

THE EFFECT OF DIETARY FAT COMPOSITION AND AGE ON RESIDUAL YOLK SAC MEMBRANE, LIVER AND GALL BLADDER BILE LIPID COMPOSITION IN BROILER CHICKS

¹S.K. Mutayoba and ²R.C.Noble. ¹*Sokoine University of Agriculture, Department of Animal Science and Production, P.O.Box 3004, Morogoro, Tanzania* and ²*Scottish Agricultural Colleges, Ayr, KAH, 58W Scotland*

SUMMARY

A study was carried out to investigate the effects of dietary fat composition and post-hatching age on lipid changes of the yolk sac membrane (YSM), liver and gallbladder bile. Two groups of ninety-day-old male broiler chicks were fed diets containing a saturated fat (tallow oil) Diet 1 and unsaturated fat (soybean oil) Diet 2 for two weeks. Twelve birds from each treatment were sacrificed on days 1, 3, 6, 9, 12 and 14 post-hatch and samples of the YSM, liver and gall bladder bile were collected. Weight changes of the chick and tissues and lipid composition were also determined during the experimental period. About 75% decrease in YSM weight occurred during the first 3 days post-hatch in both groups. However, the rate of decline was highest in chicks fed Diet 1. The weight of the YSM was negligible by day 9 post-hatch in both groups. Triglycerides (TG) formed major lipid component of the YSM at day 1 post-hatch (>60% of total lipid) but had declined to less than 2% on day 12 post-hatch. The decreases in TG were accompanied by significant increases of cholesterol esters (CE). These changes were not affected by dietary treatment. The liver constituted less than 3% of chick body weight at hatching but increased to about 6% by day 6 post-hatch and remained constant thereafter. The total lipid content of the liver increased gradually with post-hatching age. TG formed less than 4% at hatching and increased to about 50% of total lipid by day 12. In contrast CE formed about 75% of the total lipid on day 1 but declined to less than 2% on day 12 post-hatch. Similarly, decreases in phospholipids content with age were noted. Changes in liver lipid composition were influenced by both dietary fat composition and post-hatching age. Phospholipids formed the major lipid component of the gallbladder bile on day 1 post-hatch, followed by free cholesterol. Variations in gallbladder bile lipid compositions with age and dietary fat composition were observed although they were inconsistent. It was concluded from these studies that there occurs major lipid compositional

changes of the body tissues following hatching and these changes are influenced to some extent by dietary composition.

INTRODUCTION

The emergence of the chick is associated with very high concentrations of lipid in the yolk sac, liver and gall bladder bile (Entemann *et al.*, 1940). It has been observed that the lipid composition in chicks differs markedly both in distribution and fatty acid composition from that of the adult bird (Akiba *et al.*, 1988). This means that the chick undergoes dramatic physiological and biochemical changes during the early post-hatch period in order to adapt to the new environmental conditions and feeding regimes in which it finds itself. The regression of the yolk sac and lipid compositional changes of the liver are amongst many important notable changes observed during the early post-hatch period (Romanoff, 1960; Noble and Ogunyemi, 1989 and Daly and Peterson, 1990). The yolk material complements nutrient intake and fortifies efficient utilization of energy and protein by the newly hatched chick (Murakami *et al.*, 1988; Nir *et al.*, 1988; Skewes *et al.*, 1988). The normal development of the chick and the associated metabolic features are highly influenced by early feeding

regimes and to some extent by the environmental conditions. The type and range of fatty acids available in feeds are known to have a major influence on development of the chick in particular on lipid metabolism (Latour *et al.*, 1994).

In modern broiler production much emphasis is put on rapid growth rate and this is mostly achieved by feeding high lipid oriented diets very early in the chick's life. A combination of exogenous and endogenous lipid sources to the chick during this period is likely to exert pressure on the physiological processes most of which are still in developmental stages. The objective of the present study was to investigate the effects of dietary fat composition and post-hatching age on lipid composition changes of the liver, YSM and gall bladder bile.

MATERIALS AND METHODS

One hundred and eighty day old Ross 1 broiler chicks were weighed, wing banded and then randomly allocated to the two dietary treatments differing in fatty acid composition (Table 1). Each treatment was replicated three times. The feed and water

were provided *ad libitum* and standard management procedures were followed.

