Enrichment of Pork with Omega-3 Fatty Acids by Tuna Oil Supplements: Effects on Performance as well as Sensory, Nutritional and Processing Properties of Pork

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ABSTRACT : The effects of tuna oil supplementation (0, 1, 2 and 3%) to pig diets on growth and carcass yield as well as meat quality were determined in 40 crossbred pigs. Animals were fattened from 30 to 90 kg of live-weight. Twenty-four hours after slaughter, following various early- and late-post mortem measurements, loin, backfat and belly were prepared from the carcasses. Bacon was produced from the belly part by curing and smoking. Neither performance (feed intake, daily gains, feed conversion efficiency) nor carcass quality (slaughter weight, dressing percentage, lean percentage, nutrient composition of the loin) were significantly affected by tuna oil supplementation. Tuna oil also had no clear effects on early- and late-post mortem meat quality traits, water-holding capacity and tenderness of the M. *longissiumus dorsi* (LD). Colour traits of LD and backfat, and backfat firmness were not significantly affected by tuna oil, either. However, there was a certain trend to elevated fat contents of LD (and bacon), but not of backfat, with increasing levels of tuna oil in feed. Pigs receiving elevated proportions of tuna oil expressed lower VLDL cholesterol and triglyceride concentrations in blood plasma, whereas the cholesterol content of LD, backfat and bacon did not reflect this trend. Effects of tuna oil on fatty acids in LD, backfat and bacon were often small in extent, except those concerning the long-chain polyunsaturated fatty acids. With 3% tuna oil in the diet, the contents of the particularly desired omega-3 fatty acids, C20:5 and C22:6, were 0.1 and 0.2 g/kg in LD. The corresponding values for backfat and bacon were 2.6 and 12.6 g/kg, and 1.3 and 9.2 g/kg, respectively. Tuna oil supplementation was associated with significant adverse effects on flavour and overall acceptance of bacon (not significant in LD although numerically the same trend was noted), but these effects on sensory ratings were limited in extent. Also shelf life of the products, determined as TBA value after different storage periods at 4°C in LD, backfat and bacon, was significantly reduced. Overall, the present study suggests that omega-3 fatty acids may be enriched in pork by feeding tuna oil with few undesired side-effects, particularly those on sensory perception and shelf life, suggesting immediate consumption of the products is advisable. Most economically important traits (performance, slaughter and physical meat quality) remained unaffected. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 11 : 1622-1633)***

Key Words : Tuna Oil, Omega-3 Fatty Acids, Performance, Meat Quality, Sensory Evaluation, Pork

INTRODUCTION

Animal fat and cholesterol consumption is assumed to increase the incidence of coronary heart diseases mainly by elevating blood cholesterol levels (Sahaphong, 1990; Voet and Voet, 1990). These diseases are the major mortality reason in all Western civilisations, but also in countries like Thailand with an incidence of 58.5 deaths out of 100,000 persons (Public Health Policy and Planning Division, 1996). In turn omega (ω)-3 fatty acids, particularly eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6), were found to reduce hypercholesterolemia in numerous studies (e.g., Kroman and Green, 1980; Pond et al., 1992; Harris et al., 1993). Modes of action are a decrease of blood plasma concentrations of triglycerides and cholesterol and of the inclination of blood platelets to agglutinate (Rattanawongpaisarn et al., 1997). The C22:6 was also found to play an important role in the function of brain and nervous system (Ackman, 1980, cited by Huang et al., 1990). The ω-3 fatty acids cannot be synthesized in the body of mammalian species and have to be obtained from food (Jakobsen, 1995). Additionally to this favourable dietetic aspect, ω-3 fatty acids rich fats like fish oil have a high content of metabolizable energy thus also contributing to energy supply by the diet when fed to pigs in order to enrich pork with ω-3 fatty acids. This might cause side-effects on performance and carcass fatness unless pigs respond by a correspondingly lower feed intake. Furthermore, the use of fish oil might considerably increase the inclination of pork to lipid oxidation and rancidity, which may be associated with off-flavours as well as lower colour stability and nutritive value (Monahan et al., 1992), a problem which could be both enhanced or reduced by meat processing such as curing and smoking (e.g., Han and Yamauchi, 2000).

The objective of the present study was (i) to quantify the efficacy of dietary tuna oil supplementation to enrich lean and fat pork as well as a selected pork product with ω-3 fatty acids, (ii) to determine any side-effects of tuna oil on

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performance, further dietetic traits and processing properties of pork and (iii) to develop recommendations for pig feeding.

MATERIALS AND METHODS

Animals and diets

The experiment was conducted with 40 Duroc×(Large White×Landrace) pigs initially weighing 30 kg and being housed individually in pens with concrete floor of a size of 1×2 m. Applying a completely randomized design, ten pigs each (five barrows and five gilts per group) were randomly allocated to four dietary treatments comprising different levels of tuna oil (0, 1, 2 and 3%) supplemented to basal diets. The basal diets used in all treatments were two types with different crude protein contents, either a starter diet (fed from 30 to 60 kg of live-weight) or a finisher diet (fed from 60 to 90 kg of live-weight), with metabolizable energy contents of about 3 Mcal/kg DM as recommended by NRC (1998). These basal diets were composed of ground maize (53%/54% in starter/finisher period, respectively), ground rice bran (20%/22%) and a protein-mineral concentrate (27%/24%; containing fish meal, soybean meal, corn rice bran, dicalcium phosphate, sodium chloride and a vitamin-mineral premix; purchased as one batch from Sinkase Industrial Pokphand Co, Ltd., Chiang Mai, Thailand). Both types of basal diet were supplemented with butyl hydroxytoluene (BHT, 0.02% of total oil in diet) and vitamin E (α-tocopherol, 70 mg/kg diet). As recommended by Phongpiachan (1996), the respective levels of tuna oil were added as a pre-mix with filler (3% of bone meal) and then mixed with ground maize. Similarly, BHT and vitamin E were pre-mixed with ground rice bran. Subsequently all ingredients were combined to the complete diets. Although the oil was given supplementary, the animals had the opportunity to respond to the respectively increased energy density of the diet by a reduced feed intake as pigs were fed in an *ad libitum* regime. Permanent access to fresh water was provided by nipples arranged separately from the troughs. According to analyses, differences between treatment diets within fattening period were low, except in fatty acid composition, ether extract content and, consequently, in content of metabolizable energy (Table 1). As intended, starter and finisher diets within the same tuna oil treatment differed in crude protein content, but were similar in ether extract content and fatty acid profile.

