ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 8 (4): 328-341, 2009
ISSN 1682-8356
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The Effect of Different Feed Restriction Programs and Dietary L-Carnitine Supplementation on Hepatic Lipogenesis, Plasma Heterophil to Lymphocyte Ratio and Yolk IgY Content of Broiler Breeder Hens

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Abstract: Two experiments were conducted to determine effects of Everyday (ED) or Skip-a-day (SK) feeding and dietary L-carnitine on lipid metabolism and stress in broiler breeders. In Experiment 1 a 2x2 factorial design was used to compare feeding regimens (ED vs SK) and L-carnitine supplementation (0 vs 50 mg/kg). L-carnitine supplementation began at d 1 and lasted throughout the 45 weeks experimental period. SK programs were implemented from 28 days of age to 5% production. Parameters measured included *in vitro* Lipogenesis (IVL), Heterophil/Lymphocyte ratio (H/L) and yolk IgY content. Liver and blood samples were taken 1 h after feeding, at various intervals during the rearing and production periods. Both SK feeding and L-carnitine increased liver wts during rearing but differences dissipated after onset of lay. Part of the increase in liver weight in SK birds was due to higher lipid contents. L-carnitine tended to reduce liver lipid during rearing. IVL was increased by SK feeding during the rearing period. L-carnitine and SK feeding interacted to increase IVL at 20, 22 and 27 weeks. H/L was elevated at 7 weeks in SK birds, but no differences were observed after that. Neither L-carnitine nor feeding regimens affected maternal IgY transfer to egg yolks. In Experiment 2, the same effects were tested but a low density grower diet was used from 4-18 weeks. The grower diet had 9% less energy and 7% less protein than in experiment 1. Liver wt was increased in SK and L-carnitine supplemented birds up to 20 weeks. By 40 weeks, ED birds had higher liver weights than SK. Liver fat was generally higher in SK birds than ED during rearing. SK feeding increased IVL but unlike Experiment 1, L-carnitine did not. H/L ratio was elevated in SK up to 20 weeks of age after which no differences occurred. L-carnitine did not affect H/L. In conclusion, feeding regimens and L-carnitine can alter hepatic lipid synthesis. Feeding regimens like SK, incorporating lengthy periods without feed can result in elevated H/L ratios but birds are generally able to adapt to such regimens over time.

Key words: Breeders, feeding regimens, L-carnitine, heterophil/ lymphocyte ratio, lipogenesis

INTRODUCTION

The modern broiler's genetic potential for growth and tendency toward fat accumulation mean that broiler breeders must be feed restricted to prevent problems with health and reproduction (McDaniel *et al*., 1981; Hocking *et al*., 1987, 1989). Feed restriction improves the reproductive performance of broiler breeders, resulting in more eggs (Yu *et al*., 1992; Robinson *et al*., 1993), better persistence (Fattori *et al*., 1991, Robinson *et al*., 1991), fewer abnormal eggs and fewer multiple ovulations (Fattori *et al*., 1991, Yu *et al*., 1992).

During periods of fasting, lipogenic enzyme activities drop compared to fed birds (Richards *et al*., 2003; Yeh and Leveille, 1971) but these enzyme activities increase dramatically when birds are returned to feed (Yeh and Leveille, 1972; Hill *et al*., 1988; Rosebrough, 2000). Cycles of fasting and refeeding can alter expression of lipogenic genes such as Acetyl CoA Carboxylase (ACC), which is the rate controlling enzyme of lipogenesis (Rosebrough *et al*., 2002). Hillgartner *et al*. (1996) reported that the feeding induced increase in ACC synthesis was correlated with comparable changes in the abundance of mRNA species coding for ACC and concluded that nutritional regulation of ACC synthesis involved a transcriptional mechanism. Rosebrough and Steele (1985) demonstrated that repeated cycles of starvation and refeeding produced lipogenic rates that were higher than those in birds fed *ad libitum*. In birds, fat accumulates in the liver upon refeeding following a fast, for reasons which are not exactly clear (Yeh and Leveille, 1972), but possibly because mechanisms responsible for lipid mobilization from the liver do not adapt as quickly as the mechanisms responsible for increasing lipogenesis. The effects of Everyday (ED) versus Skip-a-day (SK) restriction programs on lipogenesis have not been well documented.

Feed restriction can affect indices of stress in broiler breeders (Renema and Robinson, 2004). Hocking *et al*. (1993) demonstrated that plasma Heterophil to Lymphocyte (H/L) ratios were elevated in feed restricted birds up to 16 weeks of age but did not differ by 24 weeks. Literature comparing H/L for ED and SK programs is scarce but Zuidhof *et al*. (1995) reported that H/L could be lowered in feed restricted birds by

diluting the diet with oat hulls. Maxwell *et al*. (1990) did not show differences in H/L ratio when comparing feed restricted to *ad libitum* fed birds, but they did report that basophil numbers were elevated in feed restricted birds. L-carnitine's role as a carrier of activated fatty acids across the mitochondrial membrane for oxidation has long been established (Friedman and Fraenkel, 1955). The possible effects of L-carnitine on hepatic lipid synthesis are not well defined (Xu *et al*., 2003). Maternal immunoglobulin transfer provides the chick with a valuable form of resistance against commonly encountered pathogens. L-carnitine has been shown to exhibit immuno-modulatory effects. Mast *et al*. (2000) reported that dietary L-carnitine supplementation increased antigen specific IgY (IgG) response and total Immunoglobulin (Ig) response in broilers. This evidence suggests that carnitine supplementation improves specific humoral immune responses and may enhance protective immunity after vaccination. Perhaps more significantly, Bollen and Hau (1997) showed that an increase of serum immunoglobulin Y (IgY), resulted in increased transfer of IgY to the egg. This increased Ig response due to L-carnitine may result in increased IgY levels in the yolk. L-carnitine supplementation may improve maternal immunity, potentially producing a stronger chick.

The aim of the first experiment reported herein was to determine the effects of different feed restriction programs and dietary L-carnitine supplementation on hepatic lipogenesis, H/L ratio and maternal IgY transfer in broiler breeders. A secondary objective was to determine if L-carnitine supplementation interacted in some way with feeding programs to alter metabolism and stress. A second experiment was conducted to determine the effects of feeding programs and L-carnitine on the same parameters, when low density diets were used during the rearing phase.

MATERIALS AND METHODS

Experiment 1

Stock and management: A total of 700 day old Cobb 500 broiler breeder pullets were randomly assigned to twenty 2.38x1.83 m floor pens. The 20 pen experimental units were divided into four treatments with five replicate pens of 35 pullets each per treatment. The Cobb Breeder Management Guide (Cobb-Vantress, 2005) was used as a reference for all management conditions, including the light schedule for dark-out rearing houses. The compositions of the diets utilized throughout the experiment are shown in Table 1. The starter diet was fed from 0-4 weeks of age, the grower from 4-18 weeks of age, the prebreeder from 18-22 weeks of age and the breeder I diet from 22-45 weeks of age. Pullets were weighed weekly in groups from 0-21 weeks of age. Feed allocations during rearing were determined based on BW targets set out in the guide.

Pullets were photostimulated with 13 h of light at 21 weeks at which time 60 representative birds from each treatment were housed individually in breeder cages (47 cm high, 30.5 cm wide, 47 cm deep). Photoperiod was extended by 1 h/week, each week until 16 h of light was reached. From 21 weeks of age all hens were weighed individually every week until weeks 33 and then monthly until the end of the experiment.

