Color and fatty acid profile of abdominal fat pads from broiler chickens fed lobster meal

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ABSTRACT Consumer demands for food products enriched with healthful n-3 fatty acids are steadily increasing. Feeding marine byproducts may provide an economical means of increasing the long-chain n-3 content of broiler tissues. A study was conducted to evaluate the effect of dietary lobster meal (LM) on the color and fatty acid profile of broiler chicken fatty tissue. Broilers were fed increasing levels (0, 2, 4, 6, 8, and 10%) of LM for 35 d. Fat pad samples were collected at slaughter and color and fatty acid concentrations were determined. A linear effect was found of LM on red coloration ($P < 0.05$) as dietary LM increased. Fat pad eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels also increased ($P < 0.0001$) in a linear fashion. The essential long-chain fatty acids were lower for the 10% LM diet (0.37 mg of EPA/g; 0.16 mg of DHA/g) compared with the 8% LM diet (0.51 mg of EPA/g; 0.27 mg of DHA/g). Using lobster meal as a feed ingredient resulted in broiler abdominal fat pads with a favorable increase in n-3 fatty acids.

Key words: broiler, lobster meal, fat pad, color, fatty acid

2011 Poultry Science 90:1329–1333
doi:10.3382/ps.2010-01111

INTRODUCTION

Because of increasing transportation and production costs of traditional feed ingredients, alternative ingredients are needed for North American livestock producers. Soybean meal has been the primary source of plant protein (Leeson and Summers, 2005) and limestone the primary source of calcium in broiler diets (Roland, 1986; Guinotte et al., 1991). In the past, fish meal has also been included in regional poultry diets, primarily as a source of protein and calcium. However, because of the high cost associated with fish meal, it is generally no longer included in poultry diets.

Lobster, crab, and shrimp meals are available in coastal North America as byproducts of the fishing industry. Lobster meal (LM) contains high levels of protein and calcium as well as antioxidants and long-chain n-3 fatty acids. A major concern with the use of shrimp and crab meals in poultry diets is the high level of chitin these waste products contain, which may decrease the nutritive value of the feed (Rosenfeld et al., 1997). Industry has developed a method for producing LM that is free of chitin and contains high levels of protein (44.5%) and calcium (7%).

The benefits to human health of increased consumption of dietary n-3 fatty acids have been well documented (Connor, 2000; Hu et al., 2002), and consumer demands for food products enriched with n-3 are steadily increasing. Lobster meal is a source of the essential long-chain n-3 fatty acids eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**; Daniel, 2008). The human health benefits of dietary antioxidants have also been well documented. Astaxanthin, the major carotenoid pigment found in fish and crustaceans, possesses high antioxidant activity (Guerin et al., 2003).

Feeding marine byproducts such as LM may be a useful method of increasing the content of n-3 fatty acids and antioxidants in broiler tissues. The objective of this study was therefore to evaluate the effect of dietary LM on the color and fatty acid profile of broiler chicken fatty tissue.

MATERIALS AND METHODS

The experiment was conducted as a completely randomized design with level of LM as the main factor. Lobster meal was prepared from American lobsters

©2011 Poultry Science Association Inc.
Received September 8, 2010.
Accepted February 3, 2011.
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(*Homarus americanus*) and was provided by St. Laurent Gulf Products Ltd. (Lower Caraquet, New Brunswick, Canada) as a dried, ground meal with ethoxyquin added to prevent oxidation. Increasing levels of LM (0, 2, 4, 6, 8, and 10%) were incorporated into standard broiler diets. All diets were formulated to be isonitrogenous and isocaloric and to meet or exceed NRC (1994) requirements. Starter diets contained 23% CP and 3,050 kcal of ME/kg and were fed as mash from 1 to 14 d of age (Table 1). Grower diets contained 20% CP and 3,150 kcal of ME/kg and were fed as mash from 15 to 25 d of age (Table 2). Finisher diets contained 18% CP and 3,200 kcal of ME/kg and were fed as pellets from 26 to 35 d of age (Table 3).

