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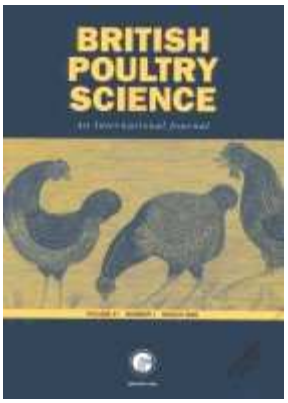

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Breast meat traits of Muscovy ducks fed on a microalga (*Crypthecodinium cohnii*) meal supplemented diet

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RUNNING TITLE: **DIETARY MICROALGA IN DUCKS**

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Abstract 1. A trial was conducted in order to increase the docosahexaenoic acid (DHA) content in the meat of Muscovy ducks (*Cairina moschata domestica L.*) fed on a diet supplemented with the microalga *Crypthecodinium cohnii*.

2. Two diets were provided to 48 male and 48 female ducks, belonging to an Italian rural strain during the last three weeks of life: a maize-soybean based diet as the control diet and the same diet supplemented with 5 g/kg microalga meal.

3. Dietary treatment did not induce differences in growth performances and slaughter traits. Similarly, chemical composition, colour, pH, oxidative stability and sensory characteristics of breast muscle were not influenced by the diet.

4. A significant increase of DHA content in breast meat of ducks fed on the *Crypthecodinium cohnii* enriched diet was observed.

INTRODUCTION

In human nutrition, the biological effect of n-3 long chain polyunsaturated fatty acids (LC-PUFAs) has received great interest as they play an active role in the prevention and management of several pathologies such as coronary heart disease, hypertension, type 2 diabetes, renal disease, ulcerative colitis, chronic obstructive pulmonary disease and Crohn's disease (Simopoulos, 2000). The main molecules of the n-3 LC-PUFAs family involved in the beneficial biological effects are eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). The current Western diet is characterised by low fish consumption, representing the main source of n-3 LC-PUFAs, and a high consumption of products from terrestrial animal origin, characterised by a large content of PUFAs of the n-6 series (Simopoulos, 2000). On the other hand, the nutritional value of these products rich in n-6 PUFAs, such

as poultry meat, could be enhanced by increasing the n-3 LC-PUFAs content. In fact, several studies aimed to enrich poultry products with n-3 LC-PUFAs indicated fish oil as the most effective source for this purpose (Hargis and Van Elswyk, 1993; Leskanich and Noble, 1997). Our previous experience showed an increase in the amount of LC-PUFAs in duck meat with dietary fish oil (Schiavone *et al.*, 2004). The recommendation of the European Union (decision 00/766/EU), concerning the ban on the use of animal proteins in animal nutrition, and the consumers' preferences, stimulated the interest in making diets for birds using exclusively vegetable ingredients.

This investigation aimed to increase the docosahexaenoic acid (DHA) content of the breast muscle of Muscovy ducks. Therefore, ducks were fed on a diet supplemented with marine microalga meal at the end of the finisher period. The microalga *Crypthecodinium cohnii*, a non-photosynthetic marine dinoflagellate, rich in DHA (Jiang *et al.*, 1999) was included in the diet and its effect evaluated at the minimum slaughter age for Muscovy ducks. Besides, meat sensory evaluation was performed to exclude the presence of a disagreeable taste.

MATERIALS AND METHODS

Animals and diets

Muscovy ducklings (*Cairina moschata domestica L.*) of both sexes of an Italian rural strain, hatched at the Experimental Avian Station of the Department of Animal Production of Pisa (Italy), were used for this trial. The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine of Pisa, Italy. All animal housing conformed to European Union guidelines.

