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Fatty Acid and Energy Metabolism in Broiler Chickens Fed Diets Containing Different Amounts of Saturated and Polyunsaturated Fatty Acids

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Abstract: The hypothesis tested was that the feeding of n-6 polyunsaturated fatty acids (n-6 PUFA) instead of Saturated Fatty Acids (SFA) results in more whole body fatty acid oxidation associated with enhanced whole body energy expenditure. To put the hypothesis to the test, broiler chickens were fed diets containing either beef tallow as source of SFA or a sunflower-oil blend as source of n-6 PUFA. Seven days old, male broiler chickens were used, they were kept individually in cages from 1-4 weeks of age. The experimental diets did not significantly affect growth performance. In broilers fed, the diet rich in n-6 PUFA, the ratio of deposition in the body to intake of digestible total PUFA which reflected n-6 PUFA was significantly decreased, pointing at preferentially increased n-6 PUFA oxidation. The ratio for n-9 Monounsaturated Fatty Acids (MUFA) was >1.0 which agrees with net de-novo synthesis but there was no diet effect. Feeding either the diet rich in n-6 PUFA or SFA did not influence energy expenditure. This study supports the idea that dietary n-6 PUFA instead of SFA are preferentially oxidized but no proof was obtained for enhanced energy expenditure.

Key words: Broilers, saturated fatty acids, polyunsaturated fatty acids, energy balance, body composition, fatty acid oxidation, fatty acid synthesis

INTRODUCTION

In broiler chickens, the feeding of diets high in n-6 Polyunsaturated Fatty Acids (n-6 PUFA) lower the amount of abdominal fat when compared to diets rich in Saturated Fatty Acids (SFA) (Pinchasov and Nir, 1992: Sanz *et al*., 1999, 2000; Wongsuthavas *et al*., 2008). Dietary n-6 PUFA instead of SFA are preferentially oxidized (Beynen and Katan, 1985) which may result in glucose being shifted from the oxidative into the lipogenic pathway.

In terms of energy metabolism, the efficiency of the conversion of glucose into fatty acids for esterification into body triacylglycerols is less than of dietary fatty acids being incorporated into body triacylglycerols (Newsholme, 1993). This implies that consumption of n-6 PUFA at the expense of SFA may increase heat expenditure. This reasoning could explain why the feeding n-6 PUFA versus SFA caused the observed decrease in abdominal fat deposition in broiler chickens. The present study with broiler chickens was carried out to determine whole body oxidation of fatty acids and energy expenditure. The birds were fed a diet either rich in SFA or n-6 PUFA.

The data obtained would allow to test the hypothesis that the feeding of n-6 PUFA instead of saturated fatty acids SFA results in more whole body fatty acid oxidation associated with enhanced whole body energy expenditure. Furthermore, it was anticipated that the data could be useful for the formulation of commercial broiler diets.

MATERIALS AND METHODS

Experimental design: About 31 days old male Arbor Acres broiler chicks were housed in groups and offered a commercial diet for 7 days before the commencement of the experiment. Then, the birds were randomly divided

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into 15 birds per dietarytreatment and were kept individually in cages. Feed was provided *ad libitum* in the form of meal. Birds had free access to clean water. The semi-purified diets were formulated to contain 20% crude protein and contained either 2% (w/w) beef tallow or a sunflower-oil blend (Table 1). Beef tallow was used as SFA rich fat and the sunflower-oil blend as an oil rich in n-6 PUFA.

The sunflower-oil blend contained various oils but sunflower oil was the main component. The fatty acid composition of the diets is shown in Table 2. The chickens were weighed at 7 and 28 days of age and feed consumption per cage was recorded for the same time interval. Feed conversion was calculated as g feed:g weight gain. The birds were closely monitored. Each day between 7 and 28 days of age, excreta were collected for the analysis of individual fatty acids to calculate apparent digestibility.

Table 1: Ingredient and analysed composition of the experimental diets

	Experimental diet	
Items	SFA	n-6 PUFA
Ingredient composition (%)		
Beef tallow	2.00	-
Sunflower-oil blend	-	2.00
Linseed oil	1.00	1.00
Tapioca starch	41.82	41.82
Soybean meal	45.00	45.00
Rice bran hulls	4.00	4.00
Limestone	0.50	0.50
Di-calcium phosphate	3.87	3.87
Salt	0.51	0.51
DL-Methionine	0.30	0.30
Premix[1]	1.00	1.00
Total	100.00	100.00
Analyzed composition (%)		
Dry matter	90.90	91.90
Crude protein (N x 6.25)	19.60	20.20
Crude fat	3.60	3.80
Crude fiber	6.00	5.90
Ash	6.70	6.20
Nitrogen free extract	55.00	55.80

