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Production of Docosahexaenoic Acid (DHA) Enriched Bacon

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North American consumers interested in improving their health through diet perceive red meat as a source of too much saturated and unhealthy fat in the diet. The purpose of this trial was to produce bacon enriched with the long-chain omega-3 fatty acid, docosahexaenoic acid (DHA). In this 25 day study, pigs were fed a standard finisher diet of canola, pea, corn, and barley, mixed with DHA, added in the form of alga biomass. Bacon content of DHA was increased to 97 mg/100 g when 1 g of DHA was added to a kilogram of feed. The pigs fed the highest diet level of alga biomass, containing 0.29% DHA, produced bacon with ~3.4 mg of DHA/g and 1.2% of the fat as omega-3 fatty acids. Feed to gain was significantly improved, and carcass quality was unaffected. However, problems of off-odors and off-flavors were reported in the bacon from the taste panel survey. Polyunsaturated fat and potential unsaturated fat oxidation as indicated by malonaldehyde levels were significantly higher in the pigs fed the higher concentrations of DHA.

KEYWORDS: Docosahexaenoic acid; bacon; alga biomass; TBARS; taste survey

INTRODUCTION

The essential dietary fats required by humans are linoleic acid (18:2n-6; LA) and α-linolenic acid (18:3n-3; ALA), which are 18-carbon chains with unsaturated double bonds starting at the sixth (omega-6) or third (omega-3) carbon from the methyl end. A balanced diet should have a ratio of ~5:1 omega-6/omega-3 unsaturated fat. The typical Western diet is closer to 16:1 (*1*, *2*). To counteract this dietary imbalance, food retailers are fortifying their products with oils rich in omega-3 fatty acids. Pork products can be enriched with omega-3 fatty acids (mainly ALA) by feeding oilseeds such as flax or canola. However, oilseeds provide only ALA, and nutritional studies have demonstrated that humans may also require the long-chain omega-3 fats eicosapentaenoic acid (EPA, 20:5n-3) and, especially, docosahexaenoic acid (DHA, 22:6n-3) for proper brain development (*3*), cardiovascular health (*4*), and immune function (*5*). Humans and pigs can convert some ALA to EPA and DHA but only at a low rate, < 10% (*5*,*6*). Dietary sources of long-chain n-3 fatty acids (EPA and DHA) are often obtained by eating cold-water marine fish. Health Canada and the U.S. Department of Health recommend that the average adult consume approximately 300 mg of long-chain omega-3 fats including DHA daily, but most North Americans rarely eat fish such as salmon (*2*). Bacon from pigs fed standard corn-based diets typically has an omega-3 to omega-6 ratio of approximately 17:1, with DHA content ranging from 1 to 4 mg/100 g (*27*). Supplementing pig diets with 15% crushed flax seed, which is high in α-linolenic (C18:3n-3), can improve the omega-6/omega-3 ratio to 5:1, but the DHA content remains relatively unchanged at approximately 5.5 mg/100 g (*7*). Long-chain omega-3 fatty acids EPA and DHA can be selectively increased in pork by feeding fish oil and fish meal byproduct (*8*). However, problems of abnormal flavors occur when the oil is added above 1% in feed, and there are concerns of environmental contaminants if the fish byproduct are not properly tested (*9*).

The problems of off-odors associated with adding extra omega-3 polyunsaturated fats are believed to be due to the polyunsaturated fat's (PUFA) susceptibility to oxidation (*10*). Feeding trials with alternate lipid sources such as lamb tallow also caused abnormal flavors (*11*). In response, it is recommended that the total amount of PUFA in a swine diet be kept below 25 g/kg of feed and include antioxidants such as vitamin E and selenium, which is a cofactor for glutathione peroxidase (*12*).

This research was performed to selectively increase the content of the long-chain omega-3 (DHA) in pork while trying to maintain a lower overall content of polyunsaturated fat to help prevent lipid oxidation and off-flavours. Pigs were fed three diets, enriched with increasing levels of DHA from microalgae *Schizochytrium* (Martek Biosciences). Microalga consumption is the main source of long-chain omega-3 fatty acids in most fish (*13*). Previous trials feeding up to 0.5% microalga DHA at different stages of production have reported significant improvements in ham and loin content of DHA, up to 70 mg/per 100 g serving (*14*,*15*). This trial investigated the effect of DHA enrichment on bacon sensory qualities and to determine a feed level required to achieve a 100 mg/serving dose. Fatty acid profiles, meat color, oxidation potentials, and texture of the DHA-enriched bacon were measured along with taste panel evaluations.

MATERIALS AND METHODS

Chemicals. Freeze-dried *Schizochytrium* microalga biomass from monoculture was purchased from Martek Biosciences Corp. 2,2′-Azobis-(2-amidinopropane) dihydrochloride (AAPH) was purchased from from

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Table 1. Approximate Composition of Experimental Mash Diets (Kilograms per Tonne) with the Estimated Final Contents of Vitamin E, Selenium, and DHA Lipid

diet	low	medium	high
dried marine algae	0.6	6.6	16
corn meal	350	350	350
pea meal	251	251	251
barley meal[a]	225	220	210
canola meal	168	168	168
finisher PMX + vitamins[b]	2.5	2.5	2.5
alfalfa/canola oil	2	2	2
vitamin E (IU/kg)	100	100	100
selenium (mg/kg)	0.5	0.5	0.5
estimated DHA (g/kg)	0.108	1.08	2.88

[a] Barley meal content adjusted to ±2.0 kg. [b] Finisher PMX vitamins: zinc, 5450 mg/kg; copper, 660 mg/kg; vitamin A, 367,200 IU/kg; vitamin D3, 40,800 IU/kg; iron, 5280 mg/kg; manganese, 2730 mg/kg, in a wheat barely soybean meal mix with 21.7% calcium.

Wako Chemicals USA, Inc. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and flourescein (disodium salt) were from Sigma-Aldrich Chemicals. Randomly methylated β-cyclodextrin (Trappsol; RMDC) was purchased from CTD Inc. (High Springs, FL).

