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Fatty Acid Composition and Sensory Evaluation of The Meat of Broilers Fed Silkworm (*Bombyx mori* L) Pupa Dietary Supplementation

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Abstract. The effects of silkworm pupa diets rich in n-3 fatty acids on fatty acid composition and quality of broiler meat were investigated. Diets containing varying amounts of dried or fresh pupa were prepared (1) basal contained 10 % fishmeal (control), (2) basal plus 10% of silkworm pupa-dried (SP), (3) basal plus 20% of silkworm pupa-dried (SP), (4) basal plus 5% of silkworm pupa-dried (SP) + 5% of silkworm pupa-fresh (SF), (5) basal plus 10% of silkworm pupa-dried (SP) + 10% of silkworm pupa-fresh (SF). One hundred and ninety five CPC 707 breed chickens of 21-days old were fed with the experimental diets. The prominent fatty acids in thigh meat tissue were oleic acid, palmitic acid, linoleic acid, stearic acid, and α-linolenic acid. Both the total amounts of n-3 fatty acids and polyunsaturated fatty acids increased, while the total amount of n-6 fatty acids decreased with increasing levels of silkworm meal in the feed, resulting in an increase in the n-3/n-6 ratio. There were no significant differences in the effects of the treatments on the sensory quality of cooked breast meat, with the exception of taste improvement, which significantly lower in the group that was fed a basal diet containing 5% each of dried and fresh pupa. In conclusion, application of silkworm pupa as fat source in feed could improve the taste and quality of broiler meat.

Keywords: Silkworm pupa, omega-3, broiler, meat quality, fatty acid composition

Abstrak: Silkworm (*Bombyx mori* L.) pupa adalah by-product dari hasil industri ulat sutra yang mengandung omega-3 asam alpha linolenat (18:3-n3) tinggi, sekitar 40% dari total asam lemak. Penelitian ini bertujuan untuk menginvestigasi pengaruh pemberian silkworm pupa pada diet ayam broiler dalam komposisi asam lemak dan kualitas daging ayam. Lima jenis diet yang digunakan adalah (1) basal yang mengandung 10% tepung ikan (control), (2) basal yang mengandung 10% pupa kering (SP), (3) basal mengandung 20% pupa kering (SP), (4) basal mengandung 5% pupa kering (SP) dan 5% pupa basah (SF), dan (5) basal mengandung 10% pupa kering (SP) dan 10% pupa basah (SF). Penelitian ini menggunakan 195 ayam broiler jantan strain CPC 707 berumur 21 hari, pemberian diet dilaksanakan selama 21 hari. Hasil penelitian menunjukkan bahwa asam oleat, palmitat, linoleat, stearat dan alpha linolenat merupakan asam-asam lemak yang dominan ditemukan pada daging paha ayam. Jumlah total omega-3 maupun asam lemak tak jenuh (PUFA) meningkat, sementara jumlah omega-6 menurun dengan penambahan silkworm pupa pada diet, menyebabkan perbandingan atau ratio n-3/n-6 menjadi tinggi. Dalam hal kualitas daging dada ayam, tidak ada perbedaan yang nyata terlihat diantara kelima perlakuan diet, dengan pengecualian pada rasa daging ayam menunjukkan nilai sensory yang rendah pada perlakuan yang mengandung 5% pupa kering dan 5% pupa basah. Kesimpulan penelitian adalah silkworm pupa dapat dijadikan sebagai sumber omega-3 pada diet untuk meningkatkan kualitas dan rasa pada daging.

Kata kunci: silkworm pupa, omega-3, broiler, kualitas daging, komposisi asam lemak.

Introduction

The importance of n-3 polyunsaturated fatty acids (n-3 PUFAs), particularly docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (20:5n-3), in human nutrition is now widely recognized, especially in the prevention and treatment of cardiovascular disease, hypertension, diabetes, and cancer, and in the development of the brain and central nervous system (Conor, 2000; Kris-Etherton et al., 2002; Simopoulos, 1999). The health benefits of PUFAs, particularly in maintaining the balance between n-6 and n-3 PUFAs, has received increasing attention (Din et al., 2004).

