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Effect of Dietary Omega-3 to Omega-6 Ratio on Growth Performance, Immune Response, Carcass Traits and Meat Fatty Acids Profile of Broiler Chickens

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Abstract

This experiment was conducted to study the effect of dietary n-3 to n-6 ratio on performance, immune response, blood parameters and fatty acids profile of broiler chickens. A total number of 192 one day old broiler chicks were randomly alloctted into 6 groups. Chicks of groups 1, 2, 3, 4, 5 and 6 were fed balanced corn-soybean diets containing n-3 to n-6 ratios of 1:1, 1:3, 1:5, 1:7, 1:9 and 1:11, respectively. Different n-3 to n-6 ratioes had no significant effect on growth performance parameters. The best dressing percentage was recorded in group 3 while no significant difference was noticed in the weight of organs except for a significant increase in the weight of gizzard in group 4. There was a variable effect of the n-3 to n-6 ratio on parameters of innate immunity. The highest lymphocyte percentage was detected in group 5. Antibody titers against Newcastle disease (ND) and Avian Influenza (AI) increased in wider ratio groups. The lowest glucose level was detected in group 4. Though serum albumin and total protein were decreased in group 3, serum globulin increased in groups 2 and 3. The lowest cholesterol content of breast meat was detected in group 3 and the highest content was detected in group 6. The cholesterol content of the thigh recorded opposite results. Narrow dietary n-3 to n-6 groups tended to record higher n-3 PUFAs content especially DHA in breast meat. While wider n-3 to n-6 ratio groups tended to deposit more SFAs, MUFAs and n-6 PUFAs than the narrower ratio groups. The best n-3 to n-6 ratio of breast meat was recorded in group 2 receiving dietary n-3 to n-6 ratio of 1:3. From the results of this study, it could be concluded that the dietary n-3 to n-6 ratio had no significant effect on growth performance of broiler chickens. The best dressing percentage was detected in group with the ratio of 1:5. The ratio of 1:3 recorded the best health state parameters.

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Introduction

The long chain omega-3 fatty acids (n-3 fatty acids) have long been recognized as an important factor in animal feeding. In man and intensively reared animals it appears that diets have become unbalanced in terms of the make-up of fat particularly polyunsaturated fatty acids (PUFAs) .The content of omega-3 (n-3) fatty acids has declined and that of omega-6 (n-6) fatty acids increased. By supplementing with fish oil (FO) or linseed oil (LSO) which is rich in n-3 fatty acids, the balance can be restored (Bezard *et al*., 1994; Manilla *et al.*, 1999 and Lopez-Ferrer *et al*., 2001).

The concentration of n-3 fatty acids in animal products depends strongly on the fatty acid composition of the diet **(**Bou *et al*., 2005). Green leaves have a surplus of the n-3 fatty acids alpha-linolenic acid (ALA) compared to the n-6 fatty acid linoleic acid (LA). In most seeds and grains, LA dominates, and the concentration of ALA is low. Some seeds have, however, high levels of ALA; *e.g.* especially linseed but also rapeseed and somewhat soybean seed. A hen living in free environments in nature will have good balance between leaves and seeds in the diet, and thus getting both n-3 and n-6 fatty acids. The feed used by modern poultry industry, however, is based on grain with a high ratio of n-6 fatty acids compared to n-3 fatty acids. This will result in a high concentration of arachidonic acid (20:4 n-6) in the meat or egg product, and less eicosapentaenoic acid (EPA, 20:5 n-3), docosapentaenoic acid (DPA 22:5 n-3) and docosahexaenoic acid (DHA 22:6 n-3). The capacity for conversion of ALA to n-3 very long chain PUFAs has been investigated (Burdge and Calder, 2005 and Bakewell *et al.*, 2006). It has been shown that this conversion is not high in humans, and it appears that young women of reproductive age have a greater capacity than men to convert the essential fatty acid ALA to DHA (Bakewell *et al*., 2006).

There has been some concern that diets enriched with n-3 PUFAs may have detrimental effects on chicken immunity and impaired resistance to infection (Fritsche *et al*., 1991; Parmentier *et al.*, 1997). However, it is not clear whether this concern is justified, given that some studies show no effect (Puthpongsiriporn and Scheideler, 2005), some show a detrimental effect (Babu *et al*., 2005), and some show an improvement (Yang and Guo, 2006). The main immune organs in poultry are the thymus, spleen, and bursa of Fabricius. During an immune response, mature lymphocytes and other immune cells interact with antigens in these tissues. Consequently, in some cases immune tissue mass can indicate immune status (Moller and Erritzoe, 2000; Grasman, 2002; Smith and Hunt, 2004). Wang *et al*. (2000) observed that feeding laying chickens diets rich in n-3 PUFAs promoted the growth of the thymus, spleen and bursa up to 4 wks of age. However, from the age of 4 wks onward, immune tissue weights began to decline, and the bursa degenerated between 4 and 8 wks of age. Nevertheless, they suggested that changes in the weights of the thymus and spleen did not correlate with the immune function. Interestingly, the same phenomenon was observed in the thymus

and spleen of mice fed n-3 PUFAs diets (Ellis *et al*., 1986; Huang *et al*., 1992). The reported effects of n-3 PUFAs on phagocytosis in animal models are inconsistent and a matter of debate. There is evidence of suppressive effects of dietary n-3 PUFAs on lymphocyte proliferation in chickens (Wang *et al*., 2000; Zhaogang *et al*., 2004; Yang *et al*., 2008), and humans (Thies *et al*., 2001; Kew *et al*., 2004).

The present study was designed to investigate the effect of different n-3 to n-6 ratios on growth performance, some blood parameters, immune tissue weight, phagocytosis activity, carcass traits and meat fatty acids profile in broiler chickens.

Materials and Methods

This study was conducted at Nutrition and Veterinary Clinical Nutrition Department, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Birds, accommodation and management

A total of 192 unsexed one day old Avian 48 chickens were used in this experiment. The chicks were individually weighed and wing-banded and randomly allocatted into 6 equal groups (32 chickens per each). The chickens were housed in a clean and well ventilated room, previously disinfected with formalin. The room was provided with electric heaters to adjust the environmental temperature according to the age of the birds. Feeds and water were supplied ad-libitum. Neomycin Sulphate, Clostin Sulphate, Spectinomycin and Cephadrin were used in the drinking water as prophylactic against the most common infectious diseases. The chickens were vaccinated against Newcastle, Infectious Bursal Disease (IBD) and Avian Influenza (AI) as presented in Table 1.

Table 1. Vaccination program of broiler chickens

Age of chickens (days)	Vaccine	Route of administration
7	ND (Hitchner)[1]	Eye drops
10	IBD (Intermediate)[2]	Eye drops
12	ND[3]	Intramuscular
12	AI[4]	Subcutaneously
17	La Sota[5] ± IBD[2]	Eye drops
27	La Sota[5] ± IBD[2]	Eye drops

[1]Newcastle Disease (ND) Hichner Fort Dodge Animal Health Batch NO: 1084264A.
[2]Infectious Bursal Disease (IBD) intermediate strain CEVA Sante Animale Batch NO: 1609T2D2A.
[3]Newcastle Disease (ND) inactivated Intervet Batch NO: A289A01.
[4]Avian Influenza (AI) H_5N_1 inactivated Vaccine, Zhaoqing Danhuanong Boilogy Medicine Co., Batch NO: 009120.
[5]La Sota, ISO S.P.A, Batch NO: 0533D.