Sampling

Samples of the residual yolk sac, liver and gall bladder bile were collected on day 1, 3, 6, 9, 12 and 14 post-hatch in 12 chicks randomly selected from each treatment after weighing. Each chick was killed by neck dislocation, followed by laparotomy to reveal the YSM, liver lobes and the gall bladder. The samples obtained were weighed and then chilled at 5°C and subsequently stored at -20°C to await further analyses.

Lipid and fatty acid analyses

The lipids were separated into major classes according to their polarity by on Silica gel G (Merck, ATG, Darmstadt, Germany) thin layer chromatoplates. A solvent system of hexane:diethyl ether:formic acid (80:20:1 v/v/v) was used and samples were left to develop for about 30 minutes in a chromatogram tank. The developed plate was air dried, sprayed with 0.1 % solution of 2,7-dichloro-fluorescein in methanol in order to facilitate the identification of each lipid moiety. This method allows lipids to resolve into separate bands identified as cholesterol esters (CE), triglycerides (TG),

free fatty acids (FFA) free cholesterol (FC) and phospholipids (PL) under UV light. Phospholipids were eluted from the gel by washing with 5-ml methanol whereas the remaining fractions were eluted individually with 5-ml diethyl ether. Washing was repeated three times.

The separated lipid fractions were transmethylated to individual fatty acids by refluxing with 4 ml of toluene:methanol:sulphuric acid mixture (20:10:1, v/v/v) in the presence of pentadecaenoic acid (C₁₅) standard (Christie *et al.*, 1970) for 30 minutes. The identification of fatty acids in each lipid fraction was done by comparing the relative retention times with known standards and carbon numbers versus semi logarithmic plots run through a Gas liquid chromatography Gaschrom P column containing 15% silicone treated with ethylene glycol succinate (EGSS-X). The relative proportions of the fatty acids were quantified by using an electronic integrator with an amplified signal (Spectro Physics, Model No 4270, U.K). The resultant peaks provided the relative proportions of the fatty acids associated with each lipid fraction according to Christie *et al.* (1970). Free cholesterol fraction was determined using a

modified charring procedure (Shand and Noble, 1980) and subsequent densitometry by a liquid scintillation counter.

Statistical analyses

All the data obtained were subjected to a T-test using Minitab Release 7.1 and was regarded to be significantly different at ($P < 0.05$).

RESULTS

Table 2 shows the mean body weight of chicks, residual yolk sac membrane (YSM) and the liver. Dietary treatment had no significant effect on weight gains. A significant regression of the residual yolk sac was observed between day 3 and 6 post-hatch, decreases being highest in chicks fed Diet 1. By day nine post-hatch the weight of the yolk sac was similar in both groups. The residual yolk sac accounted for about 6-11 % of body weight at hatching but decreased to less than 1% on day 12 post-hatch.

Triglyceride (TG), phospholipid (PL), and (cholesterol esters (CE), free cholesterol (FC) and free fatty acids (FFA)) formed about 60, 20 and 20 percent of the total lipid, respectively (Table 3). Irrespective of the diet, the residual yolk sac underwent marked lipid compositional changes during the early days post-hatch, which were

characterised by decreasing proportions of TG and PL and increases in CE and FC. However, at day 12 both TG and PL were higher in the YSM from chicks fed diet 2. In addition the total yolk sac lipid content declined very rapidly during the first 6 days post-hatch. Oleic acid was the major fatty acid component in the TG, CE and FFA fractions ranging between 45-75%, whereas, palmitic, stearic and oleic acids were evenly distributed in phospholipid fraction. The concentration of most of the fatty acids remained almost constant during the 12 days post-hatch and the effect of diet was insignificant.

The liver formed less than 3% of the chick's body weight on day 1 post-hatch, increased to about 6% by day 6 and then remained constant thereafter. The liver weight was slightly higher in chicks receiving the soyabean oil based diet.

A gradual decrease in liver lipid content with age was observed in all dietary treatments but was more drastic in chicks receiving soyabean oil based diet. Significant changes in liver lipid composition with post-hatching age were observed whereas dietary treatments had little influence (Table 4). Oleic acid was the major fatty acid of TG and CE, whereas, PL contained

substantial levels of stearic, arachidonic and docosahexanoic on day 1 post-hatch. There were marked changes in fatty acid composition with post-hatching age within the lipid fractions, which mostly occurred between the first and sixth day after hatching.

