Dietary fat type, body composition and fatty acid metabolism in broiler chickens

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(with a summary in English)

Type voedingsvet, lichaamssamenstelling en vetzuurstofwisseling bij vleeskuikens

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Promotor: Prof. dr. ir. A. C. Beynen

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To

my Father and my Mother for their eternal love

เพื่อ

เตี่ย กับแม่สำหรับความรักที่ไม่มีสิ้นสุด

List of abbreviations

ADFI	Average daily feed intake
ADG	Average daily gain
ALA	α-Linolenic acid
BT	Beef tallow
BW	Body weight
CF	Crude fibre
CP	Crude protein
DM	Dry matter
EE	Ether extract
FA	Fatty acids
FCR	Feed conversion ratio
GE	Gross energy
LA	Linoleic acid
LO	Linseed oil
ME	Metabolisable energy
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SBO	Soybean oil
SD	Standard deviation
SEM	Standard error of means
SFA	Saturated fatty acids
SO	Sunflower oil

Contents

CHAPTER 1

Scope of the thesis

The dietary ratio of saturated to unsaturated fatty acids possibly affects energy expenditure. A high ratio of polyunsaturated fatty acids (PUFA):saturated fatty acids (SFA) has been shown to increase resting metabolic rate, diet-induced thermogenesis and fat oxidation (Jones et al., 1985, 1992, Jones and Schoeller 1988, Van Marken Lichtenbelt et al., 1997). Medium chain triacylglycerols (MCT), when compared with long-chain triacylglycerols (LCT), become readily oxidized and induce satiety (Scalfi et al., 1991, Dullo et al., 1996, Stubbs and Harbron, 1996, Van Wymelke et al., 1998). It would thus appear that different types of fats may have different effects on energy balance.

Any effect of absorbed dietary fatty acids on weight gain and body composition must relate to energy balance. A positive energy balance can be caused by over-consumption of a high-fat diet. There is evidence that individual fatty acids have different effects on energy metabolism. In human subjects, a positive association between the intake of monounsaturated fatty acids (MUFA) and different indices of adiposity has been shown, whereas the intakes of PUFA and SFA were only weakly related to adiposity (Doucet et al., 1998). These data are difficult to interpret because there may be confounding factors. Controlled studies with rats have demonstrated that a diet rich in PUFA produced less accumulation of body fat than a diet rich in SFA (Shimomura et al., 1990, Dulloo et al., 1995). This result is probably due to higher diet-induced thermogenesis, elevated fat oxidation and higher sympatic activity (Shimomura et al., 1990, Matsuo et al., 1995). MUFA also seem to increase body weight more than do PUFA (Dulloo et al., 1995). Other studies also reported differences in fat accumulation, body fat distribution and oxidation rates as a result of diets varying in fatty acid composition, chain length and saturation (Hill et al., 1993)

It has been observed frequently that the addition to the diet of an oil rich in PUFA at the expense of SFA reduces the amount of abdominal fat in broiler chickens (Sanz et al., 1999, 2000a; Crespo and Esteve-Garcia, 2002a; Newman et al., 2002; Pinchasov and Nir, 1992; Villaverde et al., 2005; Zollitsch et al., 1997). Consequently, the feeding of PUFA instead of SFA with long chain length may lead to less deposition of abdominal fat. The dose-response relationship for the intake of PUFA and the amount of abdominal fat has not yet been described. Analogous to PUFA, saturated fatty acids with medium-chain length are also preferentially oxidized (Bach and Babayan, 1982). Thus, it could be hypothesized that dietary MCT would diminish abdominal fat deposition in broiler chickens.

The fatty acid composition of the diet influences the composition of body fat in broiler chickens. The relative percentage of dietary polyunsaturated fatty acids, such as linoleic (LA) and α-linolenic acid (ALA), is directly related with the percentage of these fatty acids in adipose tissue of broilers (Bavelaar and Beynen, 2003). However, the metabolic basis for the diminishing effect of PUFA on abdominal fat mass is poorly understood (Crespo and Esteve-Garcia, 2002bc, 2003; Newman et al., 2002; Villaverde et al., 2006; Sanz et al., 2000b). One possible mechanism could be that PUFA versus SFA are preferentially oxidized (Beynen and Katan, 1985) and thereby yield ATP so that carbohydrates are shifted from the oxidative into the lipogenic pathway. The conversion of glucose into triglycerides is less efficient in terms of energy deposition than is the conversion of fatty acids into triglycerides (Newsholme and Leech, 1994). Consequently, the feeding of PUFA instead of SFA may lead to less deposition of abdominal fat. At high fat intakes, β-oxidation is enhanced through increased activities of the enzymes involved (Newsholme and Leech, 1994) which could imply that preferences for individual fatty acids become masked. It would

follow that the different effect of PUFA and SFA on the deposition of abdominal fat is less with increasing fat intakes.

The dose-response relationship between the intake of PUFA and the amount of abdominal fat is described in Chapter 2. In addition, the number of fat cells per unit of surface in breast meat was measured. The iodine value of various tissues was analysed as an index of the incorporation of PUFA. High tissue concentrations of polyunsaturated fatty acids are associated with high iodine values (Gurr and Harwood, 1991).

It would be expected that the feeding of MCT-rich oils to broiler chickens would lower the deposition of abdominal fat. To test the hypothesis, broiler chickens were fed on diets containing either tallow as source of SFA, soybean oil as source of PUFA and krabok oil as source of MCT (Chapter 3).

Chapter 4 documents a study in which the hypothesis tested was that the feeding of PUFA instead of SFA would not only lead to less deposition of abdominal fat but also more heat expenditure. Broiler chickens were fed on diets in which the beef tallow component was replaced by increasing amounts of soybean oil. Heat expenditure was calculated as energy intake minus energy deposition in the whole body and energy lost with excreta.

In the study described in Chapter 5, the hypothesis tested was whether the PUFA-mediated reduction in abdominal fat mass would be associated with a decrease in whole-body fatty acid synthesis. Broiler chickens were fed on diets with different ratios of beef tallow:soybean oil. Whole-body, *de-novo* fatty acid synthesis was assessed indirectly by using two indicators: the concentration of plasma triacylglycerols (Beynen et al., 1983) and minimum *de-novo* fatty acid synthesis calculated as fatty deposition in whole carcass minus digestible fatty acid intake.

In the experiments mentioned above, the animals had free access to the experimental diets. Under conditions of *ad libitum* feeding, animals may adapt feed intakes to changes in fat absorption, fat catabolism and lipogenesis. Thus, the study described in Chapter 6 was undertaken to determine the effect of replacement of dietary SFA by PUFA under conditions of *ad libitum* and restricted feeding.

ALA is more preferentially oxidized than is LA. The hypothesis tested in Chapter 7 was that feeding a diet containing ALA instead of LA would alter the whole-body fatty acid metabolism and deposition and energy expenditure. Soybean oil was used as LA source and linseed oil was used as ALA source.

. In the last study (Chapter 8) the hypothesis tested was that a high level of ALA in a diet rich in PUFA would result in higher energy expenditure and more oxidation of ALA than a high level of ALA in a diet rich in SFA. The experimental diets were enriched with linseed oil as source of ALA and contained either beef tallow as source of SFA or sunflower oil as source of PUFA.

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CHAPTER 2

Influence of amount and type of dietary fat on deposition, adipocyte count and iodine number of abdominal fat in broiler chickens

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Summary

This study describes the relation between the type and amount of dietary fat on the deposition of abdominal fat by broiler chickens. It was hypothesized that at higher fat intakes, the well-known lowering effect of polyunsaturated fatty acids on the deposition of abdominal fat would be diminished. The experimental diets were formulated to contain three levels of added fat (3, 6 and 9%). Each level had different proportions of saturated fatty acids (SFA) and unsaturated fatty acids (UFA) by installing the ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 with the use of tallow and soybean oil. Arbor Acres chicks, aged 7 days, were fed one of the 15 experimental diets until they were aged 42 days. Feed and water were provided *ad libitum.* There was no systematic effect of dietary fat type and amount on weight gain and feed intake. The lowest SFA:UFA ratio of 1:5 produced the lowest feed conversion rates, irrespective of the amount of fat in the diet. Abdominal fat deposition was similar in the birds fed the diets containing either 3 or 6% added fat, but deposition was lower than in those fed 9% fat. A decrease in the SFA:UFA ratio of the diet was associated with a dose-dependent decrease in abdominal fat, irrespective of the amount of fat in the diet. This observation leads to the rejection of the hypothesis stated above. A decrease in the dietary SFA:UFA from 1:1 to 1:4 caused a decrease in the number of fat cells per surface unit of breast meat. It is concluded that an increased intake of soybean oil at the expense of tallow reduced abdominal fat deposition and the number of fat cells in breast meat of broiler chickens.

Keywords: fat deposition, amount of fat, fat type, adipocyte count, iodine number, broiler chickens

Introduction

The fatty acid composition of the diet influences the composition and the amount of abdominal fat in broiler chickens. The relative percentage of dietary polyunsaturated fatty acids, such as linoleic and α-linolenic acid, is directly related with the percentage of these fatty acids in adipose tissue of broilers (Bavelaar and Beynen, 2003). The addition to the diet of an oil rich in polyunsaturated fatty acids at the expense of a more saturated fat source reduces the amount of abdominal fat in broilers (Sanz et al., 1999, 2000a; Crespo and Esteve-Garcia, 2002a; Newman et al., 2002; Pinchasov and Nir, 1992; Villaverde et al., 2005; Zollitsch et al., 1997). The dose-response relationship for the intake of polyunsaturated fatty acids and the amount of abdominal fat has not yet been described.

The metabolic basis for the diminishing effect of polyunsaturated fatty acids on abdominal fat mass is poorly understood (Crespo and Esteve-Garcia, 2002bc, 2003; Newman et al., 2002; Villaverde et al., 2006; Sanz et al., 2000b). One possible mechanism could be that polyunsaturated versus saturated fatty acids are preferentially oxidized (Beynen and Katan, 1985) and thereby yield ATP so that carbohydrates are shifted from the oxidative into the lipogenic pathway. The conversion of glucose into triglycerides is less efficient in terms of energy deposition than is the conversion of fatty acids into triglycerides (Newsholme and Leech, 1984). Consequently, the feeding of polyunsaturated instead of saturated fatty acids may lead to less deposition of abdominal fat. At high fat intakes, β-oxidation is enhanced through increased activities of the enzymes involved (Newsholme and Leech, 1984) which could imply that preferences for individual fatty acids become masked. It would follow that the different effect of polyunsaturated and saturated fatty acids on the deposition of abdominal fat is less with increasing fat intakes. In this study, the above reasoning was put to the test. Broiler chickens were fed diets with five different ratios of tallow: soybean oil at three dietary fat levels and the amount of abdominal fat was determined. In this way, we would be able to report the dose-response relationship between the intake of polyunsaturated fatty acids and the amount of abdominal fat, and it would be possible to indirectly evaluate the above considerations as to metabolic effects of dietary polyunsaturated fatty acids. In addition to the amount of abdominal fat, the number of fat cells per unit of surface in breast meat were measured. The iodine value of various tissues was analysed as an index of the incorporation of polyunsaturated fatty acids. High tissue concentrations of polyunsaturated fatty acids are associated with high iodine values (Gurr and Harwood, 1991).

Material and methods

Animals and diets

A total of 390 (195 males and 195 females) 7-day-old Arbor Acres broiler chicks were used. They had been raised on a commercial diet. According to the manufacturer, the diet contained 87% dry matter, 21% crude protein, 4% crude fat, 5% crude fiber, 4% ash. Within sex, they were randomly allocated to 15 groups consisting of 13 birds each. The birds were kept in individual cages. Until the chickens were aged three weeks, the temperature in each cage was kept at 30-34 ^{o}C with 200-W electric bulbs. Feed was provided *ad libitum* in the form of meal. Animals had free access to water. The experimental diets were formulated to contain three levels of added fat (3, 6 and 9%) and five ratios of saturated to unsaturated fatty acids by mixing tallow with soybean oil. The experimental design had a 3 x 5 x 2

Table 1. Ingredient and nutrient composition of the experimental diets

Level of added fat, %	3					6					9				
***SFA:UFA*[1]**	**1:1**	**1:2**	**1:3**	**1:4**	**1:5**	**1:1**	**1:2**	**1:3**	**1:4**	**1:5**	**1:1**	**1:2**	**1:3**	**1:4**	**1:5**
Ingredient (g/100 g)															
Tallow	**2.87**	**1.45**	**0.72**	**0.28**	**-**	**5.52**	**2.85**	**1.48**	**0.65**	**0.10**	**8.15**	**4.26**	**2.25**	**1.03**	**0.22**
Soybean oil	**0.13**	**1.56**	**2.28**	**2.72**	**3.00**	**0.48**	**3.15**	**4.52**	**5.35**	**5.91**	**0.85**	**4.74**	**6.75**	**7.97**	**8.87**
Tapioca starch	46.02	46.02	46.02	46.02	46.02	43.02	43.02	43.02	43.02	43.02	40.02	40.02	40.02	40.02	40.02
Constant components2	50.98	50.98	50.98	50.98	50.98	50.98	50.98	50.98	50.98	50.98	50.98	50.98	50.98	50.98	50.98
Analysed composition (g/100 g)															
Dry matter	91.98	91.98	91.98	91.98	91.98	91.95	91.95	91.95	91.95	91.95	91.85	91.85	91.85	91.85	91.85
Crude protein	17.67	17.67	17.67	17.67	17.67	18.12	18.12	18.12	18.12	18.12	17.84	17.84	17.84	17.84	17.84
Crude fat	2.83	3.23	3.14	2.97	3.03	6.39	6.43	6.17	6.27	6.41	9.34	9.18	9.12	9.36	9.23
Crude fiber	3.14	3.14	3.14	3.14	3.14	3.22	3.22	3.22	3.22	3.22	3.13	3.13	3.13	3.13	3.13
Ash	4.14	4.14	4.14	4.14	4.14	4.37	4.37	4.37	4.37	4.37	4.22	4.22	4.22	4.22	4.22
Calculated composition (g/100 g)															
Energy: Protein ratio (g:kJ)	799.66	799.66	799.66	799.66	799.66	811.26	811.26	811.26	811.26	811.26	855.94	855.94	855.94	855.94	855.94
Calcium	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Available phosphorus	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49
Sodium	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Nitrogen free extract	64.20	63.80	63.89	64.06	64.00	59.85	59.81	60.07	59.97	59.83	57.32	57.48	57.54	57.30	57.43

[1]SFA:UFA = ratio of saturated to unsaturated fatty acids in the whole rations.

[2]The constant components consisted of the following (g): soybean meal, 41.05; rice bran, 4.0; di-calcium phosphate, 3.87; D,L-methionine, 0.3; L-lysine hydrochloride, 0.25; sodium chloride, 0.51; premix, 1.0. 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

factorial arrangement. However, extra fat was added to the diets at the expense of an identical weight of the tapioca starch component. Consequently, higher fat contents were associated with higher energy densities and therefore lower nutrient:energy ratios. The analysed (AOAC, 1975) gross energy contents of the diets containing 3, 6 and 9% added fat were 14.1, 14.7 and 15.3 MJ/kg, respectively. Table 1 shows the composition of the diets. The basal diet without added fats, but with the base content of tapioca starch, was analysed for dry matter, crude protein, crude fat, crude fiber and ash (AOAC, 1975). The macronutrient concentrations in the whole diets were then calculated. The nitrogen-free extract was calculated as residual fraction. Table 1 shows the macronutrient composition of the diets. The ratios of saturated to unsaturated fatty acids (SFA:UFA) were calculated on the basis of feed table values (NRC, 1994) for the fatty acid compositions of the feed ingredients. Individual body weights and feed intakes were determined.

Sample collection

When they were 42 days old, the broilers were stunned, slaughtered and bled at a local slaughter house. One bird per dietary group per gender was randomly taken for chemical analyses so that in total there were 30 animals. The abdominal adipose tissue from the proventriculus surrounding the gizzard down to the cloaca, breast, thigh and liver were removed and stored at –20 ^{o}C. A portion of the breast meat was stored in 10% buffered formalin solution.

Chemical and histological analyses

Feed samples were analyzed for dry matter, crude protein, crude fat, crude fiber and ash (AOAC, 1975). The nitrogen-free extract was calculated as residual fraction. The iodine number of tissue was measured according to the Hanus method (AOAC, 1975). The number of fat cells per surface unit of breast meat was counted by a histological method as described by Carson (2001).

Statistical analyses

As mentioned above, the nutrient:energy ratios of the diets with different amounts of fat were different. Therefore, only comparisons within fat levels were made. ANOVA showed that there was no influence of sex on any of the variables. The data for males and females were then pooled. All data were subjected to Duncan's multiple range test within a fat level was used to identify statistically significant differences between group means (Steel and Torrie, 1980).

Results

A total of 8 birds died during the course of the experiment. There was no association between mortality and diet composition. Table 2 shows that average daily gain (ADG) was not systematically influenced by the SFA:UFA ratio of the diet. This holds also for average daily feed intake (ADFI). At each level of added fat, the feed conversion ratio (FCR) was significantly lower at a SFA:UFA ratio of 1:5 than at a ratio of 1:1. However, there was no clear relationship between SFA:UFA ratio and FCR.

The tissue iodine number increased in the order of breast, thigh, abdominal fat and liver (Table 3). For all tissues, an increase in the dietary SFA:UFA ratio produced an increase in the iodine number, but significant differences generally were seen only for the highest versus lowest SFA:UFA ratio.

Table 2. Growth performance of broilers fed the experimental diets

Level of added fat, %	3					Pooled	6					Pooled	9					Pooled
SFA:UFA	**1:1**	**1:2**	**1:3**	**1:4**	**1:5**	**SE**	**1:1**	**1:2**	**1:3**	**1:4**	**1:5**	**SE**	**1:1**	**1:2**	**1:3**	**1:4**	**1:5**	**SE**
Initial weight, g	156	159	158	159	156	4.07	156	156	155	156	155	4.04	153	153	153	153	155	4.01
Final weight, g	1429^{c}	1407^{c}	1512^{b}	1562^{ab}	1635^{a}	12.56	1458	1570	1435	1556	1472	12.54	1542^{b}	1519^{b}	1456^{c}	1561^{b}	1709^{a}	12.79
Average daily gain (ADG), g/b/d																		
	36.3^{b}	35.7^{b}	38.7^{a}	40.1^{a}	42.3^{a}	2.01	37.2	40.4	36.6	40.0	37.6	2.01	39.7^{b}	39.0^{b}	37.2^{c}	40.2^{b}	44.4^{a}	2.05
Average daily feed intake (ADFI), g/b/d																		
	94.4	95.4	95.8	94.9	98.7	3.17	98.8^{a}	103.4^{a}	91.1^{b}	93.7^{b}	90.8^{b}	3.17	94.1	92.7	93.8	94.6	95.7	3.14
Feed conversion ratio, FCR																		
	2.60^{a}	2.67^{a}	2.48^{b}	2.37^{bc}	2.34^{c}	0.51	2.67^{a}	2.55^{ac}	2.50^{abc}	2.34^{c}	2.41^{bc}	0.51	2.37^{b}	2.38^{b}	2.52^{a}	2.35^{b}	2.16^{c}	0.50
Energy intake : gain ratio, kJ/g of wt. gain																		
	36.7^{a}	37.7^{a}	35.0^{b}	33.5^{bc}	33.1^{c}	7.21	39.3^{a}	37.5^{ac}	36.8^{abc}	34.4^{bc}	35.4^{c}	7.50	36.0^{b}	36.2^{b}	38.3^{a}	35.7^{b}	32.8^{c}	7.60

Data are means for 24-26 birds per dietary treatment and pooled SE's for each level.
^{abcd} Values within a row with different superscripts are significantly different; $P<0.05$

Table 3. Iodine numbers for tissues from broilers fed the experimental diets

Level of fat, %	3					Pooled SE	6					Pooled SE	9					Pooled SE
SFA:UFA	1:1	1:2	1:3	1:4	1:5		1:1	1:2	1:3	1:4	1:5		1:1	1:2	1:3	1:4	1:5	
Broiler tissue																		
Breast	24^{c}	32^{b}	37^{b}	37^{b}	46^{a}	1.94	27^{c}	33^{bc}	36^{abc}	40^{ab}	44^{a}	1.96	27^{c}	32^{bc}	39^{b}	41^{ab}	47^{a}	2.00
Thigh	34^{b}	43^{b}	44^{b}	48^{b}	53^{a}	2.22	36^{c}	38^{c}	42^{bc}	49^{ab}	55^{a}	2.16	32^{c}	42^{b}	46^{ab}	53^{a}	51^{a}	2.19
Fat pad	47^{c}	52^{bc}	51^{b}	54^{bc}	60^{a}	2.36	48^{c}	51^{bc}	55^{abc}	57^{ab}	59^{a}	2.38	47^{d}	53^{c}	57^{b}	59^{b}	68^{a}	2.45
Liver	85^{a}	98^{a}	99^{a}	106^{a}	100^{a}	3.20	89^{b}	101^{a}	105^{a}	101^{a}	105^{a}	3.24	80^{b}	99^{a}	102^{a}	100^{a}	108^{a}	3.21

Iodine number is expressed as g iodine consumed by 100 g of tissue; it reflects the degree of unsaturation of fatty acids as the double bonds in fatty acids react with iodine. Data are means for 2 birds per dietary treatment and pooled SE's for each level.