Experimental design: A 2x2 factorial design was used. The main effects were L-carnitine supplementation (0 or 50 mg/kg) and feeding program (ED or SK). All pens of pullets were fed *ad libitum* for the first ten d. From ten to 28 days all pullets were fed restricted amounts of feed everyday. At 28 days of age different feed restriction programs were implemented. Groups were fed using either Everyday (ED) or Skip-a-day (SK) programs from 28 days until 5% production. After 5% production all hens were fed everyday. L-carnitine supplementation (0 or 50 mg/kg) began on day 1 and continued for the duration of the experiment. The diets used for all groups were the same except that 50 mg/kg L-carnitine was added for L-carnitine supplemented groups. All groups were fed to reach the same BW as recommended in the Cobb Breeder Management Guide (Cobb-Vantress, 2005). Due to differences in efficiency of ED and SK groups, as well as L-carnitine supplemented groups, feed intake was not the same for all groups. By maintaining similar BW throughout the experiment, the effect of BW on performance was minimized as a source of variation. Maximum feed allocation was 144 g/bird which was 420 kcal ME/hen per d (Reyes and Coon, unpublished data). This was done to account for the reduced energy expenditure as a result of being housed in individual cages. Feed withdrawal began at 32 weeks and continued until the end of the experiment (45 weeks), at which time breeders were being fed 133 g/bird/day.

***In vitro* lipogenesis:** *In vitro* Lipogenesis (IVL) was measured based on the method described by Rosebrough and Steele (1987). *In vitro* lipogenesis was determined at 4, 7, 14, 20, 22 and 27 weeks of age. Prior to feeding, all birds utilized for the IVL determinations were individually weighed and marked before being returned to their pens. One hour after feeding, marked birds were killed by CO_2 asphyxiation and livers were excised, weighed and washed in phosphate buffered saline to remove blood and debris and then sliced[2] into sections of between 35-70 mg. Slice thickness was set for 3 mm. Six birds were used per treatment and three slices were used per bird. The slices were incubated in 25 mL Erlenmeyer flasks at 37°C for 2 h in 3 mL Hanks balanced salts (Hanks and Wallace, 1949) containing 10 mM HEPES and 10 mM sodium [2-^{14}C] acetate (166 MBq/mol). Incubations were conducted under a 95% O_2:

5% CO_2 atmosphere, which was obtained by gassing the vials for 15 sec. At the end of the incubation period explants were extracted in 10 mL of 2:1 chloroform: methanol for 18 h. The extract was fractionated with 2 mL 0.88% KCl and then washed according to Folch *et al.* (1957). After the washing process the bottom phase was evaporated to dryness, dispersed in scintillation fluid and counted by liquid scintillation spectroscopy. *In vitro* lipogenesis is expressed as µmoles of acetate incorporated into hepatic lipids per kg of body weight.

Liver composition: After slicing, the remaining portions of all excised livers were frozen at -20°C until further processing. Two additional birds were sacrificed and livers were excised and frozen at -20°C for determination of liver composition. These remaining portions from the livers used for IVL determination and the two extra livers were subsequently lyophilized in a Genesis SQ 12 EL Freeze drier[3] to determine the Dry Matter (DM) content. Lyophilized samples were ground before further analysis. Liver protein (N x6.25) and ether extract were then analyzed according to AOAC (1990). Dry liver wt was obtained by multiplying the DM % by the wet liver wt. Both protein and fat % were determined on a DM basis. Total protein and total fat weight was obtained by multiplying the dry liver wt by the % protein and fat in the dry liver sample, respectively.

Heterophil lymphocyte ratio: The effect of the restriction programs on the health and well being of the pullets was determined by measuring the blood Heterophil-Lymphocyte ratio (H/L). Blood H/L ratio was measured at 4, 7, 14, 20, 22, 27 and 40 weeks of age. Blood samples (2 mL), taken 1 h after feeding, by cardiac puncture, were collected into EDTA treated vials to prevent clotting. Samples were analyzed at various ages during rearing and production to determine H/L. An Abbott Cell-Dyn 3500[4] automated hematology analyzer was used to determine H/L ratio in 500 µL of the blood sample. Total White Blood Cell (WBC) and basophil counts were also obtained in this way. Recommended settings and calibrations for avian hematology were employed according to the manufacturer's operation manual.

Yolk IgY content: At 28 weeks of age, four consecutive eggs were collected from five randomly selected hens per treatment. Eggs were stored at -4°C until extraction of antibodies. Extractions were carried out in the order the eggs were collected by completing extraction of the first egg from all the hens before proceeding to the second egg.

To extract Ig from the egg yolk, a chloroform-based method described by Polson (1990) was used. The egg yolk was taken out of the eggshell and placed in a clean petri dish. The yolk membrane was washed with distilled water and then broken with the help of forceps. The yolk was allowed to run into a measuring cylinder and its volume was noted after it settled down. Twice the volume of Dulbecco's PBS (Sigma-Aldrich Inc.) was added and the contents were mixed thoroughly by shaking. Chloroform equal to the volume of egg yolk and PBS was then added and the contents were mixed vigorously, which resulted in a thick emulsion. The emulsion was then centrifuged at 1,000x g for 30 min at room temperature. After centrifugation, the mixture was separated into 3 distinct layers in the centrifuge tube: an orange-colored solution of lecithin at the bottom, a semisolid emulsion of yolk in chloroform in the middle and a watery phase of chicken serum protein on top. The watery phase on the top containing the Ig was removed, separated into aliquots and stored at -20°C until further analysis.

The levels of the total IgY in egg yolks was determined using quantitative ELISA kits[5] for IgY, following the manufacturer's instruction with slight modification. The samples were analyzed in triplicate. Each plate had its own set of standards (3.12-200 ng/mL). A working dilution of 1:50 000 was used. Reagents and buffers were prepared following the specifications of the manufacturer (Bethyl Laboratories). The working dilution of detection antibody used was 1:20,000. The samples were incubated with tetramethylbenzidine[6] for 30 min and the reaction was stopped using 2 M H_2SO_4 after 30 min. The plates were read at 450 nm of primary wavelength using an ELX 800 universal micro plate reader[7] and KC junior software. [8]The blank adjusted data were exported to an Excel file. The standard curve describing the relation between the concentration of standards and their absorbance value was generated for each plate and the concentration of antibody for each of the samples was expressed as mg per ml. Additional calculations were carried out to determine the concentration of IgY antibodies per ml of egg yolk as well as the total amount of IgY antibodies per egg yolk.

Experiment 2

Stock and management: A total of 840 d-old Cobb 500 broiler breeder pullets were randomly assigned to 24 floor pens. The 24 pen experimental units were divided into four treatments with six replicate pens of 35 pullets each per treatment. All general management procedures including pen and breeder cage size (rearing and production facilities), stocking density and lighting programs were the same as for experiment 1. In experiment 2, a low density grower diet (Table 1) was used from 4 to 18 weeks of age. The energy and protein content of the grower diet was approximately 9% and 7% lower than the standard grower diet used in Experiment 1, respectively. At photostimulation in Experiment 2, 80 representative pullets from each treatment group were individually housed.