Data and sample collection as well as measurements at the abattoir

During fattening, individual feed intake was recorded every day and pigs were weighed once every 2 weeks. Blood samples were collected at 9:00 a.m. from the jugular vein at an average live-weight of 30 (i.e., directly before the start of treatment feeding), 60 and 90 kg. Samples were immediately treated with EDTA, centrifuged at 2,500 rpm for 15 min to obtain plasma and stored at -18°C.

The animals were slaughtered at a live-weight of 90 kg. Prior to slaughter, pigs were fasted for 12 h before being transported for 7 km to the abattoir of the National Meat Technology and Training Center (Chiang Mai). They were slaughtered after 2 h of resting. Hot carcass weight was defined as the weight at 1 h post mortem (p.m.). Carcasses

Table 1. Analysed composition of the experimental diets fed in the starter and the finisher period

Tuna oil supplementation (%)	Starter period (30 to 60 kg)				Finisher period (60 to 90 kg)			
	0	1	2	3	0	1	2	3
Dry matter (DM, %)	89.4	90.3	90.6	90.6	89.8	89.4	91.3	90.9
Crude protein (% in DM)	18.7	18.3	18.1	18.0	18.5	18.6	17.7	17.7
Ether extract (% in DM)	8.56	8.63	10.48	10.50	7.86	9.56	10.03	11.13
Neutral detergent fibre (% in DM)	14.4	13.6	13.9	14.8	14.0	13.3	14.2	14.5
Metabolizable energy (Mcal/kg DM)	2.93	2.99	3.00	3.12	2.97	3.03	3.07	3.27
Fatty acids (FA, % of totally analysed FA)								
C16:0	30.33	31.75	31.15	31.59	32.03	31.03	31.57	30.53
C18:0	5.06	5.04	4.58	4.66	4.42	5.13	4.68	4.79
C20:0	0.77	0.67	0.78	0.72	0.93	0.86	0.64	0.69
C18:1	45.86	42.54	43.40	43.98	44.68	43.10	40.31	41.40
C18:2	15.35	14.06	14.56	14.57	15.01	13.70	15.36	15.57
C18:3 (ω3)	1.41	1.55	1.72	1.87	1.86	1.65	2.25	2.04
C20:4	0.58	0.70	0.82	0.89	0.88	0.73	1.10	0.95
C20:5 (ω-3; EPA)	0.07	0.50	0.63	0.69	0.10	0.79	0.93	1.14
C22:6 (ω-3; DHA)	0.03	1.69	2.99	4.25	0.05	2.34	3.12	4.11
Total saturated FA	36.16	37.46	36.51	36.97	37.39	37.66	36.90	36.02
Total polyunsaturated FA	17.34	17.50	20.72	22.27	17.75	19.32	22.77	23.81
Total ω-3 FA	1.41	3.74	5.34	6.81	1.86	4.78	6.31	7.29
Total ω-6 FA	15.93	14.76	15.38	15.46	15.89	14.44	16.46	15.52
ω-6:ω-3 ratio	11.29	3.94	2.88	2.27	8.54	3.01	2.60	2.26

were weighed again after 24 h of chilling at 4°C. At 45 min p.m. and 24 h p.m., pH (Portamess 331, Knick, Berlin, Germany) and electrical conductivity (only 24 h p.m.; LF 196, WTW, Weieim, Germany) were measured in LD between the 13th and the 14th rib. Average backfat thickness was determined as the average of the values measured by a backfat probe at the 1st rib, the last rib and the last lumbar. Thickness of subcutaneous fat layer located above the M. *longissimus dorsi* (LD) 65 mm from the dorsal mid line between the 10th and the 11th rib, was recorded in order to estimate lean percentage by the equation of Kamopas (2001), using also data on hot carcass weight and loin eye area. After chilling for 24 h, the left side of the carcass was cut perpendicular to the spine after the 6th and before the 15th rib removing the complete loin part and backfat for later analyses. The LD and backfat samples were stored at -18°C until analysis. The individual loin eye area located between the 10th and the 11th rib was copied onto transparent paper and was later planimetrically quantified. The complete loin chops of the 11th rib were dissected into lean tissue, subcutaneous fat, bone and skin in order to calculate tissue proportions. The belly part from the pigs was prepared from the left side of the carcasses between the 6th and the 15th rib after chilling for 24 h at 4°C, and was stored at -18°C until being processed to bacon as outlined by Jaturasitha (2000). Ingredients for curing of the bacon were sodium chloride (51.3%), saccharose (26.6%), nitrite powder (16.4%) and phosphate (5.7%). Cure brine was injected into the belly (10% of weight). The cured bacon was packed into plastic bags and aged at 4°C for 3 days. The cured bacon was rinsed with fresh water and then smoked at 46°C for 1 h, at 49°C for 1 h, at 52°C for 2 h and at 57°C for 30 min.

Laboratory analyses

Blood plasma samples were analysed for cholesterol and lipoprotein concentrations (HDL, LDL and VLDL cholesterol) with colorimetric methods (Jung et al., 1975). Triglyceride concentrations were determined according to Biggs et al. (1975).

The LD of the 6th and the 7th rib, cut into 2.5 cm thick slices, and backfat located on top of the 10th and 11th rib were sealed without vacuum in polyethylene bags to be chilled at 4°C for 24 h. Subsequent to 1 h of blooming at refrigerator temperature, by removing the slices from the bags, colour measurement (luminosity, redness, yellowness) were conducted using the Chroma Meter (Minolta, CR-300, Osaka, Japan). Water holding capacity of LD was assessed as drip, thawing and cooking loss (Honikel, 1987). Drip loss was determined with 2.5 cm thick slices obtained from the 7th and the 8th rib by putting the slices into plastic net bags which were placed for 24 h freely hanging inside of a pre-weighed polyethylene bag in a refrigerator at 4°C. The polyethylene bag was then weighed together with the exudate. For thawing and cooking loss, 2.5 cm thick slices from the 13th and the 14th rib were used. The samples were weighed, put into polyethylene bags and frozen at -20°C until the analysis was performed after thawing for 24 h at 4°C. Then the samples were dried with soft paper and weighed before being put into vacuumised heat resistant plastic bags for boiling at 80°C until an internal temperature of 72°C was reached. Samples were allowed to cool at ambient temperature for 30 min and then weighed again. From each cooked sample, six cores with a diameter of 12.7 mm and a length of 20 to 22 mm were obtained. Shear force was determined using a Warner-Bratzler shear device attached to an Instron universal testing machine (model 5565, Instron Ltd., Buckinghamshire, UK). A crosshead speed of 200 mm/min and a 5 kN load cell calibrated to read over the range of 0 to 50 N were applied for that purpose.