^{abc} Values within a row with different superscripts are significantly different; $P<0.05$

Table 4. Abdominal fat deposition and fat cell counts for breast meat in broilers fed the experimental diets

Level of fat, %	3					Pooled SE	6					Pooled SE	9					Pooled SE
SFA:UFA[1]	1:1	1:2	1:3	1:4	1:5		1:1	1:2	1:3	1:4	1:5		1:1	1:2	1:3	1:4	1:5	
Live weight, g																		
	1330^{c}	1375^{c}	1575^{a}	1570^{b}	1550^{c}	12.49	1740^{a}	1610^{ac}	1408^{b}	1553^{c}	1475^{bc}	12.81	1465^{c}	1560^{b}	1655^{a}	1325^{d}	1635^{ab}	12.68
Abdominal fat, g																		
	36.7	34.0	36.9	28.5	28.3	1.88	46.9	42.8	34.8	32.8	28.3	2.00	55.2	51.0	48.6	36.2	40.8	2.22
Abdominal fat, % live weight																		
	2.76^{a}	2.47^{a}	2.44^{a}	1.82^{b}	1.79^{b}	0.49	2.70^{a}	2.69^{a}	2.44^{a}	2.11^{b}	1.92^{b}	0.50	3.77^{a}	3.26^{b}	2.95^{c}	2.75^{cd}	2.49^{d}	0.57
Fat cells/cm^{2}																		
	69.0^{a}	51.5^{b}	43.5^{c}	41.0^{c}	38.5^{c}	2.29	91.5^{a}	81.5^{a}	51.5^{b}	39.5^{b}	43.0^{b}	2.62	89.5^{a}	82.0^{ab}	59.0^{bc}	44.0^{c}	42.5^{c}	2.64

Data are means for 2 birds per dietary treatment and pooled SE's for each level.
Values within a row with different superscripts are significant different; $P<0.05$

The mean final body weights of the birds selected for abdominal fat measurements (Table 4) differed from that of all birds per dietary group (Table 2). Within each fat level, the amount of abdominal fat expressed as percentage of body weight fell with decreasing SFA:UFA ratio (Table 4). The same pattern was seen for the absolute weight of abdominal fat. The percentage abdominal fat was lower for the diets containing 3 or 6% added fat than for the diets containing 9% fat. The diets with 3 or 6% fat induced similar values for the relative weight of abdominal fat. The number of fat cells in breast meat dropped when the SFA:UFA ratio decreased from 1:1 to 1:4, but there was no further drop when the ratio was decreased to 1:5 (Table 4).

Discussion

There was no clear pattern by which the dietary SFA:UFA ratio affected growth performance, but at each fat level the highest intake of UFA was associated consistently with the lowest FCR. ADFI and ADG were not systematically low and high, respectively, when the broilers were fed on the diets with the lowest SFA:UFA ratio. It is thus difficult to see why the lowest SFA:UFA ratios were accompanied by the lowest FCR's. The low FCR for birds with highest UFA intake could relate to a higher digestibility of UFA and/or to a higher protein:fat ratio in the carcass and/or to less heat production. Indeed, oils rich in UFA are digested by broiler chickens more efficiently than are fats rich in SFA (Smits et al., 2000, Wiseman et al., 1991). In birds fed a diet containing sunflower oil instead of tallow, there was no difference in carcass composition (Crespo and Esteve-Garcia, 2002a). Newman et al. (2002) have provided evidence that the feeding of sunflower oil instead of tallow to broiler chickens influences nutrient partitioning and energy expenditure. However, this study does not support convincingly that a decrease in the SFA:UFA ratio improves the FCR as there was no dose-response relationship. It should be noted that when formulating the diets the higher digestibility of soybean oil versus tallow (Smits et al., 2000) was not taken into account. Thus, a decrease in the dietary SFA:UFA ratio at the same dietary fat level would be associated with a higher digestibility of the fat component and thereby with a decrease in protein:metabolizable energy ratio of the diet. The latter may tend to increase the FCR and would then counteract any positive effect of a decreasing SFA:UFA ratio on the FCR.

In keeping with earlier work (Sanz et al., 1999, 2000a; Crespo and Esteve-Garcia, 2002a; Newman et al., 2002; Pinchasov and Nir, 1992; Zollitsch et al., 1997), abdominal fat deposition in the chickens was decreased by an increase in UFA intake. When the diet contained either 3 or 6% added fat, abdominal fat deposition was smaller than when the diet contained 9% fat. This observation may be explained by the relatively high energy density of the diet with 9% fat. However, it is clear that the effect of a decreasing SFA:UFA ratio on abdominal fat deposition was similar for the three dietary fat concentrations. Thus, our hypothesis that the effect of UFA intake on abdominal fat deposition would be smaller at higher fat intakes should be rejected.

Interesting results emerged from this study with regard to the number of fat cells per surface unit of breast meat. The diets containing either 6 or 9% fat produced similar numbers of fat cells, but these numbers were higher than when the diet contained 3% fat. Irrespective of the amount of fat in the diet, and decrease in the dietary SFA:UFA ratio from 1:2 to 1:4 caused a dose-dependent decrease in the number of fat cells. There was no further decrease in the number of fat cells in breast meat when the ratio dropped to 1:5. Possibly, polyunsaturated fatty acids regulate transcription factors, which in turn affect adipocyte development (Azain, 2004).

The present data may be important in order to enhance the quality of broilers' breast meat in terms of physical, color and sensory characteristics (Liu et al., 2004). Poultry meat quality is a relative notion, being determined by its consistency and influence on consumer health. The consistency is related to the melting point of the fat component, which is associated with its fatty acid composition (Bavelaar and Beynen, 2003). An increase in the percentage of unsaturated fatty acids causes a decrease in the firmness and an increase in the oiliness of poultry meat (Miller et al., 1990). Increasing the hardness of the tissue fat by extra saturated fatty acids can be advantageous in the marketing of broiler meat. However, an increase in saturated fatty acids at the expense of polyunsaturated fatty acids in poultry meat may lead to an increase in serum cholesterol of the consumer (Beynen, 1984). Thus, the ideal fatty acid composition of poultry meat depends on whether the emphasis is on its consistency or on consumer health. On the basis of this study, a recommendation as to the SFA:UFA ratio of the diet cannot be made because neither the consistency nor the fatty acid composition of edible broiler meat was measured. It may be noted here that the fatty acid composition of broiler meat correlates well that of broiler adipose tissue (Bavelaar and Beynen, 2003).

In conclusion, this study clearly shows that an increased intake of soybean oil at the expense of tallow reduced abdominal deposition by broiler chickens. A decrease in the dietary SFA:UFA ratio of the diet diminished abdominal fat deposition in a dose-dependent manner which was independent of the amount of fat in the diet. This study also demonstrates that the number of fat cells in broilers' breast meat depends on both the amount and type of fat in the diet.

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CHAPTER 3

Dietary soybean oil, but not krabok oil, diminishes abdominal fat deposition in broiler chickens

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Abstract

In broiler chickens we tested the hypothesis that dietary fats rich in medium-chain triacylglycerols (MCT) would diminish abdominal fat deposition as do fats rich in polyunsaturated fatty acids (PUFA). Broiler chickens were fed on diets containing either tallow, which is rich in saturated fatty acids (SFA), soybean oil, which is rich in PUFA, or krabok oil, which is rich in MCT. Krabok oil was isolated from the seeds of a tree (*Irvingia Malayana*) grown widely in tropical and subtropical areas. Growth performance was not significantly affected by the type of dietary fat. Possibly, the production of krabok oil for use in broiler rations may become economically relevant. The diets containing either soybean oil or krabok oil showed a significantly higher apparent fat digestibility than did the diet containing tallow. In keeping with earlier investigations, dietary soybean oil versus tallow significantly lowered abdominal fat deposition, the lowering being 21 %. The feeding of krabok oil instead of tallow did not affect the weight of abdominal fat, which would lead to rejection of our hypothesis.

Keywords: Tallow; soybean oil; krabok oil; broiler chicken; feed intake; abdominal fat deposition

Abbreviations: MCT, medium-chain triacylglycerols; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acid; T, tallow; SBO, soybean oil; KO, krabok oil; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio

Introduction

It has been observed frequently that the addition to the diet of an oil rich in polyunsaturated fatty acids (PUFA) at the expense of long-chain saturated fatty acids (SFA) reduces the amount of abdominal fat in broiler chickens (Sanz et al., 1999, 2000a; Crespo and Esteve-Garcia, 2002a; Newman et al., 2002; Pinchasov and Nir, 1992; Villaverde et al., 2005; Zollitsch et al., 1997, Wongsuthavas et al., 2007). The metabolic basis for the diminishing effect of PUFA on abdominal fat mass is poorly understood (Sanz et al., 2000b; Crespo and Esteve-Garcia, 2002bc, 2003; Newman et al., 2002; Villaverde et al., 2006). One possible mechanism could be that PUFA versus SFA are preferentially oxidized (Beynen and Katan, 1985) and thereby yield ATP so that carbohydrates are shifted from the oxidative into the lipogenic pathway. The conversion of glucose into triglycerides is less efficient in terms of energy deposition than is the conversion of fatty acids into triglycerides (Newsholme and Leech, 1994). Consequently, the feeding of PUFA instead of SFA acids may lead to less deposition of abdominal fat. Analogous to PUFA, saturated fatty acids with medium-chain length are also preferentially oxidized (Bach and Babayan, 1982). Thus, it could be hypothesized that dietary fats rich in medium-chain triacylglycerols (MCT) would diminish abdominal fat deposition in broiler chickens.

Krabok (*Irvingia Malayana*, Oliv. ex. A. Benn.) is a tree grown widely in tropical and subtropical areas. In Thailand, the krabok tree is commonly used for wood and charcoal production, whereas the seeds, after peeling, are consumed by people. Krabok seed oil is rich in lauric (C12:0) and myristic acid (C14:0) (Peangpra, 1977). In the light of the above-mentioned, we hypothesized that the consumption of krabok oil by broiler chickens would lower the deposition of abdominal fat. To test the hypothesis, broiler chickens were fed on diets containing either tallow, which is rich in SFA, soybean oil, which is rich in PUFA, or krabok oil, which is rich in MCT. Apart from abdominal fat weight, we also determined apparent fat digestibility.

Materials and Methods

Krabok seed oil extraction

Krabok seeds were purchased from a local market. The seeds were dehulled and ground through pass a 1-mm screen sieve of a grinding machine. Then, the meal was extracted by the Sohxlet method using hexane (AOAC, 1975). The hexane was evaporated and the residual krabok oil was used for diet formulation.

Broiler Chickens and diets

Forty-five 7-day-old Arbor Acres broiler chicks were used. They were randomly allocated to three groups of 15 birds each and kept in individual cages. Feed was provided *ad libitum* in the form of meal. Birds had free access to water. The experimental diets contained tallow, soybean oil or krabok oil. The ingredient and calculated composition of the diets is shown in Table 1.

Table 1. Ingredient and analysed composition of the experimental diets

Ingredient composition (%)	Fat source		
	Tallow	Soybean oil	Krabok oil
Tallow	2.87	0.00	-
Soybean oil	0.13	3.00	-
Krabok oil	-	-	3.00
Constant components	97.00	97.00	97.00
Analysed composition (%)			
Dry matter	92.0	92.0	91.9
Crude protein	18.0	18.0	18.0
Crude fat	3.4	3.3	4.4
Crude fiber	3.2	3.2	3.2
Ash	4.3	4.3	4.3

The constant components consisted of (g/100 g diet): tapioca starch, 46.02; soybean meal, 41.05; rice bran hulls, 4; dicalcium phosphate, 3.87; D,L-methionine, 0.3; L-lysine, 0.25; sodium chloride, 0.51; premix, 1. The premix supplied per kg of diet: vitamin A, 1,650 IU; vitamin D, 330 IU; cholecalciferol, 1,467 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; ferrous, 87.59 mg; selenium, 0.17 mg.

Table 2. Fatty acid profile of dietary fat source in broiler rations

Fatty acid	Beef tallow	Soybean oil	Krabok oil
10:0	0.0	0.0	1.8
12:0	0.0	0.0	44.4
14:0	2.0	0.2	43.7
16:0	19.8	12.9	4.5
16:1	0.9	0.1	0.6
18:0	38.1	4.6	0.4
18:1 n-9c	22.0	22.7	3.6
18:2 n-6c	0.8	53.1	0.5
18:3 n-6	0.4	0.0	0.0
18:3 n-3	0.3	3.6	0.0
20:0	0.4	1.4	0.0
20:1 n-9	0.4	1.1	0.0
SFA	63.0	19.3	95.0
MUFA	23.7	24.0	4.2
PUFA	1.6	56.7	0.5

Data collection

Feed intake of the birds was recorded daily and body weight was measured weekly. Excreta were collected quantitatively during the entire experimental period. At the end of experiment (28 days of age), the birds were stunned and slaughtered at a local slaughterhouse. Five birds per treatment were randomly chosen for measurement of organ weights. The abdominal adipose tissue (from the proventriculus surrounding the gizzard down to the cloaca), breast, thigh, liver, spleen, heart, gizzard, small intestine and large intestine from each broiler were collected and weighed. Intestines were weighed without contents. The length of small and large intestine were measured.

Chemical analysis

The experimental diets were analyzed for dry matter, ash, crude fat, crude fiber and crude protein (AOAC, 1975). In excreta dry matter and crude fat were determined. The dietary fat sources were saponified and methylated according to the procedure of Metcalfe et al. (1966) followed by gas chromatography to determine fatty acid composition (Javadi et al., 2004)

Statistical analysis

Average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and apparent digestibilities for dry matter and crude fat were calculated. Data were subjected to Duncan's multiple range test (Steel and Torries, 1980) using a computer program (SPSS for windows 9.0, SPSS Inc., Chicago, IL, 1998). The level of statistical significance was pre-set at $P < 0.05$.

Results

Chemical and fatty acids composition in experimental diets

Table 1 shows that the analysed proximate composition of the diets was similar. As would be expected, the tallow used was rich in SFA and soybean oil rich in PUFA (Table 2). The krabok oil was high in SFA, but this fraction consisted mainly of C12:0 and C14:0.

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Growth performance, fat digestibility and abdominal fat deposition

Growth performance was not significantly affected by the type of dietary fat. However, ADFI and FCR tended to lowered by feeding soybean oil. Indeed, we found that the fat component of the diet containing soybean oil was digested more efficiently than that of the diet with tallow (Table 3). However, krabok oil also was digested better than tallow, but the birds fed the diet with krabok oil did not display a tendency towards a lower ADFI. It would thus appear that the digestibility of the fat component of the diet is not a major determinant of ADFI by broiler chickens with ad libitum access to feed (Table 3).

Table 3. Effect of dietary fat source on growth performance and apparent digestibility of dry matter and crude fat

Growth performance	Fat source			Pooled
	Tallow	Soybean oil	Krabok oil	SEM
Initial BW, g	184	182	178	0.71
Final BW, g	655	647	660	4.11
ADFI, g/d	45.4	41.8	46.6	0.35
ADG, g/d	22.4	23.0	23.0	0.21
FCR (feed intake:weight gain)	2.08	1.97	2.07	0.02
Digestibility, % of intake				
Dry matter	95.1	95.5	96.1	2.51
Crude fat	76.3^{b}	82.0^{a}	81.7^{a}	2.52

Results are for 15 birds per treatment.
Values with different superscript letter differ significantly ($P<0.01$).

Organ weight

The weights of other organs were not influenced, pointing at a rather specific effect of soybean oil on abdominal fat. Contrary to our expectation, the feeding of krabok oil instead of tallow did not affect the weight of abdominal fat (Table 4).

Discussion

Feed intake

A decrease in ADFI in broiler chickens fed a diet high in PUFA has been reported earlier (Atteh et al., 1983; Sklan and Ayal, 1989; Huang et al., 1990), but the effect does not appear to be consistent (Skrivan et al., 2000; Wongsuthavas et al., 2007). A lower feed intake by birds fed a PUFA-rich could be explained by the higher digestibility of the fat component (Corino et al., 1980; Brue and Latshaw, 1985), implying a higher dietary content of metabolizable energy and thus less feed needed to meet the energy requirement.

Table 4. Effect of dietary fat source on organ weights

Organ weight, % of body weight	Fat source			Pooled SEM
	Tallow	Soybean oil	Krabok oil	
Live weight, g	747	637	647	9.76
Breast meat	10.17	10.15	10.19	0.33
Thigh meat	5.79	5.29	6.03	0.21
Abdominal fat	1.19^{ab}	0.94^{b}	1.38^{a}	0.07
Liver	2.84^{ab}	3.22^{a}	2.38^{b}	0.14
Heart	0.75	0.75	0.69	0.04
Spleen	0.13	0.12	0.12	0.01
Gizzard	3.01	3.06	2.54	0.19
Intestine (small and large)	8.18^{b}	9.94^{a}	7.92^{b}	0.31
Intestinal length, cm per 100 g of body weight				
Small intestine	16.6	20.4	19.0	1.02
Large intestine	2.5	3.4	2.8	0.22
Intestinal length (cm)				
Small intestine	124	130	123	2.84
Large intestine	19	22	18	1.04

Results are for 5 birds per treatment.

^{ab} Values in the same row with the different superscripts differ ($P<0.05$).

Fat digestibility, liver and intestine weight

Krabok oil was digested better than tallow. It has been reported earlier that MCT-rich fats are digested more efficiently by broiler chickens than are fats high in SFA (Young et al., 1963; Zheng et al., 2006). In keeping with earlier investigations (Keren-Zvi et al., 1990; Mossab et al., 2000; Wongsuthavas et al., 2007), dietary soybean oil versus tallow was found to significantly lower abdominal fat deposition, the lowering being 21 %.

An interesting finding emerged in that krabok oil versus soybean oil significantly diminished the relative weight of liver and intestine as shown in Table 4. The basis and impact of this observation are not known.

Conclusions

In conclusion, the observation that the feeding of krabok oil versus tallow did not lower abdominal fat leads to rejection of our hypothesis. It would appear that the presence in dietary fat of fatty acids that are preferentially oxidized is not a determinant of abdominal fat deposition in broiler chickens. The data do indicate that krabok oil can be used as energy source for broiler chickens without a negative affect on growth performance. Possibly, the production of krabok oil for use in broiler rations may become economically relevant.

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CHAPTER 4

Energy expenditure by broiler chickens fed diets containing various blends of beef tallow and soybean oil

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Summary

Replacement of dietary saturated fatty acids (SFA) by polyunsaturated fatty acids (PUFA) has been consistently shown to reduce the amount of abdominal fat in broiler chickens, but the metabolic basis for this effect is unknown. It was hypothesized that the feeding of PUFA instead of SFA would induce more heat expenditure, this effect being associated with less deposition of abdominal fat. Broiler chickens were given one of five diets in which the beef tallow component, which is rich in SFA, was replaced by increasing amounts of soybean oil, which is rich in PUFA. The variable fat content of the diets was 3 % (w/w). There were neither significant nor systematic effects on weight gain and feed:gain ratio. The amount of body fat was reduced significantly ($P<0.05$) when about 75 % of the tallow was replaced by soybean oil, but there was no further decrease after the incorporation of more soybean oil into the diet. Calculated energy expenditure, either expressed as absolute amount or percentage of intake, trended to enhance but was not significantly affected by the amount of soybean oil in the diet.

Keywords: broiler chickens, growth performance, energy expenditure

Introduction

Various studies have demonstrated that replacement of dietary saturated fatty acids (SFA) by polyunsaturated fatty acids (PUFA) reduces the amount of abdominal fat in broiler chickens (Sanz et al., 1999, 2000a; Crespo and Esteve-Garcia, 2002a; Newman et al., 2002; Pinchasov and Nir, 1992; Villaverde et al., 2005; Zollitsch et al., 1997; Wongsuthavas et al., 2007). The metabolic basis for the diminishing effect of PUFA on abdominal fat mass is poorly understood (Crespo and Esteve-Garcia, 2002bc, 2003; Newman et al., 2002; Villaverde et al., 2006; Sanz et al., 2000b). One possible mechanism could be that PUFA versus SFA acids are preferentially oxidized (Beynen and Katan, 1985) and thereby yield ATP so that carbohydrates are shifted from the oxidative into the lipogenic pathway. The conversion of glucose into triglycerides is less efficient in terms of energy deposition than is the conversion of fatty acids into triglycerides (Newsholme and Leech, 1994). As a consequence, the feeding of PUFA instead of SFA would not only lead to less deposition of abdominal fat associated with more heat expenditure. This reasoning was tested in the present study. Broiler chickens were fed on diets in which the beef tallow component, which is rich in SFA, was replaced by increasing amounts of soybean oil, which is rich in PUFA.

Materials and methods

Animals and Experimental diets

Seven-day-old Arbor Acres broiler chicks were randomly allocated to five groups of 15 birds each and kept in individual cages. The five experimental diets were formulated to contain 3 % of added fat. As indicated in Table 1, the amount of soybean oil was increased stepwise at the expense of beef tallow. The birds had free access to feed and water.

Table 1. Ingredient and analysed composition of the experimental diets

Items	Diet code				
	1	2	3	4	5
Ingredients (g/100 g diet)					
Tallow	2.87	1.45	0.72	0.28	-
Soybean oil	0.13	1.56	2.28	2.72	3.00
Constant components	97.00	97.00	97.00	97.00	97.00
Macronutrients (g/100 g diet)					
Dry matter	92.0	92.0	92.0	92.0	92.0
Crude Protein	18.0	18.0	18.1	18.1	18.0
Crude fat	3.36	3.49	3.42	3.26	3.33
Crude fiber	3.23	3.25	3.21	3.23	3.24
Ash	4.28	4.30	4.30	4.31	4.33
Gross energy (kJ/100 g diet)	1,613	1,619	1,612	1,632	1,636

The constant components consisted of (g/100 g diet): tapioca starch, 46.02; soybean meal, 41.05; rice bran hulls, 4; dicalcium phosphate, 3.87; D,L-methionine, 0.3; L-lysine, 0.25; sodium chloride, 0.51; premix, 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; ferrous, 87.59 mg; selenium, 0.17 mg.

Data and Sample collection

The chickens were weighed at 7 and 28 days of age. Feed consumption was recorded daily. The feed:gain ratio was calculated (g feed/g gain). Excreta were collected quantitatively during the entire experimental period. At the age of 28 days, the birds were stunned and killed. Five birds per treatment were randomly chosen for weight measurement of abdominal adipose tissue (from the proventriculus surrounding the gizzard down to the cloaca). The remaining 10 birds per dietary treatment were used to measure the energy content of whole carcass.