Table 1: Composition of diets (%) and calculated contents (%) used in both Experiment 1 and 2[1]

Ingredient	Starter	Grower Experiment 1 (Std)	Grower Experiment 2 (Low density)	Prebreeder	Breeder 1
Corn, Yellow	61.40	61.41	53.87	67.78	66.93
Soybean Meal	27.08	15.69	13.69	20.62	22.41
Wheat Midds	7.71	19.04	28.75	7.09	
Dicalcium Phosphate	1.83	1.74	1.77	1.73	1.80
Limestone	0.69	0.72	0.71	1.62	6.36
Termin-8[2]	0.30	0.30	0.30	0.30	0.30
NaCl	0.29	0.31	0.31	0.31	0.08
Poultry fat	0.25	0.50	0.25	0.25	1.67
L-Lysine Hcl	0.10				
Alimet-MHAliquid	0.10	0.07	0.07	0.08	0.19
Choline Cl-70%	0.09	0.07	0. 07	0.08	0.09
Mineral premix[3]	0.06	0.06	0.06	0.06	0.06
Copper sulphate	0.05	0.05	0.05	0.05	0.05
Vitamin premix[4]	0.04	0.04	0.04	0.04	0.05
Ethoxyquin	0.01				
Calculated analysis (%)					
ME, kcal/kg	2870	2820	2650	2920	2920
CP	18.99	15.16	14.22	16.23	15.95
Crude Fat	2.82	3.27	2.98	2.96	4.15
Ca	0.95	0.90	0.90	1.25	3.10
Total P	0.74	0.75	0.77	0.69	0.64
Avail. P	0.45	0.45	0.45	0.42	0.41
Analyzed					
ME, kcal/kg[5]		2965	2721		
CP (%)[6, 7]	18.71	15.28		16.59	16.21
CP (%)[6, 8]	18.59		14.30	16.46	15.81

[1] L-carnitine was supplemented at 50 mg/ kg into these diets for the appropriate treatments groups, [2]Mold inhibitor (Anitox Corp), [3]Mineral mix provided per kilogram of complete diet: Cu, 55 mg; I, 7.3 mg; Fe, 366 mg; Mn, 310 mg; Zn, 321 mg; K, 2.23 g; Mg, 1.09 g; Se, 0.48 mg, [4]Vitamin mix provided per kilogram of complete diet: vitamin A, 30,800 IU; vitamin D_3, 9,250 IU; vitamin E, 153.9 IU; vitamin B_{12}, 0.154 mg; riboflavin, 46.2 mg; niacin, 185 mg; pantothenic acid, 84 mg; menadione sodium bisulfite, 16.2 mg; folic acid, 12.3 mg; pyridoxine HCl, 46.2 mg; thiamine HCl, 20.5 mg; biotin, 9.3 mg; choline, 2,944 mg; niacin, 185 mg, [5]Analyzed values for Experiment 2, [6]Corrected to 90% DM, [7]Analyzed protein values for Experiment 1, [8]Analyzed protein values for Experiment 2

Experimental design: A 2x2 factorial design was also used in experiment 2. The main effects were L-carnitine supplementation (0 or 50 mg/kg) and feeding program (ED or SK). With the exception of yolk IgY not being measured in Experiment 2, the experimental design, parameters and methods of collection described for Experiment 1 were the same for Experiment 2.

Statistical analysis: Statistical analysis was carried out using the same procedures for both experiments. Data analysis was performed using JMP IN 5.1[8] statistical analysis software. Chicks were assigned to treatments on day one in a completely random manner.

Data were analyzed as a 2x2 factorial design using two-way ANOVA, with feeding program and L-carnitine supplementation as main effects. If interactions were significant, means were separated using Tukey's Studentized range test. If no interaction was observed, main effects were tested. All statements of significance are based on testing at $p \leq 0.05$.

Animal use: All procedures were carried out in accordance with Animal Use Protocol No. 03008 for the experiment, which was approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC).

RESULTS

Experiment 1

Liver weight: Liver Weight (LW) shows a gradual increase in all groups from 7 through 40 weeks of age (Table 2). The Relative Liver Weight (RLW) was calculated as the LW/ BW multiplied by 100. No interactions were observed at any age for LW or RLW. Therefore, only main effects will be discussed. At 7 weeks of age SK feeding resulted in a 12% increase ($p \leq 0.05$) in LW compared to ED pullets. SK feeding also increased RLW compared to ED feeding. L-carnitine supplemented pullets (2.19%) had higher RLW than non-supplemented pullets (2.07%). At weeks 14 SK feeding increased LW and RLW compared to ED feeding. The increase in LW was approximately 14% in SK pullets. L-carnitine supplementation also produced a significant ($p \leq 0.05$) increase in LW and RLW at 14 weeks compared to breeder pullets fed diets without carnitine supplementation. At 20 weeks of age the SK

Table 2: Absolute and relative liver weights of broiler breeder hens fed using either Everyday (ED) or Skip-a-day (SK) feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 1)

		Weeks 7		Weeks 14		Weeks 20		Weeks 22		Weeks 27		Weeks 40	
Program	L-Carnitine	LW[1] (g)	RLW[2] (%)	LW[1] (g)	RLW[2] (%)	LW[1] (g)	RLW[2] (%)	LW[1] (g)	RLW[2] (%)	LW[1] (g)	RLW[2] (%)	LW[1] (g)	RLW[2] (%)
ED	None	18.60	1.93	25.10	1.56	43.00	1.53	50.20	1.62	59.70	1.71	84.0	2.02
ED	50 mg/kg	21.20	2.13	27.30	1.72	44.40	1.65	54.40	1.79	64.70	1.84	90.9	2.14
SK	None	22.60	2.21	31.60	1.81	47.20	1.76	52.00	1.74	66.30	1.85	80.6	1.93
SK	50 mg/kg	22.20	2.26	35.90	2.11	51.20	1.85	59.40	1.91	64.40	1.79	86.0	2.04
SEM		0.50	0.03	0.70	0.02	0.70	0.02	1.30	0.04	1.20	0.03	1.8	0.04
Main effect means													
ED		19.90	2.03	26.20	1.64	43.70	1.59	52.30	1.71	62.20	1.77	87.4	2.08
SK		22.40	2.23	33.70	1.96	49.20	1.81	55.70	1.82	65.40	1.82	83.3	1.98
	None	20.60	2.07	28.30	1.69	45.10	1.65	51.10	1.68	63.00	1.78	82.3	1.98
	50 mg/kg	21.70	2.19	31.60	1.92	47.80	1.75	56.90	1.85	64.60	1.81	88.4	2.09
Source of variation		Probability											
Program		0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.20	0.15	0.18	0.51	0.19	0.26
L-Carnitine		0.30	0.02	0.04	<0.01	0.07	0.04	0.03	0.05	0.51	0.62	0.06	0.16
Program x L-carnitine		0.16	0.14	0.48	0.20	0.37	0.67	0.53	0.99	0.16	0.18	0.82	0.98

[1]Absolute liver weight, [2]Relative liver weight calculated as (LW/BW)*100

Table 3: *In vitro* lipogenesis of broiler breeder hens fed using either everyday (ED) or skip-a-day (SK) feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 1)

Program	L-Carnitine	Weeks 7	Weeks 14	Weeks 20 IVL[1] (µmoles/kg BW)	Weeks 22	Weeks 27
ED	None	78.70	81.90	87.70^{c}	111.00^{c}	67.20^{b}
ED	50 mg/kg	100.30	118.90	186.10^{b}	227.90^{b}	135.60^{a}
SK	None	144.30	155.70	146.70^{bc}	93.80^{c}	45.30^{b}
SK	50 mg/kg	179.20	165.80	393.50^{a}	302.40^{a}	192.60^{a}
SEM		7.10	7.50	12.80	9.00	8.90
Main effect means						
ED		89.50	100.40	136.90	169.50	101.40
SK		161.70	160.70	270.10	198.10	119.00
	None	111.50	118.80	117.20	102.40	56.20
	50 mg/kg	139.70	142.40	289.80	265.20	164.10
Source of variation		Probability				
Program		<0.01	<0.01	<0.01	0.12	0.32
L-Carnitine		0.09	0.12	<0.01	<0.01	<0.01
Program x l-carnitine		0.25	0.37	<0.01	0.01	0.03