The food industry has responded to demands for foods of superior health quality by modifying the nutritional profiles of foods in order to reflect recommended dietary guidelines. The most practical method to manipulate the composition of fatty acids in meat products is to modify the animal diet. Practical application of n-3 fatty acid research in the poultry industry could enhance the dietary availability of n-3 PUFAs for consumers (Balevi and Coskun, 2000).

Typical broiler diets are cereal based, and the added fat is mostly rendered fat and vegetable oils. The natural diet for poultry living in wild environment consists of seeds, plants, insects, etc. n-3 PUFA supplementation to broilers results in improved health and performance of the animal and improved product quality for human consumption.

Bou et al. (2005) reported that the concentration of n-3 PUFAs in animal products depends strongly on the fatty acid composition of the animal diet. However, the feed used by the modern poultry industry is based on grain with a high ratio of n-6 compared to n-3 fatty acids. This results in high concentrations of arachidonic acid (20:4n-6) in meat or egg products and lower concentrations of eicosapentaenoic (EPA, 20:5n-3), docosapentaenioc (DPA, 22:5n-3), and docosahexaenoic acids (DHA, 22:6n-3) (Simopoulos, 2002; 2004).

Silkworm pupa (*Bombyx mori* L.) is a by-product obtained from the silk industry and is available in large quantities in Thailand, China, India, and Japan. It contains about 40% total lipid content with ALA as a prominent fatty acid. The silk rolling industry is one of the most important industries in Thailand. The process of rolling silk cocoons produces by-products such as chrysalis (pupa), with the amount of waste exceeding 1,500 metric tons/year. Silkworm pupa oil may contain a balanced n-6/n-3 ratio. It has great potential as a high nutritive value and cheap ingredient for broiler feeds. This study was conducted to investigate the effects of diets containing different amounts of silkworm pupa on muscle fatty acid composition in male broilers and to produce broiler meat with higher concentrations of n-3 fatty acids.

Materials and Methods

Animals and diets. A total 195 three-week-old male commercial broilers (CPC 707) obtained from a local farm in southern Thailand were used in this study. Chicks with almost similar body weight were selected and randomly and evenly assigned into five treatment groups (39 chicks/group). Dried silkworm (SP) and fresh silkworm (SF) pupa powder were donated from a local farm in Thailand. Raw SF pupa was dried at 40 °C. Broiler chickens were fed one of the following protein-based diets (1) basal diet containing 10% fishmeal (control), (2) basal diet containing 10% dried silkworm (10% SP), (3) basal diet containing 20% fresh silkworm (20% SP), (4) basal diet containing 5% dried silkworm + 5% fresh silkworm (5% SP + 5% SF), and (5) basal diet containing 10% dried silkworm + 10% fresh silkworm (10% SP + 10% SF). In the four test diets, pupa powder completely replaced fishmeal. The compositions of the diets are given in Table 1.

Feed and water were supplied *ad libitum* throughout the 21-day experimental period. Growth performance, feed conversion, and body parameters were measured in terms of percentage weight gain, survival (%), and feed conversion ratio (FCR). At the end of the experiments, broilers were sent for slaughter and processing to determine carcass yield, abdominal fat content, and yield from other parts of the body. Carcasses were chilled, and abdominal fat, skin, and thighs were collected and stored at -20 °C for fatty acid analysis, and breast muscle was collected and stored for sensory analysis. Animal care and use practices during this experiment conformed to the Guide for Care and Use of Agricultural Research and Teaching (FASS, 1990).

