The dietary omega -3 PUFA alter the metabolic and the immunologic serum profile in Mangalitza pigs in extensive rearing system

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SUMMARY

The purpose of the study was to observe the influence of the dietary ecological oil on the metabolic and plasma immunological profile of the finishing Mangalitza pigs reared in an extensive system. The experiment was conducted on 12 pigs assigned to two groups: a control group fed on a conventional diet and an experimental group whose diet contained flax oil as source rich in omega 3 fatty acids. Blood samples were aseptically collected by jugular venipuncture from all animals. Gas chromatography was used to determine the detailed FAs composition. BS-130 Chemistry analyzer (Bio-Medical Electronics Co., LTD, China) was used to determine the biochemical parameters. The linolenic acid content of the flax oil (41.47%) increased 3.76 times the dietary level of this fatty acid in the compound feed for the experimental group compared to the control group. Very significant differences (P= 0.003) were observed for the tryglicerides (<2.33 in group E compared to group C). Except for the creatinin, whose values were significantly (P<0.001) lower in group E (1.36 mg/dL) compared to (1.69 mg/dL) in group C, the values for the protein and enzyme constituents increased in the flax oil group. An increase in plasma IgM and IgG concentration above the control level was observed after linseed diet ingestion, which was significant in the case of IgM (P=0.018). Our results are important because they can be used as reference for biochemical determinations in humans due to the similarities existing between the human and pig organism.

Keywords: flax oil, omega-3 PUFA, metabolic profile, immunoglobulin G and M, Mangalitza pigs

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INTRODUCTION

Nutrition and any feeding deficiency are associated to effects on the health state of the farm animals, which can increase the susceptibility of the animal organism to infectious diseases (Calder et al. 2002, Habeanu et al., 2010). The animals fed adequately are more resilient to infections because of the better integrity of the tissues, to the higher production of antibodies and to the stronger immune status (Shurson et al., 2007).

The omega 3 fatty acids were the focus of research during the recent period due to their beneficial effect to prevent infections and inflammations (Blok et al., 1996). Some authors (Nuernberg et al, 2005, Wood et al., 2004, Habeanu et al., 2008) revealed the possibility to increase the concentration of beneficial omega 3 fatty acids in animal tissues by using various types of oleaginous sources. Thus, the linseeds may inhibit the production of pro-inflammatory cytokines and of lipid mediators derived from the arachidonic acid (n-6 fatty acid). This effect of the linseeds is due to the presence of the α-linolenic fatty acid and of the lignans which influence by various mechanisms the inflammatory response, while the lignans also have an antioxidant effect (Flax Council of Canada, 2007). Simopoulos and Cleland, (2003) showed that the feeding recommendations can be changed towards a new direction: reduce the consumption of linoleic fatty acid (C18:2n-6), and increase the consumption of linolenic acid (C18:3n-3). A low ratio of these acids may have beneficial effects on the health (Nuernberg et al., 2005).

The biochemical parameters of plasma are important markers to evaluate the subclinical health as a valuable tool in modern preventive medicine (Dubreuil et al., 1997). Several biochemical variables are strongly influenced by the chronic disease and nutritional status and nutritional deficiencies (Friendship, 1984). At the same time, the metabolic profile of the blood may be correlated with the rearing technology (Parvu et al., 2003). Organic breeding of pigs is an alternative to this system. The demand for meat obtained from pigs reared in organic farms is increasingly high, especially in countries with higher living standards, whose citizens have incomes that allow them to choose quality (Bacila et al. 2010).

The knowledge on the interactions between feeding and health in Mangalitza pigs are limited. The effect of the dietary polyunsaturated fatty acids given to Mangalitza pigs of the tissular structure of the dietary lipids in relation with the other nutrients also is yet to be fully ascertained. This is a traditional, rustic, genetically consolidated pig breed which can readily adapt to the extensive rearing systems (the current trend worldwide is to gradually replace the intensive systems by extensive rearing systems, which are sustainable and environmentally friendly).