Experimental design and feeding program

Birds recived one of the six experimental diets during the experimental period (6 weeks experiment). A basal diet formulated to meet the requirements of broiler chickens as recommended in Cobb-Vantress (2008). The different proportions of

ingredients to meet the requirements at different production periods and chemical analysis of the basal diet are presented in Table 2.

Table 2. Ingredients and chemical analysis of the basal diet

Ingredients	Starter diet (0-10 d)	Grower diet (10-28 d)	Finisher diet (28-42 d)
Yellow corn, ground	55.00	59.86	62.09
Soybean meal (44% CP)	33.59	28.95	25.78
Corn gluten meal	4.12	3.50	3.82
Oil	2.80	3.48	4.29
Ground Limestone	1.41	1.20	1.15
Dicalcium phosphate	2.00	1.85	1.72
L- Lysine	0.23	0.29	0.28
DL- Methionine	0.15	0.17	0.17
Common salt	0.40	0.40	0.40
Vitamin and mineral mix[1]	0.30	0.30	0.30
Chemical analysis[2]			
ME (Kcal/Kg)	2991	3087	3175
CP (%)	22.01	20.00	18.99
Lys (%)	1.27	1.20	1.12
Met (%)	0.52	0.51	0.50
NFE (%)	58.48	57.23	57.80
CF (%)	3.59	3.42	3.29
Ash (%)	2.96	2.72	2.54
Ca (%)	1.08	0.96	0.90
P_a (%)	0.51	0.48	0.45
Na (%)	0.17	0.17	0.17
EE (%)[3]	5.45	6.24	7.09

[1]Vitamin and Mineral mix: Protoba Mix (4) produced by Eltoba Co. for Premixes & Feed, El Sadat City, Egypt. Each 3 Kg contains Manganese 100000 mg, Zinc 600000 mg, Iron 30000 mg, Copper 10000 mg, Iodine 1000 mg, Selenium 200 mg, Cobalt 100 mg, Vitamin A 12000000 IU., Vitamin D_3 3000000 IU., Vitamin E 40000 mg, Vitamin K_3 3000 mg, Vitamin B_1 2000 mg, Vitamin B_2 6000 mg, Vitamin B_6 5000 mg, Vitamin B_{12} 20 mg, Niacin 45000 mg, Biotin 75 mg, Folic acid 2000 mg and Pantothenic acid 12000 mg.

[2]According to NRC (1994), [3]According to NRC (1984).

The content of n-3 and n-6 fatty acids of the used ingredients according to (NRC, 1994) is presented in Table 3. Six experimental diets were prepared to contain different ratio of n-3 to n-6 fatty acids. This was achieved by mixing linseed oil (LSO) and sunflower oil (SFO) with different proportions in the basal diets according to Table 3 and algebraic equations were used to determine the actual proportions of LSO and SFO in starter, grower and finisher diets and tabulated in Table 4. The six experimental diets consisted of diet 1, 2, 3, 4, 5 and 6 containing n-3 to n-6 ratios of 1:1, 1:3, 1:5, 1:7, 1:9 and 1:11 respectively.

Table 3. The n-3 and n-6 content of the used feedstuffs

Ingredients	n-3 (%)	n-6 (%)
Yellow corn	0.08	1.82
Soybean meal	0.07	0.47
Corn gluten meal	0.00	1.16
Sunflower oil	1.10	67.30
Linseed oil	53.30	12.70

Table 4. The inclusion rate (%) of LSO[1] and SFO[2] in starter, grower and finisher diets of different experimental groups

Oil types	Groups No.					
	1	2	3	4	5	6
	Starter diets					
SFO	0.00	1.48	1.977	2.228	2.38	2.481
LSO	2.80	1.32	0.832	0.572	0.42	0.319
Total	2.80	2.80	2.80	2.80	2.80	2.80
	Grower diets					
SFO	0.21	1.927	2.505	2.799	2.975	3.093
LO	3.27	1.553	0.975	0.681	0.505	0.387
Total	3.48	3.48	3.48	3.48	3.48	3.48
	Finisher diets					
SFO	0.48	2.47	3.145	3.485	3.69	3.826
LSO	3.81	1.82	1.145	0.805	0.60	0.464
Total	4.29	4.29	4.29	4.29	4.29	4.29

[1]Linseed oil, [2]Sunflower oil.

Measurmrnt of growth performance

Body weight and feed intake of broiler chickens were recorded weekly. Relative growth rate (RGR), feed conversion ratio (FCR), protein intake (PI) and protein efficiency ratio (PER) were calculated.

Evaluation of immune response

Immune response of birds was estimated by a group of parameters including phagocytic activity, phagocytic index, lysozyme activity, bactericidal activity, differential leukocytic count, Haemagglutination inhibition (HI) tests for newcastle disease and avian influenza antibodies as well as lymphocyte transformation test.

Phagocytic activity and phagocytic index

These parameters were determined according to Kawahara *et al.* (1991). Fifty micrograms of *Candida albicans* culture was added to 1 mL of citrated blood collected at the end of experiment (42 days). Treated blood samples were put in shaker water bath at 23–25^{o}C for 3–5 hrs. Smears of blood were made and then stained with Giemsa stain. Phagocytosis was estimated by determining the proportion of macrophages which contain intracellular yeast cells in a random sample of 300 macrophages and expressed as percentage of phagocytic activity

(PA). The number of phagocytized *candida* cells was counted in the phagocytic cells to calculate the phagocytic index (PI) according to the following equations:
PA = (Macrophages containing yeast/total number of macrophages) × 100
PI = Number of cells phagocytized/number of phagocytic cells.

Lysozyme activity

Serum lysozyme activity was measured with the turbidimetric method described by Engstad *et al.* (1992); using 0.2 mg/mL lyophilized *Micrococcus lysodekticus* as the substrate in phosphate buffer adjusted to pH 5.75. Fifty microlitres of serum was added to 3 mL of bacterial suspension. The 540 nm absorbance was measured by spectrophotometer after-mixture (A_0) and after incubation for 30 min at 37°C (A). The result was expressed as one unit of lysozyme activity was defined as a reduction in absorbency of 0.001/min. Lysozyme activity = $\{(A_0-A)/A\}$.

Bactericidal activity

Serum bactericidal activity to *Aeromonas hydrophila* strain was determined according to Rainger and Rowley (1993). Briefly, a 300 µL of *Aeromonas hydrophila* suspension (1.5×10^3 cells/mL) and 300 µL of fresh serum were mixed in sterile tubes. A blank consisted of 300 µL of bacterial suspension and 300 µL of sterile PBS. The tubes were incubated at 28 °C. A 50 µL sample was removed at 0 ,1 ,2 ,3, 4 hrs, different dilutions were plated on nutrient agar for 24 hrs at 28°C , and colony forming units (CFU) were counted. The results were recorded as survival index (SI) according to Wardlaw and Unlles (1978). Values were calculated as follows:
SI = CFU at end/CFU at start × 100.

Differential leukocytic count

This test was done at the end of experiment. The blood film was prepared according to the method described by Lucky (1977). Ten drops from May-Grunwald stain stock solution were added to equal amount of distilled water on a dry unfixed smear then mixed and left for 1 min for staining. The dye was decanted without rinsing. Diluted Giemsa stain was poured over the film as counter stain and left for 20 mins then rinsed in water current and examined by oil emersion lens. The percentage and absolute value for each type of cells were calculated according to Jain (1986).

