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Broiler Chick Body Weight and Lipid Compositional Changes of the Yolk Sac and Liver as Influenced by Dietary Fat Sources

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Abstract: A study to determine the effects of dietary fat composition and post-hatching age on lipid changes of the yolk sac membrane (YSM) and the liver. 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 also lipid composition were determined during the experimental period. About 75% decrease in YSM weight occurred during the first 3 days post-hatch and it was negligible by day 9 post-hatch in both groups. Triglycerides (TG) was the 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 3 and 6% of chick body weight at hatching and at day 6 post-hatch respectively and remained constant thereafter. Changes in total lipid content and lipid composition were noted and were influenced by both dietary fat composition and post-hatching age. It was concluded from these this study major lipid compositional changes occur in the chick body tissues following hatching and these changes are influenced to some extent by dietary composition.

Key words: Lipid changes, yolk sac membrane, dietary fat, broiler chicks

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 the many important notable changes observed during the early post-hatch period (Romanoff, 1960; Noble and Ogunyemi, 1989; Daly and Peterson, 1990; Yadgary *et al*., 2010). 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.

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

Ingredient (g kg^{-1})	Diet 1	Diet 2	Fatty acid	Tallow oil	Soybean oil	Diet 1	Diet 2
Barley	100.0	100.0	16:0	24.8	10.2	21.8	14.3
Maize	250.0	250.0	16:1	5.6	<0.1	3.9	1.0
Wheat	245.0	245.0	18:0	21.5	3.1	15.0	4.8
Herring meal	50.0	50.0	18:1	43.8	28.5	33.4	25.1
Soybean meal	220.0	220.0	18:2	3.9	50.2	19.6	46.4
Grass meal	50.0	50.0	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	5.0	5.0	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	50.0	-					
Soybean oil	-	50.0					

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. 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 (C_{15}) 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 were 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 day 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. 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.

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 characterized 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.

Table 2: Effect of post-hatching and dietary fat composition on body, YSM and liver weight changes

		Age (days)				
Parameter	Diets	1	3	6	9	12
Body weight, g	1	48.0±1.2	60.3±1.2	99.8±1.5	169.8±3.00	277.1±5.90
	2	46.7±1.4	61.1±2.0	102.9±2.1	162.3±3.70	253.0±6.70
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±0.30	21.2±0.80
	2	2.5±0.5	6.2±0.2	12.9±0.5	17.0±0.60	22.0±0.90

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

		Age (days)				
Lipid fraction	Diets	1	3	6	9	12
Cholesterol esters (CE)	1	5.2±0.8	37.9±3.1	42.2±2.5	56.1±1.5	77.4±3.20
	2	7.4±1.2	24.3±2.3	23.1±3.2	64.3±2.2	61.2±2.50
Triglycerides (TG)	1	67.6±4.2	37.9±3.5	42.5±3.2	28.3±2.1	6.2±1.20
	2	66.5±3.1	42.9±2.4	51.2±4.4	13.6±1.2	12.33±2.6
Free fatty acids (FFA)	1	2.1±0.4	5.2±1.2	2.8±0.2	3.9±0.9	1.2±0.30
	2	2.7±0.3	4.0±0.2	3.0±0.5	2.1±0.6	6.7±3.20
Phospholipids (PL)	1	18.6±3.1	9.1±1.3	9.4±0.6	6.7±2.3	3.4±0.90
	2	19.5±0.9	22.1±1.5	14.3±0.9	2.1±0.7	5.2±0.60
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	-	-

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.

DISCUSSION

The body weight of chicks and the weight of the residual YSM obtained on day 1 post-hatch in the present study were within the range reported by others (Noble and Ogunyemi, 1989; 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; Latour *et al*., 1994; Khan *et al*., 2002). 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 soyabean oil based diet. The reason for this is unclear, but it is probable that were able to use energy from the soybean oil readily thus reducing the role of yolk lipid as source of energy. 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) and Knizetova *et al*. (1989) 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; 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 in the liver on day 1 post-hatch observed in the present study concur with the trend previously described for the neonatal chick by (Noble and Connor, 1984; Noble *et al*., 1988). 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; Hill, 1983). The decline of liver CE was accompanied by differential increases in TG and PL concentrations. This could have probably been due to

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

		Age (days)				
Lipid fraction	Diets	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

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; 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 distribution of the major fatty acids in the lipid fractions of the liver, 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 was probably due to the distribution of the yolk lipids during the early-post-hatch days (Jain *et al*., 1972; Noble *et al*., 1988). The similarity in fatty acid compositions of CE in the liver and yolk sac membrane on day 1 post-hatch is an indication of the interrelationship which exists among these tissues (Svanberg, 1971). Additionally most of the cholesterol in the liver was derived from the yolk and rate of transfer was highest during the last 2-3 days of incubation (Entemann *et al*., 1940; Zehava and Smith, 2011). However, the fatty acid composition of the TG and PL fractions of the liver differed extensively from those of the yolk lipids. The changes with age in fatty acid composition of the major lipid fractions of the liver 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 are also attributed to the changing 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.

Conclusion: 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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