[a,b]Means within a column without a common superscript differ significantly ($p \leq 0.05$), individual means were only tested when interaction was significant ($p \leq 0.05$), [1]*In vitro* lipogenesis, values are presented as µmoles sodium [2-^{14}C] acetate incorporated into hepatic lipids/kg body weight

pullets still had 12% larger livers than ED birds and RLW was also higher. L-carnitine resulted in a near significant ($p \leq 0.07$) increase in LW and a significant increase in RLW. Photostimulation occurred at 21 weeks of age. At 22 weeks of age, SK breeder pullet livers were no longer significantly larger than the livers from ED pullets. The difference in RLW was also no longer significant. L-carnitine supplementation, however, still resulted in an increase ($p \leq 0.05$) in LW and RLW compared to non-supplemented birds. At weeks 27, neither feeding program nor L-carnitine had any effect on LW or RLW. By 40 weeks there were still no effects on LW or RLW associated with feeding program but L-carnitine resulted in a 6.1 g increase ($p = 0.06$) in LW compared to non-supplemented hens. L-carnitine did not affect RLW at 40 weeks.

***In vitro* lipogenesis:** At 7 and 14 weeks of age no interactions were observed for IVL between feeding programs and L-carnitine supplementation (Table 3). At both 7 and 14 weeks of age, SK feeding resulted in a significant increase in IVL compared to ED. L-carnitine did not have a significant effect on IVL before 20 weeks. At 20, 22 and 27 weeks of age there was a significant interaction between feeding program and L-carnitine levels. At 20 weeks of age, pullets fed SK with 50 mg/kg L-carnitine had a higher level of IVL (393.5 µmoles/ kg BW) than any of the other treatment combinations. Pullets fed ED with 50 mg/ kg additional L-carnitine had higher IVL (186.1 µmoles/kg BW) than pullets fed ED without additional L-carnitine (87.7 µmoles/kg BW). The IVL for breeder pullets fed SK (146.7 µmoles/ kg BW) with diets without carnitine supplementation did not differ from the IVL from the ED pullets fed diets with carnitine supplementation.

At 22 weeks of age the SK pullets fed additional L-carnitine still had significantly higher rates of IVL than any other group. L-carnitine supplemented pullets fed ED had higher rates of IVL than either of the non-supplemented groups. The rate of IVL did not differ between the non-carnitine supplemented pullets fed either ED or SK at 22 weeks. At 27 weeks of age,

Table 4: Effect of Everyday (ED) or Skip-a-day (SK) feeding programs and L-carnitine (0 or 50 mg/kg) on liver composition of broiler breeders at 7, 20, 27 and 40 weeks of age (Experiment 1)

		Weeks 7				Weeks 20				Weeks 27				Weeks 40			
		Fat		Protein		Fat		Protein		Fat		Protein		Fat		Protein	
Program	L-Carnitine	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]
ED	None	8.9	0.44	59.5	2.90	11.0	1.36	59.70	7.37	19.80	3.35	59.10	9.90	27.80[b]	7.30[b]	50.00	13.30
ED	50 mg/kg	7.0	0.39	55.6	3.05	7.9	0.92	65.00	7.59	19.80	3.66	56.00	10.40	34.40[a]	10.20[a]	43.00	12.70
SK	None	9.8	0.59	58.9	3.54	14.2	1.97	59.50	8.28	21.70	4.04	58.40	10.80	30.30[ab]	7.20[b]	47.20	11.30
SK	50 mg/kg	6.8	0.38	63.4	3.60	9.8	1.39	58.50	8.25	20.80	3.71	58.50	10.50	26.30[b]	6.70[b]	45.30	11.50
SEM		0.6	0.04	1.8	0.07	0.6	0.08	1.20	0.15	0.70	0.16	1.00	0.20	1.20	0.30	1.20	0.40
Main effect means																	
ED		7.9	0.42	57.5	2.98	9.4	1.14	62.40	7.48	19.80	3.51	57.60	10.20	31.10	8.70	46.60	13.00
SK		8.3	0.49	61.2	3.56	12.0	1.67	59.10	8.27	21.20	3.87	58.50	10.60	28.30	7.00	46.30	11.40
	None	9.4	0.51	59.3	3.22	12.6	1.66	59.60	7.83	20.70	3.70	58.80	10.40	29.00	7.30	48.60	12.30
	50 mg/kg	6.9	0.39	59.4	3.32	8.9	1.15	61.80	7.92	20.30	3.69	57.30	10.40	30.30	8.40	44.20	12.10
Source of variation		Probability															
Program		0.69	0.28	0.34	0.01	0.02	0.01	0.22	0.03	0.34	0.26	0.65	0.23	0.25	0.01	0.90	0.07
L-Carnitine		0.03	0.08	0.96	0.48	0.01	0.01	0.43	0.78	0.76	0.97	0.47	0.99	0.58	0.06	0.08	0.83
Program x L-carnitine		0.60	0.26	0.27	0.75	0.54	0.65	0.26	0.72	0.73	0.32	0.44	0.33	0.03	0.01	0.29	0.61

[a,b]Means within a column without a common superscript differ significantly ($p \leq 0.05$), individual means were only tested when interaction was significant ($p \leq 0.05$), [1]Liver fat and protein expressed as a percentage of dry liver weight, [2]Total liver fat and protein in grams

L-carnitine added to diets of SK fed birds still resulted in the highest rate of IVL although this was no longer significantly greater than the ED fed L-carnitine supplemented group. Both ED and SK groups fed 50 mg/ kg L-carnitine had higher rates of IVL than ED or SK groups without additional L-carnitine. The rate of IVL did not differ between non-carnitine supplemented groups.

Liver composition: No interactions were observed for liver fat or protein content at 7, 20 or 27 weeks of age (Table 4). Feeding program did not alter liver fat % or total liver fat at 7 weeks. While liver protein % was not affected by feeding program, total liver protein was higher ($p \leq 0.05$) in SK (3.56 g) than in ED pullets (2.98 g) due to greater LW in SK pullets. L-carnitine supplementation resulted in lower liver fat % and marginally lower ($p = 0.08$) total liver fat. Protein % and total protein was not affected by L-carnitine. By 20 weeks of age, SK fed pullets (12.0%) had higher liver fat % than ED fed pullets (9.4%). Total liver fat was close to 50% greater in SK than ED fed pullets (1.67 vs 1.14 g). L-carnitine caused a decrease in liver fat% from 12.6-8.9% compared to non-supplemented pullets at 20 weeks. Total liver fat was also lower in carnitine supplemented birds. Protein % was not affected by feeding program but SK fed birds had higher total protein ($p \leq 0.05$) due to larger livers. L-carnitine did not affect liver protein % or total protein.

By week 27 neither feeding program nor L-carnitine resulted in any differences in liver fat % or total liver fat. Protein % and total protein were also unaffected. At 40 weeks there were significant ($p \leq 0.05$) interactions between feeding program and L-carnitine for fat % and total fat. L-carnitine supplemented birds fed ED had higher liver fat % than non-supplemented birds fed ED and supplemented birds fed SK. Total liver fat was higher in L-carnitine supplemented birds fed ED than all other groups. Protein % and total protein was not affected by feeding program or L-carnitine supplementation.