Haemagglutination inhibition (HI) test for newcastle disease (ND) and Avian influenza (AI) antibodies

Blood samples were taken on days 7, 17, 33 and 42 for ND antibodies and days 7, 33 and 42 for AI antibodies. Blood samples were left without anticoagulant to clot. The serum was separated by centrifugation at 3000 rpm for 10 min.

Microtechnique of HI test was done according to Takatasy (1955). Geometric mean titer (GMT) was calculated according to Brugh (1978).

Blood samples

At the end of the experimental period, blood samples were taken from 5 birds of different groups. The blood samples were left to drop on the side of the tube to prevent destruction of RBCs. Each blood sample was left to coagulate at room temperature. Separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 10 min. The clear serum was transferred carefully to clean and dry vials and kept in deep freezer until analysis for determination of serum glucose, total serum protein, albumin and globulin according to Trinder (1969), Doumas *et al.* (1981), Reinhold (1953) and Coles (1974) respectively.

Carcass characteristic

At the end of the experimental period, 5 chickens from each replicate of different groups were randomly selected and scarified to calculate the carcass and dressing percentages. Liver, heart, gizzard, spleen, bursa, thymus gland, abdominal fat and total edible carcass (TEC) and relative weight of each organ were calculated as follows: Relative weight = (organ weight/live body weight) × 100.

Determination of lipid parameters of tissues

Samples from the breast and thigh muscles were taken only from lean meat. No visible fat was taken within the samples. Samples were kept at –20°C till the extraction process was performed. Lipid extraction was performed according to the method described by Hara and Radin (1978). Meat samples were cut into small pieces and then triturated in a mortar. One gram of triturated meat was added to 18 mL of Hexan-Isopropanol mixture 3/2, vol/vol, vortexed gently and left over night. The mixture was filtered by filter paper. The residues were washed 2 times by 2 mL of Hexan-Isopropanol mixture and filtered by filter paper. Separation of non-lipid components was performed by addition of 12 mL of aqueous sodium sulphate solution (1 g of anhydrous sodium sulphate ± 15 mL of distilled water). Two layers were formed after vortexing for 1-2 minutes. The upper hexan-rich layer was taken for further analysis. The hexan-rich layer was poured onto a clean, previously weighed petri dish and left for complete evaporation of the solvent. The increase in weight was the weight of lipid in 1 g of sample. Samples for total cholesterol content were taken at lipid extraction process before addition of sodium sulphate solution. The total cholesterol was estimated using the method described by Allain *et al.* (1974). The obtained results were proportional to total cholesterol content of meat. 50 µg of each extracted lipid sample of breast meat was added to 3 mL of benzene and 5 mL of freshly prepared 1% sulphoric acid-methanol. The mixture was put into screw-capped test tube, heated to 90°C for

90 mins in hot air oven. Tubes were left for cooling at room temperature. 2 mL of distilled water was added. The tube was vortexed gently and left to form two layers. The upper layer was collected and filtered through anhydrous sodium sulphate in filter paper and then became ready for gas chromatography analysis. For separation of each fraction of fatty acid methyl ester the method previously described by Radwan (1978) were used. The resulting fatty acid methyl ester was analyzed on HP (Hwellet Packard) 6890 GC gas chromatography equipped with HP-5 (5% diphenyl, 95% dimethyl polysiloxane (30 m × 0.32 mm i.d), 0.25 mm film thickness). The injector/detector temperatures were 220/250. The carrier gas was nitrogen with the gas flow of 1 mL/min. A temperature programming was used. The initial temperature was 150°C and held for 2 mins. The temperature was then elevated 10°C/min until temperature 1 (200°C). The temperature was then elevated 5°C/min until temperature 2 (250°C) and held for 9 min. The total runtime was 26 mins. The areas under the curve proportional to amounts of fatty acids in lipid samples was determined and the fatty acid percentages and concentrations in tissue were calculated

Statistical analysis

The analysis of variance for the obtained data was performed using Statistical Analysis System (SAS, 1987) to assess significant differences.

Results and Discussion

Growth performance parameters

The effects of different dietary n-3 to n-6 ratio on growth performance parameters are presented in Table 5. It was noticed that there was no significant difference in body weight gain (BWG) among different experimental groups. However the best BWG was recorded in group 3 followed by group 1 which received diets containing n-3 to n-6 ratios of 1:5 and 1:1, respectively. While the lowest BWG was recorded in group 4 which received a diet containing n-3 to n-6 ratio of 1:7. The results are supported by those obtained by Zhang *et al.* (2005), Haug *et al.* (2007) and Geier *et al.* (2009). Also, Febel *et al.* (2008) who found that broiler diets containing different energy sources; sunflower oil, linseed oil, and soybean oil did not affect growth performance of broilers significantly.

Regarding FCR and PER throughout the experimental period, the statistical analysis of the obtained data revealed that there was no significant difference in FCR and PER between the different experimental groups. However, group 6 recorded the best result followed by group 1 and then group 5 and 4. This means that there was a tendency to improve the FCR and PER in groups of wide n-3 to n-6 ratio. This may be attributed to the lesser feed intake which was recorded in these groups in the absence of significant difference in total BWG between all experimental groups. Our results are in accordance with those of Geier *et al.* (2009) who found that FCR was not affected by n-3 level. These results are also supported

by those obtained by Febel *et al.* (2008) who recorded that growth performance were not affected by using different sources of energy (SO, SBO or LSO).

Moreover, it was noticed that there was no significant difference in the performance index throughout the experimental period between the different experimental groups. These results may be explained as there was no significant difference among the different experimental groups in both FCR and final BWG throughout the whole experiment and performance index underwent the same phenomenon.

Table 5. Effect of different dietary n-3 to n-6 ratios on growth performance parameters of broiler chickens throughout the experiment[1]

Items	Groups No. (n-3: n-6 ratio)					
	1 (1:1)	2 (1:3)	3 (1:5)	4 (1:7)	5 (1:9)	6 (1:11)
BWG[2]	2458±51.85	2403.18±42.10	2461.44±44.10	2374.77±50.60	2392.8±38.23	2386.3±47.17
TFI[3]	4652.1±67.44	4652.4±77.65	4774.0±67.45	4534.3±72.56	4592.2±69.78	4490.2±81.46
FCR[4]	1.92 ± 0.04	1.96 ± 0.03	1.96 ± 0.04	1.94 ± 0.04	1.93 ± 0.03	1.91 ± 0.04
PER[5]	2.72 ± 0.06	2.66 ± 0.05	2.66 ± 0.05	2.69 ± 0.06	2.68 ± 0.04	2.74 ± 0.05
PI[6]	134.5 ± 5.62	128.08 ± 4.48	130.93 ± 4.51	128.81 ± 5.36	128.34 ± 4.05	131.2 ± 5.08

[1]Values are means ± standard error. No significant difference was observed between treatments in each item (*P>0.05*).
[2]Body Weight gain (g bird^{-1}); [3]Total feed intake (g bird^{-1}); [4]Feed conversion ratio; [5]Protein efficiency ratio; [6]Performance index.

Innate immunity

Table 6 summarizes the effect of n-3 to n-6 ratio on some parameters of innate immune response of broilers. The analysis of variance of the obtained data revealed that group 5 which received a diet containing n-3 ton-6 ratio of 1:9 significantly (*P<0.05*) improved phagocytic activity when compared with all other experimental groups. The obtained data indicated that wide n-3 to n-6 ratios up to 1:9 may have better phagocytic activity than narrow ratio groups. Our results are supported by those obtained by Al-Khalifa *et al.* (2012) stated that the percentage of monocytes engaged in phagocytosis was significantly lower after feeding 60 g Kg^{-1} of fish oil (FO) than after feeding 50 g Kg^{-1} of FO. Regarding phagocytic index, the best result was recorded in group 2 fed a diet containing n-3 to n-6 ratio of 1:3. This improvement was significant when compared with groups 1, 3, 5 and 6 and insignificant when compared with group 5.