Fat firmness was determined as outlined by Jaturasitha et al. (1996) in molten backfat of which 10 ml were filled into 15 ml glass vessels. Fat was allowed to congeal for 45 min at room temperature, and then was kept at -18°C until analysis. Prior to firmness measurements, the samples were temperated in a water bath at 4°C for 30 min. Penetrating force was measured by a cylindrically shaped stainless steel penetrometer stick (diameter of 5 mm) attached to the Instron machine. Penetrometer depth was set to two thirds of total fat depth. Different from Warner-Bratzler shear force measurements, a 100 N load cell was applied which was calibrated for the range of 0 to 10 N.

Feed, LD (homogenised from the 11th and the 12th rib without connective tissue and tendon) and bacon (homogenised from the 11th and the 12th rib) were analysed for contents of dry matter, protein and ether extract as outlined in AOAC (1990). Feed was additionally analysed for contents of total ash, neutral detergent fibre (NDF) and gross energy (adiabatic bomb calorimetry, Janke & Kunkel GmbH & Co, Staufen, Germany) according to AOAC (1990). Bacon was further subjected to analysis of NaCl, phosphate, nitrate and nitrite according to methods outlined by AOAC (1995). Cholesterol concentrations were determined in LD, bacon and backfat after extraction of the fat from the tissues according to Folch et al. (1957). The extracted fat was saponified as described by Abell et al. (1951) in order to eliminate triglycerides. In the extracted and saponified fat total cholesterol was determined in the residual extract according to Jung et al. (1975), similar as in blood plasma. In the extracted but not saponified fat from the tissues also triglyceride contents were measured as outlined by Biggs et al. (1975).

The fatty acid composition of feed, LD, bacon and backfat was analyzed by the method of Folch et al. (1957).

Fat from tissues was extracted by chloroform and methanol (2:1 v/v) whereas fat from feed was extracted by a Soxhlet apparatus. Methyl esters were prepared by the method of Morrison and Smith (1964). Analysis of fatty acid methyl esters was accomplished with a gas chromatograph (model GC-14B, Shimadzu, Tokyo, Japan) equipped with a 0.25 mm×30 m×0.25 μm wall-coated fused wax capillary column. The temperature of the oven was programmed with an initial temperature of 220°C, held for 10 min, and a final temperature of 230°C, held for 13 min. The temperature was increased at a rate of 5°C/min. The injector and the detector temperatures were 250°C and 280°C, respectively. Helium was used as carrier gas, and flow rate was 1 ml/min when measured at the outlet terminal. Split ratio of injector was approximately 1:50. Eluting peaks were identified by comparison with retention time of known standards (purified fatty acids, GL Science, Tokyo, Japan).

Thiobarbituric acid (TBA) number was analyzed in LD, bacon and backfat stored at 4°C after being kept at –18°C and defrozen at refrigerator temperature during 12 h. TBA number was determined as malondialdehyde by the Pearson method (Rossell, 1994). Storage periods were 0, 5, 10 and 15 d for LD, 0, 3, 6, 9 and 12 d for bacon and 0, 3, 6 and 9 d for backfat.

Sensory assessments

LD and bacon (not subjected to prior storage at 4°C) were evaluated by a sensory panel. Slices of LD, 2.5 cm thick, were obtained from the 14th and the 15th rib and were used without connective tissue and tendon. Bacon was cut to 3 mm slices. Both were roasted in a convector oven at 200°C for 10 min (equivalent to an internal temperature of about 70°C in LD), then cut into small pieces (1.25× 1.25 cm for LD, 1×1 cm for bacon) and served on pre-warmed plates. Six panelists (three male, three female) judged samples twice a week by the facial hedonic method, and each time they evaluated eight samples. The panelists had been selected by training and testing their perception of differences in meat taste as outlined by Viriyajari (1992). Panelists had to score flavour, juiciness and texture/tenderness, and to judge their overall level of acceptance. For all variables a scale from 1 to 5 was used, providing specific attributes for the respective variables to be tested. Additionally, panelist were advised to record any impression of off-flavour of meat.

Statistical analysis

All data obtained were subjected to analysis of variance using SPSS for Windows considering dietary treatment as effect. For the blood plasma variables, in a separate analysis of variance, also diet, live-weight class of the pigs and the interaction of both were included. Comparisons among dietary treatment means were carried out by the Tukey procedure. The tables give the least square mean values for the dietary treatments, the corresponding standard errors of the means (SEM) and probabilities of error (p-level).

RESULTS AND DISCUSSION

Effects on growth and slaughter performance

In the present study, the fattening period had been sub-divided into a starting and a finishing period, where also different basal diets were fed. Pigs did not significantly differ in initial live-weight, daily gains, feed intake and conversion ratio both within the sub-periods of fattening as well as over the whole period (Table 2). There were weak tendencies for decreased daily gains in the period from 60 to 90 kg of live-weight with any inclusion of tuna oil into the diet, accompanied by a higher intake of metabolizable energy. This illustrates that tuna oil had no adverse effects on growth rate of the pigs but also that the extra energy provided by the lipids of the tuna oil was not converted into a net increase in body mass accretion and did not result in a more favourable feed conversion ratio, either. The results suggest that this probably was not simply reflecting the fact that the maximum growth rate was already reached with the unsupplemented control diet or that there were palatability problems with the supplemented diet. Pongpiachan (1996) even claimed that oil supplementation at an appropriate level would improve palatability of animal feed and hence weight gain in animals. However, no effects on growth performance of growing pigs were also found with mackerel oil supplementation by Ruiter et al. (1978). Finishing pigs, by contrast, repeatedly expressed lower growth rates when receiving canola oil with (Leskanich et al., 1997) or without (Myer et al., 1992; Warnants et al., 1996; Soler-Velasquez et al., 1998) fish oil. Economic calculations based on the present results considering feed conversion efficiency and costs valid in Thailand in 2002 demonstrate that, due to the expensive tuna oil, the respective feed costs per unit of gain will be 17.4, 19.9, 22.4 and 23.3 Baht for the four treatment groups, i.e. 1.14, 1.29 and 1.34 times higher with 1, 2 and 3% tuna oil in the diet than when feeding the control diet. Total feed costs per pig will add up to 1043, 1198, 1343 and 1400 Baht for the diets containing 0, 1, 2 and 3% tuna oil.