Animal Feeding. Animals used in this study were cared for according to Canadian Council of Animal Care (*16*) guidelines. Barrows were selected from the Lacombe Research Center (LRC) F1 pig herd produced from Duroc/Large White mating (Hypor, SK). The pigs were genetically tested and determined not to carry the *RN*- or *Hal* gene mutations (*17*). Before the experimental diets were administered, pigs were fed the grower diet without DHA biomass according to NRC nutrient requirements (*18*) and included 35% corn, 25% peas, 19% barley, 17% canola, and vitamin premix including 100 IU/kg vitamin E and 0.5 mg/kg selenium. The barrows ($n=20$) were selected at 80 kg and then individually penned in two climate-controlled rooms at the LRC piggery for 24 h without food but free access to water. The near-isonitrogenous experimental diets (**Table 1**) were prepared by adjusting the barley content in the grower diet containing ~16% crude protein with the dried alga biomass (containing ~18% DHA and ~17% crude protein) at a commercial feed mill by the tonne (Wetaskiwin Co-op Feeds, AB). The prepared diets were stored in individual hoppers at the Lacombe piggery. The pigs were started on their experimental diets and fed ad libitum. Feed samples were collected from the hoppers for fatty acid analysis (**Table 2**). Pigs were fed for 25 days in three dietary groups including 0.06, 0.6, and 1.6% of microalga biomass and 100 IU/kg of tocopherol acetate. Once the animals reached ~110 kg they were transferred to the station abattoir for slaughter. Final weights were measured just prior to slaughter.

Carcass and Meat Quality Measures. Carcasses were split, weighed, and cooled for 24 h at 4 °C and then cut according to Canadian Meat Council guidelines (*19*) at the LRC. The backfat and loin depths were estimated using the Destron PG-100 probe (Markham, ON) at the Canadian grading site (between the third and fourth last rib, 7 cm off the midline), and percentage lean meat yield was estimated with the quadratic equation (*20*). The pH of the loin was determined at the rib probe site in the loin muscle after 24 h of cooling using a hand-held Fisher Scientific Accumet 1002 pH-meter equipped with a Xerolyte spear-type electrode (Ingold MesstechnikAG, Udorf, Switzerland). Meat color reflectance CIE L^* (brightness), a^* (red−green axis), and b^* (yellow−blue axis) was measured on the surface of a 2.5 cm loin chop cut from the probe site after a 20 min bloom using a Minolta CR-300 color meter (Minolta Canada Inc., Mississauga, ON) calibrated to a white tile. Backfat softness was measured on the second subcutaneous fat layer between the first and second thoracic vertebrae with a Rex LG2400 durometer (Rex Gauge Co., Inc., Buffalo Grove, IL) in accordance with specification standard D2240-05.

Preparation of Bellies for Bacon Process. The bellies were deboned and cut into (~35 × 48 cm) portions, labeled with permanent food grade ink, and refrigerated at 4 °C. To estimate firmness, the bellies were placed with the skin side down, over a 3 in. diameter bar for 2 min at 4 °C. The degree of bend was measured by the cosine angle 4.25 cm from the top center of the suspension tube to the bottom of the belly cut edge (**Figure 1**). Belly lean meat color was measured using a Minolta color meter from the dorsal cut site. Uncooked bellies were transported to a commercial packing plant (Innisfail Meats, Innisfail, AB) for processing into bacon. The bellies were injected with a commercial brine mixture to 115% of green weight and allowed to drain for 24 h at 4 °C. The commercial brine consisted of 1.5% salt, 0.3% sugar, 0.3% sodium phosphate, 0.055% sodium erythrobate, and 0.012% sodium nitrite. The injected bellies were hung by a bacon comb attached at the flank end and then heat processed with hickory smoke according to the commercial protocol of the plant. Following the smoking process, the bacon was rinsed with water for 10 min, drip-dried, pressed, and chilled overnight at 4 °C. The chilled bacon was transferred back to the LRC and assigned coded numbers; the skin was removed, and the meat was then sliced to yield approximately 7 slices per 3 cm. Bacon slices on the air-exposed surface ends were discarded. Sliced bacon was vacuumed-packed (200 g/pack), labeled on the plastic with their identification number, and then distributed in the taste survey and stored at −20 °C.

Table 2. Fatty Acid Methyl Ester (FAME) Composition of the Basal Diet Mixed with the Dried Marine Alga Product (Milligrams of FA per Gram of Feed)[a]

	grower	low	medium	high	DHA Gold
DHA biomass (%)	0	0.06	0.6	1.6	100
% DHA added	0.00	0.01	0.11	0.29	18.00
C14:0	0.04	0.10	0.29	0.56	40.39
C16:0 palmitic acid	4.83	6.45	7.97	8.95	112.52
C16:1-9c	0.17	0.38	0.48	0.48	1.20
C18:0 stearic acid	0.97	1.70	1.89	1.91	2.48
C18:1-9c	18.10	12.30	13.53	13.39	0.73
C18:1-11c	1.39	0.95	1.04	1.03	0.59
C18:2n-6 linoleic acid	16.87	15.80	16.81	16.56	0.34
C20:0/C18:3n-6	0.21	0.14	0.16	0.17	1.57
C18:3n-3 linolenic acid	5.05	2.60	2.89	2.86	0.19
C20:1-11c	0.76	0.48	0.52	0.54	0.13
C20:2n-6	0.04	0.05	0.05	0.06	0.08
C20:3n-6	0.00	0.01	0.02	0.03	1.92
C20:3n-3	0.02	0.01	0.02	0.01	0.00
C20:4n-6 arachidonic acid	0.00	0.03	0.05	0.06	2.75
C20:5n-3 eicosapentaenoic acid	0.00	0.01	0.04	0.12	6.47
C22:4n-6	0.00	0.01	0.01	0.01	0.51
C22:5n-6 decosapentaenoic acid	0.01	0.04	0.45	1.15	79.40
C22:5n-3	0.00	0.01	0.03	0.04	2.19
C22:6n-3 docosahexaenoic acid	0.01	0.11	1.20	3.13	222.19
total	48.47	41.18	47.43	51.05	475.64
SAT	5.84	8.25	10.14	11.41	155.39
MONO	20.42	14.10	15.57	15.44	2.65
PUFA	22.00	18.69	21.56	24.04	316.04
n-3	5.08	2.75	4.18	6.17	231.04
n-6	16.92	15.94	17.39	17.87	85.00
n-6/n-3	3.33	5.81	4.16	2.90	0.37
PUFA/SAT	3.77	2.27	2.13	2.11	2.03

