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Original article

The effect of diets supplemented with fish broth and fish oil on the health of weaners

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Abstract

The aim of this study was to determine the effect of fish-based feed materials, as a source of readily available protein contained in fish broth and essential polyunsaturated fatty acids (PUFAs) found in fish oil, on the health of piglets and rearing results. The experiment was conducted on a commercial pig fattening farm. The study involved a total of 80 weaners with an approximate body weight of 15 kg. The experiment was carried out over a period of 40 days. Feed samples were subjected to laboratory analyses. Blood samples were collected from experimental group animals to determine serum biochemical and immunological parameters. The body weight gains of weaners, mortality rates and average feed intake per animal were calculated for the entire experimental period. The addition of fish broth and fish oil significantly improved the n3:n6 fatty acid ratio in diets. The presence of EPA and DHA in the experimental diet could have had a positive health effect on piglets, comparable with that exerted by therapeutic doses of zinc often administered to pigs of this age group. During the experiment, feed conversion ratio (FCR) gain was considerably reduced in the experimental group, with similar daily gains in the control and experimental group.

Key words: weaners, fish broth, fish oil, essential polyunsaturated fatty acids (PUFAs), zinc

Introduction

The performance of weaner piglets is determined by a variety of factors, including their health, the date of weaning, environmental conditions and feed quality. Feed additives used in weaner nutrition, such as symbiotics, organic acids, antioxidants, enzymes, plant (herbal) supplements and extracts and flavor and aroma enhancers improve production results and contribute to the animals' health. As a preventive measure, many farms administer diets supplemented with increased, even therapeutic, doses of zinc to piglets transferred from the farrowing house to the weaner house. Feeding fish-based feed materials to pigs in different age groups produces satisfactory results, owing to the high quality of readily available fish meal protein and the health benefits of essential polyunsaturated fatty acids (PUFAs) found in fish oil

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(Simpoulos 1991, Högberg et al. 2003). The above observation has been confirmed by previous studies where fish-based feed materials were fed to growing-finishing pigs, sows and piglets. Diets supplemented with 3% fish oil with a high PUFA content had a beneficial influence on the health of growing-finishing pigs and contributed to modifying the fatty acid composition of fat by increasing the concentrations of desirable n-3 PUFAs (Bakuła 2004). Gilts fed complete diets supplemented with fish oil were characterized by a more desirable composition and quality of sow colostrum fat, higher fertility and fecundity, a higher milk yield, as well as higher litter weight on day 21 and lower piglet mortality rates (Bakuła et al. 2006). In another experiment, the health of piglets was positively affected by diets containing very high (therapeutic) levels of zinc and high concentrations of PUFAs, in particular n-3 PUFAs (Bakuła et al. 2007). The present study expands on previous research by examining another group of pigs – weaners.

The aim of this study was to determine the effect of diets supplemented with fish broth (untypical fish-based feed material) and fish oil on the health and body weight gains of weaners in comparison with animals administered commercial feed of the same type.

Materials and Methods

The experiment was conducted under field conditions, on a commercial pig fattening farm. The study involved weaners with body weights of approximately 15 kg (the offspring of 20 Polish Landrace x Polish Large White sows from the same farrowing house, mated to Duroc boars; the sows nursed the piglets for 25 days, and the piglets stayed in pens until day 31). The experiment was carried out over a period of 40 days. Two groups were formed, control (C) and experimental (E), each of 40 weaners, 20 females and 20 males, kept in separate pens in the same building, under identical conditions.

The weaners were fed a starter diet with the addition of fish broth – a by-product of fish simmering during canned fish production and fish oil (experimental diet – ED), and a commercial starter diet from a renowned supplier which has been routinely applied in the farm for many years (control diet – CD). The animals had *ad libitum* access to feed in automatic feeders.

