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## The Effect of Different Feed Restriction Programs and Dietary L-Carnitine Supplementation on Reproductive Performance, Efficiency, Frame Size and Uniformity in Broiler Breeder Hens

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**Abstract:** Two experiments were conducted to determine the effect of Everyday (ED) or Skip-a-day (SK) feed restriction programs and L-carnitine supplementation on breeder reproductive performance. In Experiment 1 a 2 x 2 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 day 1 and lasted throughout the 45 week experimental period. SK feeding programs were implemented from 28 days of age to 5% production. Feed allocation was adjusted to ensure equal BW between groups. At 21 weeks, 60 pullets from each treatment combination were housed individually. Feeding ED improved the feed conversion ratio by 0.24 units for 21 week pullets, resulted in 3 days earlier attainment of Sexual Maturity (SM), produced 4.6 more total eggs and 5.0 more settable eggs than SK fed pullets. Uniformity was less for ED fed pullets (2.07 higher CV). Egg size was increased by 1.16g with dietary L-carnitine. Body composition was not affected by either feeding regimen or L-carnitine. In Experiment 2, the same effects were tested but a low density grower diet was used from 4-18 weeks. L-carnitine was supplemented from day 1 and SK programs began at day 28 and extended to 5% production. Feed allocation was adjusted to maintain equal BW and 80 pullets per treatment were individually housed at 21 weeks. L-carnitine and ED feeding through 21 wk improved the FCR by 0.06 and 0.12 units, respectively. Feeding ED resulted in 5.8 days earlier SM, 4.7 more total eggs and 4.4 more settable eggs than SK. Uniformity was not affected by feeding regimen or L-carnitine. Carcass fat was reduced and carcass ash was increased by L-carnitine supplementation at 22 weeks. It was concluded that ED fed breeders are more productive than SK fed breeders primarily because of earlier SM. ED fed breeders are more efficient than SK breeder pullets because there are less nutrients wasted for tissue replenishment. Feeding breeder pullets ED with low energy density diets helped eliminate uniformity differences for pullets fed ED and SK feeding regimens. Breeders fed L-carnitine during 21 wk rearing period improved the FCR by 0.06 units for both Experiment 1 and 2. While, the effect of L-carnitine on total egg production was not significant, L-carnitine supplemented birds produced 3.9 and 2.7 more total eggs at 45 weeks than non-supplemented birds in Experiments 1 and 2 respectively. The consistency of the results and the associated p-values ( $p = 0.12$ ;  $p = 0.13$ ) for total egg production in the two experiments suggest that L-carnitine may have some beneficial effects on egg production. Breeders fed carnitine also showed significant increases in EW in Experiment 1 and near significant ( $p = 0.13$ ) increases in EW for the second experiment. Carnitine was unable to attenuate the negative effects of SK feeding associated with the lengthy fasting periods.

**Key words:** Breeder, feed restriction, L-carnitine, performance

### INTRODUCTION

Generations of selection have resulted in increased body weight, feed efficiency, appetite and, unfortunately, increased fat deposition in chickens (Siegel, 1984). This tendency toward fat deposition results in undesirable reproductive complications in breeder stock (McDaniel *et al.*, 1981). Most producers employ some form of feed restriction in order to improve reproductive performance of their flock. Many of these restriction programs involve skip-a-day feeding regimens. The positive effects of feed restriction include reduced body weight, delays of Sexual Maturity (SM), increased egg production, reduction in number of unsettable eggs and increased liveability during the laying period (McDaniel *et al.*, 1981; Pearson and Herron, 1982; Pym and Dillon, 1974; Siegel and

Dunnington, 1985; Katanbaf *et al.*, 1989a, b, c). L-carnitine is endogenously synthesized from methionine and lysine. It plays a major role in the mitochondrial oxidation of long-chain fatty acids to produce energy via  $\beta$ -oxidation and oxidative phosphorylation (Borum, 1983). Methionine and lysine are generally the first and second limiting amino acids in broiler breeder diets (Harms, 1992) and so endogenous production of L-carnitine may not be sufficient to support maximal fatty acid transport into the mitochondria under certain conditions. Several studies in avian species demonstrated a growth improvement by feeding additional dietary L-carnitine (Rabie *et al.*, 1997a, b). Research has shown (Kita *et al.*, 2002) that the improvement in body weight gain caused by dietary

L-carnitine supplementation might be partially controlled by the change in plasma insulin-like growth factor-I (IGF-I) concentrations. IGF-I is a potent growth stimulator.

Chiodi *et al.* (1994) reported that the embryo contains high levels of carnitine in the early stages of development. Rabie *et al.* (1997c) showed that egg yolks from L-carnitine supplemented hens contained higher levels of carnitine than control hens. The activity of carnitine palmitoyl transferase-1 (CPT-1) in the yolk sac membrane is the highest yet reported for any animal tissue (Murray *et al.*, 1999). The young chick is heavily reliant on  $\beta$ -oxidation of fatty acids for energy. CPT-1 in the yolk sac membrane is resistant to inhibition by malonyl-CoA. This fact, along with CPT-1's extremely high activity in the yolk sac membrane, is indicative of the exceptionally high capacity for  $\beta$ -oxidation in the developing embryo and young chick.

Due to the stress related side effects of feed restriction (for review, see: Rosales, 1994), efforts have been made to identify feeding regimens that can reduce body weight and delay SM while, not necessarily causing great hunger. Qualitative feed restriction works using diet formulations with reduced nutrient densities. This dilution of the diet allows for greater quantities of feed and extended eating time, which may in turn reduce the frustration of the feeding motivation and the feelings of hunger. Low density diets may be fed in sufficient quantities to fill the gastro-intestinal tract and promote feelings of satiety (Whittaker *et al.*, 1998, 1999) while maintaining acceptable body weight.

In the first experiment the aim was to determine the effects of Everyday (ED) vs Skip-a-day (SK) feeding programs and L-carnitine supplementation on the reproductive performance and efficiency of broiler breeder hens using standard broiler breeder grower diets. In the second experiment the aim was to determine the effects of feeding programs and L-carnitine when low density diets were used during the rearing phase. A further aim was to determine if SK fed breeders benefit more from L-carnitine supplementation than ED fed breeders. It was hypothesized that the need for mobilization and oxidation of large amounts of fatty acids during the fasting period would be beneficially influenced by L-carnitine supplementation.

## MATERIALS AND METHODS

**Experiment 1:** Stock and management. A total of 700 day old Cobb 500 broiler breeder pullets were randomly assigned to 20 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 light schedules 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. Forty pullets per treatment were weighed individually at 4, 7, 14 and 20 weeks of age to obtain estimates of flock uniformity. Shank and keel lengths were measured on 20 birds per treatment at various ages using a vernier caliper as described by Leeson and Summers (1984).