Chemical analysis

The experimental diets were analyzed for dry matter, ash, crude fat, crude fiber and crude protein (AOAC, 1975). Bomb calorimetry analysis was done to determine gross energy in diets, homogenised whole carcass and excreta. Carcass and excreta were dried prior to energy measurement at 60 ^{o}C for 72 h in a forced-hot air oven. An adiabatic bomb calorimeter was used with benzoic acid as thermochemical standard. The total amount of energy that was lost as heat (energy 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 energy in whole carcass at the end of the 21-days feeding period minus the baseline energy content in the whole body. To determine baseline body energy content, 10 seven-day old chickens were used and their values were averaged.

Statistical analysis

Data were subjected to Duncan's multiple range test (Steel and Torrie, 1980) using the program of Microsoft Excel (Windows XP®). The level of statistical significance was preset at $P < 0.05$.

Results and Discussion

In keeping with our previous investigation (Wongsuthavas et al., 2007), there were no significant diet effects on weight gain, feed intake and feed:gain ratio (Table 2). Moreover, the gradual replacement of tallow by soybean oil did not induce systematic trends on weight gain and feed:gain ratio. When feed intake was not expressed in grams but gross energy, the inclusion of extra soybean oil into the diet was associated with significantly less energy intake. The lower energy intake by the birds fed soybean oil at the expense of tallow may be explained by the higher digestibility of soybean oil (Preston et al., 2001; Mossab et al., 2000; Leeson and Atteh, 1995), implying a higher dietary content of metabolizable energy and thus gross energy needed to meet the energy requirement. In fact, feed intake expressed as grams did show a tendency towards lower intakes with increasing dietary inclusion levels of soybean oil, although feed intake for the second inclusion level would appear to be aberrant.

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Table 2. Effects of experimental diets on growth performance and weight of abdominal fat tissue

Items	Diet code					Pooled
	1	2	3	4	5	SE
Initial BW, g	184	176	182	187	182	4.10
Final BW, g	655	668	613	631	647	9.80
ADFI, g	45.4	47.2	44.8	42.4	41.8	3.00
ADG, g	22.4	23.4	21.0	21.8	22.1	2.38
Feed : gain	2.07	2.02	2.18	2.04	1.80	0.63
Abdominal fat, % of final BW	1.19[a]	1.07[a]	0.81[b]	0.88[b]	0.94[b]	0.27

[a-b] Values in the same row with the different superscripts differ significantly (P<0.05)
Performance data are for 15 birds per dietary treatment. Abdominal fat data are for 5 birds per dietary treatment.

The amount of abdominal fat was reduced significantly (P<0.05) when about 75% of the tallow was replaced by soybean oil. Contrary to our earlier work (Wongsuthavas et al., 2007), there was no further decrease after the incorporation of more soybean oil into the diet. The lowering of abdominal fat was in the order of 20-30%. Thus, this study confirms the well-known effect that substitution of PUFA for SFA in the diet of broiler chickens diminishes the deposition of abdominal fat.

As would be expected on the basis of the growth performance data, there was no significant diet effect on the amount of energy stored in the carcass. The energy balance data did show that increasing intakes of soybean oil were associated with decreasing amounts of energy in the fat fraction of excreta. This effect can be explained by the fact that soybean oil is digested more efficiently by broilers than is tallow (Preston et al., 2001; Mossab et al., 2000; Leeson and Atteh, 1995) and substantiates the lower energy intakes with increasing inclusion levels of soybean oil.

Table 3. Influence of experimental diets on energy balance

Items	Diet code					Pooled
	1	2	3	4	5	SE
Energy balance (kJ/21 days)						
Intake	13,996	14,627	13,620	13,237	13,039	88.66
Stored in the body	5,112	5,323	4,691	4,540	4,641	52.92
Expenditure	5,853	5,750	5,942	5,741	5,665	57.69
Total excreta	3,010	3,548	2,986	2,956	2,721	41.88
Excreta as fat	368[a]	306[b]	258[c]	256[c]	252[c]	17.17
Fat-free excreta	2,642	3,241	2,728	2,700	2,469	39.87
Energy in whole body (kJ)						
Final	7,929	8,140	7,508	7,357	7,458	793.35
Energy balance (% of intake)						
Stored in the body	36.6	36.4	34.4	34.3	35.6	4.52
Expenditure	41.9	39.3	43.6	43.3	43.5	4.93
Total excreta	21.5	24.3	21.9	22.3	20.9	3.57
Excreta as fat	2.6[a]	2.1[a]	1.9[b]	1.9[b]	1.9[b]	1.46
Fat-free excreta	18.9	22.2	20.0	20.4	19.0	3.40

[a-b-c-d-e] Values in the same row with the different superscripts differ significantly (P<0.01)
Initial energy content of whole body was 2,817 kJ (SEM = 45.508 kJ, n =10).
Data are for 10 birds per dietary treatment.

The hypothesis tested in this study was that replacement of dietary tallow by soybean oil would increase energy expenditure. Table 3 shows that calculated energy expenditure, either expressed as absolute amount or as percentage of intake, trended to enhance energy expenditure was systematically related to the amount of soybean oil in the diet. It should be around that different dietary fatty acids may differently affect energy expenditure, at least in mice (Javadi et al., 2004). Studies in humans have shown that diets with a high PUFA:SFA ratio may increase energy expenditure. The diet with a high PUFA: saturated fatty acids (SFA) ration trended to increase the thermogenic effect food compared with a diet with a low PUFA:SFA ratio. Furthermore, the results of these studies suggest that with a high intake of PUFA there is an increased contribution of fat oxidation to the thermogenic effect of food whereas the contribution of carbohydrates is decreased. BMR was not affected by the fat type (Jones and Schoeller, 1988), but in another study polyunsaturated fat increased BMR (Van Marken Lichtenbelt et al., 1997). In any event, it appears that the lowering of abdominal fat in broiler chickens as caused by consumption of soybean oil is associated with increased energy expenditure. Abdominal fat only is a small portion of total body fat in broiler chickens, whereas the effect of intake of PUFA instead of SFA had consistent effect on whole body fat was decreased (Crespo and Esteve-Garcia, 2002bc, 2003; Newman et al., 2002; Villaverde et al., 2006; Sanz et al., 2000b). Alternatively, another mechanism, such as inhibition of de-novo fatty acid synthesis induced by high intakes of PUFA (Zheng et al., 2006; Ide et al., 1996; Clarke et al., 1976), is responsible for the observed reduction of abdominal fat in the chickens fed the diets rich in soybean oil.

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CHAPTER 5

Assessment of *de-novo* fatty acid synthesis in broiler chickens fed diets containing different mixtures of beef tallow and soybean oil

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Abstract

Replacement of dietary saturated fatty acids (SFA) by polyunsaturated fatty acids (PUFA) has been consistently shown to reduce the amount of abdominal fat in broiler chickens, but the metabolic basis for this effect is unknown. It was hypothesized that the feeding of PUFA instead of SFA would inhibit whole-body *de novo* fatty acid synthesis. As indexes of *de novo* fatty acid synthesis, we used the concentration of plasma triacylglycerols and minimum fatty acid synthesis calculated as fatty deposition minus digestible fatty acid intake. Broiler chickens were given one of five diets in which the beef tallow component, which is rich in SFA, was replaced by increasing amounts of soybean oil, which is rich in PUFA. The variable fat content of the diets was 3% (w/w). There were neither significant nor systematic effects on weight gain and feed:gain ratio. The amount of abdominal fat was reduced significantly when about 75% of the tallow was replaced by soybean oil, but there was no further decrease after the incorporation of more soybean oil into the diet. The decrease in abdominal fat was associated with a decrease in the level of plasma triacylglycerols, but it was not associated with minimum *de novo* fatty acid synthesis in the whole body.

Keywords: dietary fatty acids, broilers, abdominal fat, fatty acid deposition, fatty acid synthesis

Introduction

Numerous investigations have demonstrated that substitution of dietary polyunsaturated fatty acids (PUFA) for saturated fatty acids (SFA) reduces the amount of abdominal fat in broilers (Sanz et al., 1999, 2000a; Crespo and Esteve-Garcia, 2002a; Newman et al., 2002; Pinchasov and Nir, 1992; Villaverde et al., 2005; Zollitsch et al., 1997, Wongsuthavas et al., 2007). The mechanism underlying the diminishing effect of PUFA on abdominal fat mass is not known (Crespo and Esteve-Garcia, 2002bc, 2003; Newman et al., 2002; Villaverde et al., 2006; Sanz et al., 2000b). We have put forward (Wongsuthavas et al., 2007) the following possible mechanism. PUFA versus SFA acids are preferentially oxidized (Beynen and Katan, 1985) and thereby yield ATP so that carbohydrates are shifted from the oxidative into the lipogenic pathway. The conversion of glucose into triglycerides is less efficient in terms of energy deposition than is the conversion of fatty acids into triglycerides (Newsholme and Leech, 1984). As a consequence, the feeding of PUFA instead of SFA would not only lead to less deposition of abdominal fat, but be associated with more heat expenditure. However, we had to reject our hypothesis on the basis of a recent experiment in which energy expenditure was calculated for broilers fed on diets with different PUFA:SFA ratios (Wongsuthavas et al., 2007).

An alternative hypothesis explaining the observed PUFA-induced reduction in abdominal fat in broiler chickens would be inhibition of *de-novo* fatty acid synthesis. There is evidence that the feeding of PUFA instead of SFA will inhibit *de-novo* fatty acid synthesis in the body (Zheng et al., 2006; Sanz et al., 2000b; Ide et al., 1996; Clarke et al., 1976). In this study, we tested whether a PUFA-mediated reduction in abdominal fat mass would be associated with a decrease in whole-body fatty acid synthesis. Broiler chickens were fed on diets in which the beef tallow component, which is rich in SFA, was replaced by increasing amounts of soybean oil, which is rich in PUFA. Whole-body fatty acid synthesis was assessed indirectly by using two indicators. First, we measured the concentration of plasma triacylglycerols. Studies with isolated hepatocytes indicate that the concentration of plasma triacylglycerols is an index of *de-novo* fatty acid synthesis (Beynen et al., 1983). Secondly, we calculated minimum *de-novo* fatty acid synthesis as fatty deposition in whole carcass minus digestible fatty acid intake.

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Materials and methods

Animals and Experimental diets

Seven-day-old Arbor Acres broiler chicks were randomly allocated to five groups of 15 birds each and kept in individual cages. The five experimental diets were formulated to contain 3 % of added fat. As indicated in Table 1, the amount of soybean oil was increased stepwise at the expense of beef tallow. Fatty acid composition of the experimental diets is shown in Table 2. The birds had free access to feed and water.

Data and Sample collection

The chickens were weighed at 7 and 28 days of age. Feed consumption was recorded daily. The feed:gain ratio was calculated (g feed/g gain). Excreta were collected quantitatively during the entire experimental period. At the age of 28 days, the birds were stunned and killed. The birds were killed at 08.00 am after a three-hour fasting period. Five birds per treatment were randomly chosen for weight measurement of abdominal adipose tissue (from the proventriculus surrounding the

gizzard down to the cloaca) and blood sampling. The remaining 10 birds per dietary treatment were used to measure fatty acid composition of whole carcass.

Table 1. Ingredient and analysed composition of the experimental diets

Items	Diet code				
	1	2	3	4	5
Ingredients (g/100 g diet)					
Tallow	2.87	1.45	0.72	0.28	-
Soybean oil	0.13	1.56	2.28	2.72	3.00
Constant components	97.00	97.00	97.00	97.00	97.00
Macronutrients (g/100 g diet)					
Dry matter	92.0	91.8	91.98	91.98	91.98
Crude Protein	18.0	18.0	18.1	18.1	18.0
Crude fat	3.4	3.5	3.4	3.3	3.3
Crude fiber	3.2	3.3	3.2	3.2	3.2
Ash	4.3	4.3	4.3	4.3	4.3

The constant components consisted of (g/100 g diet): tapioca starch, 46.02; soybean meal, 41.05; rice bran hulls, 4; dicalcium phosphate, 3.87; D,L-methionine, 0.3; L-lysine, 0.25; sodium chloride, 0.51; premix, 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; ferrous, 87.59 mg; selenium, 0.17 mg.

Table 2. Fatty acid composition of the diets

Fatty acid composition, %	**Diet code**				
	1	2	3	4	5
C16:0	27.63	26.31	26.48	26.50	25.61
C18:0	32.47	23.45	16.26	11.55	7.95
C18:1 n-9	9.06	16.56	15.74	16.24	15.81
C18:1 n-7	2.69	2.79	2.09	1.68	1.45
C18:2 n-6 c	8.11	11.02	15.44	16.00	19.70
C18:3 n-6	0.40	0.26	0.00	0.00	0.00
C18:3 n-3	1.13	1.52	2.03	2.09	2.65
ΣSFA	68.31	55.38	48.09	45.50	41.33
ΣMUFA	15.93	26.47	28.92	32.04	31.91
ΣPUFA	9.64	12.80	17.47	18.09	22.35

ΣSFA= C8:0+C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0+C22:0+C24:0
ΣMUFA=C14:1+C15:1+C16:1+C18:1n-9+C18:1n-7+C20:1n-9
ΣPUFA=C18:2 n-6+18:3 n-6+18:3 n-3

Chemical analysis

The experimental diets were analyzed for dry matter, ash, crude fat, crude fiber and crude protein (AOAC, 1985). Total fat in dried excreta and carcass were extracted as described previously (Horwitz, 1975). Each sample was added to a flask and 2 ml of ethanol was added. Subsequently, 10 ml of HCl (8 mol/L) was added; the contents were gently mixed, and the flask was placed in a water-bath of 80 ^{o}C for 30-40 min. The tubes were cooled down, 10 ml of ethanol (96%, w/w) and 25 ml of petroleum ether (boiling point between 40 and 60 ^{o}C) were added and the tube was shaken for

one min. The fat-containing upper layer was decanted into a 150-ml round-bottom flask. The extraction procedure was repeated twice with 15 ml of diethyl ether and 15 ml of petroleum ether and the lipid extract was evaporated to dryness under N_2 in a water-bath of 40 ^{o}C. The round-bottom flasks with the lipids were dried overnight at 60 ^{o}C and the total lipids were measured gravimetrically. Total lipids were saponified and methylated according to the procedure of Metcalfe et al. (1966) followed by gas chromatography for determination of fatty acid composition. Concentrations of plasma triacylglycerols and cholesterol were analysed according to the procedure of (Javadi, 2005)

Calculation of digestible fatty acid intake, fatty acid deposition and minimum de novo synthesis

The total digestible fatty acid intake was calculated as fatty acid intake (g/21 days) × apparent fatty acid digestibility (fraction of intake). The deposition of fatty acids was calculated with the following formula: Fatty acid deposition (g/21 days) = carcass content of fatty acid at the end of the study – carcass content of fatty acid at the start of the study. To determine baseline body fatty acid content, ten 7-day old chickens were used and their values were averaged. The ratio of fatty acid deposition and digestible fatty acid intake was calculated for selected individual fatty acids and groups of fatty acids. Minimum *de novo* fatty acid synthesis was calculated as fatty deposition minus digestible fatty acid intake.

Statistical analysis

Data were subjected to Duncan's multiple range test (Steel and Torrie, 1980) using the program of Microsoft Excel (Windows XP®). The level of statistical significance was preset at $P < 0.05$.

Results and discussion

In keeping with our previous investigation (Wongsuthavas et al., 2007), there were no significant diet effects on weight gain, feed intake and feed:gain ratio (Table 3). The amount of abdominal fat was reduced significantly ($P<0.05$) when about 75 % of the tallow was replaced by soybean oil. Contrary to our earlier work (Wongsuthavas et al., 2007), there was no further decrease after the incorporation of more soybean oil into the diet. The lowering of abdominal fat was in the order of 20-30%. Thus, this study confirms the well-known effect that substitution of PUFA for SFA in the diet of broiler chickens diminishes the deposition of abdominal fat.

Table 3. Effects of experimental diets on growth performance and weight of abdominal fat tissue

Items	Diet code					Pooled
	1	2	3	4	5	SE
Initial BW, g	184	176	182	187	182	4.10
Final BW, g	655	668	613	631	647	9.80
ADFI, g	45.4	47.2	44.8	42.4	41.8	3.00
ADG, g	22.4	23.4	21.0	21.8	22.1	2.38
Feed : gain	2.07	2.02	2.18	2.04	1.80	0.63
Abdominal fat, % of final BW	1.19^{a}	1.07^{a}	0.81^{b}	0.88^{b}	0.94^{b}	0.27

$^{a-b}$ Means in the same row with different letters are significantly difference ($P<0.05$)
Performance data are for 15 birds per dietary treatment. Abdominal fat data are for 5 birds per dietary treatment.

The concentration of triacylglycerols in plasma was measured as an index of *de-novo* fatty acid synthesis. Table 5 illustrates that the replacement of beef tallow by soybean oil caused a significant reduction in plasma triacylglycerols ($P<0.05$). The reduction was already maximal when the diet with lowest inclusion level of soybean oil was fed. A decrease in plasma triacylglycerols after substitution of dietary PUFA for SFA has been observed earlier in broiler chickens (Crespo and Esteve-Garcia, 2001; Crespo and Esteve-Garcia, 2003). Thus, in this study the decrease in abdominal fat was associated with a lowering of plasma triacylglycerols. The implication could be that the diminished deposition of abdominal fat was caused by inhibition of *de-novo* fatty acid synthesis. The replacement of tallow by soybean oil produced a dose-dependent lowering of plasma cholesterol concentrations (Table 4). This observation confirms earlier work (Newman et al., 2002).

Table 4. Influence of experimental diets on blood plasma lipid concentration

Blood plasma lipid concentration	Diet code					P-Values
	1	2	3	4	5	
Cholesterol, μmol/l	3,650	3,435	3,356	3,144	2,794	0.069
Triacylglycerol, μmol/l	2,751[a]	2,110[b]	2,092[b]	2,106[b]	1,985[b]	0.016

[a-b] Means in the same row with different letters are significantly difference (n=5)

Table 5 shows that the total SFA and PUFA content of whole carcass differed between the dietary groups. The replacement of SFA by PUFA lowered the SFA content of the whole body and increased that of PUFA. Other investigators have also reported that replacement of dietary SFA by PUFA had marked effects on total body fatty acid composition (Pinchasov and Nir, 1992; Crespo and Esteve-Garcia, 2002ab). The UFA were the predominant fatty acids in whole carcass for all of the treatments. However, for the diet with the highest PUFA level, PUFA deposition in the whole body was higher than for the other dietary groups (Table 5).

Table 5. Influence of increasing levels of PUFA on fat and fatty acid deposition in whole carcass

Items	Diet code					P-Values	Pooled SE
	1	2	3	4	5		
Fat deposition in final whole carcass, g							
	27.97	27.48	26.13	26.63	25.74	0.6086	3.5541
Fatty acid composition, % of whole carcass							
C16:0	24.59[a]	23.49[ab]	23.15[b]	24.74[a]	22.90[b]	0.0080	1.3424
C18:0	20.53[a]	18.54[b]	17.19[b]	9.81[c]	7.72[d]	0.0001	1.7648
C18:1 n-9	37.93[b]	40.46[a]	40.79[a]	40.76[a]	31.90[c]	0.0001	1.2936
C18:2 n-6	8.06[c]	11.19[b]	11.66[b]	12.00[b]	13.73[a]	0.0001	1.2329
C18:3 n-3	0.39[d]	0.71[c]	0.72[c]	0.82[b]	0.88[a]	0.0001	0.0650
∑SFA	45.38[a]	42.29[b]	40.61[b]	34.81[c]	30.80[d]	0.0001	2.3043
∑MUFA	43.93[b]	46.80[a]	46.89[a]	46.64[a]	37.88[c]	0.0001	1.7093
∑PUFA	9.13[c]	12.21[b]	12.89[b]	13.01[b]	14.35[a]	0.0001	1.2886

ΣSFA= C8:0+C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0+C22:0+C24:0

ΣMUFA=C14:1+C15:1+C16:1+C18:1n-9+C18:1n-7+C20:1n-9

ΣPUFA=C18:2 n-6+18:3 n-6+18:3 n-3

[a-b-c-d] Means in the same row with different letters are significantly difference (n=10)

The dietary fat type had marked effects on the apparent digestibility of individual fatty acids. Palmitic, oleic, linoleic and alpha-linolenic acid were digested more efficiently when present in soybean oil than in beef tallow. A combination of different factors may be responsible for the observed diet-induced differences in apparent digestibility of identical fatty acids. As mentioned above, the total crude fat digestibility for soybean oil was greater than that for beef tallow, which may relate to enhanced micelle formation after feeding soybean oil. An improved micelle formation may favorably influence the digestion of all fatty acids in the diet. The position of a given fatty acid in the triacylglycerol molecule also plays a role. Fatty acids at the 2 position of glycerol in triacylglycerol molecules are better digested than those at the 1, 3 position (Bracco, 1994; Innis and Dyer, 1997; Nelson and Innis, 1999). During digestion, the pancreatic lipase action specifically removes fatty acids at the 1, 3 position while the resulting monoacylglycerol molecule is efficiently incorporated into micelles (Lien, 1994), leading to preferential absorption of fatty acids at the 2 position of the glycerol backbone of triacylglycerols. The intake level of a given individual fatty acid and its faecal excretion of endogenous origin will also affect the calculated apparent digestibility. A low intake level in combination with a high endogenous excretion will by itself lead to a low apparent digestibility. When comparing the digestibilities of palmitic and stearic acid for the diets with soybean oil and beef tallow, the values for the diets with soybean oil diet may biased to lower values because the intake levels were lower. On the other hand, the apparent digestibility for linoleic acid on the diets rich in soybean oil diet may be biased towards a higher value (Table 6).