ED, CD and broth samples were analyzed to determine the content of: dry matter, crude ash, total protein, crude protein soluble in pepsin and hydrochloric acid *in vitro* (the percentage content of protein soluble in pepsin and hydrochloric acid in total protein was calculated), crude fat, crude fiber, gross energy, chlorides, calcium, sodium and potassium ions, by standard methods (Regulation 2004, Journal of Laws 04.271.2688). The content of zinc, copper, iron, vitamins A, E and D in feed was determined in an accredited chemical laboratory of the Sea Fisheries Institute in Gdynia with the use of standard methods (Regulation 2004, Journal of Laws 04.271.2688). The amino acid content of protein was determined using the AAA-T 339M amino acid analyzer in hydrolyzates prepared as described by Pazourek and Kalova (1988). Fatty acids were identified and their concentrations were determined in fat extracted from feed. After the methylation of fatty acids in an acidic environment (Peisker 1964), fatty acid methyl esters were separated using a PYE Unicam 104 gas chromatograph (Shantha and Napolitano 1992) equipped with a flame ionization detector (FID). The results were recorded using a Philips recorder with a tape speed of 0.3 m/h. The peaks of fatty acid methyl esters were identified by comparing their retention times with the retention times of standard fatty acids from a mixture of known composition (Applied Science Corporation).

Blood samples for laboratory analyses were collected from the cranial vena cava *(vena cava cranialis)* from 20 weaners (10 females and 10 males) in each group on experimental days 1, 15 and 40 (the last day of the experiment).

Triglyceride concentrations, total cholesterol, HDL cholesterol and LDL cholesterol levels (LDL(mmol/l) = total cholesterol – HDL cholesterol – (triglicerides/2.2), calcium, total phosphorus, urea levels and total iron concentrations were determined in the blood serum using the EPOLL-20 BIO analyzer, BioSystem Reagents & Instruments and Pointie Scientific Polska reagent kits. Total protein levels were determined by the spectrophotometric method described by Lowry (Lowry et al. 1951). The following immunological parameters were determined by the spectrophotometric method using BioSystem Reagents and Instruments and Pointie Scientific Polska reagent kits according to the manufacturers' instructions: immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG). Globulin fractions were precipitated with polyethylene glycol 10000 kDa. C-reactive protein (CRP) was determined by the Biosystem Latex immunoturbidimetric assay.

The body weights of weaners (males and females separately) were determined individually on the first day of the experiment. Successive body weight measurements were performed on experimental days 15 and 40. Weight gains were calculated for the entire experimental period, mortality rates and average feed intake per animal were also determined. The results were processed statistically using the Student t-test.

Results

Innovative starter feed containing 7% fish broth and 4% fish oil was used in the experiment (Table 1). The total protein content of ED was within the norms recommended for weaners by Feed and Nutrition (Ensminger et al. 1990) (18-20%), and it was higher than in CD (Table 2). The percentage content of protein soluble in pepsin and HCl was similar in both groups, yet its share of total protein was 6.9% lower in ED than in CD. The diets were characterized by a similar content of the investigated amino acids, excluding histidine whose concentrations in ED were three-fold higher than in CD. Crude fat levels in ED were by 3.48% higher than in CD, but they were within the maximum allowable limits recommended for animals in the studied age group (max. 7%). Higher fat concentrations increased the energy value of ED. The analyses of mineral elements revealed higher calcium and phosphorus levels in ED and a very significant difference in zinc concentrations between the diets (201.9 mg/kg in ED and 2253.4 mg/kg in CD). Both diets contained high levels of vitamins A and E, but their concentrations in ED were nearly 50% lower than in CD. Vitamin A levels in both diets and vitamin E concentrations in CD exceeded the recommended values. The maximum recommended concentrations of vitamin D were exceeded in ED (Table 2).

Table 1. Selected parameters of chemical composition of degreased fish broth.

Parameter		Result
Dry matter	%	10.49
Crude ash	%	1.42
Total protein	%	7.46
Crude fat	%	0.4
Chlorides	%	0.9
Na	g/kg	3.19
K	g/kg	2.96
P	g/kg	1.27

The addition of fish broth and fish oil was responsible for the presence of PUFAs in ED, which were not observed in CD, and they significantly improved the n-3/n-6 fatty acid ratio (CD – 1:13.40, ED – 1:2.05) (Table 3).