Pullets were photostimulated with 13 hours of light at 21 weeks at which time 60 representative birds from each treatment were housed individually in breeder cages. Each cage had an individual feeder and nipple drinker system. Photoperiod was extended by one hour per week each week until 16 h of light was reached. From 21 weeks of age all hens were weighed individually every week until week 33 and then monthly until the end of the experiment.

**Experimental design:** A 2 x 2 factorial design was used. The main effects were L-carnitine supplementation (0 or 50 mg/kg) and feeding program (ED or SK). All pens were fed ad libitum for the first ten days. From ten to 28 days all pens were fed restricted amounts of feed everyday. At 28 days of age different feed restriction programs were implemented. Groups were fed using either ED or SK programs from 28 days until 5% production. 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 body weights 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 body weights throughout the experiment, the effect of body weight on performance was minimized as a source of variation. Feed allocation after housing (21 weeks) for Experiment 1 is shown in Table 2. Maximum feed allocation was 144 g/bird which was 420 kcal ME/hen/day (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 week 32 and continued until week 41, at which time breeders were being fed 136 g per bird per day. Mortality was recorded on a daily basis throughout the experimental period.

**Reproductive performance:** Egg production was recorded daily and egg weights were measured twice weekly, throughout the production period. Each of the first three eggs from every hen was individually weighed

Table 1: Composition of diets (%) and calculated contents (%) used in both Experiment 1 and 2<sup>1</sup>

Ingredient	Starter	Grower Experiment 1 (Std)	Grower Experiment 2 (Low density)	Prebreeder	Breeder I
Corn, Yellow	61.40	61.41	53.87	67.78	66.93
Soybean Meal	26.83	15.44	13.44	20.37	22.16
Wheat Midds	7.71	19.04	28.75	7.09	
CDP <sup>2</sup>	1.83	1.74	1.77	1.73	1.80
Limestone	0.69	0.72	0.71	1.62	6.36
Termin-8	0.30	0.30	0.30	0.30	0.30
Salt 96+%	0.29	0.31	0.31	0.31	0.08
Poultry fat	0.25	0.50	0.25	0.25	1.67
Microsystem Soy	0.25	0.25	0.25	0.25	0.25
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 <sup>3</sup>	0.06	0.06	0.06	0.06	0.06
Copper sulphate	0.05	0.05	0.05	0.05	0.05
Vitamin premix <sup>4</sup>	0.04	0.04	0.04	0.04	0.05
Ethoxyquin	0.01				
<b>Calculated analysis (%)</b>					
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
Calcium	0.95	0.90	0.90	1.25	3.10
Total phosphorous	0.74	0.75	0.77	0.69	0.64
Avail. phosphorous	0.45	0.45	0.45	0.42	0.41
<b>Analyzed</b>					
ME, kcal/kg <sup>5</sup>		2965	2721		
CP (%) <sup>6, 7</sup>	18.71	15.28		16.59	16.21
CP (%) <sup>6, 8</sup>	18.59		14.30	16.46	15.81

<sup>1</sup>L-carnitine was supplemented at 50 mg/kg into these diets for the appropriate treatments groups; <sup>2</sup>Calcium diphosphate; <sup>3</sup>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; <sup>4</sup>Vitamin mix provided per kilogram of complete diet: vitamin A, 30,800 IU; vitamin D<sub>3</sub>, 9,250 IU; vitamin E, 153.9 IU; vitamin B<sub>12</sub>, 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; <sup>5</sup>Analyzed values for Experiment 2; <sup>6</sup>Corrected to 90% DM; <sup>7</sup>Analyzed protein values for Experiment 1; <sup>8</sup>Analyzed protein values for Experiment 2

Table 2: Feed allocation schedule for broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 1)

Age (weeks)	ED <sup>1</sup>	SK <sup>2</sup>	ED + 50 mg/kg L-carnitine	SK + 50 mg/kg L-carnitine
Feed (g/bird/day)				
22	111	113	111	112
23	113	116	113	115
24	118	120	118	120
25	122	122	122	122
26	131	131	131	131
27	140	140	140	140
28	144	144	144	144
29	144	144	144	144
30	144	144	144	144
31	144	144	144	144
32	143	143	143	143
33	142	142	142	142
34	142	142	142	142
35	141	141	141	141
36	141	141	141	141
37	140	140	140	140
38	139	139	139	139
39	138	138	138	138
40	137	137	137	137
41	136	136	136	136
42	136	136	136	136
43	135	135	135	135
44	134	134	134	134
45	133	133	133	133

<sup>1</sup> Everyday; <sup>2</sup> Skip-a-day

to determine early egg weight. Relative egg weight was calculated as the mean egg weight divided by the BW at housing. All soft shelled, double yolk and cracked eggs were recorded. Settable eggs were defined as eggs weighing more than 50 g with a hard intact shell and one yolk. Age at first egg (sexual maturity) and peak were recorded. Peak was determined as a five day rolling average. The first three eggs produced by each hen were weighed to gauge early Egg Weight (EW). Subsequently, two eggs from each hen were weighed each week to determine overall mean EW for the entire production period. Relative EW was calculated as: (overall mean EW / 21 weeks BW)\*100. Feed conversion to Body Weight (BW) at 21 weeks of age was calculated in terms of total feed g/kg BW, g protein per kg BW and kcal/g BW. Efficiency of feed conversion to eggs at 45 weeks of age was calculated as total feed intake (g) per egg, total protein (g) per egg and total energy (kcal) per egg.

All hens were artificially inseminated at week 32, 36, 40 and 44. One week's worth of eggs was collected from each hen to determine fertility and hatchability at each interval. Semen was collected from same age, separately reared broiler breeder males using the abdominal massage method as described by Burrows and Quinn (1937). Semen was pooled and sperm cell concentration determined using an IMV Micro-Reader<sup>2</sup>, using an optical density of 381 nm (King and Donoghue, 2000). Semen was diluted to  $5 \times 10^7$  sperm/50  $\mu$ L using Beltsville Poultry Semen Extender to ensure all hens were inseminated with the same number and volume of sperm cells. Each hen was inseminated with 50  $\mu$ L of diluted semen. Semen was diluted to  $5 \times 10^7$  sperm/50  $\mu$ L prior to insemination to allow detection of variation in fertility levels. While this low number of sperm cells does not produce exceptionally high fertility levels, by not filling the sperm host glands in the hen it allows for differences among treatments to be determined. All eggs were collected for one week after each insemination and set in Jamesway<sup>3</sup> machines for incubation and hatching. All un-hatched eggs were broken out to determine fertility status. Fertility was calculated as the number of fertile eggs per 100 eggs set. Hatchability was calculated as the number of chicks hatched per 100 eggs set and hatchability of fertile eggs was calculated as the number of chicks hatched per 100 fertile eggs set. All hatching chicks were weighed after drying.