Heterophil to lymphocyte ratio: There were no interactions of feeding regimen and carnitine supplementation at any age for H/L ratios (Table 5). At 7 weeks of age SK feeding increased ($p \leq 0.05$) the H/L ratio from 0.17-0.26 compared to ED feeding. L-carnitine did not affect H/L ratio at 7 weeks. After 7 weeks of age, no effects of feeding program or L-carnitine on H/L ratio were significant.

Yolk IgY content: The overall mean IgY concentration was 1.14 mg/mL for 28 weeks old hens (Table 6). The overall mean total IgY per egg yolk was 16.17 mg. The interaction term was not significant for IgY content of egg yolks. There were no effects of feeding program or L-carnitine supplementation on IgY concentration or total IgY content of the egg yolk.

Experiment 2

Liver weight: No interactions were determined at any age between feeding program and L-carnitine for LW or RLW in Experiment 2 (Table 7). At 7 weeks of age LW was not affected by feeding program or L-carnitine but RLW was higher for SK pullets than for ED. No main effects were significant at 14 weeks of age. At 20 weeks of age SK feeding resulted in greater ($p \leq 0.05$) LW and RLW than ED feeding. Feeding supplemental L-carnitine produced an increased ($p \leq 0.05$) LW and RLW in pullets compared to feeding diets without carnitine supplementation. At weeks 22 and 27, no significant main effects were observed. At 40 weeks of age, hens

Table 5: Heterophil: lymphocyte ratios of broiler breeder hens fed using either Everyday (ED) or Skip-a-day (SK) feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 1)[1]

Program	L-Carnitine	Weeks 7	Weeks 14	Weeks 20	Weeks 22	Weeks 27	Weeks 40
ED	None	0.16	0.24	0.16	0.15	0.35	0.33
ED	50 mg/kg	0.18	0.17	0.15	0.27	0.44	0.31
SK	None	0.22	0.26	0.20	0.33	0.30	0.36
SK	50 mg/kg	0.29	0.21	0.21	0.42	0.45	0.63
SEM		0.02	0.04	0.01	0.05	0.10	0.06
Main effect means							
ED		0.17	0.21	0.16	0.21	0.40	0.32
SK		0.26	0.25	0.21	0.38	0.38	0.49
	None	0.19	0.26	0.18	0.24	0.33	0.34
	50 mg/kg	0.24	0.19	0.18	0.35	0.44	0.47
Source of variation		---------- Probability ----------					
Program		0.05	0.92	0.23	0.09	0.57	0.14
L-Carnitine		0.25	0.11	0.94	0.28	0.58	0.29
Program x L-carnitine		0.52	0.55	0.67	0.86	0.71	0.21

[1]Differential cell counts were obtained using an Abbott Cell-Dyn 3500 (Diamond Diagnostics Inc. Holliston, Massachusetts) automated hematology analyzer

Table 6: Levels of IgY in yolks from 28 weeks old broiler breeder hens fed using either everyday (ED) or skip-a-day (SK) feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 1)

		Yolk IgY[1,2]	

Program	L-Carnitine	Concentration (mg/mL)	Total (mg)
ED	None	1.43	20.46
ED	50 mg/kg	1.03	14.84
SK	None	1.07	15.02
SK	50 mg/kg	1.02	14.36
SEM		0.06	0.94
Main effect means			
ED		1.23	17.65
SK		1.05	14.69
	None	1.25	17.74
	50 mg/kg	1.03	14.60
Source of variation		--------- Probability ---------	
Program		0.17	0.12
L-Carnitine		0.10	0.10
Program x L-carnitine		0.19	0.19

[a,b]Means within a column without a common superscript differ significantly ($p \leq 0.05$), individual means were only tested when interaction was significant ($p \leq 0.05$), [1]IgY levels were determined using a chicken IgY quantitative ELISA kit. [2]Antibodies were extracted from egg yolk (4 eggs/hen) using a chloroform based method. The antibody levels were determined per milliliter of egg yolk which then was used to calculate total antibody per egg yolk

that were fed ED during the rearing period had heavier livers and also higher RLW than those that were fed SK. L-carnitine had no effect on LW or RLW at 40 weeks.

***In vitro* lipogenesis:** The rate of IVL was increased ($p \leq 0.05$) at 7 weeks of age by using SK rather than ED restriction feeding (Table 8). SK feeding increased IVL by approximately 55% at 14 weeks of age compared to ED. The IVL for SK pullets at 20 weeks of age was 62% higher than same age pullets fed ED. The effect of SK feeding was no longer evident at 27 weeks. L-carnitine had no significant effects on IVL at any age and no interactions were observed at any age.

Liver composition: No significant ($p \leq 0.05$) interactions were observed for fat or protein content of the liver at 7, 20 or 27 weeks of age (Table 9). At 7 weeks of age liver fat % was not affected by feeding program or L-carnitine. The total fat in the liver from 7 week old SK fed pullets was higher compared to ED pullets due to increased LW. Liver protein % was higher in ED (60.6%) fed pullets than SK (57.4%) fed pullets but total protein was similar. L-carnitine supplementation also resulted in higher liver protein % (60.4 vs 57.5%).

Differences in total protein were not significant. At week 20 there were no effects of feeding program or L-carnitine on liver fat % or total fat. Protein % was not influenced by the main effects but total liver protein was higher in SK (8.42 g) fed pullets compared to ED (7.46 g) fed pullets.

At 27 weeks of age, liver fat % and total liver fat was unaffected by feeding program or L-carnitine. Protein % was higher ($p \leq 0.05$) in ED hens than in SK hens but total liver protein did not differ. While, protein % was unaffected by L-carnitine, total protein was higher in non L-carnitine-supplemented birds. Fat % and total fat was not affected by feeding program or L-carnitine at 40 weeks of age. Protein % was also not affected. There was a significant interaction between feeding regimen and L-carnitine for total protein at 40 weeks. The total protein in livers was higher in non-supplemented ED fed hens (12.9 g) than in any other treatment group. The livers from L-carnitine supplemented ED (11.9 g) and SK (11.8 g) hens contained more total protein than SK breeder hens fed diets without carnitine supplementation (10.8 g).

Heterophil to lymphocyte ratio: At 7 weeks of age, there was a significant interaction for feeding regimen and carnitine for H/L ratio (Table 10). Non L-carnitine - supplemented pullets fed ED and L-carnitine supplemented pullets fed SK had higher H/L than L-carnitine supplemented ED pullets. After 7 weeks no

Table 7: Absolute and relative liver weights of broiler breeder hens fed using either everyday (ED) or skip-a-day (SK) feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 2)