Table 5 shows the proportions of the major lipid components of the gall bladder bile. PL was the major lipid group on day 1 post-hatch, and the PL levels were higher in chicks receiving Diet 1 throughout the experimental period. Slight increases in PL levels with post-hatching age were noted in chicks receiving Diet 2. The other lipid fractions did not show any consistent trend. Palmitic, stearic and oleic acids were higher in gall bladder bile from chicks receiving Diet 2.

DISCUSSION

The body weight of chicks and the weight of the residual YSM obtained on day 1 post-hatch in the present study was within the range reported by others (Noble and Ogunyemi, 1989 and Daly and Peterson, 1990). The rapid regression of the residual YSM in weight during the first 6 days post-hatch is in agreement with findings reported by (Romanoff, 1960; Noble and Ogunyemi, 1989 and Latour et al., 1994). These

observations suggest that the rate of yolk assimilation is probably high during the early post-hatch period than in the embryonic period. During the present study the regression rate of the YSM was slightly lower in birds receiving Diet 2. The reason for this is unclear, but it is probably possible that were able to use energy from the soybean oil readily thus reducing the role of yolk lipid as source of energy less effective. However, the lower weight gain observed in chicks receiving the soybean oil based diet imply that complete regression of the yolk sac during the early post-hatch period, is amongst the major factors which affects the chick's ultimate ability to attain its normal physiological functions and growth. Additionally Chamblee (1991) suggested that yolk sac absorption was a prerequisite for initiating growth in broilers. The lipid composition of residual YSM observed in present study is consistent with findings reported elsewhere (Noble and Ogunyemi, 1989). The increase of CE after hatching has been attributed to a slow absorption rate of this fraction when compared to other lipid groups (Romanoff, 1960; Noble and Moore, 1967 and Noble and Ogunyemi, 1989). The insignificant changes of fatty acid composition within the major

lipid fractions accord findings reported by Noble and Ogunyemi, (1989). This indicates that unlike lipid uptake there seems to be no preferential absorption of the fatty acids.

The high levels of CE present in the liver on day 1 post-hatch during this study are similar to those previously described in the neonatal chick (Noble and Connor, 1984 and Noble *et al.*, 1984). The rapid decline in CE with post-hatching age observed presently was also reported by Svanberg, (1971) who showed that the concentration of CE in the liver were high between 12 hours and 6 days after hatching. The changes in the level of CE during the early post-hatch period might be due the changing role of the liver with respect to lipid metabolism after hatching, whereby the liver becomes the major site of fat synthesis (Leveille *et al.*, 1975; Annison, 1983 and Hill, 1983). The decline of liver CE was accompanied by differential increases in TG and PL concentrations. This could have probably been due to dietary influences since the liver lipid composition is usually influenced amongst other factors by diet composition (Marion, 1965; Sim *et al.*, 1973; Shapira *et al.*, 1978 and Rogel and Watkins, 1987).

The higher PL levels observed in the liver of chicks receiving the soybean oil based diet conforms to previous findings of Giordani *et al.*, (1988) who showed that increasing dietary unsaturated fatty acids leads to a decline in TG. These observations are usually attributed to the positioning of the fatty acids during TG synthesis by the liver. Position *sn*-1 of the glycerol is usually esterified with a saturated acid whilst *sn*-2 is esterified with an unsaturated fatty acids. Hence, synthesis of TG might be inhibited during the post-hatch period in chicks by lack of enough acids needed for esterification at the *sn*-2 position due to the efficient uptake of the unsaturated fatty acids from the digestive tract (Klopfenstein and Clegg, 1980).