**Metabolizable energy determination:** The ME value of the grower diet was determined for 10 week old pullets using an acid insoluble ash marker (Celite<sup>®</sup>). Ten separate birds, reared according to the Cobb Breeder Management Guide (Cobb-Vantress, 2005), were fed the standard grower diet with 2% Celite<sup>®</sup>. After 2 days of acclimation to the diets and cages, droppings from each

of the ten individually caged pullets were collected in pans under the cages on three consecutive days. The three daily samples were combined, lyophilized in a Genesis SQ 12 EL Freeze drier<sup>5</sup>, finely ground and analyzed for acid insoluble ash and gross energy using a Parr adiabatic bomb calorimeter<sup>6</sup>. Acid insoluble ash and gross energy content of the diet was also determined. Metabolizable energy content was calculated using the equation described by Scott and Balnave (1991). The diet ME value is presented in Table 1.

**Carcass composition:** In order to determine the effect of different feed restriction programs on carcass composition, ten pullets per treatment were sacrificed by CO<sub>2</sub> asphyxiation at 4, 7, 14, 22, 27 and 40 weeks of age. Each breeder carcass was frozen at -20°C before autoclaving. The carcasses were placed in trays, covered with foil and autoclaved at 120°C for 15 h in an AMSCO 3053 sterilizer<sup>7</sup>. The carcasses were homogenized after autoclaving using a Waring 4L blender<sup>8</sup>. Sub-samples were collected after grinding and lyophilized in a Genesis SQ 12 EL Freeze drier<sup>9</sup>. Carcass protein, ash and fat were analyzed according to AOAC (1990). DM was determined as a % of total wet carcass weight. The percent carcass protein, ash and fat were reported on a dry matter basis. Dry BW was calculated by multiplying the proportion of DM by the total wet carcass weight. Total carcass protein, ash and fat were calculated by multiplying the proportion of each component by the dry BW.

**Nitrogen retention:** A 21 day Nitrogen (N) balance study was conducted from 84-05 d of age to compare N retention between pullets on different feeding programs, with or without supplemental L-carnitine. At 84 d of age 40 pullets, of similar BW (1285±16 g), reared according to breeder guidelines were randomly assigned to one of four treatment groups in a 2 x 2 factorial design. Each bird was individually caged with its own feed trough and a nipple drinker. The main effects tested were feeding program (ED or SK) and L-carnitine supplementation (0 or 50 mg/kg). Diets were the same as those described above (Table 1). All pullets were given the same total amount of feed, which was determined by following the guidelines proposed in the Cobb Breeder Management Guide (Cobb-Vantress, 2005) for birds of that age. At the start of the 21 day trial period, an additional 10 pullets of similar BW (1291±27 g) were sacrificed by CO<sub>2</sub> asphyxiation for whole body N determination. This constituted the baseline N content. The ten pullets from each treatment group were sacrificed by CO<sub>2</sub> asphyxiation at the end of the 21 day study period for carcass N determination. All carcass N determinations were carried out as described above for carcass composition. N retention was calculated as:

[(whole body N at end of trial - whole body N at start of trial) / total N intake during 21 day trial] \* 100

**Experiment 2:** Stock and management. A total of 840 days 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 stocking density and lighting programs were the same as for Experiment 1. Pullets were photostimulated with 13 h of light at 21 weeks of age. Photoperiod was subsequently increased by 1 h each week until a 16 hour photoperiod was reached. At photostimulation, 80 representative pullets from each treatment group were individually housed.

**Experimental design:** In Experiment 2 a 2x2 factorial design was used. The main effects were L-carnitine supplementation (0 or 50 mg/kg) and feeding program (everyday or skip-a-day). All other aspects of the experimental design were the same as those used for Experiment 1. However, in experiment 2 a low density grower diet (Table 1) was used from 4-18 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. The feed allocation after housing (21 weeks) is shown in Table 3. Maximum feed allocation and feed withdrawal was the same as for Experiment 1.

All weighing and measurement of performance parameters that was conducted in Experiment 1 was conducted in the same way in Experiment 2. Nitrogen retention was not determined in Experiment 2. Metabolizable energy of the low density grower diet used in Experiment 2 was determined as described above for standard grower in Experiment 1. The low density grower diet energy level is presented in Table 1.

**Statistical analysis:** Statistical analysis was carried out using the same procedures for both experiments. Data analysis was performed using JMP IN 5.1<sup>10</sup> statistical analysis software. Chicks were assigned to treatments on day one in a completely random manner. From the beginning of the trial until 21 weeks of age the pen was treated as the experimental unit. After 21 weeks birds were individually caged and each bird served as an experimental unit. 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).

Table 3: Feed allocation schedule for broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 2)

Age (weeks)	ED <sup>1</sup>	SK <sup>2</sup>	ED + 50 mg/kg L-carnitine	SK + 50 mg/kg L-carnitine
			Feed (g/bird/day)	
22	110	110	110	110
23	112	113	112	113
24	118	120	118	120
25	122	122	122	122
26	131	131	131	131
27	140	140	140	140
28	144	144	144	144
29	144	144	144	144
30	144	144	144	144
31	144	144	144	144
32	143	143	143	143
33	142	142	142	142
34	142	142	142	142
35	141	141	141	141
36	141	141	141	141
37	140	140	140	140
38	139	139	139	139
39	138	138	138	138
40	137	137	137	137
41	136	136	136	136
42	136	136	136	136
43	135	135	135	135
44	134	134	134	134
45	133	133	133	133

<sup>1</sup> Everyday ; <sup>2</sup> Skip-a-day

## RESULTS

**Experiment 1:** Body weight, uniformity and frame size. The BW data from Experiment 1 is presented in Table 4. Feed intake was adjusted weekly after weighing, to maintain equal BW between treatment groups. No interactions were observed for BW. Neither feeding program nor L-carnitine supplementation affected BW at any age ( $p \leq 0.05$ ). All treatment groups were fed identical amounts everyday during the first 4 weeks (starter period). After four weeks there was a near significant ( $p = 0.06$ ) increase in BW when L-carnitine was added to the starter diet. Estimates of uniformity are presented as CV for each group in Table 5. The CV for SK fed pullets was generally less (2.07) than that of ED fed pullets during the rearing period, independent of L-carnitine supplementation. After pullets were individually caged at 21 weeks of age, all birds had access to their own feeder and therefore, feed consumption did not differ between individuals within a treatment group. This is seen in the consistent reduction in CV of all groups after housing. Individual caging at 21 weeks prevented further comparisons in uniformity between treatment groups. Frame size measurements are presented in Table 6.