[a] Alga biomass content of DHA ~18% (v/v). SAT, saturated fatty acid calculated as (14:0 + 16:0 + 18:0 + 20:0). MONO, monounsaturated fat calculated as (16:1-9c + 18:1-9c + 18:1-11c + 20:1-11c). PUFA, polyunsaturated fat calculated as (18:2-n6 + 18:3-n3 + 20:2-n6 + 20:3-n6 + 20:3-n-3 + 20:4-n6 + 20:5-n3 + 22:4-n6 + 22:5-n6 + 22:5-n3 + 22:6-n3); n-6, omega-6 fatty acids total calculated as (18:2-n6 + 20:2-n6 + 20:3-n6 + 20:4-n6 + 22:4-n6 + 22:5-n6); n-3, omega-3 fatty acid total calculated as (18:3n-3 + 20:3-n3 + 20:5-n3 + 22:5-n3 + 22:6-n3).

Fatty Acid Analysis (FAME) of Processed Raw Bacon. Feed fatty acid methyl esters (FAME) were prepared according to the method of Sukhija and Palmquist (*21*). For bacon fatty acids, 30 g of sliced bacon composed of nearly equal amounts of muscle and fat were first pureed using a Robot Coupe Blixir BX3 (Robot Coupe USA Inc., Ridgeland, MS) and mixed, and then 500 mg was extracted with 2:1 chloroform/methanol (20:1 solvent/sample ratio). To 10 mg of extracted lipid was added 1 mL of internal standard (1 mg/mL methylnonadecaenoic acid in toluene) together with 3 mL of 5% methanolic HCl and heated to 80 °C for 1 h (*22*). Samples were then cooled, and 1 mL of 0.88 potassium chloride

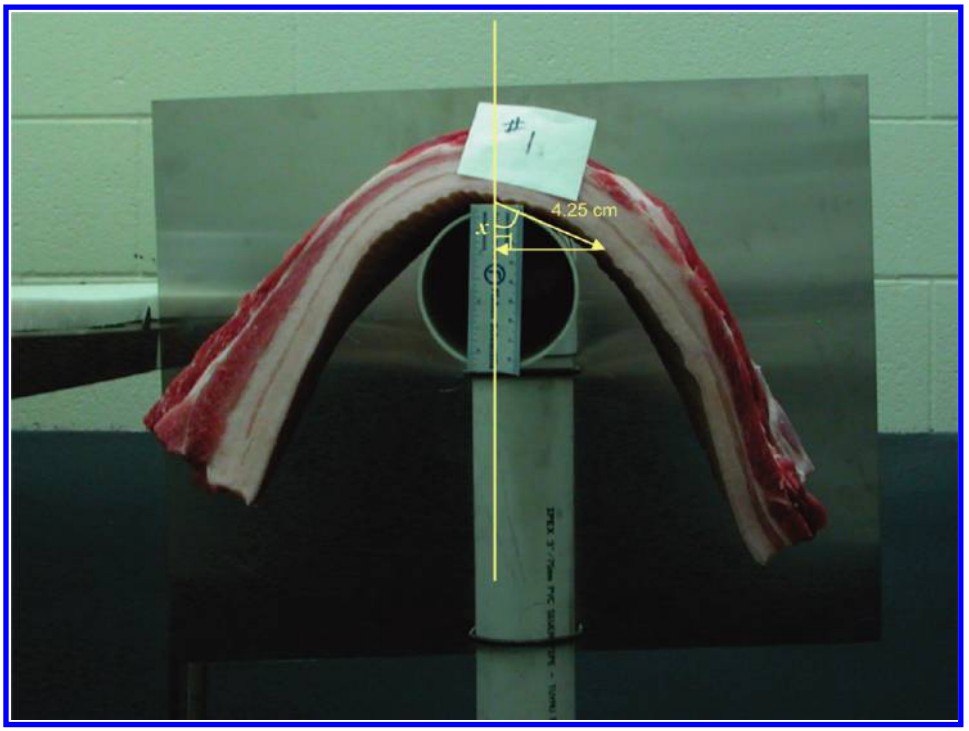

Figure 1. Flex hang method of determining belly softness. Bellies were deboned and chilled to 4 °C and then placed on the 7 cm diameter support bar for 2 min before taking an image analysis. The degree of flex was determined by measuring from the front edge of the belly above the center suspension tube to 4.25 cm to the bottom edge of the belly section and then measuring the length of drop *x* to find the cosine angle.

was added; FAME were extracted with 3 × 3 mL of hexane and dried over sodium sulfate. Feed and belly FAME were analyzed using a Varian 3800 GC (Varian, Walnut Creek, CA) equipped with a Varian 8400 autosampler and a 30 m SP2340 capillary column (Supelco, Bellefonte, PA). The system was operated under constant pressure (15 psi) using hydrogen as the carrier gas and a 20:1 split ratio. The injector and detector were held at 250 °C, and the FAME were quantified using a flame ionization detector. Samples were injected (1 μL, 0.5 μg/μL), and the column temperature was held initially at 50 °C for 30 s, increased to 170 °C at 25 °C/min, held for 3 min, increased to 180 °C at 2 °C/min, and then increased to 230 °C at 10 °C/min. Chromatograms were integrated using Varian Star Chromatography Workstation software. Peaks were identified using a GC reference standard (GLC463 from Nu-Check-Prep, Elysian, MN). The iodine value (IV) of the combined fatty acids was calculated using the equation

$$\mathrm{IV}_{\mathrm{mixture}} = \sum 100 \times \frac{A_{\mathrm{f}} \times 253.81 \times \mathrm{db}}{\mathrm{MW}_{\mathrm{f}}}$$

where A_{f} = the percentage of FAME, 253.81 is the MW of two iodines, db = number of double bonds, and MW_{f} is the MW of FAME in the triglyceride form (*23*) modified from the recommended method of AOAC (*34*). Total IV = %C16:1 (0.95) + %C18:1 (0.86) + %C18:2 (1.732) + %C18:3 (2.616) + %C20:1 (0.795) + %C20:2 (1.57) + %C20:3 (2.38) + %C20:4 (3.19) + %C20:5 (4.01) + %C22:4 (2.93) + %C22:5 (3.68) + %C22:6 (4.64).