Table 6. Individual fatty acid digestibility, g/100g fatty acid intake

Items	Diet code					P-Values
	1	2	3	4	5	
Fatty acid composition						
C16:0	73.85^{b}	76.69^{b}	81.30^{a}	80.49^{a}	81.40^{a}	0.0001
C16:1	42.58^{b}	50.12^{b}	25.34^{b}	63.51^{a}	60.34^{a}	0.0001
C18:0	69.77	71.87	75.04	73.40	72.71	0.1852
C18:1 n-9	75.42^{b}	82.80^{a}	82.69^{a}	80.55^{a}	79.48^{a}	0.0001
C18:2 n-6	82.02^{b}	76.29^{c}	83.89^{ab}	82.09^{b}	85.46^{a}	0.0001
C18:3 n-3	79.72^{b}	73.94^{c}	85.67^{a}	81.24^{b}	85.79^{a}	0.0001
∑FA	73.77^{e}	75.90^{d}	80.18^{b}	79.26^{c}	80.44^{a}	0.0001
∑SFA	72.29^{b}	73.73^{b}	78.04^{a}	78.29^{a}	79.70^{a}	0.0005
∑MUFA	77.16^{b}	80.25^{a}	76.04^{b}	81.42^{a}	79.69^{a}	0.0001
∑PUFA	80.87^{b}	75.12^{c}	84.78^{a}	81.67^{b}	85.63^{a}	0.0001
∑UFA	76.96^{c}	79.81^{bc}	83.56^{a}	81.66^{ab}	82.55^{ab}	0.0004

ΣSFA= C8:0+C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0+C22:0+C24:0
ΣMUFA=C14:1+C15:1+C16:1+C18:1n-9+C18:1n-7+C20:1n-9
ΣPUFA=C18:2 n-6+18:3 n-6+18:3 n-3
[a-b-c-d-e] Means within a row with on common superscripts differ significantly; n=15

The calculated intake of digestible fatty acids reflects the amount in the diet and feed intake, combined with the measured apparent digestibility. The deposition in the body of fatty acids was calculated as based on the fatty acid composition of the total body fat that was gained during the entire feeding period. As would be expected, the chickens fed the diets rich in soybean oil deposited more linoleic acid and those fed

Table 7. Effect of dietary fat type on digestible fatty acid intake, fatty acid deposition, deposition:intake ratio and minimum *de-novo* fatty acid synthesis during the whole feeding period

Fatty acids	Diet code					P-values	Pooled SE
	1	2	3	4	5		
Digestible intake, g							
C16:0	3.47^{a}	2.76^{b}	2.74^{b}	2.37^{c}	2.17^{c}	0.0001	0.2840
C18:0	6.62^{a}	3.35^{b}	2.18^{c}	1.18^{d}	0.61^{e}	0.0001	0.3135
C18:1 n-9	5.94^{a}	5.59^{b}	5.61^{b}	5.30^{c}	5.28^{c}	0.0001	0.1484
C18:2 n-6 c	2.02^{e}	8.27^{d}	11.24^{c}	12.40^{b}	13.70^{a}	0.0001	0.1821
C18:3 n-3	0.13^{e}	0.52^{d}	0.74^{c}	0.80^{b}	0.88^{a}	0.0001	0.0242
∑FA	23.87^{ab}	23.25^{b}	24.42^{a}	23.13^{b}	23.25^{b}	0.0491	1.0798
∑SFA	10.19^{a}	6.32^{b}	5.20^{c}	3.84^{d}	3.10^{e}	0.0001	0.5892
∑MUFA	6.25^{a}	5.87^{b}	5.90^{b}	5.56^{c}	5.53^{c}	0.0001	0.1645
∑PUFA	2.33^{e}	9.05^{d}	12.03^{c}	13.25^{b}	14.63^{a}	0.0001	0.2036
Deposition, g							
C16:0	5.96^{a}	5.83^{a}	4.15^{b}	5.09^{ab}	4.52^{a}	0.0159	1.3554
C18:0	6.09^{a}	5.91^{a}	4.24^{b}	1.99^{c}	1.28^{c}	0.0001	0.8604
C18:1 n-9	8.55^{ab}	10.04^{a}	7.44^{b}	8.15^{ab}	5.15^{c}	0.0001	2.0188
C18:2 n-6	1.99^{c}	3.31^{ab}	2.72^{b}	3.03^{b}	3.69^{a}	0.0001	0.6718
C18:3 n-3	0.06^{c}	0.20^{a}	0.15^{b}	0.20^{a}	0.22^{a}	0.0001	0.0447
∑FA	23.74^{ab}	24.93a	18.30^{c}	20.04^{bc}	20.37^{bc}	0.0147	4.6774
∑SFA	12.11^{a}	11.81^{a}	8.44^{b}	7.14^{bc}	5.83^{c}	0.0001	2.1086
∑MUFA	9.89^{ab}	11.59^{a}	8.50^{b}	9.24^{b}	6.28^{c}	0.0003	2.3810
∑PUFA	2.27^{c}	3.57^{ab}	2.99^{b}	3.24^{ab}	3.78^{a}	0.0001	0.6825
Deposition : intake, g							
C16:0	1.72^{ab}	2.12^{a}	1.55^{b}	2.18^{a}	2.08^{a}	0.0417	0.5399
C18:0	0.92^{b}	1.77^{ab}	1.98^{a}	2.04^{a}	2.32^{a}	0.0458	1.0361
C18:1 n-9	1.44^{b}	1.80^{a}	1.33^{b}	1.54^{ab}	0.97^{c}	0.0003	0.3729
C18:2 n-6	0.99^{a}	0.40^{b}	0.24^{c}	0.24^{c}	0.27^{c}	0.0001	0.1238
C18:3 n-3	0.51^{a}	0.37^{b}	0.20^{c}	0.25^{c}	0.25^{c}	0.0001	0.1245
∑FA	0.99^{ab}	1.07^{a}	0.75^{c}	0.87^{bc}	0.87^{bc}	0.0070	0.1952
∑SFA	1.19^{b}	1.88^{a}	1.65^{a}	1.93^{a}	1.88^{a}	0.0129	0.5170
∑MUFA	1.58^{b}	1.98^{a}	1.45^{bc}	1.66^{ab}	1.13^{c}	0.0011	0.4201
∑PUFA	0.98^{a}	0.39^{b}	0.25^{c}	0.25^{c}	0.26^{c}	0.0001	0.1080
Minimum *de novo* fatty acid synthesis							
SFA	1.92^{b}	5.49^{a}	3.24^{b}	3.30^{b}	2.73^{b}	0.0087	2.0348
MUFA	3.64^{b}	5.72^{a}	2.60^{b}	3.68^{b}	0.75^{b}	0.0063	2.1975
SFA/(SFA+MUFA)	0.35^{b}	0.49^{a}	0.55^{a}	0.47^{ab}	0.78^{a}	0.0022	0.0228

ΣSFA= C8:0+C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0+C22:0+C24:0

ΣMUFA=C14:1+C15:1+C16:1+C18:1n-9+C18:1n-7+C20:1n-9

ΣPUFA=C18:2 n-6+18:3 n-6+18:3 n-3

[a-b-c-d-e] Means within a row with on common superscripts differ significantly; n=15

on diets rich in beef tallow had deposited more stearic acid in their whole body. Similar data have been shown for mice (Javadi et al., 2004) and goats (Yeom et al., 2005). The increased deposition of palmitic acid in the birds fed the diet rich in beef tallow did not reach statistical significance (Table 7).

To obtain clues as to preferential deposition of fatty acids, the ratio of deposition of a given fatty acid or group of fatty acids to the intake of digestible fatty acid or group of fatty acids was calculated. The deposition:intake ratio for fatty acid 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 linoleic acid in the chickens fed the diets rich in soybean oil is consistent with the well-known preferential oxidation of linoleic acid (Slim et al., 1996; Sanz et al., 1999; Sanz et al., 2000b) and the fact that linoleic acid cannot be synthesized by chickens (Schafer, 2001). The deposition:intake ratio for the essential polyunsaturated fatty acids, linoleic and alpha-linolenic acid, cannot be higher than 1. Indeed, the ratios for alpha-linolenic acid were below and so was the ratio for linoleic acid in the broilers fed diets rich in soybean oil. The chickens fed diets high in beef tallow had a group mean deposition:intake ratio for linoleic acid that was just above 1, but was not significantly higher than 1. The extremely high deposition:intake ratio for stearic acid in chickens fed diets high in soybean oil is explained by a relatively low intake and high net synthesis of this fatty acid as shown in Table 7.

The birds fed soybean oil instead of beef tallow had a higher deposition:intake ratio for SFA, but lower ratios for MUFA and PUFA. The diet effect on the ratios for SFA and MUFA can be explained by the calculated minimum synthesis of these groups of fatty acids. Feeding the diet containing soybean oil stimulated the synthesis of SFA, but depressed that of MUFA. The higher synthesis ratio for SFA:(SFA + MUFA) in chickens fed the diets rich in soybean oil indicates that there was selective synthesis of SFA in these birds. This might point at *de novo* fatty acid synthesis being regulated to balance the amount of saturated and unsaturated fatty acids in the body because the intake of linoleic acid was very high in the birds fed the diets rich in soybean oil (Table 7).

In conclusion, the gradual replacement of beef tallow by soybean oil had no effect growth performance, but rather improved the digestibility of all individual fatty acids. Feeding the diets with increasing levels of soybean oil produced a markedly increased deposition of linoleic acid in the whole body. For groups of fatty acids, the ratio of deposition in the whole body to the intake of digestible fatty acids was calculated. It then became clear that the type of dietary had marked, specific effects on the synthesis and oxidation of fatty acids. The decrease in abdominal fat as induced by the feeding of soybean oil instead of beef tallow was associated with a decrease in plasma triacylglycerol concentrations, but not with a decrease in calculated minimum *de novo* fatty acid synthesis.

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CHAPTER 6

Comparison of restricted and *ad libitum* feeding of diets containing either beef tallow or soybean oil with regard to energy balance and digestion and deposition of individual fatty acids in broiler chickens

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Abstract

Seven-day-old broiler chicks were fed diets containing either beef tallow (BT) or soybean oil (SO) under restricted or *ad libitum* feeding conditions. It was found that the dietary fat source had no effect on growth performance. However, the SO diet tended to improve the feed conversion ratio and decreased average daily feed intake under the restricted feeding regimen. Energy expenditure of broilers fed the SO diet tended to be higher were fed restricted or *ad libitum* feeding. Energy loss with excreta was lower on the SO diet than on the BT diet. The birds fed the SO diet showed higher apparent digestibilities for the sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA); this especially the case for *ad libitum* feeding. The ratio of fatty acid deposition to intake of digestible fatty acids was increased for C18:0 in the birds fed SO diet, but the ratio for oleic acid (C18:1 n-9) was decreased. In the birds fed the diet with SO instead of BT, the deposition:intake ratio was raised for SFA, but diminished for MUFA. The increased deposition:intake ratio for SFA may be explained by an enhanced *de novo* synthesis of SFA, whereas the decreased ratio for MUFA was associated with a diminished synthesis of MUFA. The decreased ratio of deposition:intake for PUFA in the birds fed SO may be explained by preferential oxidation of PUFA.

Keywords: beef tallow, soybean oil, energy balance, fatty acid digestibility, *de novo* fatty acid synthesis

Introduction

Previous reports show that broiler chickens fed diets enriched with polyunsaturated fatty acids (PUFA) have less deposition of abdominal fat (Sanz et al., 1999; Crespo and Esteve-Garcia, 2001, 2002a) or total body fat (Sanz et al., 2000) than do their counterparts fed diets containing saturated fatty acids (SFA). Body fat accumulation is the net result of fat absorption from the diet, endogenous fat synthesis (lipogenesis) and fat catabolism through lipolysis and β-oxidation. Thus, if for diets either rich in PUFA or SFA, the amount of absorbed fat would be identical, the lower body fat deposition on the high-PUFA diet may be attributed to increased fat catabolism or diminished endogenous fatty acid synthesis or to a combination. An increased rate of fatty acid oxidation appears to be main mechanism involved (Crespo et al., 2002b; Sanz et al., 2000). In rats fed diets rich in PUFA instead of SFA, there was enhanced fat catabolism and reduced fatty acid synthesis (Shimomura et al., 1990). However, the issue is more complex and the assumption of identical fat absorption is not correct. Becker et al. (1979) reported fat absorption is increased on diets rich in PUFA instead of SFA.

Based on literature data it would be expected that the decrease in body fat as seen in broilers fed diets rich in PUFA versus SFA is associated with enhanced fat absorption, enhanced fat catabolism and decreased lipogenesis. We have put forward (Wongsuthavas et al., 2007) the following possible mechanism. PUFA versus SFA acids are preferentially oxidized (Beynen and Katan, 1985) and thereby yield ATP so that carbohydrates are shifted from the oxidative into the lipogenic pathway. The conversion of glucose into triglycerides is less efficient in terms of energy deposition than is the conversion of fatty acids into triglycerides (Newsholme and Leech, 1984). As a consequence, the feeding of PUFA instead of SFA would not only lead to less deposition of abdominal fat, but be associated with more heat expenditure.

In the experiments mentioned above, the animals were had free access to the experimental diets. Under conditions of *ad libitum* feeding, animals may adapt feed intakes to changes in fat absorption, fat catabolism and lipogenesis. Thus, the present study was undertaken to determine the effect of replacement of dietary SFA by PUFA under conditions of *ad libitum* and restricted feeding. In the broiler chickens, we measured energy balance and the digestion and deposition of individual fatty acids.

Materials and methods

Experimental treatments and design

A total of hundred 1-day-old male Arbor Acres broiler chicks were housed in groups and they were offered a commercial diet for 7 days before commencement of the experiment. Then, the birds were randomly distributed so that there were 50 birds per feeding regimen (*ad libitum* and restricted feeding) and per experimental diet containing either beef tallow (BT) or soybean oil (SO). Thus there were 25 birds per treatment and they were kept in individual cages. Birds had free access to clean water. The experimental diets were semi-purified and formulated to contain 18% crude protein. The variable fat source was added to the diets so that the amount of digestible fat was identical. The values for fat digestibility were based on a previous experiment. Table 1 shows the ingredient and analysed composition of the diets and the fatty acid composition is given in Table 2. The experimental treatments were according to a 2x2 factorial, completely randomized design.

Table 1. Ingredient and analysed composition of the experimental diets

Item	Experimental diet	
	BT	SBO
Ingredients composition		
Tallow	2.87	-
Soybean oil	0.13	2.79
Tapioca starch	45.52	45.52
Soybean meal	41.05	41.05
Hull rice bran	4.00	4.00
Di-Calcium Phosphate	3.87	3.87
Lime stone	0.50	0.50
DL-Methionine	0.30	0.30
L-Lysine hydrochloride	0.25	0.25
Sodium Chloride	0.51	0.51
Premix	1.00	1.00
Total	100	99.79
Analyzed composition		
Dry matter	91.98	92.17
GE (kJ/kg)	16,864	16,584
Crude Protein (N x 6.25)	18.12	18.16
Energy: Protein Ratio (g:kJ)	930.68	913.22
Crude fat	4.20	3.90
Crude fiber	3.23	3.24
Ash	4.30	4.31
Calculated composition		
Nitrogen free extract	63.45	63.58
Calcium	1.03	1.03
Available phosphorus	0.72	0.72
Methionine	0.50	0.50
Methionine + Cystine	0.73	0.73
Lysine	1.20	1.20

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; ferrous, 87.59 mg; selenium, 0.17 mg.

Table 2. Fatty acid composition of the broiler diets

Fatty acid, g/100 g methyl esters	Fat source	
	BT	SBO
C14:0	3.45	0.28
C16:0	26.87	26.93
C16:1	0.85	0.07
C18:0	28.55	9.29
C18:1 n-9	19.61	29.87
C18:2 n-6 c	16.63	24.90
C18:3 n-3	1.07	1.73
C20:0	0.39	0.89
ΣSFA	59.97	39.86
ΣMUFA	21.28	30.17
ΣPUFA	17.85	26.94
Σn-9	19.61	29.93
Σn-6	16.78	25.21
Σn-3	1.07	1.73
Unidentified fatty acid	0.90	3.03

ΣSFA= C8:0+C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0+C22:0+C24:0
ΣMUFA=C14:1+C15:1+C16:1+C18:1n-9+C18:1n-7+C20:1n-9
ΣPUFA=C18:2 n-6+18:3 n-6+18:3 n-3

Data and sample collection

Chicks were weighed at 7 and 28 days and feed consumption per each cage was recorded for the same period. The feed:gain ratio was calculated (g feed:g gain). Animals were closely monitored and daily mortality was recorded.

Each day during the period of 7-28 days of age excreta were collected for analysis (apparent crude fat digestibility, fatty acid digestibility).

Chemical analysis

The experimental diets were analyzed for dry matter, ash, crude fat, crude fiber, crude protein and the fatty acid profile according to the methods of the AOAC (1985). Excreta were analyzed for individual fatty acids to calculate their digestibility. Total lipids were extracted according to the method of Folch et al. (1957).

Bomb calorimetry

Bomb calorimetry was applied to determine the gross energy content of the diets, of homogenized whole carcass 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 weight x energy content) of the 21 days feeding period. The same procedure was used to calculate the water, protein, fat and ash retention (Javadi et al., 2005).

Statistical analysis

Data were subjected to analysis of variance a 2x2 factorial in completely randomized design (Steel and Torrie, 1980) by using the Microsoft Excel program (Windows xp®). Body weight, ADFI, ADG and FCR (15 replications per treatment) were used for statistical analysis. Whole carcass data of the broilers (10 replicates per treatment) were statistically analysed for group differences (Steel and Torrie, 1980). Statistical significance of differences between diets and feeding regimens were assessed using Duncan's multiple range test (Steel and Torrie, 1980).

Results and discussion

Average daily feed intake (ADFI)

ADFI was 33.49, 33.31, 47.69 and 45.18 g/d for birds fed the BT and SO diets under restricted and *ad libitum* conditions, respectively (Table 3). Under ad libitum feeding conditions, the birds fed the SO diet had lower ADFI than those fed the BT diet ($P<0.05$). The observed lower ADFI in birds fed the high-SO diet corroborates earlier work (Mossab et al., 2000; Atteh et al.1983). PUFAs in plant oils, especially, soybean oil, are better digested by chickens than the SFAs in tallow (Leeson and Atteh, 1995) so that under *ad libitum* feeding conditions chickens will have to consume less feed on diets containing SO than on diets containing BT in order to meet their energy requirement.

Table 3. Effects of experimental diets and feeding regimen on growth performance

Item	Restricted		P-value	*Ad libitum*		P-value
	BT	SBO		BT	SBO	
Number of birds	25	25	-	25	25	-
Days in experiment	21	21	-	21	21	-
Mortality, %	0	0	-	0	0	-
Initial BW, g	116.40	116.12	0.9089	114.08	116.92	0.3253
Final BW, g	484.84	465.36	0.1732	545.64	542.44	0.8472
ADFI, g	33.49	33.31	-	47.69[a]	45.18[b]	0.0450
ADG, g	17.79	17.82	0.3562	20.55	20.26	0.7860
Feed : gain	1.89	1.87	0.2072	2.34	2.29	0.1467

[a-b] Values in the same row with different superscript are significantly different.

Average daily gain (ADG)

ADG was 17.79, 17.82, 20.55 and 20.26 g/d for the birds fed the BT and SO diets under *ad libitum* feeding conditions, respectively (Table 3). There was no significant difference ($P>0.05$) between the diet groups. This may be explained by the maximum weight gain in the early stage of growth. Wongsuthavas et al. (2007) reported earlier that birds fed either diets with SO or BT did not differ with regard to ADG.

Feed : gain ratio

The feed:gain ratio of birds fed restricted amounts of feed was lower than in those fed *ad libitum* (Table 3**).** Birds fed the SO diet had a more favorable feed:gain ration than the birds fed the BT diet, there was no statistically significant difference ($P>0.05$). This outcome agrees with data published by Mossab et al. (2000), indicating that supplemental SO in a basal diet improved the feed:gain ratio in turkeys and broiler chickens. Wongsuthavas et al. (2007) also showed that the feed:gain ratio

in birds fed diets containing SO was greater than in birds fed diets containing BT. It is tempting to speculate that high PUFA intake, through altered eicosanoid synthesis, influences immunity, leading to more efficient growth.

Apparent digestibility of fat and gross energy

The data in Table 4 show that the SO diet tended to increase apparent fat digestibility and apparent gross energy digestibility when compared with the BT diet. This result is explained by the higher digestibility of PUFA versus SFA (Becker et al. 1979). An increased digestibility of dietary fat will be associated with an increased digestibility of gross energy in the diet.

Energy balance

The various aspects of the energy balance are shown in Table 4. It is clear that energy intake on the SO diet was lower than on the BT diet (11,823.30 and 11,602.04 kJ, restricted; 16,684.57 and 15,767.90 kJ, *ad libitum*) (P>0.05). Energy expenditure was calculated as the difference between the energy intake and energy stored and excreted in the excreta. The SO diets tended to increase energy expenditure (P>0.05) and to reduce energy loss with excreta (P<0.05). Energy storage was not significantly (P>0.05) affected by the type of fat in the diet. The percentage of energy intake that was expenditures was higher under supplementations of SO than, but the difference was not statistically significant.