Similar to growth performance, effects of tuna oil on carcass quality were mostly not statistically significant. Chilled carcass weights (24 h at 4°C) were around 70 kg, equivalent to an average dressing percentage of 78%, with the hot carcass weights (i.e., 1 h p.m.) being greater by 2.4 kg on average. The average carcass length was 75.7 cm and did not differ between treatments. There were certain numerical differences between groups in most carcass traits, but these non-significant changes also were not always systematic with respect to the level of tuna oil supplementation. If any, data indicates a slightly higher fatness of carcasses (lean percentage) and carcass parts

Table 2. Effect of tuna oil supplementation on performance and carcass quality

Tuna oil supplementation (%)	0	1	2	3	SEM	p-level
Performance						
Initial live-weight (kg)	30.3	30.4	30.5	31.3	0.25	0.535
Final live-weight (kg)	90.7	90.4	90.5	90.2	0.05	0.079
Days on feed	74.8	73.7	77.7	77.6	1.95	0.854
Average daily gains (kg)						
30 to 60 kg live-weight	0.71	0.77	0.74	0.73	0.018	0.687
60 to 90 kg live-weight	0.89	0.87	0.85	0.81	0.029	0.836
30 to 90 kg live-weight	0.81	0.82	0.79	0.78	0.021	0.898
Feed intake (kg/d; as fed)						
30 to 60 kg live-weight	1.79	1.90	1.82	1.82	0.029	0.591
60 to 90 kg live-weight	2.67	2.78	2.79	2.57	0.069	0.655
30 to 90 kg live-weight	2.20	2.32	2.27	2.16	0.039	0.535
ME intake (Mcal/d)	5.80	6.31	6.25	6.27	0.111	0.363
Feed conversion ratio (kg/kg gain)	2.74	2.83	2.90	2.77	0.050	0.680
Carcass quality						
Hot carcass weight (kg)	71.9	71.8	74.2	76.0	0.50	0.143
Chilled carcass weight (kg)	69.0	69.3	71.9	69.3	0.50	0.145
Dressing percentage	77.6	76.8	78.8	78.0	0.30	0.145
Carcass length (cm)	74.8	76.1	75.9	76.0	0.30	0.145
Lean percentage	62.2	62.0	61.5	57.7	0.98	0.339
Average backfat thickness (cm)	2.19	2.54	2.48	2.45	0.056	0.130
Loin eye area (cm^2)	46.5	47.0	44.0	42.0	0.84	0.123
Loin chop composition (%)						
Lean tissue	65.6	65.3	65.5	59.6	1.15	0.170
Fat tissue	14.7	15.7	16.8	18.7	0.79	0.309
Bone	14.0	14.5	12.9	16.4	0.55	0.176
Skin	5.7	4.6	4.8	5.3	0.22	0.276

(backfat thickness, loin eye area, loin chop composition) at cost of muscle accretion of the pigs receiving the highest level of tuna oil. Accordingly, the lean to fat tissue ratio in the loin chop declined from 4.5:1 without supplementation to 3.2:1 with 3% tuna oil ($p<0.05$). Although not apparent from daily gains, this shift would indicate that the extra energy supply by tuna oil was indeed used to increase body fat synthesis. In other pig fattening experiments, neither fish oil (Leskanich et al., 1997) nor plant oil (Miller et al., 1990) or whole oilseed supplementation (canola seed, Busboom et al., 1991 and Myer et al., 1992; linseed, Romans et al., 1995) had significant effects on carcass quality whereas the use of animal fat was found to clearly increase backfat thickness in the study of Miller et al. (1990).

Effects on blood plasma levels of lipoproteins

Baseline levels of cholesterol-related traits measured in blood plasma before the start of treatment feeding at 30 kg of live-weight involuntarily differed between the later experimental groups in LDL cholesterol, but not sytstematically with respect to the tuna oil groups (Table 3). The supplementation of tuna oil was found to reduce plasma VLDL cholesterol and triglycerides concentration ($p<0.05$) at 60 and 90 kg live-weight, respectively, whereas this was only apparent as a non-significant trend in total and LDL cholesterol. Plasma HDL cholesterol remained unaffected by tuna oil. Also the ratios of HDL and LDL cholesterol to total cholesterol did not differ significantly. Pigs resemble humans in lipid metabolism and their response to dietary lipids thus is an appropriate model to simulate effects in humans (discussed in Kreuzer et al., 2002). Accordingly, our results confirm the basically favourable effects of dietary fish oil supplements also found in human blood plasma (e.g., Hamazaki et al., 1996). However, the results are in contrast to some other reports (Fehily et al., 1983; Layne et al., 1996), particularly concerning the variation in plasma triglyceride concentration. Also other dietary lipids are effective in modifying plasma lipid profile of pigs. Soybean oil and coconut oil were found to have favourable and unfavourable effects, respectively, on lipid fractions in blood plasma of pigs relative to an unsupplemented control (Kreuzer et al., 1997), and there were interactions of fats with dietary fibre in cholesterol-related traits (Kreuzer et al., 2002). Li et al. (1999) reported a different response of blood cholesterol level to soybean oil and to lard supplementation in piglets.

