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Fatty Acid Metabolism in Broiler Chickens Fed Diets Either Rich in Linoleic or Alpha-Linolenic Acid

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ABSTRACT

The study with broiler chickens aimed to test the hypothesis that the feeding of the n-3 Polyunsaturated Fatty Acid (PUFA), Alpha-Linolenic Acid (ALA) results in more whole-body fatty acid oxidation than the feeding of the n-6 PUFA, Linoleic Acid (LA). It was reasoned that the increased fatty acid oxidation, if any, would be associated with enhanced whole-body energy expenditure. Broiler chickens were fed diets containing either a soybean-oil blend as source of LA or a linseed-oil blend as source of ALA. Seven-day-old, male broiler chickens were used; they were kept individually in cages from 1 to 4 weeks of age. Energy expenditure was calculated on the basis of the whole-body energy balance. For individual fatty acids, the apparent digestibility and deposition in the body was determined. The ALA diet raised the ratio of deposition in the body to intake of digestible LA and diminished that of ALA. This points at preferential oxidation of ALA, at the expense of LA, in the birds fed the ALA diet. Feeding the high-ALA instead of the high-LA diet did not influence energy expenditure when expressed as percentage of energy intake. This study supports the idea that dietary ALA versus LA is preferentially oxidized, but contrary to the hypothesis it was associated with unchanged energy expenditure.

Key words: Poultry nutrition, dietary fat type, energy expenditure, body composition, fatty acid deposition, minimum fatty acid synthesis

INTRODUCTION

The intake of n-6 polyunsaturated fatty acids (PUFA), instead of Saturated Fatty Acids (SFA), has specific effects on intermediary metabolism in broiler chickens. High intakes of the n-6 PUFA, Linoleic Acid (LA), lower the deposition of abdominal fat (Pinchasov and Nir, 1992; Sanz *et al*., 1999, 2000a, b; Newman *et al*., 2002; Wongsuthavas *et al*., 2008). The underlying mechanism is not yet clear, but ideas have been put forward and interesting observations have been made. One possible mechanism is based on the fact that dietary n-6 PUFA are preferentially oxidized when compared with SFA (Beynen and Katan, 1985). This 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. Studies in broiler chickens showed dietary n-6 PUFA increased ß-oxidation and inhibited *de novo* fatty acid synthesis (Sanz *et al*., 2000b). The observed inhibition of lipogenesis does not corroborate the above theoretical considerations.

The n-3 PUFA, Alpha-Linolenic Acid (ALA), is more preferentially oxidized than the n-6 PUFA, LA (Cunnane and Anderson, 1977). In the light of the above reasoning it could be suggested that a high intake of ALA versus LA may enhance whole-body fatty acid oxidation and increase heat expenditure. The present study was carried out to verify the above reasoning by testing the hypothesis that a high intake of ALA in the form of linseed oil enhances whole-body fatty acid oxidation in broiler chickens. In an earlier study with broiler chickens, the feeding of linseed oil caused an increase in both fatty acid oxidation and *de novo* synthesis (Crespo and Esteve-Garcia, 2002). Whole body fatty acid metabolism involves the absorption, *de novo* synthesis, oxidation and deposition of fatty acids. In order to obtain complete insight, the four aspects of fatty acid metabolism were determined in this study.

MATERIALS AND METHODS

Experimental design: The study was performed at the Rajamangala University of Technology-Isan in January-February 2007. Thirty 1-day-old, male Arbor Acres broiler chicks were housed in groups and offered a commercial diet for 7 days before commencement of the experiment. Then, the birds were randomly divided into 15 birds per treatment that were kept individually in cages. Experimental diets were provided *ad libitum* in the form of meal. Birds had free access to clean water. The semi-purified, experimental diets were formulated to contain 21% crude protein. The variable fat source in the diets was 0.52% (w/w) of linseed-oil blend plus 2.48% soybean-oil blend or 2.73% linseed-oil blend plus 0.27% soybean-oil blend as shown in Table 1. Linseed-oil blend was used as source of ALA and soybean-oil blend as source of LA. The composition of the two blends of oils was unknown, but either linseed oil or soybean oil was the major component. The diet with soybean oil contained 21.9% LA and 2.1% ALA, whereas the diet with linseed oil contained 8.8% LA and 15.1% ALA (Table 2).

Chicks were weighed at 7 and 28 days of age and feed consumption per cage was recorded for the entire period. The feed conversion ratio (FCR) was calculated for the whole period as g feed:g weight gain. Each day (from 7 to 28 days of age) excreta were collected and pooled per broiler for chemical analysis.

Chemical analysis: 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).