Measurements were taken at 10, 20 and 28 weeks. There were no significant interactions between feeding program and L-carnitine for shank or keel length at any age. No main effect differences were seen for shank length at any age. At 10 weeks of age L-carnitine supplementation resulted in an increase in keel length from 13.49-13.78 cm compared to non-supplemented pullets. No main effect differences were observed for keel length at any other age.

**Reproductive performance:** The reproductive performance of hens from Experiment 1 is shown in Table 7. The experiment was terminated at 45 weeks of age. Therefore, all values presented are for production up to and including 45 weeks of age. No interactions were observed for any of the parameters measured. Therefore, only main effects will be discussed. Sexual Maturity (SM) was defined as the age at first oviposition. Birds fed using SK programs took three days longer to reach SM than birds fed ED. No differences existed in age at SM for L-carnitine main effect. The feeding program main effect was significant ( $p = 0.05$ ) for total egg production. Hens fed ED during the rearing period

Table 4: Body weights at various ages of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 1)

Program	L-Carnitine	4 Weeks <sup>1</sup>	7 Weeks	14 Weeks	20 Weeks	22 Weeks <sup>2</sup>	27 Weeks	40 Weeks
		BW (g)						
ED <sup>3</sup>	None	524	827	1513	2212	2645	3380	4011
ED	50 mg/kg	530	826	1508	2215	2682	3379	4081
SK <sup>4</sup>	None	521	813	1526	2225	2636	3391	4051
SK	50 mg/kg	531	816	1542	2213	2594	3456	4121
SEM	2.2	3.2	2.7	12.9	11.3	13.6	27.1	
<b>Main effect means</b>								
ED		527	827	1511	2213	2664	3380	4046
SK		526	814	1534	2219	2615	3424	4086
	None	522	815	1520	2218	2641	3385	4031
	50 mg/kg	531	821	1525	2213	2638	3418	4101
<b>Source of variation</b>		Probability						
Program		0.81	0.18	0.12	0.84	0.18	0.26	0.56
L-Carnitine		0.06	0.38	0.34	0.85	0.75	0.34	0.44
Program x L-carnitine		0.69	0.31	0.41	0.78	0.64	0.26	0.88

<sup>1</sup>Values shown represent means of six pens per treatment at week 4, 7, 14 and 20; Birds were individually housed at 21 weeks. Values shown represent means of 80 individual birds per treatment at week 22, 27 and 40; <sup>3</sup>Everyday; <sup>4</sup>Skip-a-day

Table 5: Coefficients of variation<sup>1</sup> (%) of body weight of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 1)

Age (weeks)	Feeding Regimen			
	ED <sup>2</sup>	SK <sup>3</sup>	ED + 50 mg/kg L-carnitine	SK + 50 mg/kg L-carnitine
	CV (%)			
7	11.7	11.1	10.1	10.3
14	14.9	10.2	12.7	10.0
20	13.9	12.9	14.7	11.1
22	11.4	10.8	11.5	9.7
27	6.9	5.5	5.6	5.1
40	6.5	7.2	6.7	6.6

<sup>1</sup>CV determined by individually weighing 40 birds per treatment at each interval; <sup>2</sup> Everyday; <sup>3</sup>Skip-a-day

Table 6: Shank and keel lengths<sup>1</sup> of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 1)

Program	L-Carnitine	10 Weeks		20 Weeks		28 Weeks	
		Shank	Keel	Shank	Keel	Shank	Keel
ED <sup>2</sup>	None	6.83	13.51	8.91	17.28	8.87	18.12
ED	50 mg/kg	6.87	13.85	8.79	17.01	8.85	18.14
SK <sup>3</sup>	None	6.81	13.47	8.88	17.05	8.92	18.02
SK	50 mg/kg	6.89	13.71	8.81	17.19	9.01	18.33
SEM	0.03	0.07	0.04	0.10	0.04	0.09	
<b>Main effect means</b>							
ED		6.85	13.68	8.85	17.15	8.86	18.13
SK		6.85	13.59	8.85	17.12	8.96	18.18
	None	6.82	13.49	8.89	17.17	8.90	18.07
	50 mg/kg	6.88	13.78	8.80	17.10	8.93	18.24
<b>Source of variation</b>		Probability					
Program	1.00	0.52	0.95	0.89	0.19	0.72	
L-Carnitine	0.28	0.04	0.20	0.75	0.65	0.10	
Program x L-carnitine	0.71	0.72	0.74	0.31	0.22	0.11	

<sup>1</sup> Values are means of 20 measurements per treatment; <sup>2</sup>Everyday ; <sup>3</sup>Skip-a-dayTable 7: Reproductive performance of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 1)<sup>1</sup>

Program	L-Carnitine	Age at sexual maturity (d)	Total eggs /hen	Settable eggs /hen <sup>2</sup>	Abnormal eggs /hen <sup>3</sup>	First three EW <sup>4</sup> (g)	EW (g)	Relative EW <sup>5</sup> (%)	Fertility <sup>6</sup> (%)	Chick weight <sup>7</sup> (g)
ED <sup>8</sup>	None	177.0	96.4	92.6	1.31	49.34	59.19	2.18	62.4	43.2
ED	50 mg/kg	175.3	102.5	98.6	1.37	50.23	59.67	2.24	60.6	43.3
SK <sup>9</sup>	None	178.2	94.0	90.0	1.42	50.10	59.19	2.26	64.2	43.6
SK	50 mg/kg	180.2	95.2	91.1	1.43	50.02	61.04	2.38	64.8	44.0
SEM	0.5	1.1	1.1	0.09	0.33	0.25	0.02	1.7	0.3	
<b>Main effect means</b>										
ED		176.2	99.5	95.6	1.34	49.79	59.43	2.21	61.5	43.2
SK		179.2	94.9	90.6	1.43	50.06	60.12	2.32	64.5	43.8
	None	177.6	95.2	91.3	1.37	49.73	59.19	2.22	63.3	43.4
	50 mg/kg	177.8	99.1	94.9	1.40	50.12	60.35	2.31	62.7	43.6
<b>Source of variation</b>		Probability								
Program		<0.01	<0.01	<0.01	0.65	0.69	0.17	<0.01	0.90	0.29
L-Carnitine		0.61	0.12	0.20	0.86	0.54	0.02	<0.01	0.98	0.52
Program x L-carnitine		0.45	0.33	0.33	0.93	0.47	0.17	0.30	0.19	0.72