[1]The premix supplied per kg of diet: vitamin A, 1,650 IU; vitamin D, 330 IU; vitamin E, 11 IU; vitamin K, 0.55 mg; thiamine, 198 mg; riboflavin, 3.96 mg; niacin, 3.30 mg; pyridoxine, 3.85 mg; vitamin B_{12}, 0.01 mg; calcium pantothenic acid, 10 mg; folacin, 0.61 mg; biotin, 0.17 mg; choline, 1,430 mg; manganese, 65.99 mg; iodine, 0.39 mg; potassium, 330 mg; zinc, 43.97 mg; copper, 8.80 mg; iron, 87.59 mg; selenium, 0.17 mg

Table 2: Contents of main fatty acids in the experimental diets

	Experimental diet	
Fatty acid (%) of total fatty acids	SFA	n-6PUFA
C16:0 (palmitic acid)	22.08	20.46
C18:0 (stearic acid)	32.79	20.70
C18:1 n-9 (oleic acid)	19.28	27.62
C18:2 n-6 (linoleic acid, LA)	12.44	21.18
C18:3 n-3 (alpha-linolenic acid, ALA)	12.87	12.72
ΣSFA	59.77	45.43
ΣMUFA	24.30	28.54
ΣPUFA	15.93	24.95

ΣSFA = C10:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0

ΣMUFA = C16:1 + C17:1 + C18:1 n-7 + C18:1 n-9 + C20:1 n-9 + C22:1 n-9

ΣPUFA = C18:2 n-6 + C18:3 n-3 + C18:3 n-6 + C20:5n-3

Chemical analyses: The diet and faeces samples were dried at 60°C for 72 h in a forced-hot air oven and were then analyzed for crude protein, crude fiber and ash (Yeom *et al*., 2005). The dried whole carcass samples were analysed for moisture and fat (Javadi *et al*., 2004).

Total fat in the dried samples (diets and whole carcass) were extracted as described previously (Javadi *et al*., 2004). Total lipids were saponified and methylated followed by gas chromatography for the determination of fatty acid composition (Javadi *et al*., 2004).

Calculation of energy expenditure: Bomb calorimetry was used to determine the gross energy content in the diets, homogenates of whole carcasses and faeces. An adiabatic bomb calorimeter was used with benzoic acid as a thermochemical standard. The total amount of energy that was lost as heat (heat production or heat expenditure) was calculated with the formula: Energy lost as heat = energy intake-energy in excreta-energy stored in body. Energy stored in the body was determined as total energy at the end of the 21 days feeding period minus the energy in the body at the beginning (= mean body energy content) of the 21 days feeding period. An additional six birds were used to analyse the energy in the body at the beginning of the experiment. The procedures have been describe elsewhere (Javadi *et al*., 2004).

Calculation of deposition

Intake ratio for fatty acids: The total digestible fatty acid intake was calculated as fatty acid intake (g/3 weeks) x apparent fatty acid digestibility (fraction of intake). The deposition of fatty acids was calculated with the following formula.

Fatty acid deposition (g/3 weeks) = carcass content of fatty acid at the end of the study-carcass content of fatty acid at the start of the study. The ratio of fatty acid deposition and digestible fatty acid intake was calculated for selected individual fatty acids and groups of fatty acids.

Statistical analysis: The data collected were subjected to analysis of variance for a completely randomized design using the 1985 SAS software program.For body weight, Average Daily Feed Intake (ADFI), Average Daily Gain

(ADG) and Feed Conversion Ratio (FCR) there were 15 replicates per treatment for whole carcass data there were 10 replicates per treatment. Statistical significance of differences between tretments was assessed using Duncan's multiple range test. A $p<0.05$ was pre-set as criterion of statistical significance.

RESULTS

Growth performance: There was no mortality in each treatment group. For the feeding period of 21 days, ADFI and ADG did not differ between the two dietary treatments (Table 3). FCR of the birds fed the PUFA diet was lower than that of those fed the SFA diet but the difference just failed to reach statistical significance.

Body composition and energy balance: Table 4 shows that water content of whole carcass of the birds fed the n-6 PUFA diet was significantly higher than that of their counterparts fed SFA diet.

The group-mean level of body fat was lower in birds fed the n-6 PUFA diet. Energy balance was similar for the birds fed either the SFA or n-6 PUFA diet. Energy expenditure, energy loss with in excreta and energy storage did not differ between the two dietary treatments.