Concerning lysozyme activity (LA), group 5 significantly (*P<0.05*) improved LA when compared with groups 2, 3 and 4 about 100%, 100% and 200% respectively, while insignificantly (*P>0.05*) increased LA when compared with other groups. The obtained data are in agreement with Zhang *et al.* (2005) who recorded that broiler chicks fed conjugated linoleic acid (CLA) showed faster carbon clearance rate (phagocytic ability) and higher lysozyme activity than non-supplemented group. This may support the results of the current study as the CLA is chemically related to linolenic acid which increased from group 1 to group 6 in the current

study. On the other hand the data revealed that the narrowest ratio (group 1) showed the best value of bactericidal activity when compared with other groups. However, wider ratio between n-3 to n-6 generally improves the bactericidal activity than narrow ratio except that recorded by group 1. Studies in the literature reporting effects of n-3 PUFAs on phagocytosis are inconsistent with some studies showing enhanced phagocytosis (Ebeid *et al.*, 2008), some showing a decrease (Babu *et al.*, 2005), and some showing no effect (Puthpongsiriporn and Scheideler, 2005).

Table 6. Effect of different dietary n-3 to n-6 ratios on innate immune response of broiler chickens[1]

Item	Groups No. (n-3 : n-6 ratio)					
	1 (1:1)	2 (1:3)	3 (1:5)	4 (1:7)	5 (1:9)	6 (1:11)
Phagocytic activity (%)	22.2±0.49^{c}	23.9±0.69^{b}	24.4±0.43^{b}	24.9±0.23^{b}	26.7±0.3^{a}	20.7±0.56^{d}
Phagocytic index	1.7 ± 0.04^{c}	2.08±0.08^{a}	1.46±0.05^{d}	1.92±0.06^{ab}	1.9±0.06^{b}	1.88±0.05^{b}
Lysozyme activity	0.05±0.01^{ab}	0.03±0.01^{bc}	0.03±0.01^{bc}	0.02± 0.00^{c}	0.06±0.00^{a}	0.05±0.00^{ab}
Bactericidal activity (%)	40.8± 0.66^{a}	38.8±0.66^{ab}	37.6±0.4^{b}	38.6±0.93^{ab}	39.6±0.75^{ab}	39.6±0.75^{ab}

[1]Values are means ± standard error, Means with different letters at the same raw differ significantly (*P<0.05*).

Differential leukocytic count

The effect of dietary n-3 to n-6 ratio on differential leukocytic count of broilers at the end of experiment is illustrated in Table 7. The analysis of variance of the obtained data showed that the different ratios of n-3 to n-6 did not significantly affect the percentage of the monocytes. Regarding lymphocyte ratio, the highest value was recorded in group 5 together with group 2 and 3. This increase was insignificant when compared with group 4 and significant when compared with the other groups. The higher lymphocyte percentage may indicate a higher specific (acquired) immune response, as lymphocytes are responsible for both humeral (B-lymphocytes) and cell-mediated immunity (T-lymphocytes).

The statistical analysis of data revealed that group 6 recorded the highest basophil count among different groups. This increase was significant when compared with groups 2 and 4 and insignificant when compared with the other groups. While group 3 recorded the highest value in eosinophil percentage which was significant when compared with 1 and 4 and numerical when compared with the other groups. Concerning neutrophils which are considered as the first line of defense against pathogens (Weir and Stewart, 1993), group 1 recorded the highest count followed by group 4 and then group 6. These groups were significantly higher than the other experimental groups (2, 3 and 5).

The analysis of variance of the data revealed that group 3 recorded the highest value in total leukocytic count among different groups. This increase was significant when compared with all the other groups. From the above results, it may be concluded that groups 2 and 3 which received diets containing n-3 to n-6

ratios of 1:3 and 1:5, respectively had the best results in total and differential leukocytic count, which may be of value in the immune status of the bird. The increases in total leukocytic count and both lymphocytes and monocytes may increase the birds capacity to combat pathogens. The results are similar to those of Hamdy *et al.* (2003) who found that total leukocytic count increased when LSO was mixed with SO with the ratio of 1:1. This ratio was in the range of groups 2 in the current study. The lymphocytes and monocytes also increased in the same group. These findings were very similar to those of the current study.

Table 7. Effect of different dietary n-3 to n-6 ratios on total and differential leukocytic count of broiler chickens[1]

Item	Groups No. (n-3 : n-6 ratio)					
	1 (1:1)	2 (1:3)	3 (1:5)	4 (1:7)	5 (1:9)	6 (1:11)
Total WBCs ($\times 10^3$ /mm^3)	22.8±0.47^{bc}	23.9±0.55^{b}	25.5±0.43^{a}	21.5±0.27^{cd}	21.0±0.52^{d}	22.9±0.57^{bc}
Lymphocytes %	44.6±0.56^{bc}	46.4±0.34^{a}	46.3±0.33^{a}	45.9±0.35^{ab}	46.7±0.4^{a}	43.5±0.67^{c}
Monocytes %	1.4±0.16	2.0±0.33	1.7±0.21	1.8±0.36	1.3±0.15	1.3±0.15
Basophils %	5.4±0.27^{ab}	4.8±0.29^{bc}	5.7±0.33^{a}	4.0±0.30^{c}	5.9±0.31^{a}	6.3±0.26^{a}
Eosinophils %	8.1±0.46^{b}	9.2±0.49^{ab}	9.5±0.43^{a}	8.2±0.39^{b}	9.1±0.31^{ab}	8.9±0.31^{ab}
Neutrophils %	40.5±1.02^{a}	37.6±0.85^{b}	36.8±0.61^{b}	40.1±0.71^{a}	37.0±0.39^{b}	40.0±0.77^{a}

[1]Values are means±standard error, Means with different letters at the same raw differ significantly ($P<0.05$).

Antibody titer against ND and AI vaccines

Table 8 summarizes the effect of different n-3 to n-6 ratio on HI antibody titer against ND and AI virus vaccines which represent parameters of humeral immune response of broilers. The analysis of variance of the obtained data revealed that there was no significant difference between experimental groups in antibody titer against both ND and AI virus at the 7th day of the experiment before vaccination with ND and AI vaccines, respectively. This result may refer to the true assumption of the uniformity of maternal immunity of the chickens.

Regarding the HI titer against ND vaccine there was no significant difference between experimental groups at day 17th of the experiment after two successive immunization against ND virus (Hitchner at day 7 and killed vaccine at day 12). However, a numerical increase in antibody titer was recorded in groups 3 and 4 that received diets containing n-3 to n-6 ratios of 1:5 and 1:7, respectively. On the other hand, it was noticed a significant ($P<0.05$) increase in antibody titer against ND virus at 33 day of the experiment by group 4 when compared within groups 2, 3 and 6 and an insignificant improvement with other experimental groups. However, at the end of the experiment (day 42), it was noticed that there was no significant difference between the experimental groups in antibody titer against ND virus. The overall view of all these results referred to a pattern that the antibody titer against ND virus decreased with narrower n-3 to n-6 ratio within chickens aging more rapidly than those recorded by wider n-3 to n-6 groups.