Fatty acid analysis. To determine fatty acid composition, 25 mg of TL was saponified with 0.5 M methanolic sodium hydroxide and subsequently methyl esterified with 14% BF_3 in methanol to obtain the corresponding fatty acid methyl esters (FAMEs) according to the Official Methods of American Oil Chemist Society (AOCS, 1990). Quantitative analysis of FAMEs was carried out by gas-liquid chromatography (GLC) with a Shimadzu GC 14B instrument (Kyoto, Japan).

Sensory analysis. Prior to sensory evaluation, fresh breast meat was cut to the size of 1.5 x 1.5 x 4 cm and steamed at 100 °C to achieve an internal temperature of 85°C. The heating time required to attain a temperature of 85 °C was determined beforehand by inserting thermocouples connected to a temperature recorder into the thermal centers of the samples.

Table 1. Ingredients and nutrient composition of experimental diet (%)

Ingredients	Diet				
	Control	10% SP	20% SP	5%SP+5%SF	10%SP+10%SF
Corn	63.41	54.28	55.80	56.33	59.42
Soybean meal (44% protein)	21.06	26.27	16.30	25.29	15.68
Fish meal (58% protein)	10.00	-	-	-	-
Silkworm pupae (SP)	-	10.00	20.00	5.00	10.0
Silkworm pupae (SF)	-	-	-	5.00	10.0
Palm oil	3.50	5.50	4.00	3.80	1.00
Dicalcium phosphate 18%	0.35	1.50	1.40	1.50	1.40
Limestone	0.10	1.00	1.10	1.00	1.10
Methionin	0.18	0.05	-	0.05	-
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin premix[1]	0.50	0.50	0.50	0.50	0.50
Mineral premix[1]	0.50	0.50	0.50	0.50	0.50
Crude protein	20.00	20.00	20.00	20.00	20.00
Calcium	0.86	0.85	0.86	0.85	0.86
Phosphorus	0.42	0.42	0.42	0.42	0.43
Lysine	1.13	1.23	1.34	1.22	1.33
Methionine + Cystine	0.86	0.86	0.95	0.86	0.96
Tryptophan	0.23	0.29	0.31	0.29	0.31
Threonin	0.78	0.84	0.89	0.84	0.89

[1]Vitamin and mineral premix provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 5,000 UI; vitamin E, 30 mg; vitamin K3, 3 mg; vitamin B2, 8 mg; vitamin B6, 5 mg; vitamin B12, 11 µg; folic acid, 1.5 mg; biotin, 150 µg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; MN, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; and ethoxyquin, 150 mg

After the samples were heated, they were chilled in cold water to about 15 °C. The cooked samples were then wrapped in aluminum foil and kept in a water bath at a temperature of 40 °C until use. Sensory profile of three samples of cooked breast meats was determined by 18 trained assessors who used the attributes of appearance, color, odor, taste, and texture. The assessors were selected and trained according to the ISO standard (1993). For data analysis hedonic scales with nine categories were used on the ballot: the first category was labeled with "dislike extremely" and the ninth category was labeled with "like extremely."

Statistical analysis. Data were analyzed by one-way ANOVA followed by Duncan's multiple-range test. Statistical significance is indicated as "a" or "b" for *P* values < 0.05. The sensory data were subjected to ANOVA, with treatment and panelist as the main effects. When main effects were significant at $P < 0.05$, treatment means were compared using Tukey's honestly significant difference (HSD) test.

Results and Discussion

The effects of silkworm meal, SP, and SF on weight gain, feed intake, feed conversion ratio, and survival rate on broilers are shown in Table 2.

Final body weight and dressing percentage (carcass yields) were not affected by the composition of the diets. Sklan and Ayal (1989) reported no differences in growth rates or FCR of broiler chickens fed various dietary fats of different origin. The inclusion of fish oil in poultry diets has also been reported to have no effect on feed intake, live weight, or FCR (Hulan et al., 1989; Phetteplace and Watkins, 1990) compared to a control diet without added fat. These observations were consistent with a number of findings obtained in the present study. For instance, dietary fat did not influence live weight, feed intake, and FCR (Table 2).