Thiobarbituric Measure of Thiobarbituric Acid Reactive Substances (TBARS) in Raw Processed Bacon. Raw ground bacon samples (10 g) were extracted with 30 mL of trichloroacetic acid (75 g of TCA/L in water) with propyl gallate (1 g/L) and EDTA (1 g/L) and mixed with an Ultra Turrax. The dispersion was filtered through a Whatman no. 42 filter; 2.5 mL of the extract was mixed with 2.5 mL of thiobarbituric acid (TBA) (2.88 g/L) and heated to 94 °C for 40 min. The samples were immediately cooled, and the absorbance was measured at 531 nm. TBARS values were determined relative to a standard curve of malonaldehyde generated with 1 g/L of tetraethoxypropane and 20−90 mM TBA solution (*24*).

Antioxidant Potential in Raw Bacon As Determined by the Oxygen Radical Absorbance Capacity (ORAC) Method. Bacon slices were ground from visually equivalent portions of fat and lean, then frozen at −80 °C for 24 h, and vacuum-dried on a rotary evaporator for 48 h. Freeze-dried bacon (1 g) samples were homogenized with 20 mL of hexane followed by centrifugation to recover the upper hexane fraction. The hexane was evaporated under nitrogen and the residue dissolved in 10 mL of acetone/water/acetic acid (AWA: 70:29.5:0.5) by mixing for 30 s at 37 °C for 5 min and then at room temperature for another 10 min. The solution was centrifuged at 3500 rpm for 15 min, and the solvent layer was transferred to a flask and diluted with 25 mL of 0.075 M phosphate buffer at pH 7.0. To measure hydrophilic ORAC, 20 μL of the recovered acetone/water/acetic acid solution was used directly in the ORAC assay (*25*). The ORAC assay mixed 20 μL of sample with 200 μL of 14 μM fluorescein in 75 mM phosphate buffer, pH 7.0, followed by 75 μL of 8.6 mg/mL AAPH. The assays were monitored over time in a 96-well fluorescent plate reader at 37 °C with shaking in a Perkin-Elmer luminescence spectrometer model LS-50B with software package FL Winlab (PerkinElmer, Waltham, MA). The excitation wavelength was set at 485 nm and the emission wavelength at 520 nm. The antioxidant capacity was determined against a standard curve using from 1 to 500 μM Trolox in place of the samples.

Home Usage Test Survey of Bacon. Bacon bellies were sliced, vacuum-packaged in 200 g aliquots, frozen at −20 °C, and distributed to volunteer panelists (n = 40) along with a 25-point home use test (HUT) survey. Panelists were asked to cook their bacon using any method (88% fried) and then to rate the color, smell, and taste of the bacon before, during, and after cooking. Panelists were also asked to describe if any off-odors were detected. Finally, panelists were asked if they would buy this bacon (yes or no). The individual question responses were rated on a 5-point scale from 1, very unpleasant (2, unpleasant; 3, acceptable; 4, pleasant), to 5 (very pleasant). The negative question of off-odors was rated on a scale from very strong (4) [strong (3), moderately (2), slightly (1)] to none at all (0). The questionnaire results were analyzed using a Chi square comparison of pooled responses within each of the three diet treatments. For Pearson's correlation analysis, the survey questions on the cooked bacon smell, taste, and off-odor were compared against the averaged taste panel numerical scale per sample.

Statistical Analysis. The data were analyzed utilizing the Proc GLM and ANOVA procedures including a comparison of multiple group means using Duncan's test of the 9.1 SAS for Windows program (SAS Institute Inc., Cary, NC). Pooled standard error of the mean for FAME analysis

was calculated using least-squares means comparisons. The individual sample scores were analyzed using the Pearson CORR procedure with the fixed effect of the diets tested for significant differences. Differences between treatment means were considered to be significant at $P < 0.05$, and trends were declared when $P < 0.1$. Responses to the HUT surveys were grouped according to diet and compared using the Chi-squared goodness of fit test with significance accepted at $P < 0.05$. Standard deviations were calculated as a binomial attribute variable.

RESULTS

Animal Performance and Carcass Characteristics. This trial was performed to assess the effect of microalga DHA supplements on pig performance and basic sensory qualities of processed pork products such as bacon. Supplementing feed with microalga DHA up to 1.6% of the diet appeared to have no negative effect on feed intake, feed efficiency, or lean yield of the pig carcasses in comparison with the low-biomass diets (**Table 3**). Although no DHA biomass was added to the grower diets, DHA and DPA were still present at low concentrations of approximately 0.001%. (**Table 2**). The animals were individually penned and were not restricted access to their feed or water, and palatability of the microalga-mixed feed appeared to not be an issue as the consumption of treated feed was equal between diet groups. Feed conversion rates (F:G) were significantly different between diet groups (**Table 3**), and there was weak but significant positive correlation between increasing dietary DHA biomass and average daily gain ($r = 0.55$ $P = 0.012$). The percent lean carcass yield as determined using the Destron probe and the total carcass weight, minus the offal and head, were not significantly affected by the DHA diets. The pH of the loin was determined at the third to fourth last rib probe site after 24 h of cooling and was not significantly affected. Loin color was also measured at the probe site and was found to be not significantly affected by the diets.

Table 3. Effect of Increasing Dietary DHA on Carcass Quality Parameters of Growth and Lean Yield Percent As Determined Using a Destron Probe, pH, Meat Color L^*, a^*, and b^*, Measured Using a Minolta Color Meter, Backfat Softness Measured with a Durometer, and Belly Softness Measured by the Flex Hang Method As Described under Materials and Methods[a]

diet	low	medium	high
kill weight (kg)	107.95 ± 3.82	113.30 ± 4.86	111.50 ± 3.12
gain (kg)	22.61 ± 3.42 a	24.10 ± 2.80 ab	27.22 ± 2.38 b
carcass weight (kg)	82.36 ± 2.68	84.48 ± 4.75	80.72 ± 1.82
feed/gain	3.18 ± 0.43 ab	3.29 ± 0.36 a	2.74 ± 0.27 b
lean yield (%)	55.13 ± 1.97	56.84 ± 3.83	55.66 ± 1.65
pH	5.75 ± 0.62	6.16 ± 0.98	5.98 ± 0.74
L^*	52.11 ± 2.00	50.78 ± 2.23	51.27 ± 2.57
a^*	8.50 ± 0.60	8.47 ± 0.32	8.17 ± 1.06
b^*	5.21 ± 0.49	4.72 ± 0.68	4.71 ± 0.22
backfat softness[b]	52.80 ± 2.89	57.65 ± 6.41	54.49 ± 4.51
belly flex[c]	71.40 ± 4.43 ab	76.60 ± 5.81 a	69.00 ± 4.24 b

[a] Values are expressed as mean (± SD). Least-squares means in the same row with different letters are significantly different ($P < 0.05$). [b] Softness expressed as range from 0 (soft) to 100 (hard). [c] Belly flex values represent the cosine angle as shown in **Figure 1**.