Table 8. Effect of different dietary n-3 to n-6 ratios on antibody titer against ND and AI virus of broiler chickens[1]

Item	Groups No. (n-3 : n-6 ratio)					
	1 (1:1)	2 (1:3)	3 (1:5)	4 (1:7)	5 (1:9)	6 (1:11)
HI[2] (ND[3]) 7 days	3.3±0.33	4.0±0.0	3.8±0.30	4.3±0.33	3. 7±0.33	3.7±0.33
HI (ND) 17 days	3.4±0.24	3.8±0.20	4.0±0.32	4.2±0.37	3.6±0.24	4.4±0.40
HI (ND) 33 days	3.6±0.24^{abc}	3.4±0.24^{bc}	2.6±0.40^{c}	4.6±0.51^{a}	4.0±0.32^{ab}	3.4±0.24^{bc}
HI (ND) 42 days	3.6±0.24	3.8±0.37	3.4±0.24	4.2±0.20	4.4±0.40	3.8±0.37
HI (AI[4]) 7 days	2.33±0.33	2. 7±0.33	2.3±0.33	3.3±0.67	3.0±0.01	2.7±0.67
HI (AI) 33 days	2.8±0.02^{b}	3.2±0.20^{ab}	4.2±0.20^{a}	3.4±0.24^{ab}	3.2±0.58^{ab}	3.4±0.24^{ab}
HI (AI) 42 days	2.8±0.37^{ab}	2.6±0.24^{b}	3.6±0.24^{a}	3.6±0.24^{a}	3.4±0.24^{ab}	2.6±0.24^{b}

[1]Values are means±standard error, Means with different letters at the same raw differ significantly ($P<0.05$).
[2]Haemagglutination inhibition, [3]Newcastle Disease, [4]Avian Influenza

Regarding HI titer against AI virus it was noticed that group 3 fed a diet containing n-3 to n-6 ratio of 1:5 had the best antibody titer at 33 day of the broiler chicken age, while at the end of the experiment, group 3 and 4 recorded the best AI antibody titer (3.6). The previous findings are in harmony with those of Ahmed *et al.* (2009) who found that 40% flaxseed diet showed significant increase in antibody titer against sheep RBCs 3 days post injection. However, at day 10 post injection, a lower antibody titer was recorded in 10% flaxseed diet group when compared with the control group. This may indicate that n-3 PUFAs improve antibody response early after immunization but after that, antibody may decline more rapidly in higher n-3 groups. These results are also supported by Sijben *et al.* (2001) and Parmentier *et al.* (2002). From the overall results concerning immune response, it can be concluded that the innate immune response parameters showed variable results. While concerning acquired immune response, the narrower n-3 to n-6 ratio groups tended to reduce humeral immune response within aging of the broiler chicken when compared with chicken groups fed on wider ratio diets.

Serum units

Table 9 summarizes the effect of different dietary n-3 to n-6 ratio on some blood serum parameters at the end of the experimental period. It was noticed that there was a linear decline of blood serum glucose concentration within widening of n-3 to n-6 ratio from group 1 to 4. The low serum glucose level indicates a higher glucose clearance from the blood to the cells. Glucose is used mainly for energy production for maintenance and also for growth and deposition of weight. This explanation was supported by the results of FCR in the present study, in which group 4 recorded the best FCR followed by group 3 which were significantly higher than group 2 *i.e.* there was a linear improvement of FCR from group 2 to group 4. This may be explained as more glucose was needed for more deposition of weight in groups 3 and 4, which was reflected in a higher glucose clearance from the blood, and therefore lower serum glucose level.

Regarding total serum protein, no significant difference was noticed between the experimental groups. However, a linear numerical decrease in total serum protein was noticed from group 1 (4.74) to group 3 (4.58). The same pattern was existing in serum albumin. This may be explained as the higher FCR in group 3 which means more deposition of meat i.e. more deposition of protein in tissue leading to some extent, to depletion of albumin from the blood and subsequently a decrease in total protein value. The analysis of data showed that serum globulin increased in group 2, 3 and 5 which was significant when compared with group 1 and 6 and numerical when compared with group 4. There was no significant difference between group 1 and group 6 in serum globulin values.

Table 9. Effect of different dietary n-3 to n-6 ratios on serum glucose and protein parameters of broiler chikens [1]

Item	Groups No. (n-3 : n-6 ratio)					
	1 (1:1)	2 (1:3)	3 (1:5)	4 (1:7)	5 (1:9)	6 (1:11)
Glucose (g/dl)	154.8±1.74^{a}	154.4±0.74^{ab}	149.2±2.24^{bc}	142.8±1.02^{d}	149.2±2.06^{bc}	146.8±0.06^{cd}
Total protein(g/dl)	4.74±0.06	4.68±0.04	4.58±0.08	4.76±0.18	4.80±0.09	4.50±0.18
Albumin (g/dl)	2.78±0.1^{a}	2.38±0.04^{ab}	2.22±0.06^{b}	2.68±0.19^{a}	2.52±0.17^{ab}	2.54±0.18^{ab}
Globulin (g/dl)	1.96±0.12^{b}	2.30±0.03^{a}	2.36±0.07^{a}	2.08±0.1^{ab}	2.28±0.08^{a}	1.96±0.11^{b}
Albumin/Globulin	1.45±0.14^{a}	1.04±0.03^{bc}	0.95±0.05^{c}	1.31±0.13^{ab}	1.12±0.12^{abc}	1.32±0.13^{ab}

[1]Values are means ± standard error. Means with different letters at the same raw differ significantly (*P<0.05*).

Concerning albumin/globulin ratio, the lower results were recorded in group 3 which was significant when compared with groups 1, 4 and 6 and insignificant when compared with groups 2 and 5. These values were recorded after the higher serum globulin recorded in group 3 in addition to the lower serum albumin due to a better FCR, as previously explained. The higher serum globulin and lower serum albumin may be preferable as it may mean a higher growth in addition to a higher immune state which was recorded in group 3. On the other hand, narrower n-3 to n-6 ratio may determinately affect the immune response of broiler chickens.

Carcass traits and immune organs

Table 10 summarizes the effect of dietary n-3 to n-6 ratios on relative weights of organs at the end of the experiment (day 42). Ratio 1:5 (group 3) showed a numerical improvement in dressing percentage when compared with all the other experimental groups except for the highest ratio (group 6) which showed a significant (*P<0.05*) reduced dressing percentage when compared with those obtained by groups 1, 2 and 3. The three groups of narrower n-3 to n-6 ratios showed higher dressing percentages than the wider ratio groups. This may be a unique result among the available researches, like Lopez-Ferrer *et al.* (1999, 2001), as most of them recorded that inclusion of PUFAs had no significant effect on dressing percentage.

Regarding relative organ weight, no significant effect was noticed among the different treatments within an exception of the increase in relative weight of gizzard in group 4 which was significant when compared with groups 1, 2, and 3. The overall view of the carcass traits revealed that n-3 to n-6 ratio had no significant effect on weights of organs with an exception of the gizzard. However, dressing percentage and total edible carcass (TEC%) were greater in the groups of narrow n-3 to n-6 ratios. The previous findings are in agreement with that of Hassan (2004) who found that no significant effect was noticed in the weights of organs due to the different n-3 to n-6 ratios (1:5, 1:10 and 1:20).