The high concentrations of PL and FC in the gall bladder bile observed in the present study conformed to findings reported by Noble and Connor, (1984). The concentration of CE in the gall bladder of less than 5 percent on day 1 post-hatch observed in the present study was unexpected since Noble and Connor, (1984) showed that the chick embryo contained about 30 percent CE on day 19 of incubation. Consequently, it was anticipated that the chick might emerge with substantial levels of

Table 1: Ingredient and fatty acid composition of the diets and dietary fat sources

Ingredient g/kg	Diet 1	Diet 2	Fatty acid	Tallow oil	Soybean oil	Diet 1	Diet 2
Barley	100	100	16:0	24.8	10.2	21.8	14.3
Maize	250	250	16:1	5.6	<0.1	3.9	1.0
Wheat	245	245	18:0	21.5	3.1	15.0	4.8
Herring meal	50	50	18:1	43.8	28.5	33.4	25.1
Soybean meal	220	220	18:2	3.9	50.2	19.6	46.4
Grass meal	50	50	18:3	0.1	7.6	3.5	6.0
Limestone	5.3	5.3	20:3	<0.1	0.3	0.9	0.7
Dicalcium phosphate	21.7	21.7	20:4	0.1	<0.1	<0.1	<0.1
Vit/mineral mix	5	5	20:5	<0.1	<0.1	0.8	0.1
Salt	2.5	2.5	22:5	<0.1	<0.1	0.2	<0.1
Amprol mix	0.5	0.5	22:6	<0.1	<0.1	0.7	0.7
Tallow oil	50	-					
Soybean oil	-	50					

Table 2: The effect of post-hatching and dietary fat composition on body, SM and liver weight changes

Parameter	Diets	Age (days)				
		1	3	6	9	12
Body weight, g	1	48.0 ± 1.2	60.3 ± 1.2	99.8 ± 1.5	169.8 ± 3.0	277.1 ± 5.9
	2	46.7 ± 1.4	61.1 ± 2.0	102.9 ± 2.1	162.3 ± 3.7	253.0 ± 6.7
YSM remnants, g	1	15.7 ± 0.2	3.7 ± 0.7	0.8 ± 0.3	0.3 ± 0.01	0.1 ± 0.01
	2	15.4 ± 1.6	5.0 ± 0.4	0.3 ± 0.1	0.1 ± 0.03	0.1 ± 0.01
Liver weight, g	1	2.6 ± 0.3	6.2 ± 0.3	9.3 ± 1.1	16.7 ± 0.3	21.2 ± 0.8
	2	2.5 ± 0.5	6.2 ± 0.2	12.9 ± 0.5	17.0 ± 0.6	22.0 ± 0.9

Table 3: The distribution of the major lipid fractions in the residual YSM as influenced by age and dietary fat composition

Lipid fraction	Diet	Age (days)			
		1	3	6	12
Cholesterol esters (CE)	1	5.2 ± 0.8	37.9 ± 3.1	42.2 ± 2.5	56.1 ± 1.5
	2	7.4 ± 1.2	24.3 ± 2.3	23.1 ± 3.2	64.3 ± 2.2
Triglycerides (TG)	1	67.6 ± 4.2	37.9 ± 3.5	42.5 ± 3.2	28.3 ± 2.1
	2	66.5 ± 3.1	42.9 ± 2.4	51.2 ± 4.4	13.6 ± 1.2
Free fatty acids (FFA)	1	2.1 ± 0.4	5.2 ± 1.2	2.8 ± 0.2	3.9 ± 0.9
	2	2.7 ± 0.3	4.0 ± 0.2	3.0 ± 0.5	2.1 ± 0.6
Phospholipids (PL)	1	18.6 ± 3.1	9.1 ± 1.3	9.4 ± 0.6	6.7 ± 2.3
	2	19.5 ± 0.9	22.1 ± 1.5	14.3 ± 0.9	2.1 ± 0.7
Free cholesterol (FC)	1	5.7 ± 0.4	8.4 ± 0.5	6.7 ± 0.3	-
	2	4.8 ± 0.3	6.4 ± 0.7	7.2 ± 0.6	-

Table 4: The liver lipid composition as influenced by post-hatching age and dietary fat composition