In addition, weight gain was not different among chickens fed the various fat diets in comparison with that in the control group. However, higher weight gain and FCR were observed in the chickens fed the 5% SP + 5% SF diet. The effect of different types of fat on FCR could be related to the degree of unsaturation.

The effects of dietary fat sources on the fatty acid composition of thigh meat are given in Tables 3. The fatty acid composition of thigh meat, skin, and abdominal fat reflect the composition of the fatty acids included in the diet. 18:1n-9 was the predominant fatty acid in all tissues; it was followed by 16:0, 18:2n-6, 16:1, 18:0, and 18:3n-3. Ajuyah et al. (1991) reported that 18:1n-9 was the major fatty acid of carcass and muscle fats because 18:1n-9 was the predominant fatty acid in all diets. In the present study, chickens fed 5% SP + 5% SF and 10% SP + 10% SF diets had higher concentrations of 18:3n-3 due to the addition of SF in the diet. The concentration of 18:2n-6 content was not different among the treatment groups, accounting for 15–17% of fatty acid. The 22:6n-3 content was therefore higher in the control group than in the other groups due to the presence of fish oil in the diet. The n-6:n-3 ratio was also lower in 5% SP + 5% SF and 10% SP + 10% SF treatment groups than in the other treatment groups.

The fatty acid composition of thigh meat was affected by dietary fat sources. 18:3n-3 content was highest in the 5% SP + 5% SF and 10% SP + 10% SF diet groups, and it was significantly higher in the thigh muscle of broilers ($p < 0.05$; Table 3).

In terms of appearance, color, odor, and texture, sensory analysis showed no significant differences among the treatment groups in the overall sensory quality of cooked breast muscle (Table 4).

The content of other n-3 fatty acids, namely 20:5n-3 (EPA), 22:5n-3 (DPA), and 22:6n-3 (DHA), also increased with silkworm oil intake; this may have resulted from elongation and desaturation of 18:3n-3 to EPA, DPA, and DHA. The decrease in broiler muscle n-6 fatty acid and 20:4n-6 (AA) content in the 5% SP + 5% SF treatment group may be explained by competition between 18:2n-6 and 18:3n-3 for incorporation into tissue membranes and desaturation and elongation of the 18-carbon fatty acids to 20-carbon fatty acids. The n-6:n-3 ratio is significantly lower after SF treatment, resulting in more favorable n-3.

Table 2. Effect of dietary silkworm pupa on feed consumption, body weight gain, feed conversion ratio and survival ratio of broiler after 21-day feeding period[1]

Diet	Feed consumption (kg/chick) (a)	Weight gain (kg/chick) (b)	ADG (kg/chick/day)	FCR (b/a)	SR
Control	2.43	1.44	0.0686	1.69	0
10% SP	2.54	1.22	0.0579	2.09	0
20% SP	2.48	1.04	0.0495	2.39	0
5%SP+5%SF	2.59	1.34	0.0636	1.94	0
10%SP+10%SF	2.5	1.22	0.0582	2.04	0

Table 3. Effect of dietary silkworm pupa on fatty acid composition (mg/100 g) in thigh meat of broiler after 21-day feeding period [1]

