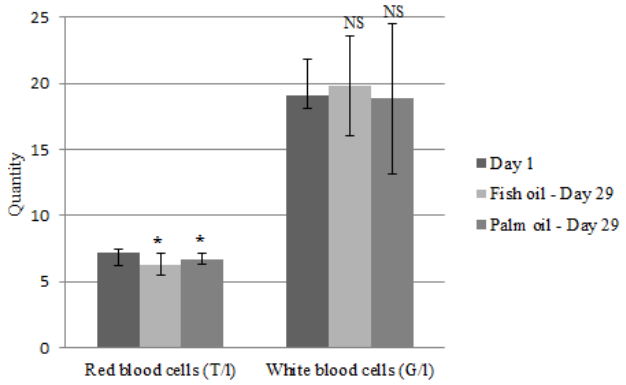
[#] *Doubek; 2003*

The effect of diet enriched with 2.5% fish and palm oil, respectively on the hematological parameters is presented in Figure 1. There is shown data from the first day of experiment and day 29. For feeding experiment is an experimental period of 29 days relatively short time to be fully reflected

the influence of diet. For this reason, these are only preliminary results obtained during the experiment.

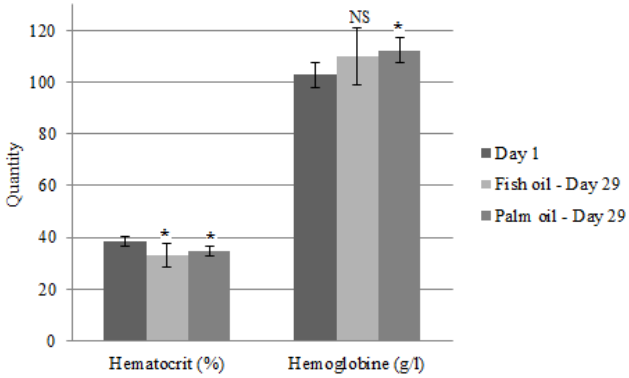
Figure 1 The effect of diet enriched with 2.5% fish and palm oil, respectively on the hematological parameters

A) Comparison of initial levels of red and white blood cells and levels at day 29



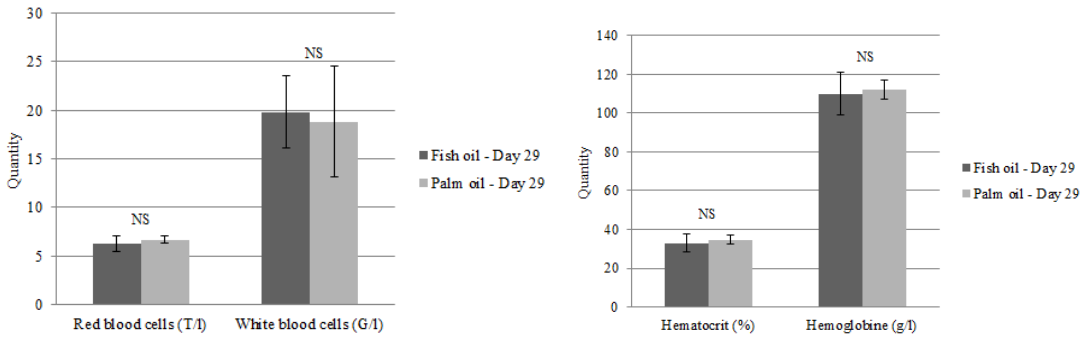
*Legend: NS – non significant difference, * - significant difference, t-test for paired samples*

B) Comparison of initial levels of hemoglobin and hematocrit and levels at day 29



*Legend: NS – non significant difference, * - significant difference, t-test for paired samples*

C) Comparison of diet enriched with 2.5% fish and palm oil, respectively at day 29



Legend: NS – non significant difference, t-test for paired samples

Diet enriched with 2.5% fish oil as well as palm oil decreased ($P<0.05$) level of red blood cells at day 29 compared with day 1. The level of white blood cells was not affected ($P>0.05$) by neither of the two diets (Figure 1A). The level of hematocrit was decreased ($P<0.05$) by diet enriched with 2.5% fish oil as well as palm oil at day 29 compared with day 1. Diet enriched with 2.5% fish oil caused non significant ($P>0.05$) difference of level of hemoglobin between day 1 and 29. But the level of