A total of 1,512 male broiler chicks (Ross; 1 d of age) were housed in 36 floor pens (0.075 m^2/bird). Each of 6 experimental diets was fed to 2 replicate pens in 3 rooms. Feed and water were provided ad libitum. To monitor growth performance, weight gain was recorded on d 14, 25, and 35 and feed consumption was recorded by period (d 1–14, 15–25, and 26–35). Throughout the trial, birds were managed in accordance with Canadian Council for Animal Care (1993) guidelines.

At 35 d of age, 2 birds/pen were killed by cervical dislocation and fat pads were collected from 2 birds/pen for 2 of the 3 production rooms. Fat pad color was measured using a MiniScan XE Plus colorimeter (Hunter Associates Laboratory, Reston, VA). Lightness (L^*), redness (a^*), and yellowness (b^*) were measured. Following color measurement, fat pads were stored at −80°C for further analysis. Total lipids were extracted according to Folch et al. (1957). Briefly, 1-g fat pad samples were homogenized in chloroform:methanol:water in an 8:4:3 ratio. Following separation, the lower phase was removed and dried under a stream of nitrogen at room temperature. Dried lipid samples were placed in dichloromethane with 0.01% butylhydroxytoluene. Fatty acid methyl esters (FAME) were prepared directly from ≤100 mg of the isolated lipid using H_2SO_4 in methanol (Budge et al., 2006). The FAME were then extracted into hexane and concentrated to 50 mg/mL for gas chromatographic analyses. The FAME were analyzed with a PerkinElmer Autosystem (PerkinElmer, Waltham, MA) equipped with a flame ionization detector using a DB-23 column (30 m × 0.25 mm i.d.; Agilent, Santa Clara, CA). Helium was used as the carrier gas and the following temperature program was employed: 153°C for 2 min, hold at 174°C for 0.2 min after increasing at 2.5°C/min, and hold at 220°C for 3 min after increasing at 2.5°C/min. Up to 66 FAME

Table 1. Dietary composition of experimental starter diets containing lobster meal

	Lobster meal (%)					
Item (% unless noted)	0	2	4	6	8	10
Ingredient						
Corn	44.81	45.73	46.66	47.59	48.46	49.38
Soybean meal	38.01	35.86	33.71	31.56	29.43	27.28
Wheat	10.0	10.0	10.0	10.0	10.0	10.0
Lobster meal[1]	—	2.0	4.0	6.0	8.0	10.0
Poultry fat	3.30	2.79	2.28	1.77	1.28	0.77
Limestone	1.62	1.44	1.27	1.09	0.92	0.74
Monodicalcium phosphate	0.89	0.85	0.80	0.76	0.72	0.67
Methionine premix[2]	0.41	0.40	0.38	0.36	0.35	0.33
Lysine HCl	—	—	—	—	0.001	0.003
Iodized salt	0.39	0.36	0.33	0.30	0.27	0.24
Coban[3]	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[4]	0.025	0.025	0.025	0.025	0.025	0.025
Vitamin–mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50
Calculated analysis						
ME (kcal/kg)	3,050	3,050	3,050	3,050	3,050	3,050
CP	23.0	23.0	23.0	23.0	23.0	23.0
Calcium	1.0	1.0	1.0	1.0	1.0	1.0
Available phosphorus	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.19	0.19	0.19	0.19	0.19	0.19
Lysine	1.36	1.35	1.35	1.35	1.35	1.35
Methionine + cystine	0.95	0.95	0.95	0.95	0.95	0.95
Determined analysis						
CP	24.3	22.7	20.1	22.6	23.4	23.3
Calcium	0.87	1.27	1.52	0.96	0.95	1.12
Total phosphorus	0.58	0.57	0.50	0.56	0.56	0.58
Sodium	0.15	0.16	0.13	0.13	0.15	0.18

[1]Supplied by St. Laurent Gulf Products Ltd. (Caraquet, New Brunswick, Canada). Protein, 44%; Ca, 7%; fat, 5%; n-6, 0.32%; n-3, 1.10%; eicosapentaenoic, 0.60%; docosahexaenoic acid, 0.30%.

[2]Supplied per kilogram of premix: DL-methionine, 0.5 kg; wheat middlings, 0.5 kg.

[3]Coccidiostat (Pfizer Animal Health, London, Ontario, Canada).