The analyses of blood serum lipids indicated a statistical difference in triglyceride concentrations in gilts. Total cholesterol levels were lower in experimental gilts after 15 days of administration of feed with a higher content of crude fat, including fish oil (lower by 0.39 mmol/l, a highly significant difference). On the last day of the experiment, total cholesterol levels were by 0.47 mmol/l higher in gilts of the experimental group, compared with the control group (significant difference) (Table 4).

The analyses of serum biochemical parameters of the examined weaners showed statistically significant differences ($p \leq 0.05$) in calcium levels between young boar groups C < E on day 15, while a reverse trend was noted in gilt groups C > E on day 40. Significant differences ($p \leq 0.01$) were noted in phosphorus concentrations between young boar groups C > E on day 15, and between gilt groups ($p \leq 0.05$) and young boar groups ($p \leq 0.01$) C < E on day 40. Statistically significant differences ($p \leq 0.01$) were also observed in urea levels on day 15 and day 40 between groups of gilts and young boars C < E. Total protein levels in the

Table 2. Results of chemical analysis of feed (a starter diet for piglets with body weight in the range of 10-30 kg)

Indicator		CD	ED
Dry matter	%	86.07	87.52
Crude ash	%	5.22	7.78
Total protein	%	17.82	19.30
Protein soluble in pepsin and HCl (*in vitro*)	%	16.27	16.87
Share of soluble protein (*in vitro*) in total protein	%	91.3	87.4
Crude fat	%	2.97	6.45
Crude fiber	%	2.38	2.05
Gross energy	MJ/kg	16.38	17.21
Lysine	%	1.189	1.388
Methionine+cystine	%	0.739	0.928
Tryptophan	%	0.194	0.185
Histidine	%	0.401	1.282
Isoleucine	%	0.693	0.836
Leucine	%	1.360	1.592
Tyrosine	%	0.556	0.674
Chloride	g/kg	0.36	0.30
Ca	g/kg	4.66	10.23
P	g/kg	4.75	6.57
Ca:P		0.98:1	1.56:1
Na	g/kg	1.44	1.44
Zn	mg/kg	2253.4	201.9
Cu	mg/kg	151.5	206.6
Fe	mg/kg	212.7	171.4
Vitamin A	IU/kg	72600	31260
Vitamin E	mg/kg	124.6	56.4
Vitamin D	IU/kg	1560	2600

Key: CD – control diet, ED – experimental diet

Table 3. Long-chain fatty acid composition of fat extracted from feed (%).

Indicator	CD	ED
C18:0 stearic acid	7.01	3.40
C18:1 n-9 oleic acid	25.33	26.91
C18:2 n-6 linolenic acid	39.00	18.60
C18:3 n-3 alphalinolenic acid	2.91	2.27
C18:4 stearidonic acid	0	1.36
C20:0 arachidic acid	0.24	0.25
C20:1 gadoleic acid	0.30	3.39
C20:2 eicosadienoic acid	0	0.32
C20:3 n-6 dihomo-γ-linolenic acid	0	0.39
C20:4 n-6 arachidonic acid	0	0.68
C20:5 n-3 EPA	0	4.76
C22:0 behenic acid	0	0.26
C22:1 erucic acid	0	3.73
C22:5 n-3 clupanodonic acid	0	0.74
C22:6 n-3 DHA	0	6.57
n-6 fatty acids	39.00	19.60
n-3 fatty acids	2.91	9.58
n3:n6	1:13.40	1:2.05

Key: CD – control diet, ED – experimental diet

blood serum were not marked by significant variations between the studied groups. Lower iron concentrations were reported in the control group on day 40, and the noted difference was statistically significant in gilts ($p \leq 0.01$) (Table 5).

The results of immunological tests revealed higher concentrations of C-reactive protein (CRP) on day 15 in the control group of young boars (difference significant at $p \leq 0.05$). IgA and IgG levels were similar in both groups throughout the experiment. A significant difference in IgM concentrations was observed between the control and experimental group of young boars on day 15 and day 40 ($p \leq 0.01$ and $p \leq 0.00\%$, respectively) (Table 6).