		Weeks 7		Weeks 14		Weeks 20		Weeks 22		Weeks 27		Weeks 40	
		LW[1]	RLW[2]	LW[1]	RLW[2]	LW[1]	RLW[2]	LW[1]	RLW[2]	LW[1]	RLW[2]	LW[1]	RLW[2]
Program	L-Carnitine	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
ED	None	23.40	1.99	35.30	1.96	46.10	1.76	52.70	1.82	72.9	1.94	85.30	2.08
ED	50 mg/kg	23.30	1.86	32.40	1.85	47.20	1.91	57.30	1.98	69.6	1.87	78.10	1.92
SK	None	24.80	2.07	33.60	2.02	48.20	1.84	52.70	1.87	71.1	1.94	69.90	1.82
SK	50 mg/kg	24.10	2.08	31.60	1.93	53.10	2.08	54.90	1.99	65.8	1.84	76.90	1.91
SEM		0.40	0.02	0.70	0.03	0.80	0.03	1.40	0.06	1.1	0.04	1.90	0.04
Main effect means													
ED		23.30	1.92	33.90	1.91	46.70	1.83	54.90	1.89	71.2	1.90	81.70	2.00
SK		24.50	2.07	32.60	1.98	50.70	1.96	53.80	1.93	68.5	1.89	73.40	1.86
	None	24.10	2.03	34.50	1.99	47.20	1.80	52.70	1.85	72.0	1.94	77.60	1.95
	50 mg/kg	23.70	1.97	32.00	1.89	50.20	1.99	56.10	1.99	67.7	1.85	77.50	1.91
Source of variation		Probability											
Program		0.24	<0.01	0.31	0.27	0.02	0.06	0.69	0.76	0.23	0.88	0.04	0.09
L-Carnitine		0.48	0.25	0.06	0.12	0.08	<0.01	0.25	0.26	0.07	0.28	0.99	0.68
Program x L-carnitine		0.46	0.16	0.72	0.79	0.26	0.50	0.66	0.86	0.66	0.84	0.08	0.14

[1]Absolute liver weight, [2]Relative liver weight calculated as (LW/BW)*100

Table 8: *In vitro* lipogenesis of broiler breeder hens fed using either everyday (ED) or skip-a-day (SK) feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 2)

Program	L-Carnitine	Weeks 7	Weeks 14	Weeks 20 IVL[1] (µmoles/kg BW)	Weeks 22	Weeks 27
ED	None	132.90	105.10	104.00	181.50	121.7
ED	50 mg/kg	136.10	98.30	104.40	144.20	135.2
SK	None	183.40	144.50	147.40	213.60	103.6
SK	50 mg/kg	162.90	169.30	189.00	230.30	124.1
SEM		6.70	5.80	5.90	11.20	9.2
Main effect means						
ED		134.50	101.70	104.00	162.80	128.5
SK		173.20	156.90	168.20	222.00	113.8
	None	158.20	124.80	125.70	197.60	112.7
	50 mg/kg	149.50	133.80	146.50	187.20	129.7
Source of variation		Probability				
Program		<0.01	<0.01	<0.01	<0.01	0.16
L-Carnitine		0.52	0.44	0.08	0.65	0.11
Program x L-carnitine		0.38	0.18	0.08	0.23	0.74

[1]*In vitro* lipogenesis, values are presented as µmoles [2-^{14}C] sodium acetate incorporated into hepatic lipids/kg body weight

Table 9: Effect of Everyday (ED) or Skip-a-day (SK) feeding programs and L-carnitine (0 or 50 mg/kg) on liver composition of broiler breeders at 7, 20, 27 and 40 weeks of age (Experiment 2)

		Weeks 7				Weeks 20				Weeks 27				Weeks 40			
		Fat		Protein		Fat		Protein		Fat		Protein		Fat		Protein	
Program	L-Carnitine	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]	%[1]	g[2]
ED	None	5.0	0.31	59.2	3.66	7.2	0.85	63.2	7.52	18.4	3.64	55.8	10.9	33.3	9.29	49.1	12.9[a]
ED	50 mg/kg	4.7	0.27	61.9	3.45	7.7	0.91	62.5	7.40	19.1	3.76	54.6	10.7	28.2	6.81	51.5	11.9[b]
SK	None	5.5	0.44	55.8	4.76	9.8	1.26	63.0	8.03	15.7	3.03	59.3	11.2	25.8	5.33	54.7	10.8[c]
SK	50 mg/kg	6.4	0.41	58.9	3.73	6.9	0.95	64.0	8.82	15.4	2.78	59.1	10.5	30.9	7.23	53.1	11.8[b]
SEM		0.5	0.03	0.5	0.27	0.7	0.09	0.8	0.11	1.1	0.24	0.9	0.1	2.1	0.65	1.3	0.1
Main effect means																	
ED		4.8	0.28	60.6	3.56	7.4	0.88	62.8	7.46	18.8	3.70	55.2	10.8	30.8	8.05	50.3	12.4
SK		6.0	0.42	57.4	4.25	8.4	1.11	63.5	8.42	15.5	2.91	59.2	10.9	28.3	6.28	53.8	11.3
	None	5.2	0.37	57.5	4.21	8.5	1.06	63.1	7.77	17.1	3.33	57.5	11.1	29.5	7.31	51.8	11.8
	50 mg/kg	5.6	0.33	60.4	3.59	7.3	0.93	63.2	8.11	17.2	3.27	56.9	10.6	29.6	7.02	52.3	11.8
Source of variation		Probability															
Program		0.26	0.04	0.01	0.22	0.48	0.21	0.68	0.01	0.15	0.12	0.03	0.73	0.56	0.18	0.18	0.01
L-Carnitine		0.76	0.55	0.01	0.27	0.38	0.49	0.92	0.14	0.93	0.91	0.71	0.05	0.99	0.82	0.84	0.93
Program x L-carnitine		0.55	0.90	0.85	0.47	0.22	0.29	0.59	0.06	0.83	0.71	0.78	0.30	0.22	0.10	0.47	0.01

[a,b]Means within a column without a common superscript differ significantly ($p \leq 0.05$), individual means were only tested when interaction was significant ($p \leq 0.05$), [1]Liver fat and protein expressed as a percentage of dry liver weight, [2]Total liver fat and protein in grams

other significant interactions were observed. At 14 weeks of age, pullets fed ED (0.25) had lower ($p \leq 0.05$) H/L ratios than those fed SK (0.69). The difference in H/L was similar and still significant at 20 weeks of age. Differences in the H/L ratio between ED and SK fed pullets were no longer detected at 22, 27 and 40 weeks of age. L-carnitine supplementation did not affect plasma H/L ratio at any age.

Table 10: Heterophil: lymphocyte ratios of broiler breeder hens fed using either everyday (ED) or skip-a-day (SK) feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 2)[1]

Program	L-Carnitine	Weeks 7	Weeks 14	Weeks 20	Weeks 22	Weeks 27	Weeks 40
ED	None	0.20[a]	0.26	0.31	0.23	0.35	0.30
ED	50 mg/kg	0.12[b]	0.24	0.27	0.31	0.32	0.22
SK	None	0.14[ab]	0.66	0.52	0.50	0.44	0.44
SK	50 mg/kg	0.23[a]	0.73	0.75	0.35	0.30	0.35
SEM		0.01	0.05	0.06	0.06	0.05	0.04
Main effect means							
ED		0.16	0.25	0.29	0.27	0.34	0.26
SK		0.18	0.69	0.63	0.42	0.37	0.39
	None	0.17	0.46	0.41	0.37	0.40	0.37
	50 mg/kg	0.17	0.48	0.51	0.33	0.31	0.29
Source of variation		---------- Probability ----------					
Program		0.28	<0.01	0.01	0.21	0.73	0.08
L-Carnitine		0.79	0.80	0.46	0.77	0.36	0.26
Program x L-carnitine		<0.01	0.66	0.28	0.33	0.55	0.98

[a,b]Means within a column without a common superscript differ significantly ($p \leq 0.05$), individual means were only tested when interaction was significant ($p \leq 0.05$), [1]Differential cell counts were obtained using an Abbott Cell-Dyn 3500 (Diamond Diagnostics Inc. Holliston, Massachusetts) automated hematology analyzer

DISCUSSION

While, there is literature that compares hepatic lipid metabolism (Leveille, 1966; Yeh and Leveille, 1971, 1972; Rosebrough and Steel, 1985; Rosebrough, 2000; Richards *et al*., 2003) and H/L ratios (Hocking *et al*., 1993, 2001) of *ad libitum* and feed restricted birds, less information exists regarding differences between types of feed restriction programs. The effect of L-carnitine on hepatic lipogenesis and stress related parameters has also been poorly defined.