A total of 48 males and 48 females were distributed by sex in 6 pens, respectively. Density was 3.5 males/m^2 and 4 females/m^2. All ducks received the same diet before the beginning of the experimental trial: a starter diet (12.1 MJ ME/kg and 240 g of crude protein/kg), and a finisher diet (12.0 MJ ME/kg and 200 g of crude protein/kg). The trial began when male ducks and female ducks were 50 and 43 d old, respectively. During the 21-d experimental period the finisher diet (Control diet, C) was compared with the same diet enriched with 5 g/kg dried microalga *Crypthecodinium cohnii* (Microalga diet, MA) (Algamix – AG DHA ®). The two diets were formulated to be isoenergetic and isonitrogenous (Table 1). Each diet was randomly assigned to three pens for each sex. Olive oil was used as fat source for both diets. During the trial, individual duck body weight and feed consumption per pen were recorded weekly to calculate the feed conversion ratio (FCR).

Table 1 near here

Carcase assessment and meat quality

At the minimum slaughter age, seven 71-d old males and seven 64-d old females per dietary treatment were sacrificed by electrical stunning followed by neck-cutting, after a 12-h starving period. Eviscerated and plucked carcases were weighted after removal of the feet and abdominal fat to obtain ready to cook carcases (RCC). Breast fillets and liver were excised from refrigerated RCC (6 hours at +4°C) and weighed to evaluate slaughter traits.

Just before the dissection, pH and colour measurements of *Pectoralis major* muscles were determined. pH was measured using a Hanna Instruments 8417 pH-meter supplied with a Hamilton Biotrode electrode. Meat colour (CIELAB system: *L**, *a**, *b**) was measured on breast muscle surface using a Minolta Chroma-Meter CR-300 colour analyser.

Pectoralis major muscle was vacuum-packaged and frozen (-20°C) for analytical and sensory determinations described below. AOAC methods (1990) were used to assess moisture, ash, protein and ether extract and results were expressed as percentage on a fresh matter basis. Fatty acid composition of both breast muscle and diets was ascertained by capillary gas chromatography after lipid extraction (Folch *et al.*, 1957) and esterification (Christie, 1982), using sodium methoxide as catalyst. Fatty acid methyl esters were analysed by a Perkin-Elmer AutoSystem gas-chromatograph, equipped with a flame ionisation detector and a Supelco Omegawax 320 capillary column (30 m x 0.32 mm, 0.25 µm film). Each fatty acid peak was identified by pure methyl ester standards (Supelco and Restek Corporation, Bellefonte, PA) and data were conveyed as relative values.

Susceptibility to lipid oxidation was estimated from thiobarbituric acid reactive substances (TBARS) according to the iron-induced TBARS procedure, described by Huang and Miller (1993): 3 g of minced breast were homogenised in 57 ml of a chilled 1.15% KCl solution; 30 ml of the homogenate were incubated at 37°C in a shaking water bath with 8.34 mg $FeSO_4 \cdot 7H_2O$ (final concentration 1 mM Fe^{+3}) as oxidative agent. The iron-induced TBARS assay was performed at 0, 60 and 120 minutes of incubation and the absorbance was read at 532 nm. Liquid malonaldehyde bis (diethyl acetal) (MDA) (Aldrich Chemical Co Ltd, Dorset England) was used as the standard to determine the linear standard response and recovery. TBARS values were calculated by multiplying absorbance by a constant coefficient K (23.58), combining standard response, recovery (93.4%), molecular weight of the MDA and sample weight. TBARS values were expressed as mg MDA/kg fresh meat.

A sensory panel test was performed on *Pectoralis major* muscle, without skin, roasted in a hot air oven at 165°C until an internal temperature of 70°C, without salt or spices and skin. Cooked samples were immediately sliced into 8 pieces and randomly offered to 6 trained panellists. The trial consisted of 4 sessions and the traits assessed were: tenderness, juiciness, fibrousnesses and acceptability. A 5-point scale was used: 1 referring to very disagreeable, very tough, very dry, very fibrous and 5 to very agreeable, extremely tender, very juicy, without fibre (Cross *et al.* 1986).