Table 1: Composition of the experimental diets

	Experimental diets	
Items	LA	ALA
Ingredient composition (%)		
Linseed oil blend	0.52	2.73
Soybean oil blend	2.48	0.27
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	98.20	98.20
Crude Protein (N x 6.25)	21.40	21.70
Crude fat	5.40	6.00
Crude fiber	3.20	3.20
Ash	7.90	7.50
Nitrogen free extract	60.30	59.80

[1]The premix supplied kg^{-1} 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 major fatty acids in the experimental diets

	Experimental diets	
Fatty acid (g/100 g methyl esters)	LA	ALA
C16:0 (palmitic acid)	25.3	20.0
C18:0 (stearic acid)	15.0	15.9
C18:1 n-9 (oleic acid)	25.8	25.8
C18:2 n-6 (linoleic acid)	21.9	8.8
C18:3 n-3 (alpha-linolenic acid)	2.1	15.1

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). Six additional birds were used to measure the amount of energy in the body at the beginning of the experiment.

Calculation of deposition: intake ratio for fatty acids: The total digestible fatty acid intake was calculated as fatty acid intake (g/3 weeks)×apparent fatty acid digestibility (fraction of fatty

acid 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 the main fatty acids.

Calculation of minimum *de novo* fatty acid synthesis: Minimum *de novo* synthesis of the sum of Saturated Fatty Acids (SFA) and Monounsaturated Fatty Acids (MUFA) was estimated as the amount deposited in the body minus the intake of digestible 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 treatments was assessed using Duncan's multiple range test. A p value<0.05 was pre-set as criterion of statistical significance.

RESULTS AND DISCUSSION

Growth performance: There was no mortality in each treatment group. For the feeding period of 21 days, ADG was depressed by 10.3% when the birds were fed the high-ALA instead of the high-LA diet (Table 3). FCR was not significantly influenced by the type of diet.

Body composition and energy balance: Table 4 shows that the whole carcass of the birds fed the high-ALA diet contained 3.5% fat, whereas that of their counterparts fed the high-LA diet contained 4.9%. Energy intake and body storage were lower for the birds fed the high-ALA diet. Energy expenditure, energy loss with in excreta and energy storage, expressed as percentage of energy intake, did not differ between the two dietary treatments.

Digestibility of individual fatty acids: Table 5 shows that on the high-ALA diet the apparent digestibility of LA was lower and that of ALA was higher than on the high-LA diet.

Ratio of fatty acid deposition: intake: On the high-ALA diet, the intake of digestible ALA was higher and the intakes of palmitic acid and LA were lower than on the high-LA diet.

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 45.5±7.34 and 39.5±4.12 (p = 0.003,

Table 3: Effects of the experimental diets on growth performance

	Experimental diet			
Items	LA	ALA	SEM	p-values
Initial BW (g)	132.00	133.00	3.000	0.871
Final BW (g)	580.00^{a}	534.00^{b}	14.900	0.038
ADFI (g)	41.80	36.90	2.210	0.125
ADG (g)	21.30^{a}	19.10^{b}	0.680	0.028
Feed: gain	2.00	1.92	0.108	0.589

n = 15 per treatment. Values within a row with different superscript differ significantly (p<0.05)

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

	Experimental diet			
Items	LA	ALA	SEM	p-values
Feed intake (g/21days)	900[a]	774[b]	37.6	0.050
Body composition of whole carcass (%)				
Water	72.2	72.7	0.77	0.775
Fat	4.9[a]	3.5[b]	0.12	0.001
Protein	17.1	16.6	0.13	0.473
Ash	2.4	2.6	0.06	0.134
Calculated energy balance (kJ)				
Intake	13.969[a]	11.459[b]	842.7	0.020
Stored in the body	6.615[a]	5.248[b]	452.5	0.028
Expenditure	4.360	3.831	637.7	0.502
In excreta	2.994	2.379	213.1	0.088
In excreta as fat	296	251	29.0	0.370
In fat-free excreta	2698	2129	201.3	0.096
Energy in whole body (kJ)				
Initial body energy	570	570	26.1	0.740
Final body energy	7.185[a]	5.818[b]	451.4	0.027

n = 10 per treatment. Values within a row with different superscripts differ significantly ($p<0.05$)

Table 5: Apparent fatty acid digestibility broiler chickens fed the experimental diets

Fatty acid	LA diet	ALA diet	SEM	p-value
Apparent digestibility (% of intake)				
C 16:0	83.9	81.5	2.25	0.455
C 18:0	70.4	69.2	3.94	0.829
C 18:1 n-9	86.7	88.0	1.58	0.579
C 18:2 n-6	91.7[a]	84.4[b]	1.89	0.013
C 18:3 n-3	90.6[b]	98.8[a]	0.92	0.001

n = 10 per dietary treatment. Values within a row with different superscript differ significantly ($p<0.05$)

n = 10) for the birds fed the high-LA and high-ALA diet, respectively. The birds fed the high-ALA diet had deposited less palmitic acid, oleic acid and LA, but more ALA than the birds fed the high-LA diet (Table 6).

The deposition: intake ratios for individual of fatty acids were different for the birds fed either the high-LA or high-ALA diet in that the latter diet had produced lower ratios for oleic acid and ALA, but a higher ratio for LA (Table 6).