Table 10. Effect of different dietary n-3 to n-6 ratios on carcass traits (%) of broiler chickens[1]

Trait	Groups No. (n-3 : n-6 ratio)					
	1 (1:1)	2 (1:3)	3 (1:5)	4 (1:7)	5 (1:9)	6 (1:11)
Dressing	75.98±0.31^{a}	75.40±0.58^{a}	76.04±1.06^{a}	74.18±0.63^{ab}	74.57±0.72^{ab}	71.29±2.34^{b}
Liver	2.12±0.15	2.25±0.07	2.29±0.20	2.15±0.05	2.38±0.10	2.40±0.08
Heart	0.41±0.04	0.39±0.03	0.41±0.01	0.38±0.03	0.43±0.03	0.44±0.03
Gizzard	1.60±0.05^{b}	1.64±0.06^{b}	1.59±0.03^{b}	1.93±0.08^{a}	1.74±0.09^{ab}	1.76±0.08^{ab}
Bursa	0.102±0.02	0.108±0.01	0.100±0.01	0.106±0.01	0.100±0.01	0.108±0.02
Spleen	0.138±0.01	0.128±0.01	0.166±0.02	0.162±0.01	0.136±0.02	0.122±0.01
Thymus	0.162±0.03	0.126±0.02	0.136±0.01	0.150±0.02	0.170±0.02	0.102±0.03
Fat	2.19±0.28	2.34±0.23	1.94±0.21	1.88±0.19	1.75±0.25	1.65±0.30
Giblets	4.13±0.20	4.278±0.10	4.29±0.22	4.45±0.09	4.54±0.12	4.59±0.12
TEC[2]	80.11±0.57^{a}	79.68±0.65^{a}	80.34±0.98^{a}	78.63±0.64^{ab}	79.11±0.67^{ab}	75.88±2.25^{b}

[1]Values are means ± standard error. Means with different letters at the same raw differ significantly ($P<0.05$).

[2]Total edible carcass.

The immune tissue (Bursa, spleen and thymus gland) relative weight can in some cases reflect the immune system response and functionality. In the current study, n-3 to n-6 ratio insignificantly affected the growth of bursa, spleen and thymus, but generally the wider ratios numerically reduced it. However, some studies do suggest that feeding PUFAs to chickens (Wang *et al.*, 2002) results in increased spleen weights. The difference may be attributed to the author that used Single Comb White Leghorn layers fed sunflower oil, animal oil, linseed oil or fish oil at 5%. The results demonstrated that chicks fed 3 PUFAs-rich diets (sunflower, linseed or fish oils) had significantly higher weights of the thymus, spleen, and bursa compared with those of chicks fed the diet with animal oil. Our data are supported by those obtained by Al-Khalifa *et al.* (2012) which indicated that fish oil did not affect the weights of the spleens of broiler chickens. Chickens fed diets containing 50 g Kg^{-1} of fish oil had significantly greater thymus weights compared with chickens fed 0, 30, or 60 g Kg^{-1} of fish oil. Chickens fed a diet containing 50 and 60 g Kg^{-1} of fish oil had significantly lower bursa weights than those of chickens fed diets containing no fish oil or 30 g Kg^{-1} of fish oil.

Moreover, an insignificant (*P>0.05*) reduction of abdominal fat with increasing n-6 fatty acids concentration was noted. This reduction may be related to excessive n-6 fatty acids treatment that may stimulate fatty acid oxidation and thus enhance the metabolic rate in animals, as demonstrated in mice (West *et al.*, 1998). This finding are in agreement with those obtained by Suksombat *et al.* (2007) who reported a significant and linear reduction in the percentage of abdominal fat with increasing CLA in the diets for broiler chicken.

Some lipid parameters of tissue

Table 11 summarizes the effect of n-3 to n-6 ratios on lipid percentage of breast and thigh meat of broilers. The results revealed no significant difference in total lipid percentage due to the dietary n-3 to n-6 ratio recorded between different experimental groups in both breast and thigh meat. In addition, there was a clear evidence that the breast lipid percentage was higher than that of the thigh.

Table 11. Effect of different dietary n-3 to n-6 ratios on tissue total lipid and cholesterol concentrations[1]

Items	Groups No. (n-3 : n-6 ratio)					
	1 (1:1)	2 (1:3)	3 (1:5)	4 (1:7)	5 (1:9)	6 (1:11)
Breast lipids (%)	4.0±0.58	3.33±0.67	3.07±0.52	4.10±1.05	5.37±0.18	4.47±1.25
Thigh lipid (%)	2.27±0.27	2.67±0.28	2.53±0.32	2.17±0.03	2.60±0.68	2.13±0.07
Breast cholesterol (mg/g)	37.07±0.24^{ab}	36.93±0.29^{ab}	36.0±0.12^{c}	36.53±0.18^{bc}	37.27±0.07^{a}	37.4±0.12^{a}
Thigh cholesterol (mg/g)	38.6±0.42^{abc}	39.27±0.13^{a}	38.93±0.48^{ab}	38.27±0.18^{bcd}	37.8±0.23^{cd}	37.33±0.18^{d}

[1]Values are means ± standard error. Means with different letters at the same raw differ significantly (*P<0.05*).

It could be concluded that widening the n-3 to n-6 ratio resulted in a decrease in the breast cholesterol content from group 1 till reaching the minimum level in group 3 then elevated to reach the maximum level in group 6. This may be supported by the findings of Ayerza and Coates (2000) who found that Chia seed (n-3 PUFAs source) supplementation to layers resulted in a reduction in cholesterol content of eggs. On the other hand, Yin *et al.* (2008) found that increasing concentration of conjugated linoleic acid (CLA) supplementation to layers resulted in an increase of cholesterol content of the egg yolk. This may support the finding of the present work as CLA is chemically related to linolenic acid which increased in diets by widening the n-3 to n-6 ratio from group 1 to group 6. Concerning the thigh cholesterol content, the results revealed an approximate reduction of thigh meat cholesterol content through widening of the ratio. In contrast, group 2 recorded the highest cholesterol content of the thigh meat among all the experimental groups.

The comparison of breast and thigh cholesterol patterns showed that there was a great variation between cholesterol deposition in thigh and breast in different experimental groups. Groups recorded higher breast cholesterol tended to record a

lower thigh cholesterol content and vise versa. In addition, the widening of n-3 to n-6 ratio from group 1 to group 6 affected cholesterol deposition in thigh and breast in two opposite directions. It could be said that the complete understanding of this findings needs further researches.

Fatty acid profile of breast meat

The effect of dietary n-3 to n-6 ratios on fatty acid content (mg/g) and the percentage of total fatty acids of breast meat of broilers are illustrated in Table 12 and Table 13, respectively. An overview of the total data concerning fatty acid profile of breast meat showed that there are fatty acids haing a predominance over the remainder fatty acids in all groups. These fatty acids are Palmitic acid (C16:0), Stearic acid (C18:0), Linoleic acid (LA, C18:2 n-6) and Docosa hexaenoic acid (DHA, C22:6 n-3).

From the analysis of variance of the obtained data it could be noticed that the percentage of saturated fatty acids (SFA_S) increased numerically by widening the n-3 to n-6 ratio. The first two groups had the lower values of SFA_S among all the experimental groups. However, the last two groups, i.e. groups 5 and 6 recorded the higher SFAs percentage. Although this increase is not significant but this may be in harmony with many researches as Lopez-Ferrer *et al.* (2001) and Cortinas *et al.* (2004). This phenomenon may be attributed to the numerical increase in Palmitic acid (C16:0), the most predominant SFAs, and Stearic acid (C18:0) in the most two wider n-3 to n-6 groups 5 and 6.

It was noticed that narrow n-3 to n-6 ratio groups tended to record lower monounsaturated fatty acids (MUFAs) percentage while, wider n-3 to n-6 ratio groups tended to score higher MUFAs. This may be in harmony with Lopez-Ferrer *et al.* (2001) who found that feeding linseed oil at 2 and 4% resulted in insignificant decrease in MUFAs in broiler meat.