The present results also showed significant age-dependent changes in plasma cholesterol levels of the pigs except in HDL cholesterol (Table 3). Total cholesterol was 1.6-fold of initial when pigs reached 90 kg of live-weight. The corresponding changes for LDL and VLDL cholesterol as well as triglycerides were 3.1-fold, 0.8-fold and 0.8-fold

Table 3. Effect of tuna oil supplementation on cholesterol-related blood plasma variables (mg/100 ml)[1]

Tuna oil supplementation (%)	0	1	2	3	Avg.	SEM	p-level		
							Tuna oil	Live-weight	Inter-action
Total cholesterol							0.045	<0.001	0.572
30 kg live-weight	55.0	61.2	47.4	45.1	52.2^{y}	2.36	0.139		
60 kg live-weight	63.2	56.2	53.6	53.6	56.6^{y}	2.20	0.234		
90 kg live-weight	85.2	80.3	82.3	76.1	81.0^{z}	2.20	0.557		
HDL cholesterol							0.664	0.375	0.522
30 kg live-weight	22.2	26.1	29.1	25.8	25.8	2.10	0.841		
60 kg live-weight	25.6	27.0	21.7	29.6	26.0	1.73	0.504		
90 kg live-weight	26.3	26.9	33.2	29.2	28.9	1.65	0.401		
LDL cholesterol							0.691	<0.001	0.376
30 kg live-weight	9.4^{b}	12.2^{a}	12.0^{a}	9.6^{b}	10.8^{y}	2.07	0.020		
60 kg live-weight	13.2	12.9	18.0	11.9	14.0^{y}	1.74	0.390		
90 kg live-weight	39.4	36.0	29.5	29.8	33.7^{z}	1.58	0.352		
VLDL cholesterol							0.017	<0.001	0.370
30 kg live-weight	21.9	20.2	22.2	20.5	21.2^{z}	0.43	0.450		
60 kg live-weight	20.7^{a}	19.1^{a}	17.4^{b}	18.8^{ab}	19.0^{y}	0.41	0.022		
90 kg live-weight	19.5^{a}	17.4^{a}	17.0^{b}	17.2^{ab}	17.8^{y}	0.40	0.084		
HDL : total cholesterol							0.459	0.005	0.666
30 kg live-weight	0.45	0.39	0.49	0.51	0.46^{z}	0.027	0.763		
60 kg live-weight	0.44	0.48	0.42	0.48	0.45^{z}	0.023	0.754		
90 kg live-weight	0.31	0.35	0.40	0.38	0.36^{y}	0.023	0.214		
LDL : total cholesterol							0.792	<0.001	0.464
30 kg live-weight	0.18^{b}	0.28^{a}	0.24^{a}	0.23^{a}	0.23^{y}	0.026	0.040		
60 kg live-weight	0.24	0.22	0.31	0.21	0.25^{y}	0.022	0.483		
90 kg live-weight	0.45	0.43	0.39	0.39	0.42^{z}	0.020	0.486		
Triglycerides							0.091	<0.001	0.291
30 kg live-weight	107.8	100.7	111.1	102.6	105.6^{z}	2.12	0.450		
60 kg live-weight	103.4^{a}	95.3^{ab}	87.1^{b}	94.3^{ab}	95.0^{y}	1.97	0.022		
90 kg live-weight	93.3^{a}	87.1^{ab}	85.0^{b}	85.9^{ab}	85.9^{x}	1.19	0.084		

[1] Means within variable and live-weight class as well as average means of live-weight classes carrying no common superscripts (a-b and x-z, respectively) are significantly different at $p<0.05$.

of initial, respectively. With total and LDL cholesterol the major changes took place in the finisher period of fattening, with VLDL cholesterol in the starter period. Triglycerides permanently declined during fattening. In contrast to the present findings, Falkenberg et al. (1995) described a decrease of total, LDL and HDL plasma cholesterol in pigs with age. Generally, the shifts illustrate important changes in blood lipid profile with age.

Effects on physical properties of pork

Meat quality traits were mainly assessed in the M. *longissimus dorsi* (LD; Table 4). In all groups early- and late-post mortem pH and electric conductivity were in the range preferred, indicating a very low incidence pork of undesired pale, soft and exudative (PSE) as well as dark, firm and dry (DFD) appearance. Tuna oil supplementation had no significant effect on these traits and on LD colour as well as various traits of water-holding capacity, including sensory perception of juiciness. Also tenderness of LD, estimated by the Warner-Bratzler shear force and determined in the sensory test, did not respond to tuna oil supplementation. By contrast, texture and colour appearance of bacon was scored lower with the supplementation of tuna oil but it may be suspected that this impression was confounded with flavour perception changes which will be discussed later.

Lipid supplementation is generally known to have only small effects on physical meat quality. Accordingly, effects of a combined fish oil and canola oil supplementation was ineffective in the study of Leskanich et al. (1997). Miller et al. (1990) reported that high levels of safflower oil, sunflower oil, canola oil and animal fat did not change thawing loss and cooking loss of LD. Van Oeckel et al. (1996) isoenergetically supplied linseed at 1.9, 3.7 and 5.4% in feed which also had no effect on physical meat quality (pH, electric conductivity), except that a brighter colour occurred. Soybean oil and coconut oil were without clear effect on the same traits investigated here (Kreuzer et al., 1997). Finally, full-fat soybeans or oilseeds were found to be without effect on physical meat quality traits (St. John et al., 1987; Busboom et al., 1991; Leszczynski et al., 1992; Myer et al., 1992).