<sup>1</sup>All parameters measured up to 45 weeks of age; <sup>2</sup>Number of eggs weighing > 50 g, not including soft shells, cracks or double yolks<sup>3</sup>Includes soft shell and double yolk eggs; <sup>4</sup>Egg weight; <sup>5</sup>(Mean egg weight / BW at housing)\*100; <sup>6</sup>Hens were artificially inseminated at week 32, 36, 40 and 44 with 5x10<sup>7</sup> sperm at each insemination; <sup>7</sup>All hatched chicks from hens at 32 and 36 weeks of age were weighed; <sup>8</sup>Everyday; <sup>9</sup>Skip-a-day

produced 4.6 more eggs than hens fed SK. L-carnitine supplemented hens produced 3.9 more eggs per hen than non-supplemented hens but the difference was not significant ( $p = 0.19$ ). Settable egg production was defined as total eggs weighing 50 g or more minus soft-shelled, double yolked or cracked eggs. Feeding ED rather than SK during the rearing period resulted in an increase of settable 5.0 eggs per hen. L-carnitine supplementation did not significantly increase production of settable eggs. Main effects did not affect the production of abnormal eggs.

The first three eggs from each hen were weighed to gauge early EW. Early egg weight was not affected by main effects. Overall EW was not affected by feeding program but L-carnitine supplemented hens (60.35 g) produced larger eggs than non-supplemented hens

(59.19 g). Relative EW was calculated as (overall EW / 21 week BW) \* 100. Relative EW was greater for SK (2.32%) than for ED (2.21%) hens and also for L-carnitine supplemented (2.31%) hens versus non-supplemented (2.22%) hens. Fertility and hatchability (not shown) were not affected by feeding program or L-carnitine supplementation. Chick weight also did not differ between treatment groups, but did follow the same pattern as for EW.

The results of the 21 days N retention study are shown in Table 8. No interaction was observed for N retention from 84-105 d. Main effects did not affect N retention, although pullets fed ED retained 38.6 % N compared to SK pullets that retained 36.6%. The probability value ( $p = 0.10$ ) shows that this improvement in N retention was close to statistical significance.



Table 8: Feed conversion ratio of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine, expressed in terms of total feed intake, protein intake and energy intake (Experiment 1)

Program	L-Carnitine	N retention from 84 to 105 d %	FCR at 21 weeks (kg/kg)	Protein intake (g)/ kg BW at 21 weeks (g/kg)	Energy intake (kcal)/ kg BW at 21 weeks (kcal/kg)	Total feed intake(g)/ egg at 45 weeks (g/egg)	Total protein intake(g)/egg at 45 weeks (g/egg)	Total energy intake kcal)/egg (at 45 weeks (kcal/egg)
ED <sup>1</sup>	None	38.4	3.65	580.7	10414	335.9	53.5	967
ED	50 mg/kg	38.8	3.64	576.7	10376	317.1	50.5	920
SK <sup>2</sup>	None	36.3	3.94	624.3	11238	385.8	61.4	1119
SK	50 mg/kg	37.0	3.83	607.3	10930	356.8	56.8	1035
SEM		1.6	0.02	3.2	57.5	7.1	1.1	20.6
<b>Main effect means</b>								
ED		38.6	3.65	578.7	10395	327.0	52.0	944
SK		36.6	3.89	615.8	11084	371.3	59.1	1077
	None	37.3	3.80	602.5	10826	360.9	57.5	1043
	50 mg/kg	37.9	3.74	592.0	10653	337.0	53.7	978
<b>Source of variation</b>								
Program	0.10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
L-Carnitine	0.50	0.08	0.09	0.09	0.09	0.09	0.09	
Program x L-carnitine		0.82	0.40	0.40	0.40	0.75	0.75	0.75

<sup>1</sup> Everyday; <sup>2</sup> Skip-a-day

Table 9: Carcass fat of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine, at 7, 14, 20, 22, 27 and 40 weeks of age (Experiment 1)

Program	L-Carnitine	7 Weeks		14 Weeks		22 Weeks		27 Weeks		40 Weeks	
		% <sup>1</sup>	g <sup>2</sup>	%	g	%	g	%	g	%	g
ED <sup>3</sup>	None	25.4	71	19.8	95	28.0	280	31.1	394	35.8	529
ED	50 mg/kg	27.3	74	21.4	107	29.8	281	33.9	433	39.9	583
SK <sup>4</sup>	None	26.6	69	21.4	105	27.7	271	32.0	398	39.2	606
SK	50 mg/kg	26.3	69	22.6	108	27.5	270	31.1	457	41.8	633
SEM		0.6	2.0	0.7	4.1	0.6	6.9	0.6	9.9	0.6	14.9
<b>Main effect means</b>											
ED		26.4	73	20.6	101	28.9	281	32.5	414	37.9	556
SK		26.5	69	22.0	107	27.6	271	31.6	428	40.5	620
	None	26.0	70	20.6	100	27.9	276	31.6	397	37.5	568
	50 mg/kg	26.8	72	22.0	107	28.7	276	32.5	445	40.9	608
<b>Source of variation</b>											
Program		0.61	0.56	0.27	0.37	0.43	0.34	0.21	0.45	0.04	0.03
L-Carnitine		0.44	0.46	0.25	0.21	0.37	0.78	0.48	0.73	0.07	0.25
Program x L-carnitine		0.81	0.64	0.76	0.44	0.30	0.68	0.24	0.29	0.53	0.76

<sup>1</sup> Carcass fat expressed as a percentage of dry carcass weight; <sup>2</sup> Total carcass fat in grams; <sup>3</sup> Everyday; <sup>4</sup> Skip-a-day

Table 10: Carcass protein of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine, at 7, 14, 20, 22, 27 and 40 weeks of age (Experiment 1)

Program	L-Carnitine	7 Weeks		14 Weeks		22 Weeks		27 Weeks		40 Weeks	
		% <sup>1</sup>	g <sup>2</sup>	%	g	%	g	%	g	%	g
ED <sup>3</sup>	None	56.4	151	59.3	279	56.2	549	52.7	651	49.4 <sup>a</sup>	722 <sup>a</sup>
ED	50 mg/kg	53.7	146	59.8	290	56.9	538	52.6	667	45.7 <sup>b</sup>	664 <sup>b</sup>
SK <sup>4</sup>	None	53.2	141	59.5	281	55.4	526	53.4	659	46.9 <sup>ab</sup>	714 <sup>a</sup>
SK	50 mg/kg	54.5	142	58.7	280	57.1	542	54.7	645	47.5 <sup>ab</sup>	730 <sup>a</sup>
SEM		0.4	2.4	0.6	4.4	0.7	9.2	0.6	8.7	0.5	8.9
<b>Main effect means</b>											
ED		54.9	149	59.6	285	56.6	544	52.7	659	47.5	694
SK		53.9	142	59.1	281	56.3	534	54.1	652	47.3	723
	None	54.6	146	59.4	280	55.8	538	53.1	655	48.2	718
	50 mg/kg	54.1	144	59.3	285	57.0	540	53.7	656	46.6	697
<b>Source of variation</b>											
Program		0.76	0.44	0.96	0.85	0.94	0.58	0.19	0.51	0.78	0.12
L-Carnitine		0.39	0.81	0.49	0.94	0.43	0.82	0.89	0.89	0.12	0.26
Program x L-carnitine		0.23	0.83	0.32	0.36	0.84	0.43	0.60	0.40	0.03	0.05