Lipid fraction	Diet	Age (days)					
		1	3	6	9	12	
Cholesterol esters (CE)	1	77.5 ± 0.7	63.3 ± 4.7	30.6 ± 2.5	5.4 ± 1.4	1.6 ± 0.4	
	2	76.8 ± 0.7	66.9 ± 4.3	22.8 ± 2.8	2.4 ± 0.1	2.4 ± 0.7	
Triglycerides (TG)	1	1.3 ± 0.1	5.7 ± 1.1	25.9 ± 3.6	49.5 ± 3.1	57.1 ± 2.3	
	2	2.0 ± 0.3	6.9 ± 2.1	29.3 ± 2.5	29.4 ± 1.4	28.7 ± 3.2	
Free fatty acids (FFA)	1	5.6 ± 0.5	7.5 ± 1.0	6.3 ± 0.6	6.3 ± 0.5	5.1 ± 0.3	
	2	4.6 ± 0.2	6.0 ± 0.8	9.3 ± 0.7	9.9 ± 1.2	10.4 ± 0.9	
Phospholipids (PL)	1	8.6 ± 0.1	10.7 ± 2.7	27.5 ± 0.7	29.2 ± 3.2	28.3 ± 1.8	
	2	8.7 ± 0.5	13.2 ± 1.5	27.6 ± 1.8	44.1 ± 1.6	44.5 ± 3.6	
Partial glycerides (PG)	1	0.7 ± 0.2	1.5 ± 0.3	2.0 ± 0.4	2.2 ± 0.4	1.9 ± 0.3	
	2	0.8 ± 0.1	0.7 ± 0.1	2.0 ± 0.2	2.9 ± 0.2	2.5 ± 0.5	
Free cholesterol (FC)	1	6.3 ± 0.5	5.4 ± 1.2	7.7 ± 0.9	7.5 ± 1.0	6.0 ± 0.1	
	2	7.8 ± 0.3	7.3 ± 0.2	9.0 ± 1.3	11.5 ± 1.3	11.1 ± 0.7	

Table 5: The effect of post-hatching age and dietary fat composition on lipid composition of the chick gall bladder bile

Lipid fraction	Diet	Age (days)				
		1	3	6	9	12
Cholesterol esters (CE)	1	4.7 ± 1.0	61.3 ± 1.4	-	6.5 ± 1.5	2.9 ± 0.1
			1.5 ± 0.5	-	2.1 ± 0.3	-
Triglycerides (TG)	1	1.8 ± 0.1	2.4 ± 0.6	1.0 ± 0.2	9.3 ± 1.3	3.0 ± 0.8
	2		0.8 ± 0.1	-	1.5 ± 0.4	3.4 ± 0.6
Free fatty acids (FFA)	1	1.0 ± 0.3	3.1 ± 0.5	4.5 ± 0.6	5.9 ± 0.8	2.5 ± 0.2
	2		3.5 ± 0.5	7.5 ± 0.9	6.5 ± 0.5	2.2 ± 0.1
Phospholipids (PL)	1	68.6 ± 1.3	25.7 ± 0.3	76.8 ± 1.7	58.5 ± 1.3	70.7 ± 1.5
	2		77.4 ± 1.7	44.2 ± 0.8	52.3 ± 2.0	78.8 ± 1.8
Free cholesterol (FC)	1	24.6 ± 1.5	7.5 ± 0.3	16.9 ± 1.3	19.8 ± 0.6	20.9 ± 1.2
	2		16.0 ± 1.9	47.9 ± 1.9	37.2 ± 1.8	15.2 ± 1.6

this lipid group. These findings suggest that there might be a rapid assimilation of certain lipid groups during the last days of incubation.

The distribution of the major fatty acids in the lipid fractions of the liver and gall bladder bile, particularly within the cholesterol esters were similar to those previously observed in the yolk sac lipid (Noble and Ogunyemi, 1989). The presence of high oleic acid levels in CE of these tissues was probably due to the distribution of the yolk lipids during the early-post-hatch days (Jain *et al.*, 1972 and Noble *et al.*, 1988). However, the fatty acid composition of the TG and PL fractions of the liver and gall bladder bile differed extensively from those of the yolk lipids. The changes with age in fatty acid composition of the major lipid fractions of the liver and gall bladder bile observed in the present study are in agreement with findings reported by Jain *et al.*, (1972); Noble *et al.*, (1988) and Noble and Ogunyemi, (1989). Changes in fatty acid composition observed during the first six days post-hatch conform with the suggestion that the chick physiological changes connected with lipid metabolism is extensive during this time.

These changes were also attributed to the alterations in the

role of the liver from being mainly a depository organ during the embryonic period to one of synthesizing fat for both structural and functional purposes.

The findings of the present study revealed that there were lipid compositional changes of the body tissues in the chick soon after hatching. Dietary fat sources had little influence on the performance of the birds. However, slight differences were observed in rate of yolk sac disappearance and liver lipid composition.

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