Table 4. Influence of experimental diets and feeding regimen on energy balance

Item	Restricted		P-value	*Ad libitum*		P-value
	BT	SBO		BT	SBO	
Feed intake (g/21d)	703.18	699.60	-	995.70	950.80	0.2339
Energy in the diets (KJ)	16,864	16,584	-	16,864	16,584	-
Apparent fat digestibility,%	77.21	80.49	0.0616	74.06[a]	81.64[b]	0.0097
Apparent GE digestibility,%	72.23	75.31	0.1805	77.05	79.62	0.0643
Body composition of whole carcass (%)						
Water	71.94	72.02	0.9187	71.90	71.43	0.4417
Fat	7.49	7.01	0.3107	7.90	7.85	0.7336
Protein	14.41	13.98	0.2110	14.17	14.29	0.7986
Ash	2.57	2.80	0.0075	2.56	2.70	0.3476
Calculated energy balance (KJ)						
Intake	11,823.30	11,602.04	-	16,684.57	15,767.90	0.1469
Stored in the body	3,316.66	3,275.84	0.8975	4,237.67	4,100.40	0.6613
Expenditure	5223.28	5461.81	0.4453	8,624.19	8,403.40	0.6938
In excreta	3,283.35	2,864.40	0.1143	3,836.05[a]	3,213.61[b]	0.0432
In excreta as fat	207.04[a]	174.81[b]	0.0440	282.95[a]	185.51[b]	0.0031
In fat-free excreta	3079.88[a]	2689.59[b]	0.0001	3553.10[a]	3028.11[b]	0.0001
Energy in whole body (KJ)						
Initial body energy	1,835.89	1,912.39	0.2399	1,768.96	1,878.92	0.1368
Final body energy	5,152.55	5,188.23	0.9741	6,006.63	5,979.32	0.3144
Percentage of energy intake that is :						
Energy stored in the body	28.05	28.24	0.9461	25.40	26.00	0.6711
Energy expenditure	44.18	47.08	0.2848	51.97	53.61	0.5304
Lost in excreta	27.77	24.69	0.1805	22.99	20.38	0.0643
Lost in excreta as fat	2.08	1.79	0.0530	2.02[a]	1.40[b]	0.0066
Lost in fat-free excreta	25.69[a]	22.90[b]	0.0001	20.97[a]	19.98[b]	0.0001

[a-b] Values in the same row with the different superscripts are significantly different

Digestibility of fatty acids

Table 5 documents the apparent digestibility of individual fatty acids. The digestibility of crude fat, MUFA, PUFA, n-9, n-6 and n-3 unsaturated fatty acid was higher for the SO diet than for the BT diet. The diet difference was especially apparent for C18:3 n-3, C20:1 n-9, C20:3 n-6 and C22:1 n-9. This observation confirms the well-known fact that the SFA in BT, in particular stearic acid (C18:0), are less efficiently digested than are the PUFAs in SO. Under similar experimental conditions Wongsuthavas et al. (2007) found earlier that a gradual increase in the level of PUFA in diet for broiler chickens was associated with increased fat digestibility. The new observation is that the digestibility of SFA in the BT diet was higher than SFA in the SO diet, both under restricted and *ad libitum* feeding conditions (P= 0.001 and P=0.024).

Table 5. Effect of experimental diets and feeding regimen on apparent fatty acid digestibility

Fatty acid (% of intake)	Restricted		Root MSE	P-value	Ad libitum		Root MSE	P-value
	BT	SBO			BT	SBO		
C12:0	71.19	73.95	5.448	0.272	70.12	72.31	9.540	0.614
C14:0	92.17[a]	39.73[b]	8.726	0.000	91.88[a]	35.94[b]	15.048	0.000
C16:0	77.67	84.16	3.821	0.001	76.84	83.16	6.715	0.050
C17:0	72.81[b]	93.15[a]	3.866	0.000	71.80[b]	92.72[a]	6.748	0.000
C18:0	71.44[b]	79.09[a]	4.943	0.003	70.38	77.77	8.683	0.073
C18:1 n-9	90.57[a]	90.27[b]	0.513	0.000	90.22	89.66	3.331	0.713
C18:2 n-6	92.75[a]	89.85[b]	1.766	0.002	92.48[a]	89.22[b]	3.074	0.029
C18:3 n-3	88.81[b]	90.62[a]	2.046	0.063	88.39	90.03	3.586	0.320
C20:1 n-9	77.87[b]	81.73[a]	4.017	0.046	77.05	80.58	7.043	0.277
C20:3 n-6	98.98[b]	99.93[a]	0.139	0.000	98.95[b]	99.93[a]	0.248	0.000
C22:1 n-9	99.76[b]	99.95[a]	0.034	0.000	99.75[b]	99.95[a]	0.060	0.000
ΣFA	77.21	80.49	3.913	0.062	74.06[b]	81.64[a]	6.867	0.010
ΣSFA	78.11[a]	68.89[b]	5.388	0.001	77.30[a]	66.94[b]	9.375	0.024
ΣMUFA	91.01[b]	92.99[a]	1.592	0.012	90.68	92.55	2.798	0.152
ΣPUFA	93.51	93.47	1.294	0.943	93.27	93.06	2.264	0.835
ΣUFA	92.26	93.23	1.440	0.150	91.98	92.80	2.526	0.472
Σn-9	89.40[b]	92.99[a]	1.770	0.000	89.01[b]	92.55[a]	3.116	0.020
Σn-6	95.86[b]	97.45[a]	0.676	0.000	95.71	96.38	1.315	0.271
Σn-3	88.81[b]	94.60[a]	1.724	0.000	88.39[b]	94.26[a]	3.330	0.000

Σ FA = Σ SFA + Σ MUFA + Σ PUFA
Σ SFA = C10:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0
Σ MUFA = C16:1 + C17:1 + C18:1 n-7 + C18:1 n-9 + C20:1 n-9 + C22:1 n-9
Σ PUFA = C18:2 n-6 + C18:3 n-3 + C18:3 n-6 + C20:5n-3
Σ n-9 = C20:1 n-9 + C22:1 n-9
Σ n-6 = C18:2 n-6 + C18:3 n-6
Σ n-3 = C18:3 n-3 + C20:5n-3
[a-b] Values in the same row with different superscript are significantly different.

Intake and deposition of digestible fatty acids

The intake of digestible palmitic and stearic acid by the broilers fed the BT diet was greater than that for the broilers the SO diet, whereas the broilers fed the SO diet had a higher intake of digestible oleic and linoleic acid (Table 6). There was no diet difference for the intake of digestible alpha-linolenic acid under conditions of *ad libitum* feeding. The broilers fed the SO diet had a greater intake of digestible MUFA and PUFA, but had a lower intake of digestible SFA. When the fatty acids were

Table 6. Influence of experimental diets and feeding regimen on digestible fatty acid intake, fatty acid deposition and deposition:intake ratio during the whole feeding period

Item	Restricted		Root MSE	P-value	Ad libitum		Root MSE	P-value
	BT	SBO			BT	SBO		
Digestible fatty acid intake, g								
C16:0	6.16	6.18	0.316	0.878	8.63	8.30	1.411	0.288
C18:0	6.02[a]	2.00[b]	0.362	0.000	8.40[a]	2.68[b]	1.423	0.000
C18:1 n-9	5.25[b]	7.36[a]	0.146	0.000	7.40	9.93	1.167	0.099
C18:2 n-6	4.56[b]	6.10[a]	0.119	0.000	6.43[b]	8.24[a]	0.905	0.003
C18:3 n-3	0.28[b]	0.43[a]	0.012	0.003	0.40	0.58	0.085	0.076
ΣFA	22.80	21.96	1.199	0.247	30.97	30.27	5.268	0.162
ΣSFA	13.83[a]	7.49[b]	0.714	0.000	19.39[a]	9.89[b]	3.104	0.000
ΣMUFA	5.72[b]	7.65[a]	0.159	0.000	8.07	10.35	1.292	0.155
ΣPUFA	4.93[b]	6.87[a]	0.129	0.000	6.96[b]	9.30[a]	1.022	0.000
ΣUFA	10.17[b]	14.37[a]	0.286	0.000	14.35[b]	19.44[a]	2.311	0.011
Σn-9	5.18[b]	7.59[a]	0.154	0.000	7.30[b]	10.27[a]	1.228	0.018
Σn-6	4.75[b]	6.70[a]	0.119	0.000	6.72[b]	9.01[a]	0.932	0.000
Σn-3	0.28[b]	0.45[a]	0.012	0.000	0.40	0.60	0.088	0.178
Fatty acid deposition, g								
C16:0	7.66	7.10	1.069	0.260	9.32[a]	7.98[b]	1.274	0.031
C18:0	3.62[a]	3.02[b]	0.470	0.012	4.19[a]	2.94[b]	0.898	0.006
C18:1 n-9	12.50[a]	10.81[b]	1.760	0.045	14.63	12.54	2.320	0.059
C18:2 n-6	4.04	4.92	0.017	0.067	5.14	5.22	1.588	0.145
C18:3 n-3	0.21	0.27	0.113	0.395	0.29	0.27	0.163	0.111
ΣFA	33.40	31.88	4.573	0.466	40.12	36.15	5.374	0.116
ΣSFA	12.20	10.75	1.615	0.060	14.71[a]	11.58[b]	1.670	0.001
ΣMUFA	14.29[a]	12.19[b]	2.042	0.034	16.92[a]	14.13[b]	2.667	0.031
ΣPUFA	4.67[b]	5.72[a]	1.108	0.048	5.88	6.93	1.682	0.179
ΣUFA	18.96	17.91	2.890	0.426	22.79	21.06	3.955	0.341
Σn-9	12.50[a]	10.81[b]	1.760	0.045	14.63	12.54	2.320	0.059
Σn-6	4.09	4.98	1.022	0.069	5.19	6.28	1.596	0.146
Σn-3	0.31	0.28	0.115	0.481	0.39	0.27	0.163	0.111
Fatty acid deposition:fatty acid intake, g/g								
C16:0	1.24	1.15	0.185	0.265	1.08	0.96	0.293	0.360
C18:0	0.60[b]	1.51[a]	0.146	0.000	0.50[b]	1.10[a]	0.388	0.001
C18:1 n-9	2.38[a]	1.47[b]	0.312	0.000	1.98[a]	1.26[b]	0.423	0.001
C18:2 n-6	0.89	0.81	0.173	0.372	0.80	0.63	0.246	0.865
C18:3 n-3	0.75	0.63	0.241	0.513	0.73	0.47	0.322	0.249
ΣFA	1.46	1.45	0.211	0.700	1.30	1.19	0.332	0.842
ΣSFA	0.88[b]	1.43[a]	0.104	0.041	0.76[b]	1.17[a]	0.185	0.044
ΣMUFA	2.50[a]	1.59[b]	0.358	0.045	2.10[a]	1.36[b]	0.591	0.055
ΣPUFA	0.95[a]	0.83[b]	0.106	0.000	0.84[a]	0.75[b]	0.142	0.004
ΣUFA	1.87[a]	1.25[b]	0.218	0.003	1.59[a]	1.08[b]	0.338	0.022
Σn-9	2.41[a]	1.42[b]	0.195	0.000	2.00[a]	1.22[b]	0.409	0.001
Σn-6	0.86[a]	0.74[b]	0.115	0.000	0.77[a]	0.70[b]	0.148	0.003
Σn-3	0.75	0.63	0.241	0.513	0.99	0.45	0.322	0.249

Σ FA = Σ SFA + Σ MUFA + Σ PUFA
Σ SFA = C10:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0
Σ MUFA = C16:1 + C17:1 + C18:1 n-7 + C18:1 n-9 + C20:1 n-9 + C22:1 n-9
Σ PUFA = C18:2 n-6 + C18:3 n-3 + C18:3 n-6 + C20:5n-3
Σ n-9 = C20:1 n-9 + C22:1 n-9
Σ n-6 = C18:2 n-6 + C18:3 n-6
Σ n-3 = C18:3 n-3 + C20:5n-3
[a-b] Values in the same row with different superscript are significantly different

pooled according to their structural similarities, the SO diet was found to provide more digestible n-9 MUFA and n-6 PUFA and n-3 PUFA under restricted feeding conditions, whereas the amount of digestible n-3 PUFA was similar to that provided by the BT diet when the broilers had free access to the diets.

The birds fed BT diet had deposited more palmitic acid, stearic acid and oleic acid, but the deposition of linoleic acid was less when compared with the birds fed the SO diet (Table 6). There was no difference between the two experimental diets with regard to the deposition of linoleic acid and alpha-linolenic acid. The birds fed the SO diet deposited more PUFA than did those fed BT diet, but no significant difference was seen for the deposition of total FA. There was more deposition of n-9 MUFA in the birds fed the BT diet, but that of n-6 PUFA and n-3 PUFA was similar to the deposition of these fatty acids in the birds fed the SO diet.

The deposition:intake ratio for stearic acid in the birds fed the SO diet were greater than in their counterparts fed the BT diet. The ratio for stearic acid was extremely high for the birds fed the diet containing BT (Table 6). However, the birds fed the SO diet had a low deposition:intake ratio for oleic acid. There was no diet effect on the deposition:intake ratio for both linoleic and alpha-linolenic acid, the ratio for the two groups of birds being less than one. There were greater ratios for total FA, MUFA, PUFA and n-6 PUFA in birds fed the BT diet, but the ratio of SFA and n-9 MUFA was higher in birds fed the SO diet, whereas there was no difference between the two dietary groups concerning the deposition:intake ratio of n-3 PUFA.

In conclusion, the inclusion of SO instead of BT in the diet for broiler chickens had no negative effect on growth performance, but it improved the digestibility of crude fat. Feeding the diet with SO produced a markedly increased deposition of PUFA in the whole body. In essence, the differential effects of dietary BT and SO on energy balance and the digestion and deposition of individual fatty acids were similar under both restricted and *ad libitum* feeding conditions.

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CHAPTER 7

Growth performance, energy balance, digestion and deposition of fatty acids in broiler chickens fed diets rich in linoleic or alpha-linolenic acid

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To be submitted

Abstract

We studied the effect of high intake of either linoleic acid (LA) or alpha-linolenic acid (ALA) on growth performance, energy balance, digestion and deposition of fatty acids in broiler chickens. Soybean oil was used as LA source and linseed oil was used as ALA source. The high-ALA diet lowered average daily gain and total feed intake. LA digestibility in the birds fed the high-LA diet was greater than that in the birds fed the high-ALA diet. Likewise, ALA digestibility was greater on the high-ALA diet. The high-LA and high-ALA diets had no differential effect on energy balance, but the amount of fat in the whole carcass was decreased in the birds fed the high-ALA diet. The fatty acid deposition:intake ratio for LA was higher in the birds fed the high-ALA diet. In contrast, the birds fed the high-LA diet showed a higher ratio for ALA. The rate of whole body de novo synthesis of monounsaturated fatty acids was higher in chickens fed the high LA-diet than in their counterparts fed the high ALA-diet. Birds fed the high-ALA diet tended to have less abdominal fat when compared with birds fed the high LA-diet, whereas the birds fed the high-ALA diet had a heavier liver and a longer intestine.

Keywords: linoleic acid, alpha-linolenic avid, energy utilization, fatty acid digestibility, de novo fatty acids synthesis

Introduction

Adequate supply of omega-3 polyunsaturated fatty acids (PUFA) is required to maintain health of animals. The parent compound of the omega-3 family of PUFA is alpha-linolenic acid (ALA, C18:3 n-3), which is an essential fatty acid because it cannot be synthesized in the body of animals. ALA can be converted by animal cells into small amounts of the long-chain omega-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Most fats of animal or plant origin contain ALA, but linseed oil is extremely rich in ALA, the ALA content being around 55%. Linseed oil is commonly used to increase the ALA content of feeds (Morris and Vaisey-Genser, 2003).

Linoleic acid (LA, C18:2 n-6) is the parent fatty acid of the omega-6 family of PUFAs. LA is an essential fatty acid for animals. It occurs in most fats of plant origin, the oils such as corn oil, sunflower oil and soybean oil containing about 50% of this fatty acid. ALA and LA are important membrane constituents, but also serve as energy source. Under common conditions, the intake by animals of LA is much higher than that of ALA, implying that LA will provide more energy. LA and ALA can be desaturated and elongated in the body to yield the precursors of the numerous eicosanoids which have various hormone-like actions (Hovenier et al., 2006).

ALA is more preferentially oxidized than is LA (Cunnane and Anderson, 1997). The preferential oxidation of ALA should result in less deposition of ALA in the body and it should affect energy expenditure or storage in body. In this study, we used broiler chickens for whole carcass analysis and to measure energy balance and the digestibility and oxidation of fatty acids. The hypothesis tested was that feeding a diet containing ALA instead of LA would alter whole-body fatty acid metabolism and deposition and energy expenditure.

Materials and methods

Experimental treatments and design

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 in individual cages. Feed was provided *ad libitum* in the form of meal. Birds had freely 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) linseed oil plus 2.48% soybean oil or 2.73% linseed oil plus 0.47% soybean oil as shown in Table 1. The experimental treatments were subjected to a completely randomized design.

Data and Sample collection

Chicks were weighed at 7 and 28 days of age and feed consumption per cage was recorded for the entire period. The feed : gain ratio was calculated for the whole period as g feed:g weight gain. Animals in each group were closely monitored and daily mortalities were recorded.

Each day (from 7 to 28 days of age) excreta were collected and pooled for chemical analysis (apparent fat digestibility, fatty acids digestibility).

Table 1. Experimental diets and chemical composition

Items	*Experimental diets*	
	LA	*ALA*
Ingredient composition		
Linseed oil	0.52	2.73
Soybean oil	2.48	0.27
Tapioca starch(Cassava)	41.82	41.82
Soybean meal (40% CP)	45.00	45.00
Rice bran hull	4.00	4.00
Lime stone	0.50	0.50
Di-calcium phosphate	3.87	3.87
Salt	0.51	0.51
DL-Methionine	0.30	0.30
Premixed[1]	1.00	1.00
Total	100	100
Analyzed composition		
Dry matter	98.18	98.18
ME_n (kJ/kg)	13,827.62	13,810.05
Crude Protein (N x 6.25)	21.36	21.67
Protein : Energy Ratio (g:kJ)	647.36	637.29
Crude fat	5.39	5.99
Crude fiber	3.24	3.23
Ash	7.91	7.52
Nitrogen free extract	59.68	60.37
Calcium	1.13	1.03
Available phosphorus	0.82	0.78
Calculated amino acids		
Methionine	0.50	0.50
Methionine + Cystine	0.73	0.73
Lysine	1.20	1.20

[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; ferrous, 87.59 mg; selenium, 0.17 mg.

Table 2. Fatty acid composition of the experimental diets

Fatty acid, g/100 g methyl esters	*Experimental diets*	
	LA	*ALA*
C16:0	25.29	19.98
C18:0	15.04	15.85
C18:1 n-9	25.83	25.78
C18:2 n-6	21.91	8.76
C18:3 n-6	0.68	0.52
C18:3 n-3	2.14	15.08
∑SFA	43.32	39.59
∑MUFA	26.61	26.25
∑PUFA	25.44	24.82
∑n-9	25.89	25.81
∑n-6	23.01	9.66
∑n-3	2.43	15.16

Σ SFA = C8:0 + C10:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0
Σ MUFA = C16:1 + C17:1 + C18:1 n-7 + C18:1 n-9 + C20:1 n-9 + C22:1 n-9
Σ PUFA = C18:2 n-6 + C18:3 n-3 + C18:3 n-6 + C20:5n-3
Σ n-9 = C20:1 n-9 + C22:1 n-9
Σ n-6 = C18:2 n-6 + C18:3 n-6
Σ n-3 = C18:3 n-3 + C20:5n-3

Chemical analysis

The experimental diets were analyzed for dry matter, ash, crude fat, crude fiber, crude protein and fatty acid profile according to the methods of AOAC (1985). Excreta were analyzed for crude fat and fatty acids to calculated fat digestibility and the digestibility of individual fatty acids. Total lipids were extracted according to the method of Folch et al. (1957).

Bomb calorimetry

Bomb calorimetry was used to determine the gross energy content in the diets, homogenized 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 weight x energy content) of the 21 days feeding period. The same procedure was used to calculate the water, protein, fat and ash retention as described earlier (Javadi et al., 2005).

Statistical analysis

The data collected were subjected to analysis of variance for a completely randomized design (Steel and Torrie, 1980) by using the program of Microsoft excel (Windows xp®). Body weight, ADFI, ADG and FCR for 15 replications per treatment

were used for statistical analysis. As to data on weight of abdominal fat, breast, thigh and liver there were 5 replicates per treatment. The whole carcass data consisted of 10 replicates per treatment. Statistical significance of differences between the two diets were assessed using the Duncan's multiple range test (Steel and Torrie, 1980).

Results and discussion

Growth performance

Average daily feed intake was 41.79 and 36.85 g/d in the birds fed the high-LA and high-ALA diet, respectively. Birds fed the high-ALA diet had lower ADFI than did those fed the high-LA diet (P>0.05). The somewhat higher digestibility of energy in chickens fed the high-ALA diet may have contributed to the lower ADFI because the birds had met their energy requirement with the lower feed intake. Average daily weight gains were 21.32 and 19.07 g/d in birds fed the high-LA and high ALA-diet, respectively, as shown in Table 3. The feed: gain ratio on the high-ALA diet tended to be lower than on the high-LA diet.

Table 3. Effects of the experimental diets on growth performance

Item	Experimental diets		*SEM*	*Pooled SE*
	LA	*ALA*		
Number of birds	15	15	-	-
Days in experiment	21	21	-	-
Mortality, %	0	0	-	-
Initial BW, g	132.47	133.27	2.9972	0.8713
Final BW, g	579.72[a]	533.80[b]	14.9278	0.0382
ADFI, g	41.79	36.85	2.2090	0.1254
ADG, g	21.32[a]	19.07[b]	0.6830	0.0276
Feed : gain	2.00	1.92	0.1082	0.5894

n=15

Values within a row having different superscripts are significantly different (P<0.05).

Energy balance and digestibility of fat

Table 4 shows that the digestibilities of fat and gross energy tended to be improved in birds fed the high-ALA diet, the values being 83.2 and 83.8% and 78.32 and 78.58%, respectively. The differences did not reach statistical significance (P>0.05). As to the be composition of the whole carcass in terms of the contents of water, protein and ash there were no significant differences (P>0.05). However, the fat content of whole carcass was decreased in birds fed the diet rich in ALA instead of LA.