<sup>1</sup> Carcass protein expressed as a percentage of dry carcass weight; <sup>2</sup> Total carcass protein in grams; <sup>3</sup> Everyday; <sup>4</sup> Skip-a-day

Table 11: Carcass ash of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine, at 7, 14, 20, 22, 27 and 40 weeks of age (Experiment 1)

Program	L-Carnitine	7 Weeks		14 Weeks		22 Weeks		27 Weeks		40 Weeks	
		% <sup>1</sup>	g <sup>2</sup>	%	g	%	g	%	g	%	g
ED <sup>3</sup>	None	9.2	25	10.9	51	9.3	91	8.5	106	7.6	111
ED	50 mg/kg	9.6	26	10.9	54	9.7	92	8.1	102	7.6	112
SK <sup>4</sup>	None	9.5	26	11.0	53	9.8	93	8.7	110	7.1	109
SK	50 mg/kg	9.6	25	10.5	49	9.8	93	8.6	99	6.7	101
SEM		0.2	0.6	0.2	0.9	0.2	2.9	0.2	1.7	0.2	4.0
<b>Main effect means</b>											
ED		9.4	25	10.9	52	9.5	92	8.3	104	7.6	112
SK		9.6	25	10.8	51	9.8	93	8.7	105	6.9	105
	None	9.4	25	11.0	52	9.6	92	8.6	108	7.4	110
	50 mg/kg	9.6	26	10.7	52	9.7	93	8.4	101	7.2	106
<b>Source of variation</b>											
Program		0.72	0.93	0.41	0.50	0.36	0.65	0.31	0.68	0.10	0.35
L-Carnitine			0.48	0.53	0.38	0.79	0.52	0.93	0.37	0.11	0.31
Program x L-carnitine			0.71	0.46	0.55	0.11	0.55	0.86	0.56	0.51	0.43
										0.45	0.56

<sup>1</sup>Carcass ash expressed as a percentage of dry carcass weight; <sup>2</sup>Total carcass ash in grams; <sup>3</sup>Everyday; <sup>4</sup>Skip-a-day

Other measures of efficiency are also presented in Table 8. The interaction term was not significant for any of the parameters calculated. Feeding ED as opposed to SK resulted in a significant ( $p < 0.01$ ) improvement in Feed Conversion Ratio (FCR), protein and energy utilization from 0-21 weeks of age. Feeding ED also reduced ( $p < 0.01$ ) the total feed, total protein and total energy required per egg produced up to 45 weeks of age. L-carnitine supplementation resulted in near significant ( $p = 0.09$ ) improvements in feed, protein and energy utilization for BW at 21 weeks and for egg production to 45 weeks.

**Body composition:** Body composition data is presented in presented in Tables 9, 10 and 11. Body compositions were expressed both as percentages of Dry Matter (DM) and as total mass (g) of fat, protein and ash. Dry carcass mass (g) was obtained by multiplying the % DM by the wet carcass weight. The total mass of fat, protein and ash was obtained by multiplying the proportion of each component in the dry carcass by the total dry carcass mass (g). No significant interactions between feeding programs and L-carnitine were observed for carcass fat % or total carcass fat at any age. Additional dietary L-carnitine did not affect carcass fat content at any age. At 40 weeks however there was a near significant ( $p = 0.07$ ) increase in fat % of L-carnitine supplemented birds compared to non-supplemented birds. Feeding program did not affect carcass fat until 40 weeks of age. At that time the ED fed hens had lower fat % and less total fat than SK hens. Carcass fat % increased from 22 to 40 weeks in all treatment groups.

There were no interactions for carcass protein (Table 10) before 40 weeks of age. Neither feeding program, nor L-carnitine supplementation affected carcass protein % or total carcass protein at any age before 40 weeks. There was a decline in carcass protein% in all treatments from 22-40 weeks of age. At 40 weeks of age

there was a significant interaction between feeding program and L-carnitine supplementation for protein % and total protein. Protein% was higher in non-supplemented ED hens than in supplemented ED hens. Total carcass protein was lower in ED fed, L-carnitine supplemented hens (664 g) than in any of the other groups. Carcass ash% and total carcass ash (Table 11) showed no interactions and did not differ between treatment groups at any age. Carcass ash % declined from 22 to 40 weeks of age in all groups.

Mortality (not shown) did not differ between any of the treatment groups.

**Experiment 2:** Body weight, uniformity and frame size. The BW data from experiment 2 are shown in Table 12. In Experiment 2, a grower diet of lower density was used from 4-18 weeks of age. Feed allocation for each group was adjusted weekly according to their BW, in an attempt to keep BW as uniform as possible between groups. At four weeks of age, L-carnitine supplemented pullets were heavier ( $p < 0.01$ ) than non-supplemented pullets by 34 g. Feed intakes were the same for all groups during the first four weeks. Therefore, the improved growth of L-carnitine supplemented pullets was due to better efficiency in feed utilization. From 25 weeks of age, all groups received equal feed allocations (Table 3). Body weight did not differ between ED and SK birds at 4, 7, 14, 20 or 22 weeks. At 27 and 40 weeks of age ED hens were heavier ( $p < 0.05$ ) than SK hens by 59 g and 164 g, respectively.

The CV for each treatment group at various ages is shown in Table 13. The CV is very similar for all groups. After housing the CV was lower than before housing because all birds were individually caged.

Frame size measurements are shown for 12, 20 and 28 weeks (Table 14). The interaction term was not significant at any age and no differences existed between main effects.