Performance results indicate higher daily gains and significantly lower levels of feed consumption per kg body weight gain in the experimental group (Table 7).

Discussion

A high fish fat content increased the diet's energy value (Table 2). In ED, the main sources of fat were fish broth and fish oil containing high levels of n-3 and n-3 PUFAs. The presence of fish-specific fatty acids was not reported in CD. PUFAs, including n-3

Table 4. Selected serum lipid indicators in piglets ($n = 10, \bar{x} \pm s$).

Indicator	Sampling date	I		II		III	
	Group	C	E	C	E	C	E
Triglycerides mmol/l	Gilts	0.24	0.08[a]	0.37[a]	0.46	0.39	0.32
		0.15	0.07	0.10	0.11	0.17	0.10
	Young hogs	0.34	0.15	0.49	0.49	0.33	0.33
		0.29	0.08	0.22	0.20	0.07	0.09
HDL cholesterol mmol/l	Gilts	0.24	0.25	0.23	0.37	0.22	0.24
		0.05	0.04	0.06	0.06	0.06	0.04
	Young hogs	0.32	0.27	0.38	0.27	0.27	0.27
		0.07	0.08	0.11	0.08	0.04	0.06
LDL cholesterol mmol/l	Gilts	1.47	1.10	1.63	1.68	1.36	1.75
		0.55	0.17	0.56	0.39	0.45	0.39
	Young hogs	1.59	1.27	1.89	1.88	1.82	1.84
		0.39	0.45	0.49	0.78	0.36	0.47
Total cholesterol mmol/l	Gilts	2.28	1.99	3.78	2.86[A]	2.35[a]	2.82
		0.68	0.13	0.85	0.43	0.60	0.36
	Young hogs	2.50	2.1	2.99	3.14	3.08	3.08
		0.42	0.6	0.48	0.84	0.44	0.49

Key:
a, b – significant difference at $p \leq 0.05$; A, B – significant difference at ≤ 0.01;
C – control group, E – experimental group; I – experimental day 1, II – day 15, III – day 40

Table 5. Selected serum biochemical indicators in piglets (n = 10, $\bar{x} \pm s$).

Indicator	Sampling day	I		II		III	
	Group	C	E	C	E	C	E
Calcium mmol/l	Gilts	1.52	1.40	1.56	1.51	1.43[a]	1.63
		0.18	0.13	0.11	0.14	0.29	0.12
	Young hogs	1.35	1.39	1.49[a]	1.61	1.72	1.65
		0.09	0.16	0.15	0.11	0.08	0.20
Total phosphorus mmol/l	Gilts	1.99	1.76	2.60	2.68	1.33[a]	2.54
		0.25	0.22	0.44	0.45	0.45	0.19
	Young hogs	1.90	1.78	3.54	2.86[A]	1.11[A]	1.33
		0.05	0.13	0.37	0.34	0.15	0.15
Urea mmol/l	Gilts	3.16	3.02	3.04[A]	5.75	2.87[A]	4.72
		0.85	0.55	0.97	0.66	0.87	0.76
	Young hogs	2.66	2.92	3.05[A]	4.90	2.85[A]	4.95
		0.21	0.55	0.58	1.07	0.89	0.92
Total protein mmol/l	Gilts	56.00	58.00	49.00	99.10	58.00	63.10
		4.36	4.30	6.96	149.75	9.85	7.56
	Young hogs	53.00	59.20	52.20	54.50	62.80	61.40
		7.35	5.93	3.43	4.17	6.49	6.65
Total iron µmol/l	Gilts	18.16	17.08	20.67	19.34	15.99[A]	27.89
		5.54	6.12	8.00	7.27	4.61	10.58
	Young hogs	21.06	18.16	23.55	20.76	22.34	26.11
		6.38	3.66	11.99	5.13	10.63	7.90

Key:
a, b – significant difference at $p \leq 0.05$; A, B – significant difference at ≤ 0.01;
C – control group, E – experimental group; I – experimental day 1, II – day 15, III – day 40

Table 6. Serum immunological indicators in piglets (n = 10, $\bar{x} \pm s$).