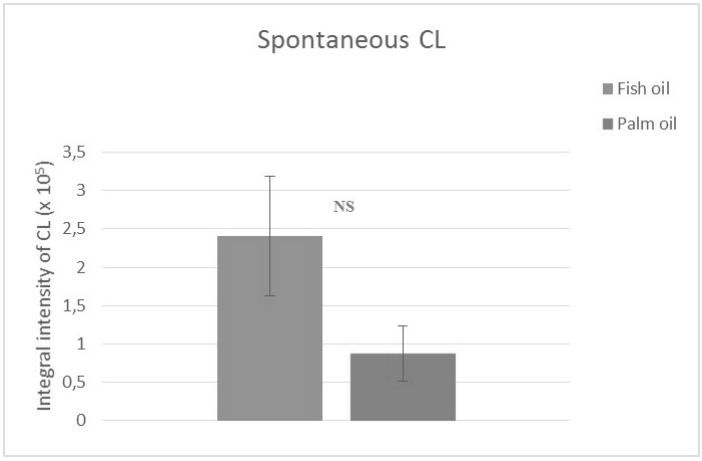
hemoglobin was increased (P<0.05) in day 29 compared with day 1 in the group fed with diet enriched with 2.5% palm oil (Figure 1B). Non-significant differences in the investigated parameters of white blood cells, red blood cells, hemoglobine and hematocrit between groups of animals fed respective diets is presented in the Figure 1C).

Chemiluminiscence of leukocytes

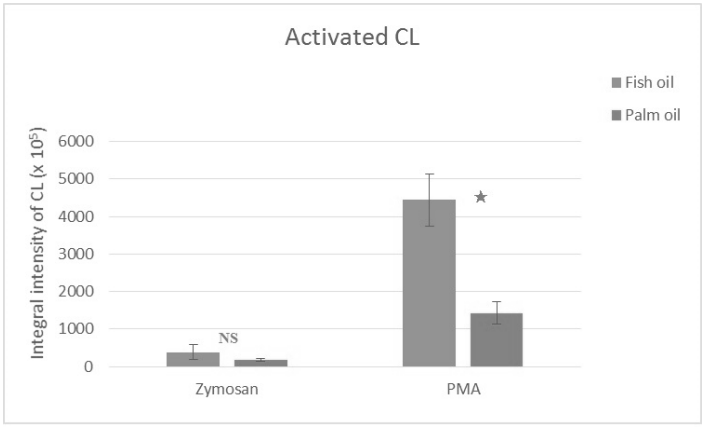
Reactive oxygen species (ROS) are critical components of the antimicrobial repertoire of phagocytic cells. ROS are also produced during normal metabolism and have many biological functions (enzymatic reaction, signal transduction etc.). However, high level of ROS is highly toxic to cells.

Figure 2 Respiratory burst. ROS produced by leukocytes were measured by chemiluminescence assay. Data are shown as mean ± SD of three pigs randomly selected from each group

A) Spontaneous chemiluminescence



B) Activated chemiluminescence



*Legend: NS – non significant difference, * - significant difference, t-test for paired samples*

The level of integral intensity of spontaneous CL as well as after stimulation by Zymozan was not affected (P>0.05) by neither of the two diets (Figure 2A). But the level of integral intensity of activated CL by PMA was increased (P<0.05) by diet enriched with 2.5% fish oil compared with control group fed with diet enriched with 2.5% palm oil (Figure 2B).

CONCLUSION

The aim of the present study was to determine the effect of diet enriched with 2.5% fish and palm oil, respectively to the overall health status of the model organism (*Sus scrofa f. domestica*). We focused on hematological parameters, which serve us as a markers of overall health status of the experimental organisms and chemiluminiscence of leukocytes which show us the level of oxidative stress of organism.

No clinical signs of disease were observed during the entire experiment and hematological analysis gave results within the reference range, that gave evidence of the animals being in a good state of health.

With the method of chemiluminiscence of leukocytes was shown that the level of integral intensity of spontaneous CL as well as after stimulation by Zymozan was not affected by neither of the two diets. But the level of integral intensity of activated CL by PMA was increased by diet enriched with 2.5% fish oil compared with control group fed with diet enriched with 2.5% palm oil. Fish oil probably created oxidative stress in organism and antioxidants (in our case tocoferol) presented in feed were not able to avoid oxidative reaction of double bounds in the molecules of fish oil.

On the other hand, we must take into account that the feeding experiment took place only 29 days and 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 lipopolysacharide (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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REFERENCES

Doubek J., Bouda J., Doubek M., Fürll M., Knotková Z., Pejřilová S., Pravda D., Scheer P., Svobodová Z., Vodička R. 2003. *Veterinary hematology*. 1st ed. Brno: Noviko. (*Veterinární hematologie*).

Isaac L. J., Abah G., Akpan B., & Ekaette I. U. 2013. Hematological properties of different breeds and sexes of rabbits. In: *Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria*. 2013, 24–27.

NseAbasi N. E., Williams M. E., Akpabio U., Edem E. A. 2014. Offiong Hematological Parameters and Factors Affecting Their Values. *Agricultural Science,* 2(1): 37–47.

Purves W. K., Sadava D., Orians G. H., Heller H. C. 2003. Life: The science of Biology. 7th ed. Sunderland, MA: Sinauer Associates and W. H. Freeman.