Table 12: Body weights at various ages of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 2)

Program	L-Carnitine	4 Weeks <sup>1</sup>	7 Weeks	14 Weeks	20 Weeks	22 Weeks <sup>2</sup>	27 Weeks	40 Weeks
		BW (g)						
ED <sup>3</sup>	None	504	888	1482	2273	2744	3571	3979
ED	50 mg/kg	539	884	1474	2256	2675	3560	3976
SK <sup>4</sup>	None	504	858	1474	2276	2699	3503	3835
SK	50 mg/kg	537	859	1409	2232	2714	3511	3793
SEM	2.0	3.1	12.4	5.0	14.2	12.6	18.9	
<b>Main effect means</b>								
ED		521	886	1478	2264	2710	3566	3978
SK		521	859	1442	2264	2707	3507	3814
	None	504	873	1478	2274	2722	3536	3907
	50 mg/kg	538	872	1442	2254	2695	3536	3884
<b>Source of variation</b>		Probability						
Program		0.89	0.14	0.16	0.31	0.93	0.02	<0.01
L-Carnitine		<0.01	0.85	0.15	0.17	0.34	0.96	0.55
Program x L-carnitine		0.85	0.26	0.26	0.20	0.14	0.72	0.60

<sup>1</sup>Values shown represent means of six pens per treatment at week 4, 7, 14 and 20; <sup>2</sup>Birds were individually housed at 21 weeks; Values shown represent means of 80 individual birds per treatment at week 22, 27 and 40; <sup>3</sup>Everyday; <sup>4</sup>Skip-a-day

Table 13: Coefficients of variation<sup>1</sup> (%) of body weight of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 2)

Age (weeks)	Feeding Regimen			
	ED <sup>2</sup>	SK <sup>3</sup>	ED + 50 mg/kg L-carnitine	SK + 50 mg/kg L-carnitine
	CV (%)			
7	11.6	12.0	10.5	11.7
14	12.6	11.8	11.8	12.0
20	11.8	12.3	10.7	11.2
22	8.8	9.7	9.7	8.5
27	7.1	5.7	6.5	6.5
40	7.5	7.6	8.5	8.4

<sup>1</sup>CV determined by individually weighing 40 birds per treatment at each interval; <sup>2</sup>Everyday; <sup>3</sup>Skip-a-day

Table 14: Shank and keel lengths<sup>1</sup> of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 2)

Program	L-Carnitine	12 Weeks		20 Weeks		28 Weeks	
		Shank	Keel	Shank	Keel	Shank	Keel
		cm					
ED <sup>2</sup>	None	7.14	13.91	8.89	17.34	9.08	18.02
ED	50 mg/kg	7.16	14.06	8.86	17.36	9.18	18.40
SK <sup>3</sup>	None	7.10	13.88	8.87	17.37	9.09	18.15
SK	50 mg/kg	7.09	13.91	8.70	17.16	9.14	18.12
SEM	0.04	0.09	0.04	0.09	0.03	0.10	
<b>Main effect means</b>							
ED		7.15	13.96	8.88	17.35	9.13	18.21
SK		7.10	13.90	8.79	17.27	9.11	18.14
	None	7.12	13.90	8.88	17.36	9.08	18.09
	50 mg/kg	7.13	13.96	8.78	17.26	9.16	18.26
<b>Source of variation</b>		Probability					
Program		0.23	0.19	0.31	0.29	0.79	0.34
L-Carnitine		0.90	0.23	0.26	0.26	0.26	0.19
Program x L-carnitine		0.80	0.24	0.43	0.20	0.69	0.41

<sup>1</sup>Values are means of 20 measurements per treatment; <sup>2</sup>Everyday; <sup>3</sup>Skip-a-day

**Reproductive performance:** No significant interactions were detected for any of the performance parameters measured (Table 15). Hens that were fed SK during rearing took 5.3 extra days to reach SM compared to ED, despite having similar BW at 22 weeks of age. L-carnitine supplementation did not affect age at SM. Hens

fed ED produced 4.7 more ( $p < 0.01$ ) total eggs per hen than SK fed hens by 45 weeks of age. A non-significant ( $p = 0.13$ ) increase of 2.7 eggs per hen was noted for L-carnitine supplemented versus non-supplemented hens by 45 weeks of age. Everyday fed hens produced 4.4 more ( $p = 0.01$ ) settable eggs per hen than SK fed hens

Table 15: Reproductive performance of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine (Experiment 2)<sup>1</sup>

Program	L-Carnitine	Age at sexual maturity (d)	Total eggs /hen	Settable eggs /hen <sup>2</sup>	Abnormal eggs /hen <sup>3</sup>	First three EW <sup>4</sup> (g)	EW (g)	Relative EW <sup>5</sup> (%)	Fertility <sup>6</sup> (%)
ED <sup>7</sup>	None	180.1	94.8	91.2	1.29	48.30	60.86	2.24	57.0
ED	50 mg/kg	180.7	98.1	94.4	1.68	48.60	60.66	2.27	60.1
SK <sup>8</sup>	None	186.3	90.8	87.7	1.19	49.47	60.48	2.26	58.4
SK	50 mg/kg	185.4	92.9	89.1	1.78	50.05	61.61	2.27	57.7
SEM	0.4	0.9	0.9	0.13	0.23	0.19	0.01	2.4	
Main effect means									
ED		180.5	96.5	92.8	1.48	48.45	60.76	2.25	58.6
SK		185.8	91.8	88.4	1.48	49.76	61.04	2.27	58.1
	None	183.2	92.8	89.5	1.24	48.88	60.67	2.25	57.7
	50 mg/kg	183.0	95.5	91.7	1.73	49.33	61.13	2.27	58.9
Source of variation									
Program	<0.01	<0.01	0.01	0.99	<0.01	0.46	0.67	0.92	
L-Carnitine		0.82	0.13	0.18	0.07	0.34	0.13	0.47	0.81
Program x L-carnitine		0.38	0.73	0.60	0.71	0.76	0.08	0.75	0.70

<sup>1</sup>All parameters measured up to 45 weeks of age; <sup>2</sup>Number of eggs weighing > 50g, not including soft shells, cracks or double yolks<sup>3</sup>Includes soft shell and double yolk eggs; <sup>4</sup>Egg weight; <sup>5</sup>(Mean egg weight/ BW at housing)\*100; <sup>6</sup>Hens were artificially inseminated at week 32, 36, 40 and 44 with 5x10<sup>7</sup> sperm at each insemination; <sup>7</sup>Everyday; <sup>8</sup>Skip-a-day

Table 16: Feed conversion ratio of broiler breeder hens fed using either everyday or skip-a-day feeding regimens, with 0 or 50 mg/kg supplemental L-carnitine, expressed in terms of total feed intake, protein intake and energy intake (Experiment 2)