Indicator	Sampling day	I		II		III	
	Group	C	E	C	E	C	E
CRP mg/l	Gilts	3.20	6.40	6.71	4.87	6.01	3.24
		2.86	6.50	5.40	4.15	7.87	2.81
	Young hogs	4.24	7.18	10.30[a]	6.66	3.57	3.82
		4.38	6.08	3.56	5.42	2.70	4.02
Ig A mg/dl	Gilts	39.10	37.48	29.87	33.17	19.97	18.63
		4.77	2.82	8.85	7.81	8.46	6.42
	Young hogs	40.04	39.16	27.54	19.27	19.86	16.42
		19.98	3.60	4.84	3.29	4.37	6.54
Ig G mg/dl	Gilts	549.56	555.28	425.65	406.41	422.47	417.71
		58.82	129.99	132.00	125.31	111.03	126.59
	Young hogs	466.92	502.74	379.05	334.30	422.61	348.17
		121.66	48.24	105.45	111.96	126.98	136.40
Ig M mg/dl	Gilts	30.54	25.58	43.70	36.62[a]	24.70	29.65
		14.34	12.34	8.70	13.27	7.51	6.96
	Young hogs	23.80	35.88	23.03	20.06[A]	23.25	20.69[a]
		5.50	13.03	11.80	5.88	8.03	5.00

Key:
a, b – significant difference at $p \leq 0.05$; A, B – significant difference at ≤ 0.01;
C – control group, E – experimental group; I – experimental day 1, II – day 15, III – day 40

Table 7. Body weight gains and feed consumption in groups (n-10).

Body weight measurements			C		E	
			Gilts	Young hogs	Gilts	Young hogs
Average body weight	(kg)	I	14.6	15.55	15.6	14.0
		II	26.00	22.56	22.15	21.1
		III	39.78	42.8	45.1	39.6
Average body weight gains per piglet over 40 days	(kg)		25.18	27.25	29.5	25.6
			26.22		27.55	
Average daily gains	(kg)		0.630	0.681	0.738	0.640
			0.656		0.689	
Feed consumption over 40 days	(kg)		1950		1400	
Feed conversion ratio (FCR)			2.01		1.58	

Key: C- control group, E – experimental group

PUFAs and the n-3/n-6 fatty acid ratio, promote good health by stimulating the immune system. In this experiment, the n-6/n-3 fatty acid ratio reached 2.05:1 in ED and 13.4:1 in CD (Table 3). According to Pepping (1999), the optimal n-6/n-3 fatty acid ratio falls in the range of 2:1 to 4:1. Simopoulos (1991) claims that the most desirable n-6/n-3 fatty acid ratio is 1:1. The presence of EPA and DHA in ED (Table 3) could have exerted a positive health effect on weaners owing to the widely acclaimed immunomodulative properties of PUFAs (Simopoulos 1991, Pepping 1999, Hogberg et. al. 2003, Bakuła 2004, Bakuła et al. 2006, 2007).