The experimental diets had no significant influence on energy balance. Energy intake, energy stored in the body, energy expenditure and energy loss with excreta were all similar for the birds fed either the high-ALA or high-LA diet.

Table 4. Influence of experimental diets on energy balance

Item	*Experimental diets*		*SEM*	*P-Values*
	LA	*ALA*		
Feed intake (g/21d)	900[a]	774[b]	37.5730	0.0498
Energy in the diets (KJ)	16,126	16,109	-	-
Apparent fat digestibility,%	83.2	83.8	6.7116	0.8477
Apparent GE digestibility,%	78.3	78.6	1.6155	0.9212
Body composition of whole carcass (%)				
Water	72.2	72.7	0.7735	0.7749
Fat	4.9[a]	3.5[b]	0.1177	0.0001
Protein	17.1	16.6	0.1329	0.4728
Ash	2.4	2.6	0.0566	0.1343
Calculated energy balance (KJ)				
Intake	13,969[a]	11,459[b]	842.6704	0.0196
Stored in the body	6,615[a]	5,248[b]	452.458	0.0278
Expenditure	4,360	3,831	637.7332	0.5024
In excreta	2,994	2,379	213.1059	0.0879
In excreta as fat	296	251	29.0080	0.3700
In fat-free excreta	2698	2129	201.2979	0.0964
Energy in whole body (KJ)				
Initial body energy	570	587	26.1075	0.7404
Final body energy	7,185[a]	5,835[b]	451.355	0.0274
Percentage of energy intake that is :				
Energy stored in the body	48.4	46.3	3.2377	0.6538
Energy expenditure	30.0	32.3	3.9096	0.6343
Lost in excreta	21.7	21.4	1.6155	0.9212
Lost in excreta as fat	2.2	2.2	0.2451	0.9555
Lost in fat-free excreta	19.5	19.2	1.5213	0.9089

n = 10

Values within a row having different superscripts are significantly different (P<0.05).

Apparent fatty acid digestibility

Table 5 shows the apparent digestibility for selected individual fatty acids and groups of fatty acids. As to the digestibility of palmitic, stearic and oleic acid (C16:0, C18:0 and C18:1n-9) there was no significant diet effect (P>0.05). The birds fed the high-LA diet displayed a significantly greater digestibility of linoleic acid (C18:2 n-6) than those fed the high-ALA diet. The apparent digestibility of ALA on the high-ALA diet was greater digestibility than on the high-LA diet. The intake level of a given individual fatty acid and its faecal excretion of endogenous origin will also affect the calculated apparent digestibility. A low intake level in combination with a high endogenous excretion will by itself lead to a low apparent digestibility. When comparing the digestibilities of ALA and LA for the high-ALA and high-LA diets, the LA value for the high-ALA diet may be biased to lower values because the intake level was low. On the other hand, the apparent digestibility for ALA on the high-ALA diet may be biased towards a higher value. The digestibility of the sums of total fatty acids, saturated, monounsaturated, polyunsaturated and n-9 MUFA (∑FA, ∑SFA, ∑MUFA, ∑PUFA and n-9 MUFA) did not differ between the two diets, but the digestibility of the n-6 PUFA was greater for the high-LA diet and that of the n-3 PUFA was greater on the high ALA-diet.

Table 5. Effect of the experimental diets on apparent fatty acid digestibility

Fatty acid	*LA diet*	*ALA diet*	*SEM*	*P value*
Apparent digestibility, % of intake				
C 16:0	83.91	81.48	2.2531	0.4553
C 18:0	70.44	69.22	3.9385	0.8291
C 18:1 n-9	86.71	87.97	1.5751	0.5794
C 18:2 n-6	91.74[a]	84.41[b]	1.8942	0.0135
C 18:3 n-3	90.55[b]	98.75[a]	0.9204	0.0001
Σ FA	83.21	83.79	6.7116	0.8477
Σ SFA	70.34	71.27	4.0549	0.8732
Σ MUFA	88.21	87.59	4.4398	0.9232
Σ PUFA	91.15	91.58	1.2567	0.8112
Σ n-9	88.21	91.18	3.6241	0.5692
Σ n-6	87.20[a]	82.37[b]	1.5013	0.0356
Σ n-3	90.55[b]	98.75[a]	0.9204	0.0001

n = 10

Σ SFA = C8:0 + C10:0 + C14:0 + C15:0 + C16:0 + C18:0 + C20:0 + C22:0 + C24:0

Σ MUFA = C15:1 + C16:1 + C18:1 n-7 + C18:1 n-9 + C20:1 n-9

Σ PUFA = C18:2 n-6 + C18:3 n-3

Values within a row having different superscripts are significantly different ($P<0.05$).

Calculation of Digestible Intake, Deposition and Minimum de novo Synthesis of Fatty acids

The intake of digestible palmitic acid, linoleic acid, SFA and n-6 PUFA of the birds fed the high-LA diet was greater than that of the birds fed the high-ALA diet, whereas the birds fed the high-ALA diet had a higher intake of digestible ALA and n-3 PUFAs (Table 6). There was no diet effect on the intake of digestible stearic acid, oleic acid, FA, MUFA, PUFA and n-9 MUFA. The birds fed the high-LA diet had a greater group-mean intake of total digestible fatty acids of the FA, MUFA and PUFA classes, but had a lower intake of digestible n-3 PUFAs.

The whole body of the birds at baseline contained an average total fat mass of 7.67 g. The calculated total fat mass in the carcass at the end of the experiment was 45.45 ± 7.34 and 39.53 ± 4.12 ($P = 0.0029$; $n = 20$) for the birds fed the high-LA and high-ALA diet, respectively. The birds fed the high-LA diet had deposited more palmitic, oleic acid LA, but less ALA when compared with the birds fed the high-ALA diet (Table 6). There was no difference between the two diets as to the deposition of stearic acid. The birds fed the high-LA diet deposited more FA, SFA and MUFA than their counterparts fed the high-ALA diet, but no significant diet effect was seen for the deposition of total PUFA. There was more deposition of n-9 MUFA and n-6 PUFA in the birds fed the high-LA diet, but that of the n-3 PUFA was lower when compared with the birds fed the high-ALA diet.

The birds with LA instead of high ALA intake had a higher deposition:intake ratio for MUFA, but the ratios for SFA and PUFA were similar for the two dietary groups. The diet effect on MUFA deposition:intake ratio can be explained by the calculated minimum synthesis of this group of fatty acids. Feeding the diet rich in LA stimulated the synthesis of MUFA. The higher synthesis ratio for SFA☹SFA + MUFA) in birds fed the high-LA diet indicates that there was selective synthesis of MUFA in these birds as shown in Table 7. This might point at *de novo* fatty acid synthesis being regulated to balance the amount of saturated and unsaturated fatty acids in the body because the intake of LA was very high in the birds fed the high-LA diet.

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

Fatty acid	*Experimental diets*		*SEM*	*P-value*
	LA	*ALA*		
Digestible fatty acid intake, g				
C 16:0	10.29[a]	7.55[b]	0.5479	0.0033
C 18:0	5.14	5.09	0.4003	0.6612
C 18:1 n-9	10.86	10.51	0.5921	0.4174
C 18:2 n-6	9.75[a]	3.43[b]	0.4080	0.0001
C 18:3 n-3	0.94[b]	6.90[a]	0.2299	0.0001
Σ FA	40.37	38.85	2.4792	0.4084
Σ SFA	14.78[a]	13.08[b]	0.9517	0.0481
Σ MUFA	11.39	10.66	0.5983	0.3936
Σ PUFA	11.25	10.54	0.5545	0.3587
Σ n-9	11.08	10.91	0.5940	0.4287
Σ n-6	9.73[a]	3.69[b]	0.4089	0.0001
Σ n-3	1.07[b]	6.94[a]	0.2302	0.0001
Fatty acid deposition, g				
C 16:0	9.39[a]	7.33[b]	0.4221	0.0042
C 18:0	3.28	3.14	0.1169	0.4565
C 18:1 n-9	15.01[a]	11.11[b]	0.7003	0.0015
C 18:2 n-6	3.39[a]	2.61[b]	0.1557	0.0034
C 18:3 n-3	0.25[b]	0.81[a]	0.0263	0.0001
Σ FA	37.78[a]	31.86[b]	1.4892	0.0020
Σ SFA	16.37[a]	14.38[b]	0.5368	0.0118
Σ MUFA	17.59[a]	12.96[b]	0.7903	0.0010
Σ PUFA	3.82	3.72	0.1786	0.6945
Σ n-9	15.61[a]	11.88[b]	0.6959	0.0020
Σ n-6	3.50[a]	2.75[b]	0.1589	0.0054
Σ n-3	0.35[b]	0.97[a]	0.0275	0.0001
Deposition: intake ratio, g/g				
C 16:0	0.91	0.97	0.0816	0.3250
C 18:0	0.64	0.62	0.0682	0.9731
C 18:1 n-9	1.38[a]	1.06[b]	0.1050	0.0370
C 18:2 n-6	0.35[b]	0.76[a]	0.0313	0.0001
C 18:3 n-3	0.27[a]	0.12[b]	0.0139	0.0001
Σ FA	0.94[a]	0.82[b]	0.0565	0.0419
Σ SFA	1.11	1.10	0.0817	0.6415
Σ MUFA	1.55[a]	1.22[b]	0.1216	0.0366
Σ PUFA	0.34	0.35	0.0237	0.5742
Σ n-9	1.41	1.09	0.1069	0.0531
Σ n-6	0.36[b]	0.75[a]	0.0320	0.0001
Σ n-3	0.33[b]	0.14[a]	0.0257	0.0001

n = 10
Σ FA = Σ SFA + Σ MUFA + Σ PUFA
Σ SFA = C10:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0
Σ MUFA = C16:1 + C17:1 + C18:1 n-7 + C18:1 n-9 + C20:1 n-9 + C22:1 n-9
Σ PUFA = C18:2 n-6 + C18:3 n-3 + C18:3 n-6 + C20:5n-3
Σ n-9 = C20:1 n-9 + C22:1 n-9
Σ n-6 = C18:2 n-6 + C18:3 n-6
Σ n-3 = C18:3 n-3 + C20:5n-3
Values within a row having different superscripts are significantly different (P<0.05).

The birds with LA instead of high ALA intake had a higher deposition:intake ratio for MUFA, but the ratios for SFA and PUFA were similar for the two dietary groups. The diet effect on MUFA deposition:intake ratio can be explained by the calculated minimum synthesis of this group of fatty acids. Feeding the diet rich in LA stimulated the synthesis of MUFA. The higher synthesis ratio for SFA☹SFA + MUFA) in birds fed the high-LA diet indicates that there was selective synthesis of MUFA in these birds as shown in Table 7. This might point at *de novo* fatty acid synthesis being regulated to balance the amount of saturated and unsaturated fatty acids in the body because the intake of LA was very high in the birds fed the high-LA diet.

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

Item	*Experimental diets*		*P-value*
	LA	*ALA*	
Minimum synthesis (g/28 days)			
SFA	1.58 ± 1.15	1.30 ± 0.72	0.3838
MUFA	6.21[a] ± 2.48	2.30[b] ± 1.16	0.0044
SFA/(SFA+MUFA)	0.20 ± 0.11	0.36 ± 0.17	0.1459

Means ± SD for 10 chickens per experimental diet.
Values within a row having different superscripts are significantly different (P<0.01).

Organ weight

Weight of breast meat, thigh meat, liver and abdominal fat were not significantly different (P>0.05) for the two diet groups as shown in Table 8. However, group-mean abdominal fat deposition was lower on the high-ALA diet. In contrast, liver weight and intestinal length were higher on the high-ALA diet. This finding might relate to the observation that the birds fed the high-ALA diet had a higher group-mean digestibility of gross energy than the birds fed the high-LA diet. A heavier liver and intestines might point at higher bile secretion, high digestive enzyme activities and greater absorption ability. Furthermore, the length of the intestine (small and large intestine) in the birds fed the high ALA-diet was longer than in the birds fed the high-LA diet (P=0.0017).

Table 8. Effects of high LA and ALA intakes on organ weights

Item	*Experimental diets*		*P value*
	LA	*ALA*	
Carcass percentage			
Breast meat	11.24	10.00	0.2914
Thigh meat	11.35	12.01	0.7002
Liver	3.01	3.38	0.3988
Abdominal fat	0.55	0.27	0.1176
Intestine length per 100g of BW	25.81[b]	34.22[a]	0.0017

n = 5

Conclusions

The main fatty acid in the high-LA diet was LA, whereas the high-ALA diet was very rich in ALA. The amounts of stearic and oleic acid in the two diets were not much different. Thus, the high-LA diet may be representative for diets rich in n-6 PUFAs, whereas the high-ALA diet may represent diets rich in n-3 PUFAs.The inclusion of ALA in the diet for broiler chickens lowered average daily gain and total feed intake. The intake of ALA instead of LA had no influence on the energy balance, but it reduced the fat content in the whole body. Feeding the high-LA diet produced a markedly increased deposition of LA in the whole body and the high-ALA diet induced a marked deposition of ALA in the whole carcass. For groups of fatty acids, the ratio of deposition in the whole body to the intake of digestible fatty acids was calculated. It then became clear that the type of dietary had marked specific effects on the synthesis and oxidation of fatty acids. In fact, the high LA-diet stimulated the whole-body synthesis of MUFA.

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CHAPTER 8

Fatty acid and energy metabolism in broiler chickens ingesting a high amount of alpha-linolenic acid with diets either rich in saturated or polyunsaturated fatty acids

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To be submitted

Abstract

The aim of this study was to investigate in broiler chickens the metabolism of alpha-linolenic acid (ALA, 18:3n-3) when incorporated into diets either rich in saturated (SFA) or polyunsaturated fatty acids (PUFA). The experimental diets were enrich with linseed oil as source of ALA and contained either beef tallow as source of SFA or sunflower oil as source of PUFA. Seven-day-old, male broiler chickens were used; they were kept in individual cages fed from 1 to 3 weeks of age. The experimental diets did not significantly affect growth performance. However, the PUFA-rich diet tended to improve the feed:gain ratio and to decrease average daily feed intake. The broilers fed the PUFA diet tended to have higher values for energy expenditure and lower values for whole body fat content. The digestibility of individual fatty acids was no different between the two dietary groups. The ratio of deposition in carcass to intake of digestible fatty acids for the whole feeding period was increased for PUFA in birds fed the SFA diet. In this study the hypothesis tested was that a high level of ALA in a diet rich in PUFA would result in higher energy expenditure and more oxidation of ALA than a high level of ALA in a diet rich in SFA.

Keywords: alpha-linolenic, saturated fatty acids, unsaturated fatty acids, energy balance, body composition, *de novo* fatty acid synthesis

Introduction

The type of fat in the diet of broiler chickens affects their fat and energy metabolism. Studies have shown that diets high in polyunsaturated fatty acids (PUFA) lower the amount of abdominal fat when compared with diets rich in saturated fatty acids (SFA) (Pinchasov and Nir 1992, Sanz et al. 1999, Sanz et al. 2000a, b). Similar results have been reported for studies with rats (Shimomura et al. 1990, Dulloo et al. 1995, Takeuchi et al.1995) and mice (Mercer and Trayhurn, 1987).

Our previous studies (Chapter 6) have indicated that broilers fed a diet containing soybean oil rich in PUFA instead of beef tallow rich in SFA had higher rates of oxidation for linoleic acid (LA), but there was no difference for α-linolenic acid (ALA). This finding was unexpected as based on earlier investigations (Cunnane and Anderson, 1997; Ide et al., 1996; Jones et al., 1985), reporting that ALA is more preferentially oxidised than is LA. The possible explanation for this discrepancy could be the low level of ALA in both the PUFA-rich and SFA-rich diet. Broiler chickens only need a relatively small amount of ALA, which is an essential fatty acid. ALA is required as a precursor for the production of eicosanoids and for the deposition in body membranes. 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 and Leech, 1984). Dietary PUFA instead of SFA are preferentially oxidized (Beynen and Katan, 1985), resulting in carbohydrates being shifted from the oxidative into the lipogenic pathway. Hence, increasing the dietary PUFA level at the expense of SFA would increase heat expenditure due to higher fat oxidation. It could thus be hypothesized that a high level of ALA in a diet rich in PUFA would result in higher energy expenditure and more oxidation of ALA than a high level of ALA in a diet rich in SFA. In the present study, this hypothesis was tested.

Materials and methods

Experimental Treatments and Design

Thirty 1-day-old male Arbor Acres broiler chicks were housed in groups and were offered a commercial diet for 7 days before the commencement of the experiment. Then, the birds were randomly divided into 15 birds per treatment and were kept in individual cages. Feed was provided *ad libitum* in form the of meal. Birds had free access to clean water. The semi-purified diets were formulated to contain 20% crude protein. The amounts of digestible, variable fats in the diets were identical as based on the digestibility measurements in a previous experiment. The diets contained either 2.01 (w/w) beef tallow plus 0.99% linseed oil or 2.00% sunflower oil plus 1.00% linseed oil (Table 1). Beef tallow was used as a fat rich in SFA, sunflower oil as an oil rich in n-6 PUFA and linseed oil contains about 55% of ALA. The experimental treatments were subjected to a completely randomized design.

Data and Sample Collection

The chickens were weighed 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 and daily mortality was recorded.

Each day between 7 and 28 days of age excreta were collected for the analysis of crude fat and individual fatty acids to calculate apparent digestibility of crude fat and individual fatty acids).

Table 1. Experimental diets and chemical composition

Items	Experimental diets	
	SFA	PUFA
Ingredient composition		
Beef tallow	2.01	-
Sunflower oil	-	2.00
Linseed oil	0.99	1.00
Tapioca starch(Cassava)	41.82	41.82
Soybean meal (40% CP)	45.00	45.00
Rice bran hull	4.00	4.00
Lime stone	0.50	0.50
Di-calcium phosphate	3.87	3.87
Salt	0.51	0.51
DL-Methionine	0.30	0.30
Premixed[1]	1.00	1.00
Total	100	100
Analyzed composition		
Dry matter	90.91	91.91
GE (Kcal/kg)	16,320	16,728
Crude Protein (N x 6.25)	19.63	20.19
Protein : Energy Ratio (g:kJ)	811.05	828.53
Crude fat	3.60	3.83
Crude fiber	5.99	5.93
Ash	6.73	6.18
Nitrogen free extract	54.96	55.78
Calcium	1.43	1.38
Available phosphorus	0.82	0.87
Calculated amino acids		
Methionine	0.50	0.50
Methionine + Cystine	0.73	0.73
Lysine	1.20	1.20

[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; ferrous, 87.59 mg; selenium, 0.17 mg

Table 2. Fatty acid composition of the experimental diets

Fatty acid	Experimental diets	
	SFA	PUFA
C14:0	1.35	0.22
C14:1	0.44	0.08
C16:0	22.08	20.46
C16:1	1.06	0.12
C17:0	2.31	2.32
C18:0	32.79	20.70
C18:1 n-9t	3.11	0.70
C18:1 n-9c	19.28	27.62
C18:2 n-6 t	0.29	0.42
C18:2 n-6 c	12.44	21.18
C18:3 n-6	0.00	0.00
C18:3 n-3	12.87	12.72
C20:0	0.51	0.44
C20:5n-3	0.33	0.49
C22:0	0.02	0.22
C24:0	0.00	0.00
Unidentified Fatty Acids	0.72	1.08
ΣSFA	59.77	45.43
ΣMUFA	24.30	28.54
ΣPUFA	15.93	24.95
Σn-9	22.39	28.34
Σn-6	12.73	21.63
Σn-3	13.20	13.32

Σ FA = Σ SFA + Σ MUFA + Σ PUFA
Σ SFA = C10:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0
Σ MUFA = C16:1 + C17:1 + C18:1 n-7 + C18:1 n-9 + C20:1 n-9 + C22:1 n-9
Σ PUFA = C18:2 n-6 + C18:3 n-3 + C18:3 n-6 + C20:5n-3
Σ n-9 = C20:1 n-9 + C22:1 n-9
Σ n-6 = C18:2 n-6 + C18:3 n-6
Σ n-3 = C18:3 n-3 + C20:5n-3

Chemical Analyses

The diet and faeces samples were dried at 60 ^{o}C for 72 h in a forced-hot air oven and were then analyzed for crude protein, crude fiber, and ash (AOAC, 1990). The dried, whole carcass samples were analysed for moisture and fat (AOAC, 1990).

Total fat in the dried samples (diets, whole carcass and faeces) were extracted as described previously (Horwitz, 1975). Each sample was added to a flask and 2 ml of ethanol was added. Subsequently, 10 ml of HCl (8 mol/L) was added; the contents were gently mixed, and the flask was placed in a water-bath of 80 ^{o}C for 30-40 min. The tubes were cooled down, 10 ml of ethanol (96%, w/w) and 25 ml of petroleum ether (boiling point between 40 and 60 ^{o}C) were added and the tube was shaken for one min. The fat-containing upper layer was decanted into a 150-ml round-bottom flask. The extraction procedure was repeated twice with 15 ml of diethyl ether and 15

ml of petroleum ether and the lipid extract was evaporated to dryness under N_2 in a water-bath of 40 ^oC. The round-bottom flasks with the lipids were dried overnight at 60 ^oC and the total lipids were measured gravimetrically. Total lipids were saponified and methylated according to the procedure of Metcalfe et al. (1966) followed by gas chromatography for determination of fatty acid composition (Javadi et al., 2004).

Calculation of Digestible Fatty Acid Intake and Fatty Acid Deposition

The total digestible fatty acid intake was calculated as fatty acid intake (g/3 weeks) × 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. Whole carcass content of fatty acid at the end (g). The ratio of fatty acid deposition and digestible fatty acid intake was calculated for selected individual fatty acids and groups of fatty acids.

Bomb calorimetry

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 weight x energy content) of the 21 days feeding period. The same procedure was used to calculate the water, protein, fat and ash retention as described earlier (Javadi, 2005).