Table 3: Growth performance of broiler chickens fed the experimental diets

	Experimental diet			
Items	SFA	n-6 PUFA	Pooled SE	p-value
Initial BW (g)	127.00	133.00	12.700	0.205
Final BW (g)	539.00	551.00	51.400	0.533
ADFI (g)	42.00	38.60	7.260	0.212
ADG (g)	19.50	19.90	2.370	0.668
FCR	2.17	1.94	0.326	0.065

n = 15 per treatment

Table 4: Body composition and energy balance in broiler chickens fed the experimental diets

	Experimental diet			
Items	SFA	n-6 PUFA	Pooled SE	p value
Feed intake (g/21d)	804	758	130.1	0.443
Body composition of whole carcass (%)				
Water	69.6^{b}	74.8^{a}	2.89	0.001
Fat	4.1	3.70	0.23	0.419
Protein	17.8	14.4	0.30	0.394
Ash	2.8	2.30	0.06	0.875
Calculated energy balance (KJ)				
Intake	13,115	12,679	661.8	0.624
Stored in the body	4,423	4,461	488.7	0.955
Expenditure	5,958	5,578	834.6	0.800
In excreta	2,734	2,640	956.3	0.830
In excreta as fat	208	193.0	62.0	0.600
In fat-free excreta	2,526	2,447	918.8	0.851
Energy in whole body (KJ)				
Initial body energy	570	570.0	116.8	0.740
Final body energy	4,993	5,031	1468.8	0.933

$^{a-b}$Values differ significantly

Digestibility of individual fatty acids: The apparent digestibility of individual fatty acids and groups of fatty acids did not differ between the dietary treatments (Table 5).

Ratio of fatty acid deposition: intake: On the SFA diet, the intake of digestible stearic acid and SFA was higher than on the n-6 PUFA diet. On the other hand, the birds fed the n-6 PUFA diet had a higher intake of digestible oleic acid, linoleic acid (LA), MUFA a nd n-6 PUFA (Table 6). There was no diet effect on the intake of digestible palmitic acid, Alpha-linolenic Acid (ALA) and n-3 PUFA.

The whole body of the birds at baseline contained on average a total fat mass of 7.67 g. Total fat mass in the carcass at the end of the experiment was 27.9±7.48 and 27.3±6.81 ($p = 0.822$, $n = 10$) for the birds fed the SFA and n-6 PUFA diet, respectively. The birds fed n-6 PUFA diet

Table 5: Effect of experimental diets on aparent fatty acid digestibility

Fatty acid (%) of intake	SFA	n-6 PUFA	Pooled SE	p value
C 16:0	79.8	83.0	6.89	0.309
C 18:0	74.1	76.4	8.98	0.578
C 18:1 n-9	88.0	86.9	4.61	0.598
C 18:2 n-6	91.7	93.6	3.26	0.204
C 18:3 n-3	92.5	94.1	4.10	0.375
ΣSFA	76.9	79.7	7.88	0.445
ΣMUFA	85.8	84.0	5.93	0.516
ΣPUFA	87.0	88.5	7.68	0.657

Table 6: Intake of digestible fatty acids, fatty acid deposition and deposition:intake ratio in broiler cickens fed the experimental diets

Fatty acid	SFA	n-6 PUFA	Pooled SE	p value
Digestible fatty acid intake, g /21 days				
C 16:0	5.1	4.9	1.050	0.672
C 18:0	7.0^{a}	4.6^{b}	1.540	0.002
C 18:1 n-9	5.7^{b}	5.0^{a}	1.020	0.001
C 18:2 n-6	3.4^{b}	5.7^{a}	0.700	0.001
C 18:3 n-3	3.4	3.5	0.130	0.640
Σ SFA	13.3^{a}	10.5^{b}	2.570	0.035
Σ MUFA	6.3^{b}	6.9^{a}	1.130	0.014
Σ PUFA	4.0^{b}	6.4^{a}	0.840	0.001
Fatty acid deposition (g/21 days)				
C 16:0	4.5	4.7	1.380	0.820
C 18:0	3.0^{a}	1.8^{b}	0.820	0.003
C 18:1 n-9	6.8	7.7	2.110	0.309
C 18:2 n-6	1.3	1.4	0.550	0.690
C 18:3 n-3	0.1	0.1	0.050	0.797
Σ SFA	7.6	6.5	1.860	0.206
Σ MUFA	6.9	7.8	2.130	0.346
Σ PUFA	1.5	1.6	0.580	0.780
Deposition:intake ratio (g g^{-1})				
C 16:0	0.89	0.95	0.402	0.961
C 18:0	0.43	0.39	0.227	0.447
C 18:1 n-9	1.18	1.54	0.458	0.110
C 18:2 n-6	0.39	0.25	0.227	0.085
C 18:3 n-3	0.03	0.03	0.075	0.837
ΣSFA	0.57	0.62	0.575	0.742
ΣMUFA	1.15	1.13	0.594	0.714
ΣPUFA	0.37^{a}	0.24^{b}	0.438	0.002

$^{a-b}$Values in the same row with the different superscripts differ significantly

had deposited less stearic acid than the birds fed the SFA diet (Table 6). There was no difference between the two diets as to the deposition of the other individual and groups of fatty acids. The deposition:intake ratios for individual and groups of fatty acids were similar for the birds fed either the SFA or n-6 PUFA diet, except for the ratios of LA and n-6 PUFA (Table 6). The birds fed the n-6 PUFA diet had a significantly lower ratio for PUFA.