The calcium to phosphorus ratio is an important consideration in feed, and according to Feed and Nutrition (Ensminger et al. 1990, Russell McDowell 1992), it should fall in the range of 1.2:1 to 1.5:1. In ED, the Ca:P ratio approximated the upper recommended limit, while it was below the guideline values in CD (Table 2). High zinc levels (2253.4 mg/kg) (Table 2) were observed in CD relative to the guideline level of 150 mg/kg (Grela and Pastuszak 2004, Regulation 2004, Journal of Laws 04.162.1704). In CD, zinc concentrations assumed a therapeutic value. High zinc doses are used in the prevention of diarrhea in piglets (Pejsak et al. 1998, Grela and Pastuszak 2004). Zinc stimulates pig growth by inhibiting the development of pathogens in the gastrointestinal tract, stimulating the secretory functions of intestinal epithelial cells (secreting substances that stimulate the immune system in the gastrointestinal tract) and modifying the absorption of selected electrolytes, thus preventing excessive dilution of the digesta and the occurrence of diarrhea. Special caution should be exercised when supplementing feed with therapeutic doses of zinc, which inhibits the absorption of copper. If administered in excessive quantities, zinc may lower HDL levels, leading to anemia. Zinc has an inhibitory effect on magnesium absorption and magnesium balance, it lowers iron absorption and increases iron turnover (Grela and Pastuszak 2004). The above probably contributed to higher serum iron levels in weaners of the experimental group on day 40 (Table 5). Zinc is not accumulated in tissues, and excess zinc is excreted in feces and, in small quantities, in urine. Excess zinc concentrations in feed could have significant environmental impacts, in particular in crops fertilized with animal slurry.

The analyzed diets revealed high variations in vitamin concentrations (higher levels of vitamin A in CD than in ED, and higher levels of vitamin D in ED than in CD). Excess vitamins are excreted from the body. The vitamin content of feed should be carefully balanced for financial reasons.

The nutritional value of protein is determined by limiting amino acids, their quantity and proportions. Feed should contain the following essential (limiting) amino acids, which cannot be synthesized by the body: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The remaining endogenous amino acids are synthesized by pigs. The biological value of protein is determined based on the ratio of lysine to methionine + cystine, threonine and tryptophan. The above proportions are presented in Table 2. In ED, protein was characterized by higher concentrations of most amino acids than in CD. A high share of fish protein in ED was correlated with much higher histidine levels (Table 2).

Cholesterol is found in cell membranes, it is a precursor of steroid hormones and bile acids. Total cholesterol levels in the blood serum are determined by the metabolic rate of lipotropic molecules which transport cholesterol between the liver and the intestines where it is absorbed during digestion. Similar HDL (high-density lipoprotein) concentrations were observed in both groups (C and E). HDL molecules (known as “good” cholesterol) transport cholesterol back to the liver. The reported lipid indicators were higher than the reference values for pigs (Winnicka 2002) (Table 4). In addition to fatty acids, other feed

nutrients, such as protein and carbohydrates, including fiber, affect cholesterol levels in the body. Urea excretion is directly proportional to dietary intake of protein, and it is also determined by the degradation rate of endogenous protein. Elevated serum urea levels in experimental gilts and young boars were noted on day 15 and 40, suggesting that feed protein was not fully utilized by the animals (Table 5). This hypothesis was validated by the values of protein digestibility coefficients (Table 2).

The total cholesterol to urea ratio was lower in the experimental group than in the control group, suggesting lower nutrient utilization in experimental animals. This hypothesis was not confirmed by the daily gains of weaners and feed intake per kg body weight gain in the experimental group (Table 7).

It can be assumed that ED supplemented with fish protein and fish oil, characterized by higher n-3 PUFA levels, had a positive effect on the health condition of animals. Lower CRP levels in the experimental group validate the above assumption (Table 6). CRP is a plasma globulin synthesized in the liver and one of the most sensitive acute phase proteins responding to tissue damage and inflammations. CRP activates the complement system as the inflammatory response. Low CRP levels testify to the animals' good health. This observation was confirmed by similar immunoglobin A and G levels in both groups, and lower IgM levels in the experimental group on day 15 and day 40 (Table 6). The results of biochemical and immunological tests indicate that the animals' health was well within the norm throughout the experimental period.

The body weight gains of weaners, in particular feed intake per kg weight gain, deserve special attention. During the experiment (40 days), feed consumption in the experimental group was reduced by 550 kg, with the same number of animals in both groups (40) and similar daily gains (C – 0.656, E – 0.689). The above directly affected average feed intake per kg body weight gain (Table 7).

Conclusions

High dietary zinc levels contributed to the good health of control group weaners, while in the experimental group, a similar effect was produced by diet supplementation with fish broth and fish oil. Feed utilization efficiency was better in the experimental group than in the control group.

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