Statistical analysis of the data of PUFAs percentage of breast meat revealed that the its higher content was detected in group 1 which was significant when compared with group 5 and numerical when compared with the other groups. It was also noticed that narrow n-3 to n-6 ratio increased total PUFAs percentage compared with the wide n-3 to n-6 ratio groups. This may be due to the higher n-3 PUFAs which was the predominant constituent of the breast muscles in these groups. The n-3 PUFAs percentage in breast meat showed no significant difference between different experimental groups. However, there was a linear decrease in n-3 PUFAs percentage from group 2 to group 5. This may indicate that widening the dietary n-3 to n-6 ratio decreased the n-3 content of the breast meat. Decreasing n-3 PUFAs content of the breast meat was attributed to the numerical decrease in DHA content from group 2 to group 5. These results are in agreement with those of Qi *et al.* (2010) who found that decreasing n-6 to n-3 ratio increases deposition of desirable n-3 long chain PUFAs in edible tissue of broilers.

Table 12. Effect of different dietary n-3 to n-6 ratios on fatty acid (mg/g) profile of breast meat[1]

Fatty Acids	Groups No. (n-3 : n-6 ratio)					
	1 (1:1)	2 (1:3)	3 (1:5)	4 (1:7)	5 (1:9)	6 (1:11)
C6:0	0.03±0.02	0.01±0.00	0.01±0.00	0.01±0.00	0.13±0.05	0.28±0.06
C8:0	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.0	0.04±0.02	0.01±0.01
C10:0	0.13±0.05	0.03±0.01	0.03± 0.00	0.04±0.00	0.06±0.04	0.07±0.05
C11:0	0.16±0.02	0.09±0.00	0.12±0.03	0.14±0.03	0.13±0.07	0.1±0.05
C12:0	0.27±0.20^{ab}	0.21±0.19^{ab}	0.18±0.10^{ab}	0.04±0.01^{a}	0.62±0.06^{a}	0.16±0.09^{b}
C13:0	0.22±0.11	0.29±0.14	0.10±0.06	0.05±0.01	0.07±0.04	0.07±0.01
C14:0	0.02±0.01	0.02±0.01	0.02±0.00	0.03±0.01	0.08±0.04	0.06±0.04
C15:0	0.00	0.00	0.00	0.00	0.02±0.02	0.02±0.02
C16:0	2.20±0.26	1.69±0.41	1.51±0.2	2.44 ± 0.65	4.80±1.77	4.06±2.13
C17:0	0.00^{b}	0.004±0.0^{ab}	0.09±0.04^{ab}	0.02±0.01^{ab}	0.11±0.06^{a}	0.07±0.03^{ab}
C18:0	0.00^{c}	1.09±0.22^{bc}	1.50±0.22^{abc}	1.96±0.47^{abc}	4.35±0.14^{a}	3.19±1.75^{ab}
C20:0	0.11±0.05	0.08±0.02	0.20±0.08	0.13±0.03	0.13±0.04	0.06±0.04
C21:0	0.10±0.05	0.10±0.05	0.28±0.07	0.27±0.05	0.23±0.06	0.17±0.06
***SFAs*[2]**	3.26±0.24	3.62±0.47	4.06±0.54	5.13±1.28	10.78±3.16	8.32±4.22
C14:1	0.06±0.01	0.05±0.03	0.04±0.01	0.07±0.01	0.06±0.01	0.04±0.02
C15: l	0.05±0.01	0.02±0.01	0.01±0.00	0.05±0.02	0.12±0.12	0.02±0.01
C16:1	0.02±0.01	0.02±0.01	0.02±0.01	0.10±0.02	0.24±0.13	0.26±0.19
C17:1	0.04±0.02^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.17±0.1^{a}	0.02±0.01^{b}
C20:1	0.11±0.05^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
C22:1	0.08±0.06	0.21±0.05	0.16±0.02	0.30±0.07	0.91 ± 0.71	0.55±0.40
***MUFAs*[3]**	0.36±0.05^{b}	0.28±0.09^{b}	0.24±0.04^{b}	0.53±0.14^{ab}	1.50±0.61^{a}	0.88±0.39^{ab}
C18:2n-6	6.21±0.75^{ab}	4.63±0.96^{b}	4.49±0.54^{b}	6.82±1.56^{ab}	10.18±0.78^{a}	8.45±3.31^{ab}
C20:4n-6	0.00^{b}	0.00^{b}	0.00^{b}	0.08±0.04^{a}	0.31 ± 0.27^{a}	0.23±0.23^{a}
C20:3 n-6	0.03± 0.01	0.02±0.01	0.01±0.0	0.00	0.24±0.24	0.26±0.26
C20:2 n-6	0.00^{b}	0.02±0.01^{b}	0.03±0.02^{b}	0.13±0.05^{a}	0.00^{b}	0.13±0.04^{a}
C22:2 n-6	0.04±0.02	0.10±0.06	0.07±0.06	0.09±0.03	0.39±0.08	0.44±0.41
Sum n-6	6.28±0.78	4.87±1.03	4.59±0.54	7.12±1.68	11.17±1.4	9.51±4.17
C18:3 n-3	0.29±0.04	0.30±0.05	0.28±0.1	0.57±0.31	2.21±1.45	2.47±1.91
C20:5 n-3	0.15±0.02	0.06±0.03	0.14±0.02	0.34±0.14	1.17±0.75	0.93±0.66
C22:6 n-3	21.44±2.71	19.47±4.10	18.21±3.44	22.38±5.56	21.93±7.22	16.99±6.79
Sum n-3	21.88±2.74	19.84±4.15	18.63±3.38	23.35±5.98	25.31±5.58	20.39±5.76
***PUFAs*[4]**	26.16±3.52	24.62±5.04	23.22±3.92	30.46±7.65	36.48±4.58	29.9±7.44
n-3 : n-6	3.48±0.01	4.18±0.46	3.99±0.28	3.23±0.12	2.43±0.69	3.01±1.02

[1]Values are means±standard error. Means with different letters at the same raw differ significantly ($P<0.05$). [2]Saturated fatty acids, [3]Monounsaturated fatty acids, [4]Polyunsaturated fatty acids.

Those data are supported by those obtained by Du *et al.* (2000) and Ajuyah *et al.* (1993) who all found that decreasing n-6 to n-3 ratio increased the n-3 PUFAs especially DHA. Besides, there were many researchers who concluded that increasing the amount of n-3 PUFAs resulted in the enhancement of deposition of n-3 PUFAs in tissues of broilers. Examples of these researches are Gonzalez-Esquerra and Leeson (2000), Skrivan *et al.* (2000), Bou *et al.* (2006), Febel *et al.* (2008) and Zuidhof *et al.* (2009).