DISCUSSION

It has been repeatedly shown that replacement of dietary SFA by n-6 PUFA lowers abdominal fat deposition in broilers (Pinchasov and Nir, 1992: Sanz *et al*., 1999, 2000; Wongsuthavas *et al*., 2008). Abdominal fat represents a small part of the total fat deposition in chicken. Studies of fat sources on whole body fat content are limited and show no significant effect of dietary PUFA source on fat deposition in broilers (Pinchasov and Nir, 1992; Crespo and Esteve-Garcia, 2002a). Likewise in the present study, there was no significant effect of dietary n-6 PUFA on total body fat.

Contrary to what would be expected, the birds fed the n-6 PUFA diet did not deposit more LA than those fed the SFA diet. In broiler chickens, the intake of LA is directly related with LA incorporation into adipose tissue (Bavelaar and Beynen, 2003) and breast meat (Smink *et al*., 2008). In two studies with broiler chickens the amount of PUFA in the diet was indeed reflected by the fatty acid composition in the whole carcass (Waldroup and Waldroup, 2005; Javadi *et al*., 2007).

The hypothesis tested was that the feeding of n-6 PUFA versus SFA would increase whole-body fatty acid oxidation and energy expenditure. The deposition:intake ratio for fatty acids reflects the net amount synthesized and/or the proportion of the digested fatty acid not oxidized. A deposition:intake ratio >1 would point at net de-novo synthesis, whereas a ratio <1 would indicate net oxidation. The low deposition:intake ratio for PUFA when compared with that of SFA or MUFA is consistent with the well-known preferential oxidation of PUFA (Beynen and Katan, 1985; Cunnane and Anderson, 1997; Jones *et al*., 1985; Yeom *et al*., 2005) and the fact that LA and ALA are essential fatty acids and by definition are not synthesized in the body.

Thus, the deposition:intake ratio for PUFA, LA and ALA cannot be >1 as was indeed found. In the birds fed the n-6 PUFA diet, the deposition:intake ratio for PUFA which mainly reflects LA was significantly lower than that for the birds fed the SFA diet. This observation agrees with preferential oxidation of PUFA in the broiler chickens fed the n-6 PUFA diet when compared with their counterparts fed the SFA diet. Thus, whole body fatty acid oxidation was higher on the n-6 PUFA diet. However, this effect was not associated with increased energy expenditure. This would lead to rejection of the hypothesis tested.

The deposition:intake ratio for MUFA which can be synthesized from glucose was not affected by the type of fat in the diet. Thus, the consumption of n-6 PUFA instead of SFA did not influence de-novo fatty acid synthesis. Results of studies in rats (Shimomura *et al*., 1990) and in broiler chickens (Sanz *et al*., 2000) indicate that dietary PUFA increase the β-oxidation and inhibit de novo fatty acid synthesis (Shimomura *et al*., 1990; Sanz *et al*., 2000). However, in a study using linseed oil as PUFA, both an increased oxidation and de novo synthesis were found (Crespo and Esteve-Garcia, 2002b). Thus, the effect of high PUFA intake on de novo synthesis of fatty acids is not clear.

ALA is more preferentially oxidized than is LA (Cunnane and Anderson, 1997; Ide *et al*., 1996; Jones *et al*., 1985). Because of the addition of 1% linseed oil to each diet, the two experimental diets contained a relatively high, identical amount of ALA. It could be suggested that the preferential oxidation of PUFA in the birds fed the n-6 PUFA diet would result in more oxidation of ALA than in the birds fed the SFA diet. However, there is no evidence for this suggestion. The deposition of ALA in the whole body was similar for the two dietary groups which also held for the deposition:intake ratio of ALA. The ratio for ALA was much lower than that for LA which corroborates the more preferential oxidation of ALA than that of LA.

CONCLUSION

In this study, the outcome of this study is in agreement with the concept that dietary n-6 PUFA are more preferentially oxidized than SFA. However, no proof was obtained for increased energy expenditure. Thus, the data obtained refute the hypothesis tested.

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