Table 4. Effect of tuna oil supplementation on physical properties of the M. *longissimus dorsi* (LD) and sensory evaluation of LD and bacon[1]

Tuna oil supplementation (%)	0	1	2	3	SEM	p-level
pH						
45 min p.m.	6.37	6.40	6.28	6.21	0.053	0.580
24 h p.m. (ultimate)	5.55	5.51	5.56	5.51	0.019	0.755
Electric conductivity (24 h p.m.)	3.05	3.20	3.22	2.88	0.330	0.985
Colour traits (24 h p.m.)						
Luminosity (L)	57.5	58.3	57.7	56.1	0.58	0.601
Redness (a)	9.1	9.7	9.2	9.7	0.29	0.817
Yellowness (b)	4.9	5.6	5.2	5.0	0.41	0.957
Water-holding capacity						
Drip loss (%)	6.7	8.6	6.7	8.6	0.77	0.712
Thawing loss (%)	15.5	15.6	15.7	13.9	0.84	0.857
Cooking loss (%)	22.4	21.3	22.4	25.3	0.74	0.280
Warner-Bratzler shear force (N)	34.1	35.3	36.7	38.5	0.10	0.400
Sensory evaluation scores[2]						
LD						
Tenderness	4.10	3.68	3.85	3.68	0.062	0.045
Juiciness	3.78	3.56	3.51	3.56	0.056	0.351
Flavour	3.71	3.36	3.33	3.31	0.057	0.042
Overall acceptance	3.95	3.58	3.60	3.60	0.056	0.057
Bacon						
Texture	3.76^{a}	3.53^{ab}	3.44^{b}	3.47^{b}	0.033	<0.001
Colour	3.73^{a}	3.34^{b}	3.37^{b}	3.43^{b}	0.036	<0.001
Flavour	3.63^{a}	3.42^{ab}	3.36^{ab}	3.31^{b}	0.038	0.001
Overall acceptance	3.70^{a}	3.39^{b}	3.34^{b}	3.28^{b}	0.035	<0.001

[1] Means carrying no common superscripts are significantly different at $p<0.05$.
[2] 1=low, 5=high (details see Materials and Methods).

Effects on ω-3 fatty acid content of pork

Tuna oil supplementation to feed substantially increased its content of ω-3 fatty acids from 1.5 to 8.1 g/kg finisher diet, and this particularly with C20:5 (EPA) and C22:6 (DHA) and, less so, with α-C18:3 (Table 1). The contents of C20:5 and C22:6 achieved with 3% tuna oil in the diet in LD (Table 5), backfat (Table 6) and bacon (Table 7) were 0.1 and 0.2 g/kg, 2.6 and 12.6 g/kg, and 1.3 and 9.2 g/kg, respectively. Without tuna oil supplementation, these two fatty acids were below the detection level and ranged intermediary with 1 and 2% tuna oil in the diet. This confirms that these fatty acids exclusively came from dietary intake. The increase in proportion of total fatty acids was at least partially counterbalanced by a decrease in the proportion of the ω-6 fatty acids (significant in bacon) and therefore resulted in a clearly improved ω-6:ω-3 ratio in all body tissues analysed.

From a dietetical point of view, a daily intake of 15 g ω-3 fatty acids with >3 double bonds is recommended for humans (Wolfram, 1989), and the present reults show that pork from tuna oil fed pigs can substantially contribute to this intake. Basically, meat can be enriched with ω-3 fatty acids through feeding fish oil (Irie and Sakimoto, 1992), fish meal with high residual oil content (Lysø and Astrup, 1987; Hulan et al., 1989; Huang et al., 1990), linseed (Romans et al., 1995) and, with lower efficacy, other plant oils such as rapeseed oil (Myer et al., 1992; Leskanich et al., 1997). However, plant oils do not contain C20:5 and C22:5 which are considered to be superior in prevention and treatment of coronary heart diseases to α-linoleic acid (C18:3) (Sahaphong, 1990). Tuna oil contains more C22:6 than C20:5. Both fatty acids are beneficial to human health. C22:6 is prevalent in brain and nervous tissues of infants; hence, C22:6 intake has an important role for the development of the brain and nervous system (McPherson and Spiller, 1996). Furthermore, C20:5 and C22:6 are precursor of prostacyclin, which can decrease platelet aggregation and velocity of blood in blood stream (Sahaphong, 1990). McPherson and Spiller (1996) reported that C20:5 is more effective than C22:6 in this respect. The first attempts to enrich land animal products with ω-3 fatty acids have been made with eggs in Australia (Farrell and Gibson, 1991). A high efficacy of transfer is proven for long (e.g., Sangsaraporn, 1994), and in some countries enriched eggs are currently marketed as health food.

Effects on other dietetic criteria of pork

Tuna oil changed the fatty acid profile not only with respect to ω-3 fatty acids but also in the proportion of other fatty acids, which was mostly not only a compensation of the altered concentration of ω-3 fatty acids, but particularly reflected the differences in the composition of the tuna oil compared to typical porcine fat. In bacon, with tuna oil every unsaturated fatty acid was significantly changed in its

Table 5. Effect of tuna oil supplementation on composition and TBA number of the LD[1]

Tuna oil supplementation (%)	0	1	2	3	SEM	p-level
Dry matter (%)	26.0	26.3	26.1	24.5	0.13	0.658
Protein (%)	21.3	21.1	21.1	21.4	0.10	0.696
Ether extract, (%)	1.66[b]	2.39[ab]	2.56[ab]	2.69[a]	0.141	0.043
Triglycerides (%)	1.97	1.81	1.70	1.71	0.087	0.685
Cholesterol (mg/100 g)	53.7	57.6	53.9	55.1	1.05	0.985
Fatty acids (FA, % of totally analysed FA)						
C16:0	30.94	31.47	30.42	31.00	0.217	0.421
C18:0	14.49	15.54	15.43	15.76	0.225	0.198
C20:0	0.48	0.37	0.41	0.44	0.019	0.337
C18:1	41.04	40.63	40.21	39.99	0.283	0.589
C18:2	8.81	7.81	8.73	7.97	0.233	0.311
C18:3 (ω-3)	1.66	1.85	1.98	2.00	0.090	0.540
C20:4	1.96[a]	1.16[b]	1.16[b]	1.12[b]	0.098	0.002
C20:5 (ω-3)	ND[2]	0.45	0.61	0.60	0.045	0.264
C22:6 (ω-3)	ND[2]	0.67[b]	1.23[ab]	1.30[a]	0.106	0.019
Total saturated FA	45.91	47.39	46.27	47.21	0.283	0.187
Total polyunsaturated FA	12.44	12.30	13.04	13.13	0.377	0.836
Total ω-3 FA	1.66[c]	2.82[b]	3.88[a]	3.94[a]	0.204	<0.001
Total ω-6 FA	10.77	8.97	9.89	9.09	0.301	0.123
ω-6:ω-3 ratio	6.57[a]	3.25[b]	2.98[b]	2.57[b]	0.313	<0.001
TBA number (mg malondialdehyde/kg)						
0 days of storage	0.11	0.14	0.16	0.16	0.012	0.569
5 days of storage	0.13	0.15	0.19	0.23	0.023	0.481
10 days of storage	0.17	0.23	0.24	0.29	0.020	0.239
15 days of storage	0.18[b]	0.24[b]	0.28[ab]	0.57[a]	0.044	0.006

[1] Means carrying no common superscripts are significantly different at p<0.05.
[2] ND=Non detected.

proportion of total fatty acids (Table 7). This was less pronounced in LD (Table 5) and backfat (Table 6). Generally, tuna oil supplementation decreased C18:1 and C18:2 (not significant in LD). However, the overall changes caused by tuna oil supplementation on the proportions of the major fatty acids in body fat were small in extent compared to studies supplementing other dietary fats to pigs (e.g., Jaturasitha et al., 1996; Kreuzer et al., 1997), and even in a shorter experiment with piglets changes in carcass fat composition were greater (Bosi et al., 2000).