[4]Antibiotic (Elanco Animal Health, Guelph, Ontario, Canada).

[5]Supplied per kilogram of diet: vitamin A, 9,750 IU; vitamin D_3, 2,000 IU; vitamin E, 25 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; DL-Ca-pantothenate, 13.5 mg; vitamin B_{12}, 0.023 mg; niacin, 29.7 mg; folic acid, 4.0 mg, choline, 801 mg; biotin, 0.3 mg; pyridoxine, 4.95 mg; thiamine, 2.91 mg; manganese, 72 mg; zinc, 80.0 mg; copper, 25 mg; selenium, 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 1,432 mg; ground limestone, 500 mg.

Table 2. Dietary composition of experimental grower diets containing lobster meal

	Lobster meal (%)					
Item (% unless noted)	0	2	4	6	8	10
Ingredient						
Corn	52.27	53.38	54.32	55.26	56.2	57.14
Soybean meal	30.27	28.08	25.93	23.78	21.62	19.47
Wheat	10.0	10.0	10.0	10.0	10.0	10.0
Lobster meal[1]	—	2.0	4.0	6.0	8.0	10.0
Poultry fat	3.90	3.33	2.81	2.3	1.78	1.26
Limestone	1.66	1.41	1.23	1.05	0.88	0.70
Monodicalcium phosphate	0.75	0.71	0.66	0.62	0.58	0.54
Methionine premix[2]	0.21	0.19	0.16	0.14	0.12	0.09
Iodized salt	0.37	0.34	0.31	0.28	0.25	0.22
Coban[3]	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[4]	0.025	0.025	0.025	0.025	0.025	0.025
Vitamin–mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50
Calculated analysis						
ME (kcal/kg)	3,150	3,150	3,150	3,150	3,150	3,150
CP	20.0	20.0	20.0	20.0	20.0	20.0
Calcium	0.92	0.92	0.92	0.92	0.92	0.92
Available phosphorus	0.40	0.40	0.40	0.40	0.40	0.40
Sodium	0.18	0.18	0.18	0.18	0.18	0.18
Lysine	1.12	1.12	1.12	1.12	1.11	1.11
Methionine + cystine	0.65	0.64	0.64	0.64	0.64	0.64
Determined analysis						
CP	19.4	20.9	19.1	20.6	20.4	20.3
Calcium	0.71	1.00	0.77	0.92	1.00	1.08
Total phosphorus	0.49	0.54	0.49	0.52	0.52	0.52
Sodium	0.11	0.16	0.13	0.16	0.17	0.17

[1]Supplied by St. Laurent Gulf Products Ltd. (Caraquet, New Brunswick, Canada).

[2]Supplied per kilogram of premix: DL-methionine, 0.5 kg; wheat middlings, 0.5 kg.

[3]Coccidiostat (Pfizer Animal Health, London, Ontario, Canada).

[4]Antibiotic (Elanco Animal Health, Guelph, Ontario, Canada).

[5]Supplied per kilogram of diet: vitamin A, 9,750 IU; vitamin D_3, 2,000 IU; vitamin E, 25 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; DL-Ca-pantothenate, 13.5 mg; vitamin B_{12}, 0.023 mg; niacin, 29.7 mg; folic acid, 1.0 mg, choline, 801 mg; biotin, 0.3 mg; pyridoxine, 4.9 mg; thiamine, 2.9 mg; manganese, 72 mg; zinc, 80.0 mg; copper, 25 mg; selenium, 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 1,543 mg; ground limestone, 500 mg.

were identified by comparison with known standards (Sigma-Aldrich Inc., St. Louis, MO) and were reported as weight percentage of total fatty acids. The main effect of LM on fat color and fatty acid composition was evaluated by one-way ANOVA using the Proc Mixed procedure of SAS (SAS Institute, 1999). Contrast statements were used to determine relationships among treatments. Differences between main effects were separated using Tukey's test. Main effects were considered statistically significant at α level of 5%.