Fatty acids	Diet				
	Control	10% SP	20% SP	5% SP + 5% SF	10% SP + 10% SF
14:0	29.3 ± 9.0[a]	21.3 ± 3.9[c]	22.0 ± 3.2[c]	21.3 ± 3.5[c]	15.9 ± 6.8[b]
14:1n-5	7.5 ± 2.9[a]	5.5 ± 1.8[ac]	4.7 ± 0.7[bc]	4.8 ± 0.9[bc]	4.0 ± 1.3[b]
15:0	4.3 ± 0.6[a]	2.7 ± 0.4[b]	2.9 ± 0.0[b]	3.1 ± 0.4[b]	TR
16:0	740.1 ± 185.0[a]	628.1 ± 93.6[a]	665.2 ± 96.5[a]	692.1 ± 109.2[a]	592.4 ± 234.5[a]
16:1n-9	11.7 ± 3.1[a]	10.7 ± 0.9[a]	12.3 ± 1.7[a]	12.2 ± 1.8[a]	10.6 ± 4.6[a]
16:1n-7	180.1 ± 76.1[a]	100.8 ± 13.4[a]	121.6 ± 27.2[a]	120.4 ± 32.5[a]	114.0 ± 45.3a
16:2n-7	11.2 ± 0.5[a]	TR	2.1 ± 0.4[b]	2.6 ± 0.1[b]	TR
17:0	6.0 ± 1.3[a]	5.7 ± 2.0[a]	5.7 ± 1.7[a]	5.2 ± 1.2[a]	5.3 ± 1.3[a]
17:1n-8	22.8 ± 2.3[a]	8.3 ± 1.7[b]	9.5 ± 2.0[b]	9.5 ± 4.2[b]	10.1 ± 2.4[b]
18:0	170.1 ± 33.6[a]	161.5 ± 21.8[a]	185.3 ± 24.9[a]	187.3 ± 24.9[a]	192.1 ± 58.8[a]
18:1n-9	1015 ± 313.6[a]	913.0 ± 117.0[a]	1052 ± 148.3[a]	986.5 ± 209.4[a]	918.2 ± 451.6[a]
18:1n-7	190.2 ± 17.4[a]	36.1 ± 4.7[bc]	40.0 ± 6.8[c]	39.9 ± 5.0[c]	33.7 ± 5.8[b]
18:2n-6	451.3 ± 86.3[a]	429.7 ± 63.4[a]	457.7 ± 19.0[a]	456.8 ± 92.6[a]	381.7 ± 163.4[b]
18:3n-6	4.1 ± 1.4[a]	3.6 ± 1.0[a]	4.0 ± 0.3[a]	6.3 ± 2.1[a]	3.7 ± 2.4[a]
18:3n-3	20.9 ± 4.7[a]	32.9 ± 5.8[c]	56.2 ± 4.6[d]	82.7 ± 19.3[e]	133.3 ± 77.9[b]
20:0	7.0 ± 0.9[a]	TR	2.7 ± 0.7[b]	2.9 ± 0.8[b]	3.6 ± 1.8[b]
20:1n-11	2.9 ± 0.7[a]	2.9 ± 0.3[a]	3.3 ± 1.5[a]	4.3 ± 0.5[a]	5.5 ± 2.6[a]
20:1n-9	8.0 ± 2.0[a]	6.8 ± 1.0[a]	7.0 ± 1.1[a]	7.6 ± 2.1[a]	6.6 ± 3.2[a]
20:2n-6	29.7 ± 1.6[a]	8.5 ± 1.6[b]	9.2 ± 1.4[b]	8.6 ± 0.7[b]	7.9 ± 3.3[b]
20:3n-6	8.7 ± 3.6[a]	9.3 ± 2.0[a]	10.5 ± 1.8[a]	10.5 ± 1.9[a]	9.3 ± 2.6[a]
20:4n-6	35.6 ± 20.2[a]	51.4 ± 12.8[b]	55.4 ± 11.0[b]	48.4 ± 16.1[b]	38.1 ± 7.2[a]
20:5n-3	11.8 ± 3.7[a]	2.9 ± 0.5[b]	5.3 ± 0.9[c]	7.7 ± 2.3[ac]	12.2 ± 3.3[a]
22:4n-3	16.4 ± 1.5[a]	3.5 ± 0.8[b]	2.7 ± 0.9[b]	TR	TR
22:5n-3	11.7 ± 5.6[a]	8.0 ± 2.0[c]	12.2 ± 1.8[ad]	13.5 ± 3.0[ad]	18.3 ± 5.1[b]
22:6n-3	51.5 ± 31.2[a]	7.7 ± 2.8[c]	11.5 ± 2.4[b]	11.9 ± 3.3[b]	12.4 ± 3.7[b]
total	3048	2461	2761	2746	2524

[a-d] Values bearing different superscripts on the same row differ significantly (P<0.05).