Minimum *de novo* fatty acid synthesis: Minimum *de novo* synthesis of MUFA was significantly diminished in the birds fed the high-ALA diet, whereas that of SFA was unaffected (Table 7).

In this study, the broiler chickens were fed ad libitum. It was found that the high-ALA diet significantly reduced ADG, but did not significantly influence FCR. The non-significant lowering of ADFI may in part be explained the higher digestibility of ALA versus LA. Furthermore, it is possible that linseed oil versus soybean oil in the diet had diminished feed acceptability. The lower feed intake by the birds fed the high-ALA diet may not interfere with the interpretation of the outcomes. The main variables are expressed as relative rather than absolute values. Energy expenditure was calculated as a percentage of energy intake, fatty acid oxidation and synthesis are assessed by the deposition:intake ratios of fatty acids.

Table 6: Effect of dietary fat type on digestible fatty acid intake, fatty acid deposition and the deposition: intake ratio during the whole feeding period

	Experimental diets			
Fatty acid	LA	ALA	SEM	p-value
Digestible fatty acid intake (g)				
C 16:0	10.3[a]	7.60[b]	0.550	0.003
C 18:0	5.1	5.10	0.400	0.661
C 18:1 n-9	10.9	10.50	0.590	0.417
C 18:2 n-6	9.8[a]	3.40[b]	0.410	0.001
C 18:3 n-3	0.9[b]	6.90[a]	0.230	0.001
Fatty acid deposition (g)				
C 16:0	9.4[a]	7.30[b]	0.420	0.004
C 18:0	3.3	3.10	0.120	0.457
C 18:1 n-9	15.0[a]	11.10[b]	0.700	0.002
C 18:2 n-6	3.4[a]	2.60[b]	0.160	0.003
C 18:3 n-3	0.3[b]	0.80[a]	0.030	0.001
Deposition: intake ratio (g g^{-1})				
C 16:0	0.91	0.97	0.082	0.325
C 18:0	0.64	0.62	0.068	0.973
C 18:1 n-9	1.38[a]	1.06[b]	0.105	0.037
C 18:2 n-6	0.35[b]	0.76[a]	0.031	0.001
C 18:3 n-3	0.27[a]	0.12[b]	0.014	0.001

n = 10 per dietary treatment. Values within a row with different superscript differ significantly ($p<0.05$)

Table 7: Effect of dietary fat type on minimum de novo synthesis of fatty acids during the whole feeding period

	Experimental diets		
Items	LA	ALA	p-value
Minimum synthesis (g/21 days)			
SFA	1.58±1.15	1.30±0.72	0.384
MUFA	6.21±2.48[a]	2.30±1.16[b]	0.004

Means±SD for 10 chickens per experimental diet. Values within a row with different superscript differ significantly ($p<0.01$)

In broiler chickens, the intakes of LA and ALA are directly related with their amounts in adipose tissue and muscle (Bavelaar and Beynen, 2003; Smink *et al*., 2008). Thus, it would be expected, as was indeed found, that the birds fed the high-LA diet had deposited more LA and those fed the ALA-diet accumulated more ALA in their body. Despite the lower intake of LA than that of ALA in the birds fed the high ALA-diet, the amount of deposition of LA was much greater than that of ALA. This points at preferential oxidation of ALA when compared with LA. The deposition:digestible 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 LA and ALA, when compared with that of palmitic, stearic or oleic acid, 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 cannot be synthesized in the body of chickens. Thus, the deposition: intake ratio for LA and ALA, cannot be higher than 1, as was indeed found. The deposition:intake ratio for ALA was lower than that for LA, corroborating the evidence that ALA is more preferentially oxidized than LA (Cunnane and Anderson, 1997).

It is clear that this study in broilers, using the method of measurement of apparent fatty acid digestibility and whole-body analysis of fatty acids, shows that ALA is indeed more preferentially oxidized than is LA. It was hypothesized that preferential ALA oxidation would lead to more energy expenditure. However, energy expenditure expressed as a percentage of energy intake was similar for the birds fed either the high-LA or high-ALA diet. MUFA are the main fatty acids synthesized from glucose. The deposition:intake ratio for MUFA was higher than 1.0, pointing at de-novo synthesis. The high-ALA diet had induced a lower deposition:ratio for MUFA than did the high-LA diet. This indicates that the high intake of ALA had inhibited *de novo* fatty acid synthesis, which was also illustrated by the calculated rate of minimum synthesis of MUFA. The observed inhibition of *de novo* synthesis of MUFA by feeding the high-ALA instead of high-LA diet is consistent with studies in isolated hepatocytes (Mikkelsen *et al*., 1993) and in broilers (Crespo and Esteve-Garcia, 2002).

In conclusion, the outcome of this study is in agreement with the concept that dietary ALA is more preferentially oxidized than LA. No proof was obtained for increased energy expenditure. Thus, the hypothesis tested was rejected.

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