The purpose of this study was to observe the effects of the dietary ecological oil on the metabolic and plasma immunological profile of the finishing Mangalitza pigs reared in an extensive system.

MATERIAL AND METHODS

Animals, feeding and sampling

The experiment was conducted on 12 finishing Mangalitza pigs for 35 days. This breed in specialised in fat production. All animals were reared in extensive production system and maintained under same management and feeding programme.

The animals were assigned randomly to two groups: a control group (C) fed on a conventional diet (corn, barley, what and soybeans) for the extensive rearing systems, formulated to meet all nutritional requirements (NRC, 1988) and an experimental group (E) whose diet included flax oil (produced by cold pressing) as rich source of n-3 polyunsaturated fatty acids and a vitamin-mineral supplement. The control diet had 13% crude protein, 4.02% ether extractives, 4.76% fibre, ash 3.44%, while the experimental diet had 14% crude protein, 4.65% ether extractives, 6.56% gross fibre and 4.28% ash. The linolenic acid content of the flax oil (41.47%) increased 3.76 times the dietary level of this fatty acid in the compound feed for the experimental group compared to the control group. The fatty acids composition of the finishing diets (control and flax oil groups) and of the flax oil are shown in Table 1a and 1b.

Table. 1a. Fatty acids composition of the flax oil (%)

C14:0 (myristic)	C16:0 (palmitic)	C16:1 (palmit-oleic)	C18:0 (stearic)	C18:1cis-9 (oleic)	C18:2n-6 (linoleic)	C18:3n-3 (α linolenic)	CLA	C18:2n-6 : C18:3n-3
0.06	5.91	0.15	2.99	20.75	27.04	41.71	0.60	0.64

Table. 1b. Fatty acids composition of the diets (%)

Fatty acids	Control group	Flax oil group
C14:0 (myristic)	0.09	0.07
C16:0 (palmitic)	12.10	9.98
C16:1 (palmitoleic)	0.14	0.09
C18:0 (stearic)	2.78	3.32
C18:1cis-9 (oleic)	23.68	26.40
C18:2n-6 (linoleic)	54.57	38.00
C18:3n-3 (α linolenic)	5.86	22.07
C18:2n-6 : C18:3n-3	9.31	1.72

At the end of the experiment blood samples were aseptically collected by jugular venipuncture from all animals (heparinized Vacutainer tubes, Vacutest®, Arzergrande, Italy) in order to determine glycol–lipids composition (glucose, cholesterol and triglycerides), proteins (total protein, albumin, creatinin and urea), minerals (phosphorus-P, calcium-Ca, magnesium-Mg), enzymes activity (alanine aminotransferase-ALT; aspartate aminotransferase- AST, and alkaline phosphatase-AP).

Measurements and analyses

The crude protein of the diet was determined using a semiautomatic classical Kjeldahl method using a Kjeltek auto 1030 – Tecator (SR 13325). The fat was extracted using an improved version of the classical method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal (SR ISO 6492). The crude fibre was determined with a classical semiautomatic Fibertec-Tecator method (STAS 959715-77) and the ash by calcinations' at 550^o until constant mass (SR ISO 5984). The nitrogen-free extractives (NFE) were calculated from the formula: NFE = DM - (CP + EE + GF + Ash). The metabolisable energy (ME) was calculated with regression equations developed by the „Oskar Kellner" Institute of animal nutrition: ME = 5.01 × DP + 8.93 EE + 3.44 GF + 4.08 DNFE.

Gas chromatography was used to determine the detailed FAs composition. Briefly, after lipid extraction from the samples, the FAs were transformed from methyl esters by transmethylation, and the components were separated in the capillary chromatograph column. BS-130 Chemistry analyzer (Bio-Medical Electronics Co., LTD, China) was use to determine the biochemical parameters.

Statistical analysis

All results are expressed as mean and standard error of the mean (SEM). Statistical differences between groups for different parameter concentrations were determined using ANOVA general linear model, GLM). P-values <0.05 were considered significant and for P-value <0.001 and <0.0001 were considered high significant. The correlation between some plasma indicators has been determined with Pearson's coefficient.