Belly Quality. The quality of the belly was measured before the bacon curing process to test for overall belly firmness and belly fat firmness. The belly firmness, as measured using the belly flex hang method, was increased in the high-DHA diet group (**Table 3**), and there was a correlation between the feed/gain ratio and belly flex ($r = 0.70$; $P < 0.001$). The degree of bend was significantly reduced by increased carcass fat ($r = -0.67$; $P < 0.01$) as determined with the Destron probe. This supports the hypothesis that thinner bellies are generally more floppy (*26*). However, belly fat softness as measured using the durometer was not significantly affected by diet. There was also no correlation between backfat softness and belly softness as measured with a durometer ($r = 0.27$; $P = 0.26$). The lean meat content of the belly and the loin probe site generally are not strongly correlated (*27*).

Bacon Flavors and Oxidation Levels. Volunteer panelists were given one package of sliced bacon in frozen, vacuum packages (~200 g/package). Each package contained an average of 10 slices. From the 40 samples distributed, 38 survey sheets were returned and 88% of the panelists chose to fry the bacon. The responses to the key general questions of overall smell of the bacon are shown in **Figure 2**. The survey questions on the taste and smells of the bacon were grouped to rate the bacon before, during, and after cooking. A significant drop in "acceptability" (* chi square $P < 0.05$) was expressed for bacon made from pigs fed

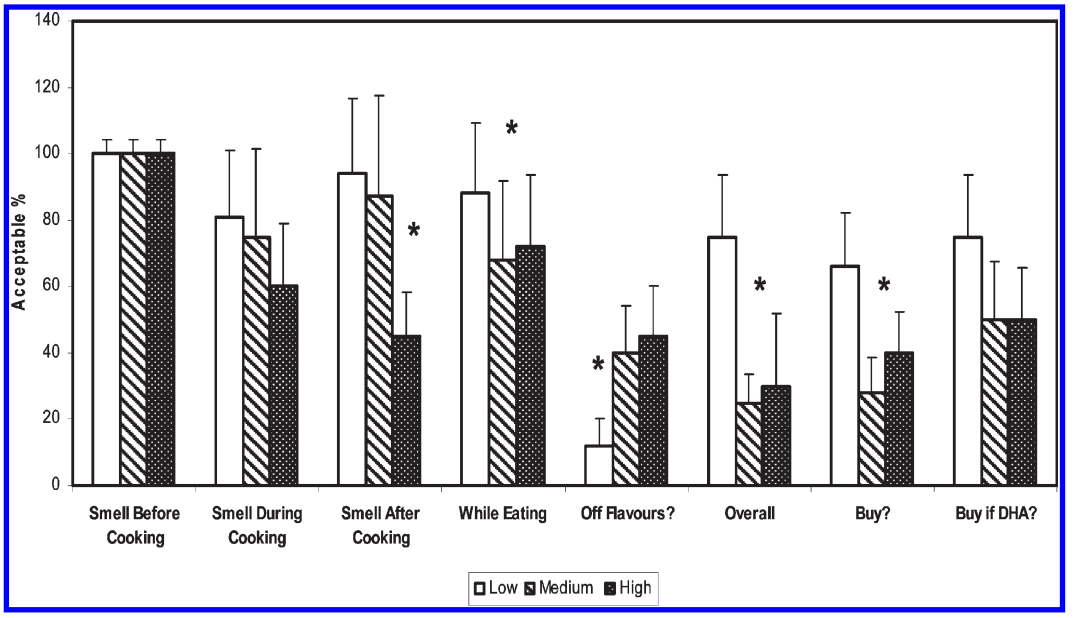

Figure 2. Percentage of bacon rated as acceptable by the home use test (HUT) survey from pigs fed either the low, medium, or high DHA microalga biomass diets. Off-flavor bars represent the percentage that would be unacceptable. ∗, chi square $P < 0.05$; significant differences in the HUT response between the bacon samples from the diet treatments. Error bars represent the binomial SD, calculated from the attribute variable proportion of percentage acceptable.

Table 4. Fatty Acid Methyl Ester Composition Means (Percentage of Total FAME Recovered) of Raw Bacon Prepared from Animals Fed Three Diets of Increasing Levels of Dried Marine Alga Biomass

	low	medium	high	SEM
C14:0	1.44	1.46	1.48	0.07
C16:0	23.54	23.52	23.64	0.44
C16:1-9c	2.40	2.33	2.30	0.12
C18:0	11.36	11.50	11.55	0.35
C18:1-9c	41.18	41.08	39.55	0.74
C18:1-11c	4.64	4.77	4.58	0.21
C18:2-n6	10.37	10.01	10.59	0.32
C20:0/C18:3-n6	0.26	0.27	0.29	0.02
C18:3-n3	1.98	1.91	2.01	0.07
C20:1-11c	0.98	0.99	0.93	0.05
C20:2-n6	0.46	0.41	0.45	0.02
C20:3-n6	0.12	0.11	0.12	0.01
C20:3-n3	0.28	0.26	0.25	0.02
C20:4-n6	0.44	0.46	0.44	0.05
C20:5-n3	0.05 a	0.05 a	0.11 b	0.01
C22:4-n6	0.08	0.08	0.09	0.01
C22:5-n6	0.05 a	0.12 b	0.29 c	0.01
C22:5-n3	0.23 a	0.23 a	0.29 b	0.02
C22:6-n3	0.15 a	0.41 b	1.02 c	0.03
total FAME (mg/g)	295.2	310.9	332.5	28.6
SAT[b]	36.34	36.49	36.67	0.67
MONO[c]	49.20 a	49.17 a	47.37 b	0.76
PUFA[d]	14.20 a	14.07 a	15.67 b	0.47
n-6[e]	11.52	11.20	11.99	0.38
n-3[f]	2.68 a	2.87 a	3.68 b	0.10
n-6/n-3	4.29 a	3.91 b	3.26 c	0.06
PUFA/SAT	0.39	0.39	0.43	0.02
TBARS[g]	0.35	0.42	0.73	0.10