RESULTS AND DISCUSSION

Fat Pad Coloration

Broiler fat pad coloration was affected by feeding increasing levels of LM for 35 d (Table 4). A linear effect was observed on both the red (a*) and yellow (b*) scores. As the level of LM increased in the diets, so did the level of corn (Tables 1, 2, and 3), explaining the linear effect observed for the yellow score. Yellow coloration of the fat pad was higher ($P < 0.05$) in broilers fed 10% dietary LM compared with those fed no LM or 2% LM. The increasing level of corn associated with increasing levels of LM in the diets may explain the difference in yellow color observed between 2 and 10% LM because lutein, a xanthophyll found in corn, is naturally deposited into the fatty tissue of broilers (Pérez-Vendrell et al., 2001). Although a linear effect was observed on red score, no treatment effect was observed ($P > 0.05$), suggesting that the LM caused no unwanted red coloration to the abdominal fat. The LM did not alter the lightness (L^*) of the fat pad in the current study.

Research with laying hens fed LM indicated that laying hens were able to deposit dietary carotenoid pigments in the egg yolk (Daniel, 2008). Results from the current study indicate that although these carotenoids were also deposited into the broiler fat pad, relatively little change occurred in the redness of the fat pad from increasing the levels of LM. The change in color of the fat pad appears to be a result of the increasing levels of corn in the diet as the LM content increased.

Fat Pad Fatty Acid Profile

The fatty acid profile of lipid extracted from broiler fat pads was also affected by feeding increasing levels of LM for 35 d (Table 5). Total saturated (29 mg/g) and monounsaturated (54 mg/g) fatty acids were not affected by level of dietary LM. Polyunsaturated fatty acids, in particular n-3 polyunsaturated fatty acids, were higher ($P < 0.05$) in fat pads from broilers fed 6% LM compared with those fed 10% LM. The 18 carbon chain

Table 3. Dietary composition of experimental finisher diets containing lobster meal

	Lobster meal (%)					
Item (% unless noted)	0	2	4	6	8	10
Ingredient						
Corn	58.28	59.14	59.98	60.83	61.67	62.52
Soybean meal	24.91	22.77	20.64	18.5	16.37	14.23
Wheat	10.0	10.0	10.0	10.0	10.0	10.0
Lobster meal[1]	—	2.0	4.0	6.0	8.0	10.0
Poultry fat	3.41	2.97	2.53	2.09	1.65	1.21
Limestone	1.62	1.44	1.27	1.09	0.91	0.74
Monodicalcium phosphate	0.72	0.67	0.63	0.59	0.55	0.51
Methionine premix[2]	0.12	0.10	0.07	0.05	0.03	0.005
Iodized salt	0.37	0.34	0.31	0.28	0.25	0.22
Coban[3]	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[4]	0.025	0.025	0.025	0.025	0.025	0.025
Vitamin–mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50
Calculated analysis						
ME (kcal/kg)	3,200	3,200	3,200	3,200	3,200	3,200
CP	18.0	18.0	18.0	18.0	18.0	18.0
Calcium	0.90	0.90	0.90	0.90	0.90	0.91
Available phosphorus	0.38	0.38	0.38	0.38	0.38	0.38
Sodium	0.18	0.18	0.18	0.18	0.18	0.18
Lysine	0.96	0.96	0.96	0.96	0.96	0.96
Methionine + cystine	0.65	0.64	0.64	0.64	0.64	0.64
Determined analysis						
CP	17.9	18.8	18.3	18.2	17.6	17.4
Calcium	0.79	0.78	0.86	0.87	1.20	1.24
Total phosphorus	0.47	0.47	0.47	0.51	0.50	0.49
Sodium	0.14	0.15	0.15	0.20	0.18	0.17

[1]Supplied by St. Laurent Gulf Products Ltd. (Caraquet, New Brunswick, Canada).

[2]Supplied per kilogram of premix: DL-methionine, 0.5 kg; wheat middlings, 0.5 kg.

[3]Coccidiostat (Pfizer Animal Health, London, Ontario, Canada).

[4]Antibiotic (Elanco Animal Health, Guelph, Ontario, Canada).