RESULTS AND DISCUSSION

Biochemical parameters of blood plasma

The mean concentration of serum biochemical constituents (glucose, triglycerides, total protein, albumin, creatinin, urea, Ca, P, Mg, ALT, AST, AP) and humoral immune parameters (Ig G and Ig M), are shown in Table 2.

The data of this study show the existence of significant (P<0.05) or very significant (P<0.01) differences between the two groups for most blood constituents in favour of the flax oil treatment, probably due to the diet. However, the level of plasma Ca in the control pigs fed on the conventional diet (no supplemental Ca) was close to that of the experimental pigs which received additional Ca, while the level of plasma P was even higher. A possible explanation might be that the extensive rearing system (close to the traditional system) feeds the animals indoors and are allowed to go out in the paddock, which allows direct contact with the sun rays, which is beneficial to vitamin D synthesis and implicitly to Ca and P metabolism. In both groups Ca has been absorbed slightly better than the normal physiological limits (Table 1). This presumes that no additional Ca as calcium carbonate or monocalcium phosphate is needed for the diets of Mangalitza pigs reared under extensive conditions due to the efficient metabolisation of this element during the finishing period. The correlation between the two parameters (Ca and P) was positive (0.65). The lower level of the phosphorus in the flax oil treatment might be due to the lower level of glucose, the inorganic phosphorus depending on glucydes metabolism. Ca to P ratio is within the normal range in both groups (1.16 for group C and 1.56 for group E).

Table 2. Metabolic profile of blood plasma in Mangalitza finishing pigs

Variable*	Normal values	Control	Flax oil	SEM	P**
Triglycerides (mg/dL)	33-50	83.80A	35.87B	7.45	0.003
Total protein (g/dL)	5.8-8.3	6.84a	7.73b	0.16	0.023
Albumin (g/dL)	2.3-4.0	3.45A	4.06B	0.09	0.006
Bilirubin (mg/dL)	0-0.5	0.07	0.09	0.01	0.630
Creatinin (mg/dL)	0.8-2.3	1.69A	1.36B	0.05	0.001
Urea (mg/dL)	8.2-24.6	22.00A	23.50B	0.22	0.003
Urea to creatinin ratio	<20	13.02A	16.79B	0.69	0.004
Calcium (mg /dL)	9.3-11.5	12.50	12.81	0.16	0.810
Phosphorus (mg/dL)	5.5-9.3	10.85A	8.50B	0.57	0.003
Magnesium (mg/dL)	2.3-3.5	3.39	3.81	0.28	0.730
Ca to P ratio	1.0-2.0	1.16a	1.56b	0.10	0.022
ALT (U/L)	21.7-46.5	57.50A	66.90B	1.76	0.010
AST (U/L)	15.3-55.3	62.80A	96.90B	8.77	0.008
AST/ALT	1.0	1.09	1.44	0.13	0.120
AP (U/L)	41.0-176.0	55.40a	84.82b	10.88	0.015
LDH (U/L)	159.6-424.7	520.85	564.25	15.95	0.220
GGT (U/I)	31.0-52.0	56.85	65.95	3.82	0.310

*references of normal values (Reference Guides, 1998. The Merk Veterinary Manual)
** a,b – different letters = significant differences between groups ($P<0.05$); A,B different letters = high significant differences between groups ($P<0.0001$, $P<0.01$, $P<0.001$).

As seen in Table 2 very significant differences (P= 0.003) were noticed for the triglycerides, which were 2.33 times lower in the flax seed group than in the control group. The fact that the nutrients are at a proper level and ratio in the case of the flax oil treatment corrects the imbalances and deficiencies since it is known that some nutrients decrease the blood and, implicitly, triglycerides level. An optimal level of vitamins, the presence of antioxidants and minerals reduce the blood triglycerides. Our results are similar to those reported by

Hellwing et al. (2007) who used a diet with bacterial protein (*Ralstonia* sp., *Brevibacillus agri,* and *Aneurinibacillus* sp).