[a] The average bacon sample was composed of 30.5 ± 6.4% fat. Means with different letters are significantly different ($p > 0.05$). Least-squares means in the same row with different letters are significantly different ($P < 0.05$). [b] SAT, saturated fatty acid calculated as (14:0 + 16:0 + 18:0 + 20:0). [c] MONO, monounsaturated fat calculated as (16:1-9c + 18:1-9c + 18:1-11c + 20:1-11c). [d] PUFA, polyunsaturated fat calculated as (18:2-n6 + 18:3-n3 + 20:2-n6 + 20:3-n6 + 20:3-n-3 + 20:4-n6 + 20:5-n3 + 22:4-n6 + 22:5-n6 + 22:5-n3 + 22:6-n3). [e] n-6, omega-6 fatty acids total calculated as (18:2-n6 + 20:2-n6 + 20:3-n6 + 20:4-n6 + 22:4-n6 + 22:5-n6). [f] n-3, omega-3 fatty acid total calculated as (18:3n-3 + 20:3-n3 + 20:5-n3 + 22:5-n3 + 22:6-n3). [g] TBARS, thiobarbituric acid measure of malonaldehyde reactive species, an indicator of unsaturated fatty acid oxidation.

Table 5. Fatty Acid Analysis (FAME Milligrams per Gram) of Raw Bacon Prepared from Animals Fed Three Diets of Increasing Levels of Dried Marine Algae Biomass[a]

	low	medium	high	pooled SEM
C14:0	4.13	4.53	4.92	0.47
C16:0	67.70	73.05	78.42	6.61
C16:1-9c	6.88	7.25	7.66	0.73
C18:0	32.61	35.48	38.30	3.03
C18:1-9c	119.16	128.72	131.64	13.01
C18:1-11c	13.30	15.02	15.26	1.54
C18:2n-6	29.86	30.65	35.29	2.89
C20:0/C18:3n-6	0.74	0.87	0.98	0.09
C18:3n-3	5.70	5.84	6.66	0.55
C20:1-11c	2.83	3.13	3.13	0.36
C20:2n-6	1.33	1.27	1.52	0.15
C20:3n-6	0.33	0.36	0.39	0.04
C20:3n-3	0.81	0.82	0.85	0.09
C20:4n-6	1.26	1.30	1.48	0.11
C20:5n-3 (EPA)	0.14 a	0.18 a	0.36 b	0.03
C22:4n-6	0.23	0.27	0.31	0.04
C22:5n-6 (DPA)	0.14 a	0.36 b	0.97 c	0.05
C22:5n-3	0.65 a	0.69 a	0.97 b	0.06
C22:6n-3 (DHA)	0.43 a	1.22 b	3.39 c	0.13
total	288.22	310.99	332.48	28.29
SAT	104.44	113.06	121.63	9.92
MONO	142.17 a	154.11 a	157.69 b	15.37
PUFA	40.87 a	42.96 a	52.19 b	3.95
n-6	33.15	34.20	39.95	3.19
n-3	7.72 a	8.75 a	12.23 b	0.77
n-6/n-3	4.29 a	3.91 b	3.26 c	0.06
PUFA/SAT	0.39	0.39	0.43	0.02
TBARS	0.34 a	0.44 ab	0.73 b	0.10
iodine value	70.48 a	71.04 a	74.55 b	0.88
ORAC	20.33	21.25	23.00	1.83

[a] The average bacon sample was composed of 30.5 ± 6.4% fat. Means with different letters are significantly different ($p > 0.05$). Iodine value = %C16:1 × (0.95) + %C18:1 × (0.86) + %C18:2 × (1.732) + %C18:3 × (2.616) + %C20:1 × (0.795) + %C20:2 × (1.57) + %C20:3 × (2.38) + %C20:4 × (3.19) + %C20:5 × (4.01) + %C22:4 × (2.93) + %C22:5 × (3.68) + %C22:6 × (4.64). ORAC, oxygen radical absorbance capacity method, assay values relative to Trolox units.

> 0.6% alga biomass (~0.11% DHA). The increase in unacceptable smell scores was higher during cooking than after cooking (**Figure 2**). Panelists were asked to describe if any off-odors were detected. Bacon from the pigs fed the 1.6% DHA diet that scored as "unacceptable" during and after cooking had odors described as "barnyard" or "piggy". Finally, panelists were asked if they would buy this bacon. The number of panelists that were willing to buy bacon made from the low-DHA feed group was 88%. There was a significant drop, to 70% and then to 30%, in panelists that would buy the bacon that was made with 0.6% or 1.6% DHA. The number of panelists that responded that they would buy the same 1.6% bacon if it was labeled as "nutritionally enhanced with DHA" improved only slightly to 45%. However, this improvement was enough to overcome the initial rate of rejection observed between bacon made from the high- and medium-DHA diet fed hogs. An important distinction observed, of the 88% of panelists that would buy the low-DHA bacon, was that 73% of those respondents would prefer it over their regular bacon. In addition, 70% of the low-DHA bacon samples were scored as having a pleasant to very pleasant overall flavor described as fresh or green. Unfortunately, although this survey asked what percentage extra they would pay for the DHA bacon, only 6 of the 40 panelists responded. Survey responses from the panelists served the medium- and high-DHA bacon samples had < 40% that would actually buy this bacon over their regular purchase, and this improved only to 50% if the bacon was labeled as "nutritionally improved with DHA".