Statistical analysis

Measurements of *in vivo* performances, slaughter traits, chemical composition, fatty acid profile and sensory analysis of meat were split by sex and analysed with a linear model including the fixed effect of diet (SPSS, 1997). Significance of differences was evaluated by t-test.

RESULTS

Fatty acid composition of microalga meal was characterised by a 30.6% DHA content. The 5 g/kg inclusion of microalga meal in MA diet resulted in a 2.3 g/kg content of DHA of total fatty acids, while DHA in the C diet was not detected (Table 1). Growth performances, slaughter traits and breast muscle traits (colour, pH and chemical composition) were not influenced by dietary treatments (Tables 2, 3 and 4). Similarly, susceptibility to lipid oxidation was not affected by the diet (Table 4). Besides, the inclusion of microalga did not influence tenderness, juiciness, fibrousness and acceptability of meat (Table 4).

Tables 2,3,4 near here

Fatty acid composition of breast meat (Table 5) was affected ($P < 0.05$) by the diet. In particular DHA content was significantly higher in birds fed on the MA diet (+ 2.9 fold in males and + 2.8 fold in females, for MA and C diet,

respectively). The improvement of DHA content in breast muscles due to MA diet significantly influenced the total n-3 PUFAs amount (+ 1.9 fold in males and + 1.8 fold in females, for MA and C diet, respectively) and the n-6/n-3 ratio (- 1.8 fold in males and – 1.9 fold in females, for MA and C diet respectively). Table 5 near here

DISCUSSION

Our results of moisture, protein and lipid contents in breast meat, 74.6 - 76.8 %, 20.2 - 21.2 %, 0.99 - 1.06 % respectively, are similar to those reported by other authors, (Baéza *et al.*, 1997; Baéza *et al.*, 2002; Koci *et al.*, 1982; Paquin, 1988).

Avian fatty acids are typically monounsaturated, due to an active hepatic delta-9 desaturase, and an oleic and palmitoleic acid predominance. Domestic granivores use dietary carbohydrate for *de novo* fatty acid synthesis. Birds usually, except for some species, lack the enzymatic capacity to introduce double bonds past the ninth carbon of the fatty acid. Thus, they cannot use stearic acid to synthesise linoleic acid or alpha-linolenic acid, which are essential fatty acids in birds (Klasing, 2000). Dietary provision of lipids was shown to influence the composition of phospholipid membranes and adipose depots. In fact, susceptibility of avian tissues and yolk lipids to fatty acid manipulation has been reviewed by Leskanich and Noble (1997) and Hargis and Van Elswyk (1993). Thus, the enrichment of poultry products with n-3 fatty acids, especially those with a number of carbons up to 20, is the main goal of fatty acid manipulation. Maldijan *et al.* (1996) showed that phospholipid fatty acid composition of eggs from chicken and duck (*Anas platyrhynchos L.*) markedly differed. The phospholipid fraction of duck eggs has higher proportions of arachidonic acid and lower proportions of DHA than chicken eggs. This shows that duck liver is quiet efficient at converting dietary 18:2 n-6 into 20:4 n-6, whereas in chicken the

conversion of 18:3 n-3 into 22:6 n-3 has priority. Thus, variations in the fatty acid profiles of yolk lipids may be derived from dietary provision of PUFAs as well as genetic differences among avian species regarding liver metabolic activities. However, in broiler chicken the capability to transfer LC-PUFAs from liver to peripheral tissue seems to be less effective than the mechanism involved in the incorporation of these in the yolk lipids. In fact Lopez-Ferrer *et al.* (2001) showed that dietary linolenic acid (28% of total dietary fatty acid) stimulates hepatic metabolic pathways involved in the elongation and desaturation of C18:3 n-3 into 22:6 n-3 (5.67%-7.16% of total fatty acid), resulting in an inefficient build-up in thigh muscles (0.25% of total fatty acid). Similarly, in turkey, the conversion of C18:3 n-3 to EPA and DHA is weak. According to Komprda *et al*. (2005) the administration of three different diets containing 1.9%, 36.5% and 1.1% alpha-linolenic acid resulted in 6.8%, 9.0% and 5.7% EPA+DHA in breast meat.