[5]Supplied per kilogram of diet: vitamin A, 9,750 IU; vitamin D_3, 2,000 IU; vitamin E, 25 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; DL-Ca-pantothenate, 13.5 mg; vitamin B_{12}, 0.012 mg; niacin, 29.7 mg; folic acid, 1.0 mg, choline, 801 mg; biotin, 0.3 mg; pyridoxine, 4.9 mg; thiamine, 2.9 mg; manganese, 72 mg; zinc, 80.0 mg; copper, 25 mg; selenium, 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 1,543 mg; ground limestone, 500 mg.

fatty acids (oleic, linoleic, and α-linolenic) decreased ($P < 0.01$) as dietary LM increased, whereas levels of the long-chain fatty acids EPA and DHA increased ($P < 0.0001$) from 0 to 8% LM. The ratio of n-6 to n-3 fatty acids decreased in a linear fashion ($P < 0.05$) as LM increased in the diet. It is important to consider this as the ratio of these 2 classes of fatty acids rather than only changes in n-3 levels because a decline in this ratio has been found to benefit human health (Simopoulos, 2008).

Increased levels of long-chain fatty acids EPA and DHA in the fat pad indicate that these essential fatty acids are partitioned to fat storage areas when broilers are fed LM. To our knowledge, no other studies report the effects of dietary LM on broiler fat pad fatty acid profile. Carrillo-Domínguez et al. (2005) fed crab meal to laying hens and found that egg yolk n-6 and n-3 fatty acids (including EPA) increased as dietary inclusion increased from 0 to 6%. Newman et al. (2002) found that the fatty acid profile established in the abdominal fat pad of broiler chickens reflected that consumed in the diet. When fed fish oil, incorporation of EPA and DHA into the abdominal fat pad reflected the proportions of these fatty acids in the diet. This appears to be the case in this study upon the incorporation of LM in the diet up to 8%. Providing LM at 10% of the diet resulted

Table 4. Effect of increasing levels of dietary lobster meal on broiler fat pad coloration

Item	Lightness (L^*)	Red (a^*)	Yellow (b^*)
Lobster meal (%)			
0	72.0	6.51	26.7^{bc}
2	71.0	7.02	25.7^{c}
4	71.4	7.10	28.9^{ab}
6	70.7	7.24	28.2^{abc}
8	68.9	7.73	26.8^{bc}
10	71.2	7.65	30.7^{a}
SE	0.67	0.38	0.95
Analysis of variance (P-value)			
Treatment	NS	NS	0.017
Linear	NS	0.021	0.010

[a–c]Means within a column with different superscripts are significantly different ($P \leq 0.05$); n = 4.

Table 5. Effect of increasing levels of dietary lobster meal on broiler fat pad fatty acid profile[1] (mg of fatty acid methyl esters/g of fat pad)

Item	SFA	MUFA	PUFA	n-3 PUFA	Ratio n-6:n-3	Oleic (18:1 n-9)	Linoleic (18:2 n-6)	α-Linolenic (18:3 n-3)	EPA (20:5 n-3)	DHA (22:6 n-3)
Lobster meal (%)										
0	27.6	54.9	17.5^{ab}	1.18^{d}	12.3^{a}	45.1^{a}	15.5^{a}	0.19^{a}	0.025^{e}	0.018^{d}
2	27.7	54.4	18.0^{ab}	1.54^{c}	10.5^{ab}	45.0^{a}	15.5^{a}	0.16^{ab}	0.123^{de}	0.063^{cd}
4	28.1	54.5	17.4^{ab}	1.69^{bc}	9.1^{ab}	44.7^{ab}	14.8^{ab}	0.15^{abc}	0.120^{cd}	0.118^{bc}
6	28.7	51.7	19.6^{a}	2.20^{a}	9.3^{ab}	41.4^{c}	16.4^{ab}	0.14^{b}	0.288^{bc}	0.135^{bc}
8	29.4	53.6	17.0^{ab}	2.07^{ab}	7.1^{b}	42.0^{bc}	13.9^{ab}	0.12^{b}	0.508^{a}	0.265^{a}
10	31.5	53.5	15.1^{b}	1.62^{c}	8.2^{ab}	42.1^{bc}	12.6^{b}	0.11^{c}	0.370^{b}	0.163^{b}
Analysis of variance (P-value)										
Treatment	0.055	0.057	0.003	<0.0001	0.039	0.002	0.001	0.001	<0.0001	<0.0001
Linear	0.004	0.048	0.016	<0.0001	0.003	0.0001	0.001	<0.0001	<0.0001	<0.0001
Quadratic	NS	NS	0.002	<0.0001	0.246	NS	0.008	NS	0.025	0.04

[a–e]Means within a column with different superscripts are significantly different ($P \leq 0.05$); n = 4.