Statistical analysis

The data collected were subjected to analysis of variance for a completely randomized design using the program of Microsoft excel (Windows xp®). For body weight, ADFI, ADG and FCR there were 15 replicates per treatment; for the weight of abdominal fat, breast, thigh and liver there were 5 replicates per treatment and for whole carcass data there were 10 replicates per treatment. Statistical significance of differences between was assessed using Duncan's multiple range test (Steel and Torrie, 1980).

Results

Growth Performance

Average daily feed intake was 42.01 and 38.63 g/d for the birds fed the SFA and PUFA diets, respectively (Table 3). Average daily weight gain was 19.51 and 19.89 g/d in birds fed the SFA and PUFA diet, respectively (Table 3). There was no significant difference between the groups. The feed:gain ratio of birds fed the PUFA diet was greater than that of birds fed the SFA diet (Table 3), but there was no significant difference. This result agrees with data from Mossab et al. (2000), showing that supplemental soybean oil in a basal diet improved the feed:gain ratio in turkeys and broiler chickens. Wongsuthavas et al. (2007) also showed that the feed:gain ration in birds fed diets rich in PUFA in the form of soybean oil was greater than in birds fed diets rich in SFA in the form of beef tallow.

Table 3. Effect of the experimental diets on growth performance

Items	Experimental diets		Pooled SE	P value
	SFA	PUFA		
Number of birds	15	15	-	-
Days in experiment	21	21	-	-
Mortality, %	0	0	-	-
Initial BW, g	127.07	133.07	12.6630	0.2050
Final BW, g	538.88	550.73	51.3827	0.5325
ADFI, g	42.01	38.63	7.2619	0.2119
ADG, g	19.51	19.89	2.3743	0.6683
Feed : gain	2.17	1.94	0.3265	0.0654

Body Composition, Energy balance and digestibility of fatty acids

Table 4 shows that the water content of the whole carcass of the birds fed the SFA diet was lower than in the birds fed the PUFA diet ($P<0.01$). The group-mean amount of body fat was lower in birds fed PUFA diet. The energy balance was similar for the birds fed either the SFA or PUFA diet. Group mean values for apparent digestibility of crude fat and gross energy were higher in birds fed the PUFA diet, but the difference was far from statistical significance.

Table 4. Influence of experimental diets on energy balance

Item	Experimental diets		Pooled SE	P value
	SFA	PUFA		
Feed intake (g/21d)	804	758	130.0610	0.4429
Energy in the diets (kJ/kg)	16,320	16,728	-	-
Apparent fat digestibility,%	81.1	83.3	6.2275	0.4564
Apparent GE digestibility,%	78.2	79.1	2.9803	0.8245
Body composition of whole carcass (%)				
Water	69.6[b]	74.8[a]	2.8904	0.0008
Fat	4.1	3.7	0.2269	0.4186
Protein	17.8	14.4	0.2959	0.3936
Ash	2.8	2.3	0.0595	0.8752
Calculated energy balance (KJ)				
Intake	13,115	12,679	661.7705	0.6237
Stored in the body	4,423	4,461	1488.6245	0.9545
Expenditure	5,958	5,578	834.5524	0.7996
In excreta	2,734	2,640	956.3229	0.8296
In excreta as fat	208	193	62.0272	0.5995
In fat-free excreta	2,526	2,447	918.7389	0.8505
Energy in whole body (KJ)				
Initial body energy	569.6	587.2	116.7564	0.7404
Final body energy	4,992.5	5,048.5	1468.7506	0.9329
Percentage of energy intake that is :				
Energy stored in the body	35.0	35.2	3.4544	0.9793
Energy expenditure	43.1	43.9	4.1805	0.9396
Lost in excreta	21.9	20.9	2.9803	0.8245
Lost in excreta as fat	1.66	1.53	0.7358	0.6413
Lost in fat-free excreta	20.2	19.4	2.9162	0.8412

[a-b] Values in the same row with the different superscripts differ significantly

Energy expenditure, the amount of energy loss with in excreta and energy storage did not differ significantly between the two dietary treatments. The digestibility of individual and groups of fatty acids as shown in Table 5 did not reveal differences between the dietary treatments.

Table 5. Effect of experimental diets on fatty acid digestibility

Fatty acid	SFA	PUFA	Pooled SE	*P* value
C 16:0	79.75	82.98	6.8894	0.3085
C 18:0	74.11	76.39	8.9756	0.5780
C 18:1 n-9	87.99	86.89	4.6136	0.5982
C 18:2 n-6	91.65	93.57	3.2585	0.2044
C 18:3 n-3	92.46	94.13	4.1003	0.3750
Σ FA	81.14	83.26	6.2275	0.4564
Σ SFA	76.93	79.68	7.8813	0.4452
Σ MUFA	85.78	84.03	5.9251	0.5164
Σ PUFA	86.98	88.53	7.6781	0.6567
Σ n-9	85.78	84.03	5.9251	0.5164
Σ n-6	91.65	93.57	3.2585	0.2044
Σ n-3	92.46	94.13	4.1003	0.3750

Digestible Fatty Acid Intake, Fatty Acid Deposition and Fatty Acid Deposition : Intake Ratio

The intake of digestible stearic acid and SFA for birds fed the SFA diet was higher than that for the birds fed the PUFA. On the other hand, the birds fed the PUFA diet had a higher intake of digestible oleic acid, LA, MUFA and sum and n-6 PUFA (Table 6). There was no diet difference for the intake of digestible palmitic acid, ALA acid and sum of n-3 PUFA. The birds fed the PUFA diet tended to have a greater intake of total digestible fatty acids (FA), MUFA, PUFA, n-9 MUFA and n-6 PUFA, but had an intake of digestible n-3 PUFA that was similar to that of the birds fed the SFA diet.

The whole body of the birds at baseline contained an average total fat mass of 7.67 g. The calculated total fat mass in the carcass at the end of the experiment was 27.85 ± 7.48 and 27.33 ± 6.81 ($P = 0.8224$, $n = 20$) for the birds fed the SFA and PUFA diet, respectively. The birds fed SFA diet had deposited more stearic acid when compared with the birds fed the PUFA diet ($P<0.01$) (Table 6). There was no difference between both diets as to the deposition of palmitic and oleic acid, LA, ALA, FA, SFA, MUFA, PUFA, n-9 MUFA, n-6 PUFA and n-9 PUFA

The deposition:intake ratios for palmitic, stearic and oleic acid, LA, ALA, FA, SFA, MUFA, n-9 MUFA, n-6 PUFA and n-9 PUFA in the birds fed the SFA diet was similar to those in the birds fed the PUFA diet. The ratio for PUFA was extremely high for the birds fed the diet containing SFA (Table 6). The birds fed SFA instead of PUFA had a higher deposition:intake ratio for PUFA.

Table 6. Effect of experimental diets on digestible fatty acid intake, fatty acid deposition and the deposition:intake ratio during the whole feeding period

Fatty acid	SFA	PUFA	Pooled SE	*P* value
Digestible fatty acid intake, g				
C 16:0	5.10	4.91	1.0535	0.6717
C 18:0	7.03[a]	4.58[b]	1.5365	0.0024
C 18:1 n-9	5.70[b]	5.02[a]	1.0195	0.0005
C 18:2 n-6	3.38[b]	3.48[a]	0.7036	0.0001
C 18:3 n-3	3.44	3.47	0.1269	0.6399
Σ FA	23.49	24.10	4.9907	0.8342
Σ SFA	13.31[a]	10.48[b]	2.5743	0.0346
Σ MUFA	6.03[b]	6.94[a]	1.1279	0.0138
Σ PUFA	4.01[b]	6.39[a]	0.8385	0.0001
Σ n-9	5.56[b]	6.89[a]	1.1279	0.0138
Σ n-6	3.38[b]	5.86[a]	0.7171	0.0001
Σ n-3	3.53	3.63	0.1269	0.6399
Fatty acid deposition, g				
C 16:0	4.52	4.66	1.3774	0.8203
C 18:0	3.03[a]	1.80[b]	0.8177	0.0034
C 18:1 n-9	6.75	7.73	2.1029	0.3088
C 18:2 n-6	1.33	1.43	0.5521	0.6903
C 18:3 n-3	0.11	0.11	0.0514	0.7972
Σ FA	20.18	19.66	5.2223	0.8253
Σ SFA	7.55	6.46	1.8589	0.2059
Σ MUFA	6.92	7.84	2.1277	0.3464
Σ PUFA	1.48	1.55	0.5753	0.7798
Σ n-9	6.92	7.84	2.1277	0.3464
Σ n-6	1.37	1.45	0.5528	0.7470
Σ n-3	0.11	0.11	0.0514	0.7972
Deposition: intake ratio, g/g				
C 16:0	0.89	0.95	0.4019	0.9606
C 18:0	0.43	0.39	0.2272	0.4470
C 18:1 n-9	1.18	1.54	0.4582	0.1104
C 18:2 n-6	0.39	0.41	0.2272	0.0853
C 18:3 n-3	0.03	0.03	0.0750	0.8370
Σ FA	0.86	0.82	0.3241	0.4906
Σ SFA	0.57	0.62	0.5749	0.7419
Σ MUFA	1.15	1.13	0.5939	0.7137
Σ PUFA	0.37[a]	0.24[b]	0.4375	0.0022
Σ n-9	1.24	1.14	0.5939	0.7137
Σ n-6	0.41	0.25	0.2272	0.0853
Σ n-3	0.03	0.03	0.0750	0.8370

Σ FA = Σ SFA + Σ MUFA + Σ PUFA

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

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

Σ PUFA = C18:2 n-6 + C18:3 n-3 + C18:3 n-6 + C20:5n-3

Σ n-9 = C20:1 n-9 + C22:1 n-9

Σ n-6 = C18:2 n-6 + C18:3 n-6

Σ n-3 = C18:3 n-3 + C20:5n-3

[a-b] Values in the same row with the different superscripts differ significantly

Organ weight

The weights of breast meat, thigh meat, liver and abdominal fat were not significantly influenced by the type of dietary fat as shown in Table 7. However, abdominal fat deposition tended to be decrease on the PUFA diet. In contrast, liver weight and intestinal length were higher on the PUFA diet. Interestingly, liver weight and intestine length of birds fed the PUFA diet were higher than in birds fed the SFA diet.

Table 7. Effect of experimental diets on organ weights

Carcass composition, %	Experimental diets		Pooled SE	P value
	SFA	PUFA		
Breast meat	11.29	10.73	2.6263	0.7473
Thigh meat	11.44	11.98	3.0884	0.7915
Liver	2.95	3.39	0.6172	0.2923
Spleen	0.09	0.09	0.0262	0.9068
Gizzard	2.23	3.17	0.4393	0.0095
Abdominal fat	0.33	0.28	0.1289	0.5566
Intestine weight	4.67	6.35	1.0935	0.0405
Intestine length per 100g of BW	26.43	34.45	5.0963	0.0376

Discussion

In this study we tested the hypothesis that that a high level of ALA in a diet rich in PUFA would result in higher energy expenditure and more oxidation of ALA than a high level of ALA in a diet rich in SFA. The data show that ALA metabolism was not affected by the background type of fat in the diet. Clearly, on the basis of the present study, the hypothesis has to be rejected.

The lowering of ADFI in birds fed the PUFA diet corroborates literature data (Mossab et al., 2000; Atteh et al., 1983) and may be explained the higher digestibility of PUFA (Leeson and Atteh, 1995) so that the chickens would have to consumed less feed to satisfy their energy requirement. Average daily gain did not differ between the diet groups. Possibly, the birds already displayed optimum growth under the conditions of the experiment. Similar results have been reported earlier by Wongsuthavas et al. (2007), showing that birds fed diets rich in PUFA had similar ADG as did birds fed diets fortified with SFA. Birds fed the PUFA diet showed a somewhat better F:G than did birds fed the SFA diet, but there was no significant difference. This result was agrees with data published by Mossab et al. (2000), showing that supplemental soybean oil in a basal diet improved F:G in turkeys and broiler chickens. Wongsuthavas et al. (2007) also showed that F:G in birds fed a high-PUFA diet was greater than in birds fed a high-SFA diet.

The calculated intake of digestible fatty acids reflects the amount in the diet and feed intake, combined with the measured apparent digestibility. The deposition in the body of fatty acids was calculated as based on the fatty acid composition of the total body fat that was gained during the entire feeding period. As would be expected, the birds fed the SFA diet deposited more saturated fatty acids and those fed the PUFA diet which had deposited more stearic acid in the whole body. Similar data have been shown for mice (Javadi et al., 2004) and goats (Yeom et al., 2005). To obtain clues as to preferential deposition of fatty acids, the ratio of deposition of a given fatty acid or group of fatty acids to the intake of digestible fatty acid or group of fatty acids was calculated. The deposition:intake ratio for fatty acid 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 in the birds is consistent with the well-known preferential oxidation of linoleic and alpha-linolenic acid (Cunnane and Anderson, 1997; Jones et al., 1985; Yeom et al., 2005) and the fact that linoleic and alpha-linolenic acid cannot be synthesized by birds and pigs (Javadi et al., 2004; Azain, 2000; Nguyen et al., 2005). The deposition:intake ratio for the essential polyunsaturated fatty acids, LA and ALA, cannot be higher than 1. Indeed, the ratios for ALA were below and so was the ratio for LA in the birds fed both diets.

Conclusion

The supplementation of ALA to either a diet rich in SFA or PUFA had no effect on growth performance, the digestibility of crude fat and individual fatty acids, *de novo* fatty acid synthesis as estimated by calculation. It was predicted that ALA oxidation would be enhanced in birds fed the high-PUFA diet, but this was not seen. Feeding the PUFA diet did produce a markedly increased deposition of LA in the whole body. An interesting finding emerged from this study in that PUFA verus SFA feeding significantly increased the relative weight and length of intestine. The basis and impact of this observation are not known.

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CHAPTER 9

General conclusions

In this section, the main conclusions from this thesis are listed and briefly substantiated.

Replacement of dietary saturated fatty acids by unsaturated fatty acids reduces the amount of abdominal fat in broiler chickens.

Figure 1, which is based on the data in Chapter 2, clearly shows that an increased intake of soybean oil at the expense of beef tallow reduced abdominal deposition by broiler chickens. Soybean oil typically is rich in polyunsaturated fatty acids (PUFA) and beef tallow is rich in saturated fatty acids (SFA). A decrease in the dietary SFA:UFA ratio of the diet diminished abdominal fat deposition in a dose-dependent manner which was independent of the amount of fat in the diet. The study also demonstrated that the number of fat cells in broilers' breast meat depends on both the amount and type of fat in the diet (Chapter 2).

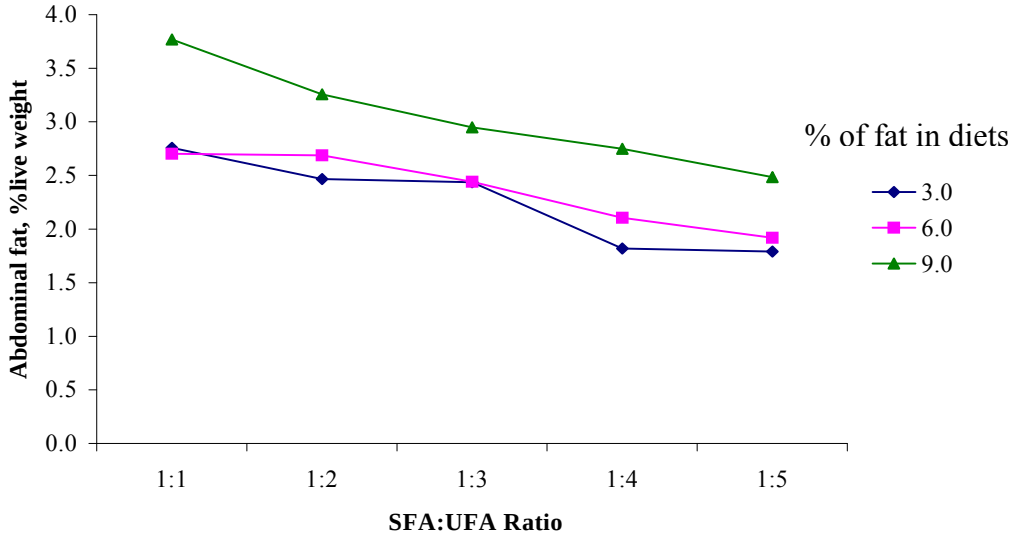

Figure 1. Relationship between the SFA:UFA ratio of the diet and the amount of abdominal fat expressed as percentage of live body weight. The relationship is presented for three levels of dietary fat (% of fat in the diet).

Dietary soybean oil, but not krabok oil, diminishes abdominal fat deposition in broiler chickens

In Chapter 3, it is shown that the feeding of krabok oil versus beef tallow did not lower abdominal fat. Krabok oil is rich in medium-chain fatty acids. It is known that medium-chain fatty acids, just like PUFA, are preferentially oxidized. The study in described in Chapter 2 and the data in Figure 1 led to the hypothesis that fatty acids that are preferentially oxidized may lower abdominal fat deposition in broiler chickens. However, the feeding of a diet rich in krabok oil did not influence abdominal fat. Krabok (*Irvingia Malayana*, Oliv. ex. A. Benn.) is a tree grown widely in tropical and subtropical areas. In Thailand, the krabok tree is commonly used for wood and charcoal production, whereas the seeds, after peeling, are consumed by people. The data presented in Chapter 3 do indicate that krabok oil can be used as

energy source for broiler chickens without a negative affect on growth performance. Possibly, the production of krabok oil for use in broiler rations may become economically relevant.

Energy expenditure is comparable for broiler chickens fed diets containing various blends of beef tallow and soybean oil

In the study described in Chapter 4, the hypothesis tested was that replacement of dietary tallow by soybean oil would increase energy expenditure. Energy expenditure, either expressed as absolute amount or as percentage of intake, was systematically related to the amount of soybean oil in the diet. It should be around that different dietary fatty acids may differently affect energy expenditure, at least this has been shown for in mice. In any event, it appears that the lowering of abdominal fat in broiler chickens as caused by consumption of PUFA-rich soybean oil is not associated with increased energy expenditure.

It is possible, but not proven unequivocally, that de novo fatty acid synthesis is decreased in broiler chickens fed diets rich in PUFA instead of SFA.

Chapter 4 documents that the lowering of abdominal fat in broiler chickens seen after the feeding of PUFA instead of SFA was associated with an increase in energy expenditure. In Chapter 5 it was then hypothesized that the feeding of PUFA instead of SFA would inhibit whole-body de novo fatty acid synthesis. As indexes of de novo fatty acid synthesis, the concentration of plasma triacylglycerols and minimum fatty acid synthesis calculated as fatty deposition minus digestible fatty acid intake were used. The decrease in abdominal fat after the feeding of PUFA-rich diets was associated with a decrease in the level of plasma triacylglycerols, but it was not associated with minimum *de novo* fatty acid synthesis in the whole body.

Both under ad libitum and restricted feeding conditions, there is evidence that dietary PUFAs are preferentially oxidized which is associated with a decrease in the ratio of PUFA deposited in the body to the intake of digestible PUFA.

In the experiments mentioned above, the animals had free access to the experimental diets. Under conditions of *ad libitum* feeding, animals may adapt feed intakes to changes in fat absorption, fat catabolism and lipogenesis. Thus, the study described in Chapter 6 was undertaken to determine the effect of replacement of dietary SFA by PUFA under conditions of *ad libitum* and restricted feeding. Energy expenditure of broilers fed the high-PUFA diet was not significantly higher when the diet was either fed restrictedly or *ad libitum*. Energy loss with excreta was lower on the SO diet than on the BT diet when fed *ad libitum*. In the birds fed the diet with PUFA instead of SFA, the deposition:intake ratio was raised for SFA, but diminished for mono-unsaturated fatty acids (MUFA). The increased deposition:intake ratio for SFA may be explained by an enhanced *de novo* synthesis of SFA, whereas the decreased ratio for MUFA was associated with a diminished synthesis of MUFA. The decreased ratio of deposition:intake for PUFA in the birds fed the high-PUFA diet may be explained by preferential oxidation of PUFA.

High intake of alpha-linolenic acid may lower body fat in broilers when compared with high intake of linoleic acid.

The PUFA alpha-linolenic acid (ALA) is more preferentially oxidized than is the PUFA linoleic acid (LA). The preferential oxidation of ALA should lead to less deposition of ALA in the body and it should affect energy expenditure or storage in body. In the study presented in Chapter 7, the hypothesis tested was that feeding a diet containing ALA instead of LA would alter the whole body fatty acid metabolism and deposition and energy expenditure. Soybean oil was used as LA source and linseed oil was used as ALA source. Apparent LA digestibility was greater in the birds fed the high-LA diet and ALA digestibility was greater on the high-ALA diet. The high-LA and high-ALA diets had no differential effect on energy balance, but the amount of fat in the whole carcass was decreased in the birds fed the high-ALA diet. The rate of whole body *de novo* synthesis of monounsaturated fatty acids was higher in chickens fed the high LA-diet than in their counterparts fed the high ALA-diet. Birds fed the high-ALA diet tended to have less abdominal fat when compared with birds fed the high LA-diet. It would appear that the outcome of the study supports the view that the preferential oxidation of ALA explains the decrease in body fat.

A high level of alpha-linolenic acid (ALA) in a diet rich in PUFA does not result in higher energy expenditure and more oxidation of ALA than a high level of ALA in a diet rich in SFA.

It was hypothesized that a high level of ALA in a diet rich in PUFA would result in higher energy expenditure and more oxidation of ALA than a high level of ALA in a diet rich in SFA (Chapter 8). The experimental diets were enriched with linseed oil as source of ALA and contained either beef tallow as source of SFA or sunflower oil as source of PUFA. Unexpectedly, the broilers fed the PUFA diet tended to have lower values for energy expenditure, but as would be expected they had a lower whole body fat content. There was no evidence for an influence of the type of fat as dietary background on ALA metabolism, including oxidation so that the hypothesis had to be rejected.