Dietary algae have been successfully used in the nutrition of laying hens (Herber and van Elswyk, 1996; Nitsan *et al*., 1999; Tallarico *et al.*, 2002), broiler chickens (González-Esquerra and Leeson, 2001; Mooney *et al.*, 1998; Sirri *et al.*, 2003a; Sirri *et al*., 2003b) to obtain enriched poultry products, characterised by a significant amount of DHA or EPA, which could contribute to the novel concept of "functional food" in human nutrition (Garg *et al*., 2006; Almeida *et al*., 2006). In biological tissues and fluids, susceptibility to lipid oxidation is related to fatty acid chain length, number of double bonds (Liu *et al*., 1997) and amount of antioxidant molecules (Surai, 1999). Our data on iron-induced TBARS showed no differences between groups in spite of the increase in DHA amount in animals fed on the diet supplemented with the microalga meal. This might be explained by the fact that the content of naturally occurring carotenoids, such as beta-carotene and

canthaxanthin in the *Crypthecodinium cohnii* (Barclay *et al.*, 1994), may provide LC-PUFAs stabilisation due to their antioxidant properties (Surai, 2002). Other authors studied the effect of other dietary microalgae on lipid stability of avian meat. No differences were found by Mooney *et al*. (1998) in breast meat TBARS values among chickens fed on 0%, 2.8% and 5.5% of dried *Schizochytrium*. Sirri *et al.* (2003a and 2003b) studied the effect of 0.0%, 0.5% and 1.0% dietary *Schizochitrium* in broiler chicken on lipid stability of both drumstick and breast meat. TBARS values of drumstick meat were not influenced by dietary *Schizochytrium*, oppositely breast meat TBARS values of the same birds receiving 1.0% *Schizochytrium* appeared to be significantly higher than those from birds fed on 0.0 and 0.5% alga meal.

The 5 g/kg inclusion of *Crypthecodinium cohnii* did not affect the sensory panel test, agreeing with the results shown by Sirri *et al*. (2003b) when *Schizochytrium* were administered to chickens. Likewise Mooney *et al*. (1998) referred no modification of flavour in fresh chicken meat, while cooked meat samples from the experimental group with the highest level of *Schizochytrium* (5.5% of diet) were judged “undesirable”.

In conclusion the 5 g/kg inclusion of microalga *Crypthecodinium cohnii* in the diet, provided for the last 21 d of life, improved DHA content in the duck meat without negative influences on sensory traits of the breast meat.

Also, the dietary microalga did not negatively influence growth performances or slaughter traits. In addition, chemical composition, colour, pH and oxidative stability of breast muscle were not influenced by the microalga supplemented diet. Muscovy duck appeared to react positively to this dietary

supplementation. Thus, the use of *Crypthecodinium cohnii* could be used as a dietary strategy to enhance n-3 PUFA in duck meat.

In conclusion this DHA-enriched meat could contribute to human nutrition, providing an increase of about three-fold in DHA content compared to meat from ducks fed on a diet without microalga meal supplementation.

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Table 1. *Ingredients, chemical and fatty acid composition of the diets*

	Experimental period *(last 21 d of life)*	
	C diet	MA diet
Ingredients (g/kg)		
Maize meal	598	602
Soybean meal	340	333
Dicalcium phosphate	20	20
Calcium carbonate	17	17
Sodium chloride	2	2
DL-methionine	2	2
L-lysine	1	1
Vitamin and mineral premix[a]	5	5
Olive oil	15	13
Crypthecodinium cohnii meal	-	5
Chemical composition (g/kg)		
Dry matter	878.1	878.6
Crude protein	200.1	202.6
Ether extract	34.4	37.3
Crude fibre	29.2	31.4
Ash	67.7	63.2
Metabolisable energy (MJ/kg)	12.09	12.07
Fatty acid composition (g/100 g fatty acid)		
C14:0	0.11	0.31
C16:0	13.89	13.92
C16:1n7	0.33	0.34
C18:0	3.06	2.81
C18:1n9ct	31.84	31.80
C18:2n6c	45.19	45.45
C18:3n3	1.99	1.72
C22:6n3	-	0.23
SFAs[b]	17.96	17.98
UFAs[c]	79.81	79.65
SFAs/UFAs	0.23	0.23
MUFAs[d]	32.38	32.45
PUFAs[e]	47.43	47.20
n6	45.48	45.22
n3	1.95	1.99
n6/n3	23.00	23.00