The level of the total protein was within the physiological range (5.8 - 8.3 g/dL; Reference Guide, 1998) in both groups (6.84 g/dL control group vs. 7.73 linseed oil group). Except for the creatinin, which displayed significantly (P<0.001) lower values in the flax oil group (1.36 mg/dL) compared to 1.69 mg/dL in group C, the total protein and the other protein constituents increased in the flax oil group. The dietary flax oil also increased the albumin level by 17%, the difference between the two groups being very significant (P=0.006). The results for protein and albumin are close to those reported by Habeanu et al. 2010. Bilirubin was slightly higher than the normal physiological levels in both groups. When it is at a proper physiological level, bilirubin acts as an antioxidant (Kaneko et al., 1997). The plasma creatinin and urea were within the normal physiological levels and the ratio of these indicators showing how the liver works was lower than 20 (13.02 in the control group and 16.79 in the flax oil group), which shows a proper functioning of the kidneys and a good hydration of the animals. The correlation between these two indicators was negative (-0.88).

AP activity is a potential marker used as a supportive measure of Ca and P adequacy (Boyd, 1983). AP level was influenced very significantly by the flax oil (>53% vs. control group, P=0.015).

The values recorded for both groups were slightly over the recommended limit for ALT and AST enzymes and more important level was noted on linseed oil group. Our results are higher for ALT and AST than those reported by Czech et al., 2009, who used a plant extract. The activity of these enzymes, slightly higher than the normal physiological levels, may suggest an overloading of the liver, but their ratio is close to 1, which removes the idea of liver dysfunction. No significant differences between the two groups were noticed for LDH and GGT enzymes (P>0.05).

The results of the present study indicate that linseed oil supplements investigated herein had the ability to potentiate the immune function by boosting the humoral immune response during the finishing period; an increase in plasma IgM and IgG concentration above the control level (Fig. 1) was observed after linseed diet ingestion, which was significant in the case of IgM (P=0.018). This fact is very important if we take into consideration that immunoglobulin M and G plays a crucial role in the clearance of invading microbes and the generation of the first and the long lasting immunity.

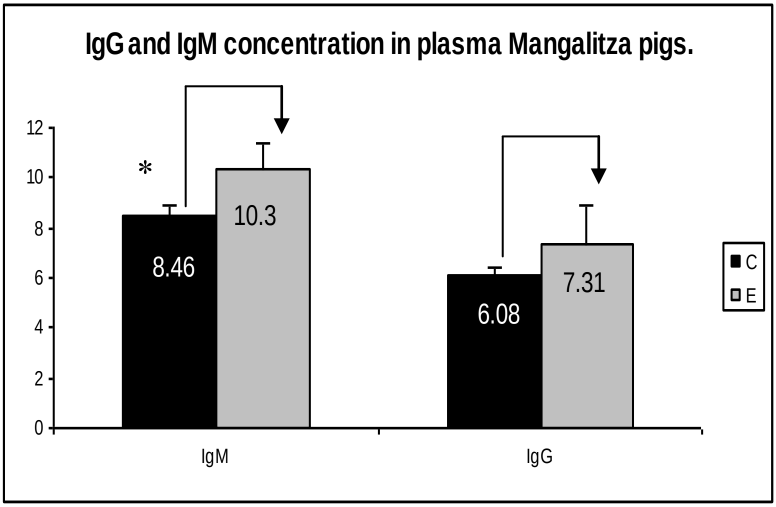

Figure 1. IgG and IgM concentration in plasma Mangalitza pigs.
* significant differences between the groups (P<0.05).

CONCLUSIONS

The use of flax oil in the diets of finishing Mangalita pigs changed significantly most of the biochemical and immunological plasma parameters. The data show that the plasma constituents are within the physiological range or slightly higher, but with no adverse effects on the health status of the animals. Our results are important because they can be used as reference for biochemical determinations in humans due to the similarities existing between the human and pig organism.

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