[1]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

in decreased levels of EPA and DHA in the fat pad, indicating that these fatty acids are being partitioned elsewhere. In conclusion, using LM as a feed ingredient resulted in broiler abdominal fat pads with a favorable increase in n-3 fatty acids.

ACKNOWLEDGMENTS

Financial support was provided by the Nova Scotia Department of Agriculture (Truro, Nova Scotia, Canada) and by Agriculture and Agri-Food Canada (Ottawa, Ontario, Canada). Lobster meal was provided by St. Laurent Gulf Products Ltd. (Caraquet, New Brunswick, Canada). The technical assistance of the Atlantic Poultry Research Centre (Truro, Nova Scotia, Canada) unit staff is greatly appreciated.

REFERENCES

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Mar. Mamm. Sci. 22:759–801.

Canadian Council for Animal Care. 1993. Guide to the Care and Use of Experimental Animals. 2nd ed. CCAC, Ottawa, Ontario, Canada.

Carrillo-Domínguez, S., M. E. Carranco-Jauregui, R. M. Castillo-Dominguez, M. I. Castro-Gonzalez, E. Avila-Gonzalez, and F. Perez-Gil. 2005. Cholesterol and n-3 and n-6 fatty acid content in eggs from laying hens fed with red crab meal (*Pleuroncodes planipes*). Poult. Sci. 84:167–172.

Connor, W. E. 2000. Importance of n-3 fatty acids in health and disease. Am. J. Clin. Nutr. 71(Suppl.):171S–175S.

Daniel, M. 2008. Evaluating by-products of the Atlantic shellfish industry as alternative feed ingredients for laying hens. MSc Thesis. Nova Scotia Agricultural College and Dalhousie University, Truro, Nova Scotia, Canada.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Guerin, M., M. E. Huntley, and M. Olaizola. 2003. *Haematococcus* astaxanthin: Applications for human health and nutrition. Trends Biotechnol. 21:210–216.

Guinotte, F., Y. Nys, and F. de Monredon. 1991. The effects of particle size and origin of calcium carbonate on performance and ossification characteristics in broiler chicks. Poult. Sci. 70:1908–1920.

Hu, F. B., L. Bronner, W. C. Willet, M. J. Stampfer, K. M. Rexrode, C. M. Albert, D. Hunter, and J. E. Manson. 2002. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. JAMA 287:1815–1821.

Leeson, S., and J. D. Summers. 2005. Commercial Poultry Nutrition. 3rd ed. University Books, Guelph, Ontario, Canada.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Newman, R. E., W. L. Bryden, E. Fleck, J. R. Ashes, W. A. Buttemer, L. H. Storlien, and J. A. Downing. 2002. Dietary n-3 and n-6 fatty acids alter avian metabolism: Metabolism and abdominal fat deposition. Br. J. Nutr. 88:11–18.

Pérez-Vendrell, A. M., J. M. Hernández, L. Llauradó, J. Schierle, and J. Brufau. 2001. Influence of source and ratio of xanthophylls pigments on broiler chicken pigmentation and performance. Poult. Sci. 80:320–326.

Roland, D. A. Sr. 1986. Eggshell quality IV: Oyster shell versus limestone and the importance of particle size or solubility of calcium source. World's Poult. Sci. J. 42:166–171.

Rosenfeld, D. J., A. G. Gernat, J. D. Marcano, J. G. Murillo, G. H. Lopez, and J. A. Flores. 1997. The effect of using different levels of shrimp meal in broiler diets. Poult. Sci. 76:581–587.

SAS Institute. 1999. SAS/STAT User's Guide. Version 6. 4th ed. SAS Institute Inc., Cary, NC.

Simopoulos, A. P. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med. (Maywood) 233:674–688.