[a] provided per kg of diet: retinol 3 mg; cholecalciferol, 45 mg; DL-α-tocopheryl acetate 30 mg; thiamine 1.5 mg; riboflavin 3 mg; pyridoxine 1.5 mg; cobalamin 0.015 mg; pantothenic acid 8.0 mg; niacin 25 mg; choline chloride 500 mg; Fe ($FeSO_4 \cdot 7H_2O$), 30 mg; Cu ($CuSO_4 \cdot 5H_2O$) 1.5 mg; Mn ($MnSO_4 \cdot H_2O$) 80 mg; Zn ($ZnSO_4 \cdot 7H_2O$) 30.0 mg; I (KI) 1.4 mg.
[b] saturated fatty acids; [c] unsaturated fatty acids; [d] monounsaturated fatty acids;
[e] polyunsaturated fatty acids.

Table 2. *Effects of microalga meal incorporation in diets for 3 weeks on growth performance of Muscovy ducks (means, n = 24)*

		Females				Males			
		C	MA	SEM	*P*	C	MA	SEM	*P*
Initial age	d	43	43			50	50		
Final age	d	64	64			71	71		
Initial LW [a]	g	1343	1369	21.05	ns	2314	2318	32.64	ns
Final LW	g	1893	1909	22.72	ns	3314	3339	41.55	ns
Total body weight gain	g	550	540	11.44	ns	1000	1021	24.34	ns
ADG [b]	g	26.20	25.71	0.54	ns	47.64	48.62	1.16	ns
FCR [c]		5.31	5.03	0.11	ns	4.36	4.00	0.11	ns

[a] Live body weight.
[b] Average daily weight gain.
[c] Feed conversion ratio (n = 3 for females; n = 4 for males).

Table 3. *Effects of microalga meal incorporation in diets for 3 weeks on slaughter traits of Muscovy ducks (means, n = 7)*

		Females *(64 d old)*				Males *(71 d old)*			
		C	MA	SEM	*P*	C	MA	SEM	*P*
LW [a]	g	1895	1918	24.89	ns	3443	3454	23.12	ns
RCC [b]	g	1171	1193	16.07	ns	2163	2181	20.72	ns
	%LW	61.78	62.23	0.46	ns	62.82	63.14	0.53	ns
BM [c]	g	244.40	249.26	4.98	ns	431.20	432.17	14.67	ns
	%RCC	20.88	20.90	0.42	ns	21.27	21.69	0.32	ns
Liver	g	27.54	28.60	1.31	ns	67.76	70.53	3.93	ns
	%RCC	2.35	2.40	0.11	ns	3.18	3.24	0.19	ns

[a] Live body weight.
[b] Ready to cook carcase.
[c] Breast muscles.