Skip-a-day feeding generally resulted in higher LW and RLW during the rearing period regardless of grower diet density, but dissipated after photostimulation. The SK feeding program was used through 5% production. The physiological changes that occur due to photostimulation apparently influence the effects of SK feeding on LW.

In two experiments, Muiruri *et al*. (1975) demonstrated that RLW was substantially higher in meal-fed chicks after feeding than it was in *ad libitum* fed chicks. Muiruri and researchers collected livers 1 h after feeding chicks which was the same time frame for liver collections of breeder pullets in Experiment 1 and 2. Rosebrough and Steel (1985) also reported an increase in RLW of fasted and refed broilers compared to *ad libitum* fed birds. Although *ad libitum* feeding of breeder pullets was not compared to SK and ED feeding regimens in the research reported herein, the results show that different types of feed restriction programs can have an influence on LW. de Beer and Coon (2007) and de Beer *et al*. (2007) previously reported that breeder pullets fed ED usually consumed their available feed in <1 h and had empty crops at 12 h after feeding. The researcher reported that breeder pullets fed a SK regimen with twice the amount of feed required more time to consume the feed and the crop would be empty 24 h after feeding. The physiological responses to fasting, such as increased glucagon and corticosterone and reduced plasma triglyceride, occurred at times coincidental with crop emptying in both ED and SK birds (de Beer *et al*., 2008). The previous research by de Beer *et al*. (2008) indicates that the true length of fast was approximately 12 h for ED breeder pullets and 24 h for SK breeder pullets. It is likely that the length of the fast and the size of the subsequent meal play a role in changing the LW.

Muiruri *et al*. (1975) suggested that much of the increased LW found in chicks fed after being feed restricted was glycogen and water rather than lipid. Leveille (1966) found a near two-fold increase in liver glycogen concentration after refeeding of meal-fed chicks. In contrast to the speculation of Muiruri *et al*. (1975), results from experiment 1 and 2 and those of other groups (Akiba *et al*., 1983; Katanbaf *et al*., 1989a) show that total fat content of the liver was increased after a meal by SK feeding compared to ED feeding. Certainly, some part of the increased LW after a meal is the result of enhanced glycogen synthesis and accumulation, but the results of these trials suggest that liver lipid also increases after a meal. The lipids present after a meal are packaged and transported out of the liver as very low density lipoproteins and used to help supply the energy demand of the bird when feed is not available. By manufacturing more lipid in the fed state, the SK birds are able to prepare for the fasting period.

Interestingly, the effects of SK feeding on liver size and composition were diminished after photostimulation. A marked increase in liver size during the pre-lay and early lay periods has been noted (Yu and Marquardt, 1974). In Experiment 1 there was a 37% increase in overall mean LW from 20-27 weeks of age. The LW of ED birds increased 43% in this time, while that of SK birds increased only 33%, despite SK feeding continuing up to 5% production. In Experiment 2 the overall mean increase in LW was 43%. In ED birds the increase was 52% while in SK birds it was only 35%. The physiological changes that occur after photostimulation and the onset of production alter the effects of feeding regimens.

Liver weight and RLW were increased by feeding L-carnitine to pullets during the rearing period in Experiment 1 but less so in Experiment 2. Lien and Horng (2001) found that liver weight was not affected by L-carnitine supplementation at 160 mg/kg in broiler chickens. Kita *et al.* (2002) tested various levels of L-carnitine addition in chicks from 0-2000 mg/kg and found no influence on liver weight. L-carnitine reduced the liver fat % and total liver fat at 7 and 20 weeks in Experiment 1 but did not have the same effect in Experiment 2. The effects of L-carnitine on LW and liver composition were not consistent in these two experiments. The lower density grower diet in Experiment 2 may have altered the effects of L-carnitine supplementation. Du *et al.* (2005) reported that L-carnitine reduced liver lipid content in laying hens after 7 weeks of supplementation at 50 or 100 mg/kg of diet. Dietary L-carnitine supplements have also been found to reduce the liver lipid content of certain fish species (Santulli and d'Amelio, 1986; Burtle and Liu, 1994).

The liver is the major site of fatty acid synthesis in the chicken (O'Hea and Leveille, 1969; Hermier, 1997). The *in vitro* lipogenesis data represents the incorporation of sodium [2-^{14}C] acetate into hepatic lipids/kg body weight in liver slices taken one hour after feeding. Skip-a-day feeding resulted in an increased rate of IVL over ED birds during the rearing period in both Experiment 1 and 2. The effects of SK feeding up to 5% production were not carried over to 27 weeks of age. Other authors (Yeh and Leveille, 1971, 1972; Rosebrough and Steele, 1985; Rosebrough *et al.*, 1988; Rosebrough, 2000) have also demonstrated the tendency of intermittent feeding to increase the rate of hepatic lipogenesis. Lipogenesis is increased up to 50-fold after feeding of previously fasted chicks (Muiruri *et al.*, 1975). The results reported herein demonstrate that the magnitude of the lipogenesis increase is dependant on the severity of the fast and perhaps the size of the subsequent meal.

The mechanisms by which feed restriction alter the rate of lipogenesis include, increased hepatic Acetyl-CoA Carboxylase (ACC) mRNA expression (Hillgartner *et al.*, 1996; Richards *et al.*, 2003), increased availability of NADPH reducing equivalents from the Malic Enzyme (ME) reaction and increased circulating triiodothyronine (T_3) levels (Rosebrough, 2000). The rate limiting step in lipogenesis occurs at ACC, which is regulated in the short-term by covalent modification (phosphorylation) and allosteric control by citrate (Hillgartner *et al.*, 1995, 1996). In the longer term, there is transcriptional regulation of ACC, which is mediated by insulin, glucagon, T_3 and glucose (Hillgartner *et al.*, 1996). Richards *et al.* (2003) found that feed restriction also increased the expression of sterol regulatory element binding protein-1, ATP-citrate lyase, fatty acid synthase, ME and stearoyl-CoA (Δ9) desaturase-1 genes compared to *ad libitum* fed birds. Together, several substrate-related, endocrine and transcriptional effects control the rate of hepatic lipid synthesis.

L-carnitine and its system of transport enzymes control the entry of long-chain fatty acids into the mitochondria for ß-oxidation. The inhibition of Carnitine Palmitoyl Transferase-1 (CPT-1) by malonyl-coenzyme (CoA), allows it to control the rate of β-oxidation and regulate the deposition or oxidation of fatty acids (Zammit, 1999). Although, the crucial role of L-carnitine in lipid transport is well understood its effects on the lipogenic pathway are still poorly defined.

In experiment 1, SK feeding and L-carnitine supplementation interacted to increase IVL at 20, 22 and 27 weeks of age. L-carnitine also caused near significant increases in IVL at 7 and 14 weeks of age in experiment 1 and at 20 weeks in experiment 2. Taken together, this data would suggest that added dietary L-carnitine has a tendency to increase hepatic lipogenesis and that under conditions of SK feeding, this increase is magnified. The mechanisms by which L-carnitine could affect lipogenesis have not been definitively identified.