[1]All measurements were done on a fresh basis; values are means six repetition per treatment. TR; trace.

Table 4. Sensory quality of cooked broiler breast meat[1]

	Diet				
	Control	10% SP	20% SP	5%SP+5% SF	10%SP+10% SF
Appearance	7.1 ± 0.5[a]	7.2 ± 0.6[a]	7.1 ± 0.6[a]	7.2 ± 0.5[a]	7.1 ± 0.6[a]
Color	7.1 ± 0.6[a]	7.1 ± 0.7[a]	7.1 ± 0.6[a]	7.1 ± 0.7[a]	7.0 ± 0.7[a]
Odor	6.4 ± 0.8[a]	6.6 ± 0.8[a]	6.6 ± 0.8[a]	6.6 ± 0.7[a]	6.7 ± 0.7[a]
Taste	6.5 ± 0.7[a]	6.8 ± 0.7[ab]	6.8 ± 0.7[ab]	6.9 ± 0.7[b]	6.8 ± 0.6[ab]
Texture	6.5 ± 0.6[a]	6.6 ± 0.8[a]	6.7 ± 0.8[a]	6.7 ± 0.5[a]	6.7 ± 0.5[a]
Overall	6.7 ± 0.7[a]	6.9 ± 0.7[a]	6.9 ± 0.7[a]	6.9 ± 0.5[a]	6.9 ± 0.6[a]

[a,b] Values bearing different superscripts on the same row differ significantly (P<0.05).
[1] Hedonic scale 1 to 9, where 1 is "dislike extremely" and 9 is "like extremely".

content in the SF broilers. The significant reduction in the n6:n3 PUFA ratio in the thigh meat of broilers fed diets containing SF is probably related to the high ALA content, which might have suppressed the metabolism of n-6 fatty acids and consequently reduced their deposition in thigh muscle. According to Ajuyah et al. (1991), the use of ALA in the poultry diet reduces the lipid content in thigh and chest meat due to possible reductions in hepatic lipogenesis stimulated by high ALA levels. Since a lower ratio of n-6:n-3 PUFA has been recommended for human nutrition, enrichment of poultry meat with n-3 PUFAs is essential for decreasing the n-6:n-3 PUFA ratio. The current study clearly revealed that muscle fatty acid content could be manipulated to decrease the n-6:n-3 PUFA ratio. In the Western diet, the intake of n-3 fatty acids is inadequate, resulting in increased platelet clotting, inflammation, cardiac disease, and cancer (Simopoulos, 2002; 2004). Barlow et al. (1990) suggested that the total n-3 content of the Western diet should be 3 g/d. Moreover, Okuyama et al. (1997) recommended a n-6:n-3 PUFA ratio of 4:1. In contrast, the n-6:n-3 PUFA ratio in the Western diet ranges from 10:1 to 21:1. Therefore, meats rich in n-3 fatty acids might have health advantages compared to regular products with low n-3 fatty acid content.

However, taste quality of broiler breast meat was significantly lower in the 5% SP + 5% SF treatment group. This may have been caused by the flavor of silkworm oil. The lowest score for taste quality was found in the control group, which may be explained by the presence of fish oil in the diet.

Conclusion

In conclusion, fatty acid composition in animal products intended for human consumption could be manipulated by nutritional supplementation to decrease the risk of heart disease and lifestyle-related diseases. The composition of fatty acids in poultry is important, and the use of silkworm pupa oil in poultry rations would subsequently affect human health in a positive manner by increasing n-3 fatty acid quantities in animal products.

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