Concentrations of major nutrients and of some dietetically undesired compounds in LD, backfat and bacon are given in Tables 5, 6 and 7. With few exceptions, effects of tuna oil on contents of dry matter, protein, lipids and cholesterol were low, with the typical differences between tissues being found. With increasing tuna oil supplementation, intramuscular (LD) fat content increased. This was balanced by a decreased water content at a relatively unchanged protein content. Bacon, instead, showed a declining protein content with tuna oil addition, without a corresponding increase in lipid content. As tuna oil also had only weak effects on carcass fatness, it can be assumed that the shifts in intramuscular fat either reflected a specific effect of tuna oil (or even of the ω-3 fatty acids supplied by tuna oil) or randomly occurred through unintended genetically-based differences. Any direct effect of the increased dietary energy density through tuna oil supplementation would probably have affected fat accretion in most body tissues to a comparable extent. Different from blood plasma cholesterol, pigs did not respond to tuna oil in cholesterol concentration of any of the tissues investigated. The same observation has been made previously with fat supplementation to pigs (Kreuzer et al., 1997) illustrating that the baseline level of cholesterol in tissue is difficult to change. Retention in the bacon of sodium, phosphate, nitrate and nitrite, which are essential for the typical taste and flavour of bacon but, at the same time, are dietetically undesired, was not affected by tuna oil in feed of the pigs from which the bacon was produced (Table 7).

Effects on flavour and processing quality of pork

Flavour scores of LD and bacon gradually and significantly decreased with the addition of tuna oil to the diet (Table 4), similar to the finding of Leskanich et al. (1997) who described abnormal flavour in pork from pigs fed with a source rich in ω-3 fatty acids. No explicit statements concerning off-flavours were given by the panellists in the present study, but it can be assumed that these were masked in the general perception of a somewhat impaired flavour of LD and bacon. The results furthermore suggest that higher tuna oil doses are more detrimental to flavour scoring than low dietary proportions, although it has

Table 6. Effect of tuna oil supplementation on backfat properties[1]

Tuna oil supplementation (%)	0	1	2	3	SEM	p-level
Penetrometer force (mN)	627	805	472	534	67.3	0.334
Colour traits (24 h p.m.)						
Luminosity (L)	77.3	77.9	78.3	76.6	0.33	0.326
Redness (a)	7.1	7.1	6.9	7.6	0.33	0.892
Yellowness (b)	4.4	3.9	4.6	4.6	0.37	0.904
Triglycerides (g/100 g)	64.8	59.8	59.5	58.5	1.20	0.271
Cholesterol (mg/100 g)	61.1	57.8	57.2	56.9	1.17	0.561
FA (% of totally analysed FA)						
C16:0	27.29	27.81	27.89	27.00	0.164	0.174
C18:0	16.22	17.30	16.17	16.53	0.205	0.189
C20:0	0.41	0.43	0.48	0.48	0.016	0.381
C18:1	39.10^{a}	37.33^{b}	38.03^{ab}	37.42^{b}	0.198	0.004
C18:2	13.33	12.11	12.10	12.37	0.184	0.055
C18:3 (ω-3)	3.08	3.07	3.13	3.18	0.061	0.922
C20:4	0.21^{b}	0.31^{ab}	0.33^{ab}	0.37^{a}	0.018	0.014
C20:5 (ω-3)	ND[2]	0.28^{b}	0.34^{a}	0.45^{a}	0.021	0.002
C22:6 (ω-3)	ND[2]	1.33^{b}	1.50^{a}	2.16^{a}	0.127	0.016
Total saturated FA	43.92	45.54	44.55	44.02	0.312	0.242
Total polyunsaturated FA	16.96	17.11	17.41	18.54	0.292	0.121
Total ω-3 FA	3.08^{c}	4.69^{b}	4.97^{ab}	5.80^{a}	0.174	<0.001
Total ω-6 FA	13.54	12.42	12.43	12.74	0.189	0.119
ω-6:ω-3 ratio	4.41^{a}	2.81^{b}	2.59^{bc}	2.27^{c}	0.108	<0.001
TBA number (mg malondialdehyde/kg)						
0 days of storage	0.48	0.81	0.90	0.14	0.129	0.065
3 days of storage	1.12	2.23	2.29	2.34	0.222	0.231
6 days of storage	1.14	2.30	2.43	3.23	0.286	0.071
9 days of storage	1.33^{b}	2.55^{ab}	2.82^{ab}	3.61^{a}	0.282	0.031

[1] Means carrying no common superscripts are significantly different at $p<0.05$.
[2] ND=Non detected.

to be stated the levels of decline in grading were generally not very high. This illustrates that a certain level of tuna oil might be still tolerable from a sensory point of view. Overall sensory acceptance appears to be closely related to flavour scoring, thus being the overriding factor for acceptance. This seems reasonable since texture-related traits are not of very high importance in pork.