Summary

This basis for the studies in this thesis is the observation that dietary polyunsaturated fatty acids (PUFA), when compared with saturated fatty acids (SFA), lowers the amount of abdominal fat in broiler chickens. The various studies performed aimed at unravelling the mechanism for the PUFA effect in terms of fatty acid and energy metabolism at the level of the whole body of broiler chickens. The central hypothesis tested was that fatty acids that are preferentially oxidized may lower abdominal fat deposition in broiler chickens.

An increased intake of PUFA in the form of soybean oil at the expense of SFA in the form of tallow reduced abdominal deposition by broiler chickens in a does-dependent fashion, the relationship being essentially independent of the fat level of the diet.

The hypothesis was tested that dietary fats rich in medium-chain triacylglycerols (MCT) would diminish abdominal fat deposition as do fats rich in polyunsaturated fatty acids (PUFA). Broiler chickens were fed on diets containing either tallow, which is rich in SFA, soybean oil, which is rich in PUFA, or krabok oil, which is rich in MCT. In keeping with earlier investigations, dietary soybean oil versus tallow significantly lowered abdominal fat deposition. The feeding of krabok oil instead of tallow did not affect the weight of abdominal fat, which would lead to rejection of the hypothesis or at least questions the general nature of the hypothesis.

Subsequently, it was hypothesized that the feeding of PUFA instead of SFA would induce more heat expenditure, this effect being associated with less deposition of abdominal fat. Broiler chickens were given one of five diets in which the beef tallow component was replaced by increasing amounts of soybean oil. The amount of body fat was reduced significantly when about 75 % of the tallow was replaced by soybean oil, but there was no further decrease after the incorporation of more soybean oil into the diet. Calculated energy expenditure, either expressed as absolute amount or percentage of intake, tended to increased but was not significantly affected by the amount of soybean oil in the diet.

Without evidence for increased energy expenditure, it then was hypothesized that the feeding of PUFA instead of SFA would inhibit whole-body *de novo* fatty acid synthesis. As indexes of *de novo* fatty acid synthesis, the concentration of plasma triacylglycerols and minimum fatty acid synthesis calculated as fatty deposition minus digestible fatty acid intake were used. Broiler chickens were given one of five diets in which the beef tallow component was replaced by increasing amounts of soybean oil. The decrease in abdominal fat was associated with a decrease in the level of plasma triacylglycerols, but it was not associated with minimum *de novo* fatty acid synthesis in the whole body. Thus, the hypothesis was neither proven nor disproven.

In the experiments so far, the animals had free *libitum* access to the experimental diets. Under conditions of *ad libitum* feeding, animals may adapt feed intakes to changes in fat absorption, fat catabolism and lipogenesis. Thus, a study described was undertaken to determine the effect of replacement of dietary SFA by PUFA under conditions of *ad libitum* and restricted feeding. Energy expenditure of broilers fed the high-PUFA diet tended to be higher were fed restricted or *ad libitum* feeding. In the birds fed the diet with PUFA instead of SFA, the deposition:intake ratio was raised for SFA, but diminished for mono-unsaturated fatty acids (MUFA). The increased deposition:intake ratio for SFA may be explained by an enhanced *de novo* synthesis of SFA, whereas the decreased ratio for MUFA was associated with

a diminished synthesis of MUFA. The decreased ratio of deposition:intake for PUFA in the birds fed the high-PUFA diet may be explained by preferential oxidation of PUFA. This study would support the central hypothesis in this thesis in that fatty acids that are preferentially oxidized may lower abdominal fat deposition in broiler chickens.

ALA is more preferentially oxidized than is LA. The hypothesis tested was that feeding a diet containing ALA instead of LA would alter the whole body fatty acid metabolism and deposition and energy expenditure. Soybean oil was used as LA source and linseed oil was used as ALA source. The high-LA and high-ALA diets had no differential effect on energy balance, but the amount of fat in the whole carcass was decreased in the birds fed the high-ALA diet. The rate of whole body *de novo* synthesis of monounsaturated fatty acids was higher in chickens fed the high LA-diet than in their counterparts fed the high ALA-diet. Birds fed the high-ALA diet tended to have less abdominal fat when compared with birds fed the high LA-diet. The outcome of this study is in agreement with the idea that ALA is more preferentially oxidized than is LA.

. In the last study the hypothesis tested was that that a high level of ALA in a diet rich in PUFA would result in higher energy expenditure and more oxidation of ALA than a high level of ALA in a diet rich in SFA. The experimental diets were enriched with linseed oil as source of ALA and contained either beef tallow as source of SFA or sunflower oil as source of PUFA. The broilers fed the PUFA diet tended to have higher values for energy expenditure and lower values for whole body fat content.

In general, it is concluded that the feeding of PUFA versus SFA lowers the amount of abdominal fat in broiler chickens through preferential oxidation of PUFA. However, the anticipated increase in energy expenditure after the feeding of high-PUFA diets could not be demonstrated convincingly.

Samenvatting

De achtergrond voor het onderzoek beschreven in dit proefschrift is de waarneming dat meervoudig onverzadigde vetzuren (PUFA) in de voeding, vergeleken met verzadigde vetzuren (SFA), de hoeveelheid buikvet bij vleeskuikens verlagen. De verschillende uitgevoerde proeven waren gericht op het ontknopen van het mechanisme dat ten grondslag ligt aan het effect van PUFA. De aandacht ging uit naar de vetzuur- en energiehuishouding, op het niveau van het totale lichaam van vleeskuikens. De centrale hypothese die werd getoetst, was, dat vetzuren die preferentieel worden geoxideerd de hoeveelheid buikvet bij vleeskuikens kunnen verlagen. Literatuurgegevens duiden op preferentiële oxidatie van PUFA en ook van middel-keten vetzuren.

Extra opname van PUFA in de vorm van sojaolie, ten koste van SFA in de vorm van rundvet, verminderde de aanzet van buikvet bij vleeskuikens volgens een dosis-afhankelijk verband. Dit verband was in essentie onafhankelijk van de hoeveelheid van in de voeding.

De hypothese is getoetst dat een voedingsvet rijk aan triglyceriden met middel-keten vetzuren (MCT), evenals vetten rijk aan PUFA, de aanzet van buikvet zou verminderen. Vleeskuikens kregen voeders met rundvet of sojaolie, of krabokolie. De vetten zijn respectievelijk rijk aan SFA, PUFA of MCT. In overeenstemming met het eerdere onderzoek verlaagde sojaolie versus rundvet de hoeveelheid abdominaal vet. Het voeder met krabokolie had echter geen invloed op het buikvet. Dit betekent dat de centrale hypothese niet wordt ondersteund of in ieder geval niet algemeen geldig kan zijn.

Vervolgens werd geredeneerd dat de opname van PUFA in plaats van SFA meer warmteproductie zou veroorzaken. Dit extra energieverlies zou dan gepaard gaan met een vermindering van het buikvet. Vleeskuikens kregen een van de vijf experimentele voeders waarin rundvet was vervangen door toenemende hoeveelheden sojaolie. De hoeveelheid buikvet was significant gereduceerd bij vervanging van 75 % van het rundvet door sojaolie, maar er was geen verdere daling na de toevoeging van meer sojaolie aan de voeding. De berekende warmteproductie, uitgedrukt als absolute hoeveelheid of als percentage van de opname, was niet significant verhoogd door de toevoeging van sojaolie aan de voeding.

Zonder bewijs voor extra warmteproductie werd verondersteld dat dat verstrekking van voeders met PUFA, in plaats van SFA, de vetzuursynthese in het lichaam zou verminderen. Een verminderde vetzuursynthese zou een lagere hoeveelheid buikvet kunnen verklaren. Als index voor de vetzuursynthese werden de plasmaconcentratie van triacylglycerolen en de berekende, minimale, totale lichaamssynthese van vetzuren gebruikt. Vleeskuikens kregen weer voeders waarin de rundvetcomponent was vervangen door toenemende hoeveelheden sojaolie. De afname van het buikvet was geassocieerd met een afname van de plasmatriacylgycerolen, maar niet met de minimale de-novo vetzuursynthese in het lichaam. De hypothese werd derhalve noch bevestigd noch verworpen.

In de eerdere experimenten was er voor de kuikens altijd voeder beschikbaar. Onder de conditie van ad libitum voedering kunnen dieren hun voeropname aanpassen aan veranderingen in vetabsorptie, vetzuuroxidatie en lipogenese. Derhalve werd een onderzoek uitgevoerd om de vervanging van PUFA door SFA te bestuderen bij kuikens die ad libitum of beperkt werden gevoerd. De warmteproductie door kuikens op de PUFA-rijke voeding was gemiddeld hoger wanneer ad libitum werd gevoerd in

plaats van beperkt. Bij de kuikens op het PUFA-rijke voeder was de verhouding aanzet:opname verhoogd voor SFA, maar verlaagd voor enkelvoudig onverzadigde vetzuren (MUFA). De toename in de aanzet:opname verhouding voor SFA kan worden verklaard door een verhoogde de-novo synthese van SFA, terwijl de afgenomen verhouding voor MUFA samenging met een verminderde synthese van MUFA. De afgenomen verhouding van aanzet:opname voor PUFA kan worden verklaard door preferentiële oxidatie van PUFA. Deze studie ondersteunt het centrale de idee in dit proefschrift dat vetzuren die preferentieel worden geoxideerd de hoeveelheid buikvet bij vleeskuikens kunnen verlagen.

Volgens literatuurgegevens wordt alpha-linoleenzuur (ALA) meer preferentieel geoxideerd dan linolzuur (LA). De hypothese werd getoetst dat de verstrekking van een voeder met ALA in plaats van LA de vetzuurstofwisseling en de aanzet en verlies van energie zou beïnvloeden. Sojaolie werd gebruikt als bron van LA en lijnzaadolie als drager van ALA. De LA- en ALA-rijke voeders hadden geen invloed op de energiebalans, maar de hoeveelheid vet in het totale lichaam was verminderd bij de kuikens op het ALA-rijke voeder. De de novo synthese van MUFA was groter voor de kuikens die het LA-rijke voeder kregen dan voor de dieren die het voeder met extra LA kregen. De kuikens op het ALA-rijke voeder hadden gemiddeld minder buikvet. De uitkomst van dit onderzoek is in overeenstemming met de opvatting dat ALA meer preferentieel wordt geoxideerd dan LA.

. In het laatste onderzoek werd nagegaan of een hoog niveau van ALA in een voeder rijk aan PUFA zou leiden tot meer warmteverlies en meer oxidatie dan een hoog niveau van ALA in een voeder rijk aan SFA. De experimentele voeders waren verrijkt met lijnzaadolie als ALA-bron en bevatten of rundvet als bron van SFA of zonnebloemolie als drager van PUFA. De vleeskuikens die het PUFA-rijke voeder kregen hadden gemiddeld hogere waarden voor warmteproductie en lagere waarden voor de hoeveelheid vet in het totale lichaam.

De algemene conclusie luidt dat de opname van PUFA, in plaats van SFA, de hoeveelheid buikvet bij vleeskuikens verlaagt door preferentiële oxidatie van de PUFA-component. Echter, de veronderstelde extra warmteproductie op een PUFA-rijk voeder kon niet overtuigend worden aangetoond.

บทสรุป

วัตถุประสงค์พื้นฐานของการศึกษาในวิทยานิพนธ์เล่มนี้ เพื่อเปรียบเทียบการใช้กรดไขมันไม่อิ่มตัวที่มีพันธะคู่หลายตำแหน่ง (PUFA) กับ กรดไขมันอิ่มตัว (SFA) ในอาหาร ที่มีผลทำให้ลดการสะสมไขมันในช่องท้องของไก่เนื้อ หลายการทดลองที่ได้ทำการศึกษาครั้งนี้ เพื่อหากลไกการทำงานของ PUFA ที่มีผลต่อ ระดับการเผาผลาญพลังงานของไขมันในร่างกายไก่เนื้อ ส่วนจุดประสงค์ของการทดลองคือ กรดไขมันชนิดใดที่ร่างกายไก่เนื้อได้นำไปเผาผลาญเป็นพลังงานก่อน โดยอาจจะส่งผลถึงการสะสมไขมันในช่องท้องของไก่เนื้อให้ลดลงได้

การได้รับกรดไขมัน PUFA จากน้ำมันถั่วเหลือง ซึ่งใช้ทดแทนไขวัวในระดับที่สูงขึ้น สามารถลดการสะสมไขมันช่องท้องของไก่เนื้อได้ โดยอาจมีความสัมพันธ์ระหว่างระดับไขมันในอาหารเข้ามาเกี่ยวข้อง

ส่วนการตั้งสมมุติฐานในการใช้ไขมันที่อุดมไปด้วยกรดไขมันสายกลาง (medium-chain triacylglycerol, MCT) อาจจะลดการสะสมไขมันช่องท้องได้เช่นเดี่ยวกับการใช้ PUFA โดยประกอบสูตรอาหารไก่เนื้อจากแหล่งไขมันต่างๆ คือ ไขวัว (อุดมไปด้วย SFA) น้ำมันถั่วเหลือง (อุดมไปด้วย PUFA) และน้ำมันกระบก (อุดมไปด้วย MCT) ผลจากการศึกษาวิจัยพบว่า การใช้น้ำมันถั่วเหลืองสามารถลดการสะสมไขมันในช่องท้องมากกว่าเมื่อเปรียบเทียบกับการใช้ไขวัว ส่วนการใช้น้ำมันกระบกทดแทนไขวัว ไม่มีผลทำให้ลดการสะสมไขมันช่องท้องได้ ซึ่งผลที่ได้จากการทดลองครั้งนี้ ได้ขัดแย้งกับสมมุติฐานที่กล่าวไว้ข้างต้น

ต่อมาได้มีการตั้งสมมุติฐานต่อไปอีกว่า การใช้ PUFA ทดแทน SFA อาจมีผลทำให้เพิ่มการเผาผลาญพลังงาน โดยจะส่งผลทำให้ลดการสะสมไขมันช่องท้องได้ ดังนั้นจึงได้ทำการทดลองโดยนำไก่เนื้อมาทดสอบอาหาร 5 สูตร โดยให้ไก่เนื้อได้รับอาหารแต่ละสูตรตลอดการทดลอง ซึ่งอาหารแต่ละสูตรทำการทดแทนไขวัวด้วยน้ำมันถั่วเหลืองในระดับที่สูงขึ้น จากการทดลองพบว่า การทดแทนน้ำมันถั่วเหลืองในระดับที่สูงขึ้นทำให้ลดการสะสมไขมันในร่างกายได้ร้อยละ 75 แต่เมื่อระดับน้ำมันถั่วเหลืองในอาหารสูงมากเกินไป ก็ไม่มีผลต่อการลดการสะสมไขมัน ส่วนการคำนวณค่าการเผาผลาญพลังงาน โดยแสดงผลจากปริมาณที่กินทั้งหมดเทียบเป็นร้อยละ พบว่า มีแนวโน้มสูงขึ้นในอาหารที่ประกอบด้วยน้ำมันถั่วเหลือง แต่ไม่มีความแตกต่างกันทางสถิติ

เมื่อปราศจากความชัดเจนของการเพิ่มค่าการเผาผลาญพลังงาน จึงได้มีการตั้งสมมุติฐานต่อไปอีกว่า การทดแทน SFA ด้วยการใช้ PUFA อาจจะยับยั้งกระบวนการสังเคราะห์กรดไขมันในร่างกายได้ ซึ่งตัวบ่งชี้ของการสังเคราะห์กรดไขมันในร่างกาย คือ ความเข้มข้นของไตรเอซิลกลีเซอรอลในพลาสม่า และการคำนวณการสังเคราะห์กรดไขมันต่ำสุด (minimum fatty acid synthesis) โดยคำนวณจากกรดไขมันที่สะสมลบด้วยกรดไขมันที่ได้รับเข้าไปในร่างกาย ประกอบด้วยอาหาร 5 สูตร โดยการใช้ไขวัว แล้วทดแทนด้วยน้ำมันถั่วเหลืองในระดับที่สูงขึ้น พบว่า การสะสมไขมันช่องท้องมีปริมาณลดลง เป็นผลเนื่องมาจากไตรเอซิลกลีเซอรอลในพลาสม่าลดลง ไม่ใช่เกิดจากการสังเคราะห์กรดไขมันต่ำสุด ดังนั้นสมมุติฐานที่ตั้งไว้ทั้งสองมีทั้งที่เป็นไปตามสมมุติฐาน และขัดแย้ง

จากการทดลองที่ผ่านมา ได้ทำการศึกษาโดยให้อาหารแบบเต็มที่ พบว่าการให้อาหารแบบเต็มที่ สัตว์สามารถปรับตัวในการกินภายใต้การให้อาหารแบบเต็มที่ โดยมีการปรับตัวจากไขมันที่ดูดซึม การเผาผลาญพลังงาน รวมทั้งการสังเคราะห์ไขมัน ด้วยเหตุนี่ ในการศึกษาครั้งนี้ ได้ทำการทดลองผลของการทดแทน SFA ด้วย PUFA ภายใต้การให้อาหารแบบเต็มที่และแบบจำกัด จากผลการทดลองพบว่า ค่าการเผาผลาญพลังงานของไก่เนื้อที่ได้รับ PUFA ในระดับสูง มีแนวโน้มสูงขึ้น ในไก่เนื้อที่ให้อาหารแบบจำกัด ส่วนไก่เนื้อที่ได้รับ PUFA ทดแทน SFA พบว่า ค่าสัดส่วนของการสะสมต่อการได้รับกรดไขมันของ SFA มีค่าสูงขึ้น แต่อย่างไรก็ตามค่าการ

กรดอัลฟ่าลิโนเลนิค จะถูกเผาผลาญก่อนกรดลิโนเลอิค เพื่อเป็นการทดสอบสมมุติฐานจึงได้ทำการศึกษาอาหารที่ประกอบด้วย กรดอัลฟ่าลิโนเลนิค ทดแทนกรดลิโนเลอิค ซึ่งอาจจะพบการเปลี่ยนแปลงของขบวนเมทาบอลิซึมของไขมันทั้งร่างกาย การสะสมไขมัน และการเผาผลาญพลังงาน โดยใช้น้ำมันถั่วเหลืองเป็นแหล่งของกรดลิโนเลอิค และน้ำมันลินซีดเป็นแหล่งของกรดอัลฟ่าลิโนเลนิค จากการศึกษาพบว่า ปริมาณกรดลิโนเลอิคที่สูง กับปริมาณกรดอัลฟ่าลิโนเลนิคที่สูงในอาหาร ไม่มีความแตกต่างระหว่างค่าความสมดุลของพลังงาน แต่ปริมาณของไขมันทั้งร่างกายของไก่เนื้อที่ได้รับกรดอัลฟ่าลิโนเลนิคลดลง ส่วนอัตราการสังเคราะห์กรดไขมันไม่อิ่มตัวที่มีพันธะเดี่ยวของทั้งร่างกายไก่เนื้อที่ได้รับกรดลิโนเลอิคมีค่าสูงกว่าไก่เนื้อที่ได้รับกรดอัลฟ่าลิโนเลนิค เมื่อพิจารณาที่ปริมาณไขมันช่องท้องพบว่า ไก่เนื้อที่ได้รับกรดอัลฟ่าลิโนเลนิคมีแนวโน้มในการสะสมไขมันช่องท้องน้อยกว่า ไก่เนื้อที่ได้รับกรดลิโนเลอิค จากผลที่ได้มีความสอดคล้องกับความรู้ที่ว่ากรดอัลฟ่าลิโนเลนิค จะถูกเผาผลาญมากกว่ากรดลิโนเลอิค

ในการทดลองสุดท้ายได้ทดสอบสมมุติฐานที่ว่าระดับของกรดอัลฟ่าลิโนเลนิคที่สูงในอาหารที่อุดมไปด้วย PUFA อาจจะทำให้เกิดการเผาผลาญพลังงาน และการเผาผลาญกรดอัลฟ่าลิโนเลนิคที่สูงขึ้น มากกว่ากรดอัลฟ่าลิโนเลนิคที่สูงในอาหารที่อุดมไปด้วย SFA สูตรอาหารทดลองประกอบด้วยน้ำมันลินซีดที่อุดมไปด้วยกรดอัลฟ่าลิโนเลนิค ผสมอยู่ในไขวัวที่เป็นแหล่งของ SFA และน้ำมันเมล็ดดอกทานตะวันที่เป็นแหล่งของ PUFA จากการทดลองพบว่า อาหารไก่เนื้อที่เป็นแหล่งของ PUFA มีแนวโน้มว่าค่าการเผาผลาญพลังงานสูงขึ้น และค่าการสะสมไขมันทั้งร่างกายลดลง

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CURRICULUM VITAE

The author of this thesis was born on March 20th, 1978 in Amphur Khamtalesor, Nakhonratchasima Province, Thailand. He received his elementary education from Anubarn Nakhonratchasima School, and primary education from Khamthalesor Witthaya School and secondary education from Nakhonratchasima Agricultural and Technology Collage in Nakhonratchasima. Following his graduation from Rajamangala Institute of Technology, Kalasin Campus in 1999 (B.Sc.), he started his study in animal nutrition at Khon Kaen University in 2000 and graduated with a M.Sc. degree in 2003. Thereafter, he started his work as lecturer at the Rajamangala University of Technology-Isan, Sakon Nakhon Campus. In the same year (2003), Prof. A.C. Beynen visited Sakon Nakhon and Dr.Chalermpon Yuangklang (Pee Tong) introduced him to Prof. A.C. Beynen and then he became his Ph.D. student in the framework of a sandwich program. The Ph.D. program involved the dietary fat type, body composition and fatty acid metabolism in broiler chickens. He will defend his Ph.D. thesis in public and obtain his Ph.D. degree on November 8th, 2007. Subsequently, he will return to Sakon Nakhon where he is appointed as a lecturer at the Faculty of Natural Resources, Sakon Nakhon, Thailand.

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