Table 13. Effect of different dietary n-3 to n-6 ratios on fatty acid (% of total FA) profile of breast meat[1]

Fatty Acids	Groups No. (n-3 : n-6 ratio)					
	1 (1:1)	2 (1:3)	3 (1:5)	4 (1:7)	5 (1:9)	6 (1:11)
C6:0	0.10±0.05	0.03±0.01	0.04±0.02	0.04±0.01	0.28±0.27	0.54±0.54
C8:0	0.05±0.02	0.07±0.03	0.08±0.04	0.04±0.01	0.08±0.08	0.01±0.01
C10:0	0.45±0.21^{a}	0.11±0.04^{ab}	0.09±0.01^{b}	0.1±0.01^{b}	0.12±0.09^{ab}	0.14±0.09^{ab}
C11:0	0.53±0.08^{a}	0.38±0.18^{a}	0.42±0.07^{a}	0.42±0.06^{a}	0.26±0.13a	0.30±0.11^{a}
C12:0	0.99±0.59	0.59±0.52	0.65±0.53	0.10±0.004	1.29±0.16	0.30±0.15
C13:0	0.75±0.35	0.88±0.36	0.45±0.31	0.12±0.01	0.15±0.08	0.16±0.07
C14:0	0.05±0.02	0.07±0.03	0.08±002	0.09±0.002	0.16±0.09	0.14±0.06
C15:0	0.00	0.00	0.00	0.00	0.04±0.04	0.03±0.03
C16:0	6.91±0.03	6.03±0.81	5.53±0.25	6.66±0.19	10.01±3.9	9.36±3.3
C17:0	0.00^{b}	0.03±0.03^{b}	0.39±0.17^{a}	0.03±0.02^{b}	0.24±0.13^{ab}	0.17±0.05^{ab}
C18:0	0.00^{b}	3.90±0.45^{ab}	5.47±0.11^{a}	5.47±0.11^{a}	9.04±2.59^{a}	7.16±2.73^{a}
C20:0	0.31±0.15^{ab}	0.26±0.003^{ab}	0.67±0.23^{a}	0.35±0.01^{ab}	0.27±0.09^{ab}	0.19±0.1^{b}
C21:0	0.28±0.14^{b}	0.45±0.30^{ab}	0.98±0.19^{a}	0.77±0.07^{ab}	0.46±0.11^{ab}	0.48±0.14^{ab}
SFAs[2]	10.43±1.10	12.82±1.96	14.89±0.88	14.24±0.05	22.43±7.14	19.08±6.38
C14:1	0.17±0.02	0.17±0.08	0.16±0.05	0.19±0.01	0.13±0.02	0.12±0.06
C15:l	0.16±0.01	0.07±0.03	0.03±0.02	0.13±0.04	0.24±0.24	0.05±0.03
C16:1	0.07±0.03	0.06±0.03	0.1±0.08	0.29±0.01	0.50±0.27	0.55±0.32
C17:1	0.12±0.06^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.36±0.22^{a}	0.05±0.05^{b}
C20:1	0.30±0.15^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
C22:1	0.28±0.24	0.72±0.10	0.60±0.05	0.82±0.01	1.90±1.50	1.35±0.75
MUFAs[3]	1.12±0.02^{ab}	1.04±0.26^{b}	0.90±0.17^{b}	1.45±0.02^{ab}	3.14±1.32^{a}	2.13±0.62^{ab}
C18:2 n-6	19.54±0.1	16.35±0.98	16.57±1.02	19.19±0.73	20.94±1.91	20.27±3.75
C20:4 n-6	0.00^{b}	0.00^{b}	0.00^{b}	0.19±0.08^{a}	0.65±0.59^{a}	0.44±0.44^{a}
C20:3 n-6	0.07±0.04	0.07±0.07	0.03±0.03	0.00	0.60±0.50	0.50±0.50
C20:2 n-6	0.00^{b}	0.07±0.04^{b}	0.10±0.05^{b}	0.30±0.08^{a}	0.00^{b}	0.30±0.06^{a}
C22:2 n-6	0.11±0.05	0.31±0.14	0.25±0.21	0.22±0.02	0.79±0.36	0.87±0.79
Sum n-6	19.72±0.19	16.81±0.99	16.96±0.95	19.94±0.53	23.01±3.25	22.43±5.51
C18:3 n-3	0.91±0.02	1.08±0.03	1.19±0.59	1.29±0.65	4.66±3.16	5.27±3.46
C20:5 n-3	0.49±0.09	0.24±0.13	0.54±0.08	1.02±0.16	2.47±1.64	2.0±1.18
C22:6 n-3	67.3±0.95	68.01±3.32	65.51±2.00	62.06±0.24	44.27±15.01	49.07±16.22
Sum n-3	68.71±0.88	69.32±3.20	67.24±1.44	64.36±0.57	51.41±10.22	56.36±11.61
PUFAs[4]	88.43±1.08^{a}	86.14±2.2^{ab}	84.21±0.84^{ab}	84.31±0.03^{ab}	72.4±7.13^{b}	78.78±6.10^{ab}

[1]Valuesare means ± standard error. Means with different letters at the same raw differ significantly ($P<0.05$). [2]Saturated fatty acids, [3]Monounsaturated fatty acids, [4]Polyunsaturated fatty acids.

The analysis of variance of data concerning the percentage of total n-6 PUFAs revealed that no significant effect was noticed due to a different n-3 to n-6 ratio but there was a tendency to increase n-6 PUFAs content of the breast meat linearly and numerically from group 2 to group 5. The previously mentioned pattern was typically the same of the most predominant n-6 PUFAs in the breast meat, LA (C18:2 n-6). The other n-6 PUFAs had no effective role in modulating that pattern. The fatty acid C22:2 n-6 percentage was less than 1% in all experimental groups. This may explain the great effect of LA on the total content of n-6 PUFAs. Concerning n-3 to n-6 ratio of the breast meat, the analysis of variance showed that there was no significant difference between different experimental groups.

However, the highest ratio was detected in group 2 followed by group 3. The n-6 PUFAs content of group 2 was 4.78 mg/g meat and that of group 3 was 4.59 mg/g meat while that of group 5 and 6 were 11.17 and 9.51 mg/g meat, respectively. There was a great evidence that n-3 to n-6 ratio increased in the groups of narrow dietary n-3 to n-6 ratio. These findings are in agreement with those of Ajuyah *et al*. (1993) and Qi *et al.* (2010) who found that lowering of n-6 to n-3 ratio resulted in lowering the n-6 to n-3 ratio in tissues of broiler chicken. In other words, increasing the n-3 to n-6 ratio increased the n-3 to n-6 ratio of tissues of broiler chicken.

The final conclusion of fatty acid analysis of the breast meat is that the best fatty acid profile was scored in group 2 followed by group 3 as they contain a lower MUFAs content, a lower n-6 PUFAs percentage and group 2 recorded a higher n-3 PUFAs percentage among all groups. Finally group 2 followed by group 3 recorded the best tissue n-3 to n-6 ratio among different experimental groups. The higher n-3 content of the meat is known to be of a great value to human consumers as it has protective effect in relation to inflammatory vascular diseases (Von Schaky and Harris, 2007 and Gogus and Smith, 2010). Studies also showed that human diets, when n-3 to n-6 ratio is well-adjusted, decrease cardiovascular drug dose. Furthermore, the balance between the n-3 and n-6 PUFAs is very important for homeostasis and normal development. The recommended n-3 to n-6 ratio for infants ranged from 1:1 to 1:2 (Simopoulos, 2002).

Conclusion

From the results of this study, it could be concluded that the dietary n-3 to n-6 ratio had no significant effect on growth performance of broiler chickens. The best dressing percentage was detected in group of the ratio of 1:5. The ratio of 1:3 recorded the best health state parameters. While the ratios 1:3 and 1:5 had the best lipid and fatty acid profile in breast meat. In summary, the current study suggests that feeding broiler chickens diets rich in n-3 PUFAs suppresses some aspects of the immune response that are considered to be important lines of defense against tumor, viral, bacterial and other infections. It remains to be determined whether this has an effect on risk of infection, which could have important implications for the poultry industry.

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