There was no significant correlation between TBARS values or iodine values and "off" or "unpleasant" flavor and smell scores (**Table 6**), but TBARS and iodine values were significantly increased in the raw bacon from the higher DHA diet group (**Tables 4** and **5**). The TBARS value, which estimated the concentration of malonaldehyde, a byproduct of fatty acid oxidation, was significantly increased in the bacon from the pigs fed the medium (0.6%) and high (1.6%) DHA diets. The 0.6% DHA biomass diet contained approximately 21 g of PUFA/kg of feed, increasing the polyunsaturated fat content of the pork to ~14% of total FAME. The high diet contained ~24 g of PUFA/kg of feed, and this significantly increased the bacon PUFA to 15.6% of total FAME. The HUT survey generally started to rate the bacon as unacceptable for abnormal flavors when the bacon PUFA content was > 15%. The PUFA acceptance value is considerably lower than the acceptable limit of 24% PUFA inner backfat content when pigs were fed soybean oil with linseed (*7*, *28*) but higher than the acceptable limit of 11.5% backfat PUFA when pigs were fed > 3% fish oil (*29*).

There was a significant correlation (**Table 6**) between the loin color parameter b^* (blue to yellow) and positive bacon smell

($r = 0.64$, $P < 0.01$) or loin pH and "off" bacon smells ($r = 0.57$; $P < 0.01$). The color and pH measures were not significantly different between diet groups (**Table 3**). Antioxidant potential of the meat, as determined by the fluorescein protection (ORAC) assay, was not correlated with diet, TBARS estimates, or taste scores.

Fatty Acid Profiles of Feed and Raw Prepared Bacon. The fatty acid profiles of the bacon were analyzed on raw freeze-dried ground slices collected from the middle of the processed bellies. The average fat content in the bacon samples was approximately 30.5 ± 6.4%. Neutral lipids and phospholipids were not independently assessed in this trial. Comparisons of unprocessed bellies, processed bacon, and cooked bacon can be found in the USDA 2008 nutrition data site (*30*). The dried alga biomass mainly increased the content of palmitic acid (C16:0), docosapentaenoic acid (C22:5n-6, DPA), and docosahexaenoic acid (C22:6n-3, DHA) in the feed (**Table 2**). As a result, DPA and DHA but not palmitic acid was increased significantly in the bacon between diets (**Tables 4** and **5**). Although no DHA biomass was added to the grower diets, DHA and DPA were still present at low concentrations of approximately 0.001%. Previous animal studies (*28*) have estimated that the ratio of omega-6/omega-3 in the diet influences the meat intramuscular fat omega-6/omega-3 ratio at $r \sim 0.77$, but this is highly influenced by the duration of feeding. Human studies of dietary influence on body fat composition are even more variable with expected correlations to $r^2 < 0.3$ (*5*). The correlation between dietary DHA and the subsequent level of DHA in the raw bacon is shown in **Figure 3**. For this trial, a linear conversion rate was estimated at $y = 0.98x + 25.71$, with the three diet levels of DHA as x (mg/100 g of feed) and the subsequent level of DHA bacon as y (mg/100 g of raw bacon).

Table 6. Pearson Correlation Coefficients *r* Comparing Bacon Taste Scores on Acceptable Smell, Acceptable Flavor, or Abnormal Off-Flavor against the Measures of Diet, Animal Performance (Lean Yield Percent, Feed/Gain), Carcass Qualities (pH, Loin Color *L**, *a**, *b**, Chroma, Hue, Backfat and Belly Fat Hardness), Bacon Lipid Composition (SAT, MONO, PUFA), and Bacon Oxidation Status (ORAC, Iodine Value, TBARS)[a]

	smell	flavor	off-flavor
diet	−0.0	−0.22	0.09
lean yield (%)	0.31	−0.26	−0.08
feed/gain (kg)	−0.04	−0.02	0.09
pH	−0.38	−0.12	0.57**
*L**	0.38	−0.01	−0.24
*a**	0.06	−0.01	−0.27
*b**	0.64**	0.31	−0.49*
chroma	0.27	0.10	−0.38
hue	0.62**	0.29	−0.28
backfat	−0.05	−0.3	0.01
belly	−0.12	−0.45*	0.14
SAT (mg/g)	0.41*	0.02	−0.11
MONO (mg/g)	−0.36	−0.19	0.29
PUFA (mg/g)	0.04	0.26	−0.31
ORAC	0.19	0.04	−0.12
iodine value	−0.19	0.06	−0.07
TBARS	−0.15	−0.04	0.21

[a] Taste panel scores from the bacon HUT survey were pooled according to each animal. Significance: *, $p < 0.05$; **, $p < 0.01$.

DISCUSSION

North American consumers interested in improving their health through diet perceive red meat as a source of too much saturated and unhealthy fat in the diet (*31*). The purpose of this trial was to produce bacon with enriched levels of DHA and to determine if it would still be acceptable for consumer perceptions of appearance flavor and texture (*32*). The 2008 USDA study (*30*) suggests that a balanced diet should contain approximately 100−350 mg of long-chain omega-3 fatty acids daily (*6*). A 28 g strip of raw bacon from corn−soybean fed pigs typically contains approximately 46% fat, with 0.5% of that fat as omega-3 fatty acids but very little DHA (*27*). In the present 25 day study, the pigs were fed a canola, pea, corn, and barley mixed diet with DHA added in the form of alga biomass. Bacon content of DHA was increased to 97 mg/100 g when 1 g of DHA was added to a kilogram of feed. Bacon made from the pigs fed the highest level of DHA biomass at 1.6% contained ~3.4 mg of DHA/g, with 1.2% omega-3 fatty acids in total fat. However, problems of off-odors and -flavors were reported in the bacon from the taste panel surveys, especially from the pigs fed the higher concentrations of DHA.

Odors are complex, and their acceptability is culturally influenced (*33*). Oxidation of omega-3 and omega-6 fatty acids can produce propanal and hexanal byproducts, which initially give odor notes of green, fresh, or alcohol (*33*, *34*), whereas the odors of fishy or rancid fat usually require longer exposure to oxidation of lipids and proteins (*35*). The low-DHA diet bacon received an acceptability rating by 88% of the volunteer respondents, and