Table 4. *Effects of microalga meal incorporation in diets for 3 weeks on physico-chemical characteristics, oxidation susceptibility and sensory traits of the* Pectoralis major *muscle from Muscovy ducks (means, n = 7 or 5 for chemical composition)*

	Females				Males			
	C	MA	SEM	*P*	C	MA	SEM	*P*
pH [a]	5.64	5.67	0.04	ns	5.75	5.69	0.03	ns
Chemical composition [a]								
Moisture - %	74.61	75.20	0.22	ns	76.77	76.57	0.09	ns
Protein - %	21.15	20.90	0.11	ns	20.59	20.17	0.20	ns
Fat - %	1.06	1.00	0.09	ns	0.99	1.02	0.06	ns
Ash -%	1.35	1.36	0.02	ns	1.23	1.26	0.02	ns
Iron-induced TBARS (mg MDA kg^{-1} meat) [a]								
0 minutes	0.54	0.41	0.10	ns	0.88	1.20	0.22	ns
60 minutes	0.97	0.83	0.11	ns	1.89	2.46	0.37	ns
120 minutes	1.62	1.41	0.15	ns	1.63	2.18	0.27	ns
Colour [a]								
*L**	44.95	45.14	0.55	ns	46.28	46.84	0.41	ns
*a**	17.71	17.98	0.54	ns	17.07	16.68	0.30	ns
*b**	5.46	5.54	0.29	ns	4.53	4.70	0.36	ns
Sensory traits [b]								
Tenderness	3.48	3.35	0.09	ns	3.35	3.21	0.13	ns
Juiciness	3.39	3.08	0.14	ns	2.73	2.78	0.17	ns
Fibrousness	3.32	3.04	0.13	ns	2.62	2.55	0.10	ns
Acceptability	3.03	2.96	0.15	ns	2.48	2.83	0.103	ns

[a] results are means of two replicate analyses.
[b] each value is the mean of the judgement of 4 panellists.

Table 5. *Effects of microalga meal incorporation in diets for 3 weeks on fatty acid composition (% of total fatty acids) of the* Pectoralis major *muscle from Muscovy ducks (means, n = 3, results are means of three replicates analyses)*

	Female				Male			
	C	MA	SEM	*P*	C	MA	SEM	*P*
C14:0	0.35	0.38	0.02	ns	0.36	0.33	0.04	ns
C16:0	24.57	24.57	0.71	ns	24.00	22.36	0.55	ns
C16:1n7	1.27	1.17	0.16	ns	2.09	1.40	0.45	ns
C18:0	12.82	12.48	0.78	ns	12.29	13.17	0.76	ns
C18:1n9ct	23.75	23.11	1.48	ns	23.73	23.31	1.73	ns
C18:1n7	2.81	2.52	0.08	ns	2.64	2.44	0.16	ns
C18:2n6c	16.85	17.03	0.45	ns	15.08	16.38	1.12	ns
C18:3n3	0.36	0.36	0.05	ns	0.33	0.34	0.03	ns
C20:4n6	8.87	9.10	0.96	ns	9.90	9.85	0.42	ns
C20:5n3	0.06	0.11	0.02	ns	0.08	0.12	0.01	ns
C22:4n6	1.99	1.80	0.21	ns	2.14	1.77	0.13	ns
C22:5n6	1.24	1.04	0.15	ns	1.20	0.96	0.11	ns
C22:5n3	0.40	0.39	0.07	ns	0.47	0.44	0.04	ns
C22:6n3	0.70	1.97	0.11	< 0.05	0.74	2.12	0.18	< 0.05
SFAs[a]	38.08	37.82	0.26	ns	37.01	36.25	0.37	ns
UFAs[b]	60.06	61.73	0.40	ns	60.28	61.54	0.75	ns
SFAs/UFAs	0.63	0.62	0.01	ns	0.61	0.59	0.01	ns
MUFAs[c]	28.55	27.80	1.52	ns	29.13	27.80	2.05	ns
PUFAs[d]	31.51	32.93	1.40	ns	31.16	33.74	1.80	ns
n6	29.98	30.10	1.25	ns	29.50	30.65	1.61	ns
n3	1.53	2.83	0.16	< 0.05	1.66	3.09	0.21	< 0.05
n6/n3	19.85	10.65	1.05	< 0.05	17.84	9.98	0.67	< 0.001

[a] saturated fatty acids; [b] unsaturated fatty acids; [c] monounsaturated fatty acids; [d] polyunsaturated fatty acids.