Shelf life and fat firmness as well as its appearance are important quality traits for processing of meat. Fat shelf life was measured by following TBA number in LD, backfat and bacon during varying storage periods at 4°C (Tables 5, 6 and 7). In all tissues investigated, tuna oil caused a significant increase in TBA number, as was also noted in pork from pigs fed rancid rice bran (Chae and Lee, 2002), and there was a clear dose-response relationship. In LD and backfat significant effects did not occur before the last measurement period was reached after 15 d and 9 d of storage, respectively, but weak tendencies were already observed earlier. In bacon the tuna oil effects on TBA number were significant from the beginning and both, absolute levels and treatment group differences, did not increase very much from then on. This illustrates that, other than in LD and backfat, fat oxidation in bacon had already occurred at the time when the storage experiment was started, either because the maximum level of oxidation possible was already reached along with curing and smoking or because the procedures applied during processing had a conserving effect. Particularly nitrite, which was added when curing the bacon, was found to have remarkable antioxidative properties in pork (Han and Yamauchi, 2000). This may explain why the TBA numbers in the bacon were similar in level to that in LD despite its far higher fat content. The TBA numbers on the respective final days of storage had been increased by 3% tuna oil to similar extents in all tissues (by the 3.2-, 2.7- and 3.5-fold of the levels of control LD, backfat and bacon). Fatty fish meal also clearly enhanced the oxidation of pork in the study of Lysø and Astrup (1987). Similar to the tuna oil in the present study, 4% soybean oil in the diet massively reduced shelf life of backfat and belly fat in pigs as assessed by the Rancimat induction period in another study (Kreuzer et al., 1997). The inclination of unsaturated fatty acids to oxidation exponentially increases with their number of double bonds, which explains why ω-3 fatty acids incorporated into pork are highly susceptible to oxidation. The findings concerning fat shelf life are therefore in line with the effects on sensory flavour scoring confirming that these were at least partially caused by oxidative off-flavours.

Tuna oil supplementation did not systematically affect penetrometer firmness of backfat (Table 6). This was not

Table 7. Effect of tuna oil supplementation on composition and TBA number of the bacon[1]

Tuna oil supplementation (%)	0	1	2	3	SEM	p-level
Dry matter (%)	48.2	44.2	47.0	44.2	0.73	0.133
Protein (%)	16.6^{a}	15.5^{ab}	14.6^{b}	14.0^{b}	0.26	0.001
Ether extract, (%)	33.7	38.5	36.0	38.9	0.94	0.192
Triglycerides (%)	27.1	31.2	25.9	25.3	1.13	0.260
Cholesterol (mg/100 g)	60.5	53.6	53.9	56.3	1.53	0.386
Sodium chloride (g/kg)	1.16	0.98	1.15	1.08	0.038	0.349
Phosphate (g/kg)	4.10	3.57	3.96	3.80	0.110	0.381
Nitrate (mg/kg)	50.6	54.9	59.2	56.8	3.71	0.885
Nitrite (mg/kg)	7.87	5.80	5.82	7.08	0.743	0.729
FA (% of totally analysed FA)						
C16:0	26.27	26.52	25.81	26.10	0.146	0.365
C18:0	16.21	17.49	16.21	16.30	0.216	0.100
C20:0	0.37^{b}	0.42^{ab}	0.41^{ab}	0.44^{a}	0.007	0.011
C18:1	39.58^{a}	38.08^{ab}	37.73^{b}	36.95^{b}	0.242	0.001
C18:2	13.44^{a}	11.58^{b}	12.42^{ab}	11.94^{b}	0.164	<0.001
C18:3 (ω-3)	3.38	3.18	3.61	3.46	0.065	0.133
C20:4	0.56^{ab}	0.48^{b}	0.57^{ab}	0.61^{a}	0.014	0.009
C20:5 (ω-3)	ND[2]	0.30^{c}	0.41^{b}	0.52^{a}	0.018	<0.001
C22:6 (ω-3)	ND[2]	1.91^{c}	2.79^{b}	3.64^{a}	0.159	<0.001
Total saturated FA	42.86	44.44	42.44	42.84	0.292	0.074
Total polyunsaturated FA	17.38^{b}	17.47^{b}	19.81^{a}	20.19^{a}	0.292	<0.001
Total ω-3 FA	3.53^{c}	5.40^{b}	6.82^{a}	7.64^{a}	0.227	<0.001
Total ω-6 FA	14.00^{a}	12.06^{b}	12.99^{ab}	12.55^{b}	0.173	<0.001
ω-6:ω-3 ratio	4.24^{a}	2.29^{b}	1.91^{c}	1.71^{c}	0.122	<0.001
TBA number (mg malondialdehyde/kg)						
0 days of storage	0.19^{c}	0.34^{bc}	0.45^{ab}	0.60^{a}	0.035	<0.001
3 days of storage	0.18^{b}	0.39^{ab}	0.44^{a}	0.60^{a}	0.037	<0.001
6 days of storage	0.19^{c}	0.34^{bc}	0.45^{b}	0.62^{a}	0.034	<0.001
9 days of storage	0.19^{c}	0.36^{bc}	0.49^{ab}	0.64^{a}	0.038	<0.001
12 days of storage	0.19^{c}	0.36^{bc}	0.53^{ab}	0.67^{a}	0.041	<0.001

[1] Means carrying no common superscripts are significantly different at $p<0.05$.
[2] ND=Non detected.

expected as 4% of soybean oil were found to make backfat softer by a factor of 6 relative to a low-fat control diet, whereas coconut oil even further increased backfat firmness (Jaturasitha et al., 1996; Kreuzer et al., 1997). However in the present study, tuna oil had a far smaller effect on the proportions of the major saturated and unsaturated fatty acids than soybean oil in the experiment of Kreuzer et al. (1997). It is also important to note that tuna oil did not change the colour properties of the backfat (Table 6), which would impair purchase decision in case of a darker and, especially, a more yellow appearance.

IMPLICATIONS

From the present results it appears that tuna oil supplementation to pig diets is basically a promising way to enrich pork with ω-3 fatty acids. The results on TBA number indicate that pork from pigs fed elevated levels of tuna oil should be consumed very rapidly and are only suitable for processing to products with long assumed shelf-life when potent antioxidative compounds such as nitrite are added. Other side-effects of tuna oil on performance, carcass and meat quality were low. As there was a dose-response relationship between dietary tuna oil level and desired respectively undesired effects, an assessment of the level of tuna oil recommended to farmers by extension or marketing organisations has to be made for each individual situation, also taking into consideration the high price of tuna oil. This disadvantage demands specific marketing strategies and markets frequented particularly by health-concerned consumers or coronary-heart disease susceptible clients.

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삭제됨:

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