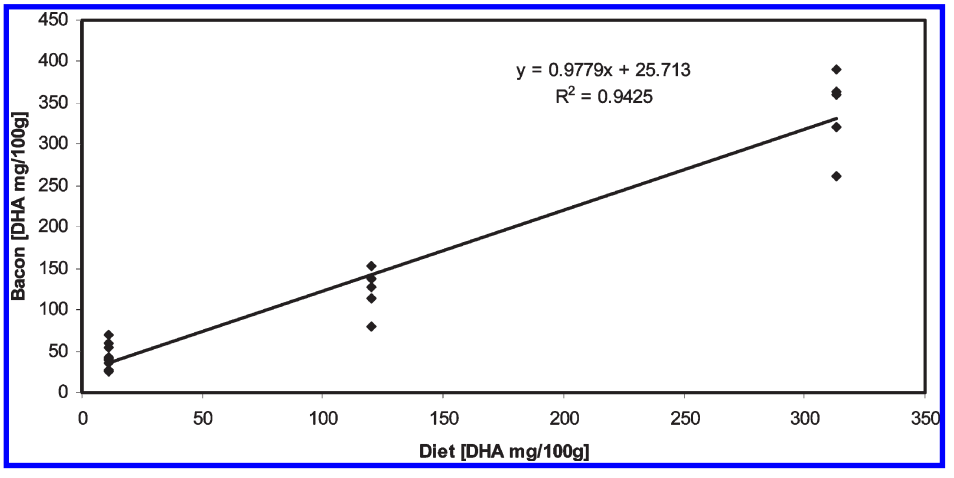

Figure 3. Retention of DHA from feed into bacon when pigs are fed ad libitum for 25 days prior to slaughter. The DHA content in the diets was averaged from the lipid analysis. The diets were mixed with algae biomass (~18% DHA) for a final DHA concentration of low (11 mg), medium (120 mg), and high (313 mg of DHA in 100 g of feed). Raw bacon FAME DHA content was measured from the total lipid extracted from freeze-dried bacon expressed as milligrams of DHA per 100 g serving, as explained under Materials and Methods.

73% described the product as very pleasant with fresh or green odor notes and preferred it over their regular bacon purchase. The bacon from the animals fed the higher dietary levels of DHA scored significantly lower for overall flavor and odor acceptability and were described as having "barnyard" or "strong pig" smells. A similar effect on pork flavor is reported when pigs are fed elevated levels of lamb tallow (*32*) or plant monounsaturated fats (*34*). Previous papers have suggested that dietary PUFA should be kept below 15% (or 28 g/kg of feed) and stearic acid (C18:0) above 15% to avoid structural, flavor, and oxidative problems (*28*). The PUFA levels in the diets used in this trial were increased from 18 to 24 g/kg of feed with the added DHA biomass, which significantly increased the PUFA content to 15.6% in the high-DHA diet bacon fat. The stearic acid content was reduced from 4.1 to 3.7% in the 1.6% DHA diet, but this did not significantly change the stearic acid content in the bacon, which averaged 12% of total FAME in all groups. Still, the bacon stearic acid content was below the recommended 15% threshold level and may have negatively affected the overall acceptability of the bacon in the home use surveys.

The bacon from the pigs fed the higher concentration of DHA did have a significant increase in TBARS values, indicating increased lipid oxidation. The TBARS values did not strongly correlate with taste scores, but taste scores did correlate with changes in the color measure b^*, which indicates potential oxidation of hemoglobin or proteins in the pork (*36*). The conditions of storage and packaging were assumed to be similar between samples, but small changes such as position in the processing line or location of the bacon slice in the belly cut could have had a large effect on the overall atmospheric exposure. Higher PUFA content in meat products generally increases the potential for oxidation and abnormal flavors during storage (*32*). The potential maximum lipid oxidation was estimated by applying a calculated iodine value (*23*), which represents the total number of double bonds available for oxidation in a fat sample. The iodine value did not significantly increase in the bacon samples until the highest concentration of DHA was fed and was not correlated with the taste panel scores. The bacon was also compared using the ORAC test, which measures total antioxidant content, but there was no significant correlation with flavor or any fatty acid changes in the bacon. Therefore, the TBARS test was the closest biochemical measure to predict off-flavors in bacon when the lipid content was modified.

Increasing the percentage of PUFA in pork can lead to processing problems (*26*). The processing structure of the bacon from pigs fed the higher levels of DHA was not significantly affected, as determined by the meat quality measures of color, pH, lean meat yield, or softness. The degree to which meat softness interferes with butchering by clogging cutting blades or slicing yield (*27*) was not tested. The softness of the belly was not significantly affected by the DHA diets but was correlated with poorer feed conversion (feed/gain) ($r = 0.70$; $P < 0.001$) and negatively correlated ($r = -0.57$; $P < 0.01$) with the overall carcass yield. Generally, bellies with lower fat content tend to flex more than high-fat bellies at 4 °C.

On the basis of this trial's estimated conversion rate of dietary DHA into bacon DHA, pigs would need to be fed a diet of 759 mg of DHA/kg of feed for 25 days prior to slaughter, to produce bacon containing 100 mg of DHA/100 g serving. This is equivalent to a 0.42% alga biomass diet, which is below the medium 0.6% biomass diet used in the current study. Judging from the taste panel survey, bacon made from pigs fed approximately 0.6% alga biomass diet may still have some consumers noticing off-flavors. The negative effects of dietary DHA on flavor acceptability limits the amount of DHA that can be increased in bacon. The abnormal flavor is believed to be due to the polyunsaturated fatty acid structure of DHA and its susceptibility to oxidation. In this trial, the pig diets were supplemented with 100 IU/kg of feed tocopherol and 0.5 mg/kg of feed selenium to help prevent lipid oxidation, but the bacon from the higher 1.6% DHA biomass diets still had a higher concentration of TBARS reactive aldehydes. Future trials to produce bacon with > 100 mg of DHA/100 g serving could attempt to prevent potential off-flavors by significantly increasing feed antioxidants such as vitamin E or selenium, reducing n-6 lipids and overall feed PUFA content or by increasing stearic acid content. Alternative sources of DHA might also be examined, because the level of C22:5n-6 decosapentanoic acid (DPA) was quite high in the alga biomass at approximately 79 mg/g and the DPA may interfere with the biological benefits expected with DHA (*37*).

The pigs in the high-DHA, 1.6% biomass diet group were fed approximately 3 kg of feed/day in the final 25 days, which is approximately 8640 mg of DHA daily. However, no detrimental health problems have been detected (*38*), and because the feed/gain ratio was actually improved relative to the low 0.06% diet, the animals appear to have benefited from the higher dietary DHA. The addition of DHA to improve the nutritional qualities of bacon may also be combined with conjugated linoleic acid (CLA), which would also improve the structure (*39*).

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