Acetyl-CoA (the immediate substrate for fatty acid synthesis) and palmitoyl-CoA (a product of lipogenesis) can be reversibly converted to acetylcarnitine and palmitoylcarnitine, respectively, via the reaction of carnitine acyltransferase enzymes. The concentration of carnitine or the activities of carnitine acetyltransferase and CPT-1 and CPT-2 could affect *de novo* lipogenesis. L-carnitine supplementation can increase circulating levels of T_3 (Buyse *et al.*, 2001). Transcriptional regulation of ACC (Hillgartner *et al.*, 1996), ME and fatty acid synthase (Roncero and Goodridge, 1992) may be mediated by T_3. The inactive pentose phosphate pathway in birds makes ME the primary source of reducing equivalents for synthetic reactions in the form of NADPH. Roncero and Goodridge (1992) showed that addition of T_3 to chick hepatocytes resulted in a 30-40 fold increase in ME activity. They also demonstrated that addition of T_3 resulted in increased fatty acid synthase mRNA in chick hepatocytes. Interestingly, they found that adding L-carnitine to the incubation medium increased the stimulatory effect of T_3 on ME and fatty acid synthase mRNA expression.

Carboxylation of acetyl-CoA in hepatocytes to form malonyl-CoA is the first step of lipogenesis, in a reaction catalyzed by ACC. According to Xu *et al.* (2003), an increase in dietary L-carnitine resulted in increased carnitine concentrations in the liver, which led to increased activity of carnitine acetyltransferase enzymes and accelerated the transportation of acetyl-CoA from mitochondria to cytosol. The rate of synthesis of malonyl-CoA increases with the increase of acetyl-CoA concentration in cytosol (Dyck *et al.*, 1998).

Richter *et al.* (1996) reported that ME activity and the rate of fatty acid synthesis was not affected by L-carnitine in adult female rats. Dias *et al.* (2001) found in seabass,

that hepatic ACC activity was increased three-fold when L-carnitine was added to their diets. Craig and Gatlin (1998) found that ACC activity was four- to five-fold higher in juvenile red drum fish when L-carnitine was added to their diets. Marquis *et al.* (1968) found that in the absence of citrate, L-carnitine depressed lipogenesis in rat livers, while, in the presence of citrate, lipogenesis was stimulated by increasing L-carnitine. When ACC activity is stimulated by the presence of citrate and cytoplasmic acetyl-CoA is high, L-carnitine may increase the rate of lipogenesis by removal of long-chain fatty acids (feedback inhibitor of ACC) from the cytoplasm.

In order to ensure the well being (Katanbaf, 1989b) and maximum performance (Katanbaf, 1989c) of broiler breeders, feed restriction is essential. The most severe feed restriction occurs during the rearing period and is associated with behaviors indicative of stress, hunger and frustration (Savory *et al.*, 1992; Hocking *et al.*, 1993, 1996). Gross and Siegel (1983) evaluated the use of H/L ratio as a measure of stress in chickens. They concluded that H/L was a good measure of the chicken's perception of stress and could be used to compare groups exposed to various types of stress. Analysis of H/L ratio data from our experiments show that early in the rearing period SK feeding increases the H/L ratio. In experiment 1 the elevated H/L ratio was only evident at 7 weeks, while in Experiment 2 this effect lasted up to 20 weeks of age. L-carnitine did not affect H/L at any time in either experiment. The H/L ratio at 14 and 20 weeks in Experiment 2 was markedly elevated. The use of lower density diets with increased feed intakes was expected to attenuate some of the stress related effects of feed restriction. The use of a lower density grower diet, however, did not improve H/L ratios. The energy and protein content of the low density diet was 9 and 7% less than the standard diet, respectively. Perhaps these small differences were not enough to benefit stress related parameters.

Gross and Siegel (1986) demonstrated that birds were quickly able to adapt to periods of fasting and that elevated H/L quickly returned to normal. They found that the H/L ratio of 4 week old broilers was dramatically increased during a 48 h fasting period. If the same birds were exposed to a similar fast a week later it was found that the H/L ratio was once again elevated but not as dramatically as during the first fasting period. Zulkifli *et al.* (1993) showed that the adaptation to 60% feed restriction took approximately 12-16 days as measured by H/L ratio. The same authors found that release from feed restriction also elicited a stress response. Similar to the findings in experiment 2, Hocking *et al.* (1993) found that in broiler breeders the H/L ratio of restricted pullets was elevated at 8, 12 and 16 weeks of age as compared to full-fed control groups. Unlike Maxwell *et al.* (1990), who found no differences in H/L ratio of *ad libitum* fed and restricted birds, but observed elevated basophil numbers, our studies found no increase in basophil numbers.

Maternal immunoglobulin transfer occurs via the egg and provides the chick with a valuable form of resistance against certain commonly encountered pathogens. In eggs, IgY, the primary maternal antibody, is present predominantly in the yolk (Leslie and Clem, 1969). Mast *et al.* (2000) found that dietary L-carnitine supplementation increased serum antigen specific IgY response and total Ig response in broilers. They suggested that L-carnitine supplementation improves specific humoral immune responses after immunization with bovine serum albumin. Bollen and Hau (1997) showed that an increase of serum IgY, resulted in increased transfer of IgY to the egg. Hamal *et al.* (2006) estimated the % dam-to-chick plasma transfer of IgY to be about 30%. Based on these findings it is possible that L-carnitine may enhance protective immunity after vaccination. Deng *et al.* (2006) also concluded that short-term supplementation of dietary L-carnitine after hatching enhanced subsequent humoral immunity in Leghorn-type chickens. They found that supplementing L-carnitine to the diet for the first four weeks resulted in increased primary antibody response to sheep red blood cell immunization at 12 weeks.

The present research demonstrated no effect of feeding regimen or L-carnitine supplementation on yolk IgY levels. The breeder hens in the present experiment were not immunized with bovine serum albumin prior to sampling and non-specific IgY responses were examined in the breeder hens. The overall mean yolk IgY concentration (1.14 mg/mL) was almost identical to that found by Hamal *et al.* (2006) in Line 1 of the two meat type breeder lines they tested (1.15 mg/mL). Based on research reported herein there is no benefit to maternal antibody transfer under normal circumstances as a result of L-carnitine supplementation.

The experiments reported herein show that both feeding regimens and L-carnitine are able to mediate changes in hepatic lipid metabolism. Skip-a-day feeding programs clearly elevate hepatic lipogenesis during the rearing period in broiler breeders. The ultimate fate of the lipid produced may depend on other factors, but there is no dramatic lipid accumulation over time in the liver of SK fed birds above ED fed birds. L-carnitine also tended to increase hepatic lipid synthesis. Yolk IgY content and H/L ratios were not affected by L-carnitine but SK feeding elevated H/L ratios during the early rearing period. Further studies regarding the ultimate consequences of elevated lipogenesis in SK fed pullets and the effects of L-carnitine on lipogenesis would be of great interest.

ACKNOWLEDGMENTS

The authors would like to thank Cobb-Vantress Inc. and Lonza Inc. for financial support for these experiments.

The authors would also like to thank the staff at the University of Arkansas Poultry Research Farm for assistance during the trials.

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Abbreviation key: ACC = Acetyl CoA Carboxylase, CPT-1 = Carnitine Palmitoyl Transferase-1, ED = Everyday fed pullets, H/L = Heterophil to Lymphocyte ratio, IVL = *In vitro* Lipogenesis, LW = Liver Weight, ME = Malic Enzyme, RLW = Relative Liver Weight, SK = Skip-a-day fed pullets, T_3 = Triiodothyronine

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