Program	L-Carnitine	FCR at 21 weeks (kg/kg)	Protein (g)/ kg BW at 21 weeks (g/kg)	Energy intake (kcal)/ kg BW at 21 weeks (kcal/kg)	Total feed intake(g)/ egg at 45 weeks (g/egg)	Total protein intake(g)/egg at 45 weeks (g/egg)	Total energy intake (kcal)/ egg at 45 weeks (kcal/egg)
ED <sup>1</sup>	None	3.86	595.1	10745	346.7	55.8	999
ED	50 mg/kg	3.78	582.8	10511	329.0	52.0	948
SK <sup>2</sup>	None	3.96	610.1	11022	358.3	56.6	1032
SK	50 mg/kg	3.92	604.6	10915	351.3	55.5	1012
SEM	0.01	1.0	18.7	3.8	0.6	11.1	
Main effect means							
ED		3.82	589.0	10628	337.9	53.4	974
SK		3.94	607.4	10969	354.8	56.1	1022
	None	3.91	602.6	10884	352.5	55.7	1016
	50 mg/kg	3.85	593.7	10713	340.2	53.8	980
Source of variation							
Program	<0.01	<0.01	<0.01	<0.01	0.03	0.03	0.03
L-Carnitine		<0.01	<0.01	<0.01	0.11	0.11	0.11
Program x L-carnitine		0.11	0.11	0.10	0.49	0.49	0.49

<sup>1</sup> Everyday; <sup>2</sup> Skip-a-day

by 45 weeks of age. L-carnitine supplementation did not significantly increase settable egg production. No main effects differences were seen in abnormal egg production, although L-carnitine supplementation tended ( $p = 0.07$ ) to increase abnormal eggs.

Early EW was 1.2 g higher in SK than in ED fed breeders but was not affected by L-carnitine supplementation. Overall mean EW and relative EW were not affected by feeding program or L-carnitine supplementation. Fertility and hatchability (not shown) were also not affected by either of the main effects.

Data describing the efficiency of feed utilization is presented in Table 16. No significant interactions were detected for any of the parameters measured. Feeding pullets using ED restriction resulted in a consistently significant improvement in feed, protein and energy utilization for BW gain during the first 21 weeks of age.

For example, ED fed pullets required 18.4 g less feed protein per kg BW gain at 21 weeks of age. L-carnitine supplementation also significantly improved the efficiency of feed, protein and energy utilization to 21 weeks of age. Supplemented birds required almost 9 g less protein for each kg BW increase during the rearing period. Everyday feeding reduced the total amount of feed, protein and energy required per egg produced compared to SK feeding. For each egg produced, ED hens consumed 53.4 g total protein while SK hens consumed 56.1 g. The improvement in feed, protein and energy utilization for egg production by L-carnitine supplemented hens was not significant.

**Body composition** Body composition data from Experiment 2 (not shown) differed in some cases from Experiment 1. For example, at 22 weeks of age, pullets

supplemented with L-carnitine had lower ( $p = 0.05$ ) carcass fat% (22.7 vs. 25.0%) and lower total fat (214 g vs. 253 g) than non-supplemented pullets. These differences did not persist through the production period and by 40 weeks there was no difference in carcass fat. No differences occurred in carcass protein% or total protein at any age. L-carnitine supplemented birds tended to have higher carcass ash% and total ash than non-supplemented birds at all ages. Significant differences ( $p = 0.05$ ) between carcass ash% of supplemented and non-supplemented birds were observed at 22 weeks and 40 weeks of age. At 22 weeks of age, L-carnitine supplemented pullets had 10.7% carcass ash while non-supplemented pullets had 9.8% ash. At 40 weeks of age supplemented hens had 9.5% carcass ash, while non-supplemented hens had 8.4%. This equates to a 15 g difference in total carcass ash at 40 weeks.

## DISCUSSION

Feed restriction programs like those tested here are essential to the welfare (Katanbaf *et al.*, 1989a) and productivity (Katanbaf *et al.*, 1989c) of broiler breeder hens. 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). Lysine and methionine (its precursors) are generally the first and second limiting amino acids in poultry nutrition and so it is possible that endogenous production of L-carnitine may not be sufficient to support maximal fatty acid transport in certain individuals under certain environmental conditions.

In both trials, feed allocations were adjusted weekly after weighing, in order to maintain equal BW between groups. In both trials SK fed birds required more feed than ED birds to reach the same BW. A difference of 6.5% in feed conversion ratio (kg feed/ kg BW), in favor of ED birds was seen in Experiment 1. In Experiment 2, when a low density grower diet was used from 4-18 weeks of age, an improvement of only 3% was noted. These results are similar to those reported by de Beer and Coon (2007), which show that ED fed pullets grow more efficiently than SK fed pullets. Leeson and Summers (1985) also found that BW was lower in birds reared on SK compared to ED programs with equal intakes. Katanbaf *et al.* (1989a) found that pullets fed ED were 8% heavier than their SK fed counterparts at 21 weeks despite equal intakes. Powell and Gehle (1976) reported that ED fed pullets weighed 11% more at 22 weeks of age than SK pullets. They fed more to ED birds but not sufficiently more to explain the increased BW of the ED birds. Bennett and Leeson (1989) compared growth in ED and SK breeders and found that by 20 weeks there was a 100 g difference in BW in favor of ED breeders.

Katanbaf *et al.* (1989a) showed that pullets fed either every second day, or every third day had higher

circulating xanthophyll levels than everyday fed pullets. They attributed this difference to greater lipid (site of xanthophyll storage) mobilization during the fasting period in these two groups. The deposition and mobilization process is not perfectly efficient, which explains some of the reduced efficiency in SK compared to ED fed birds. The differences in efficiency of growth between ED and SK birds were not as large when lower density grower diets were used in Experiment 2.

These two experiments demonstrated (Table 8, 16) that feeding L-carnitine to broiler breeder pullets, during severe feed restriction, improved feed utilization during the rearing period. While the improvement in feed utilization was statistically significant in Experiment 2 ( $p < 0.01$ ) and close to significant in Experiment 1 ( $p = 0.08$ ), the actual improvement in FCR was less than 2% in both cases. While the primary function of L-carnitine in the body is to shuttle fatty acids into the mitochondria for  $\beta$ -oxidation, it may have other effects that influence efficiency in broiler breeders.

Kita *et al.* (2002) demonstrated that the improvement in body weight gain caused by dietary L-carnitine supplementation in broilers was mediated by increases in plasma insulin-like growth factor-I (IGF-I) concentrations. It is known that IGF-I is a potent growth stimulator. Rosebrough and McMurtry (1993) proved that variations in dietary protein and energy intake result in changes in plasma IGF-I concentration. In the work of Kita *et al.* (2002), plasma IGF-I increased with increased dietary L-carnitine and they attributed improved weight gain partially to this factor.

The benefits of L-carnitine in terms of feed conversion and growth are controversial. Musser *et al.* (1999) concluded that in sows, feeding 50 mg/ kg L-carnitine in the diet throughout gestation increased sow body weight and last rib fat depth gain. Like the breeders in our study, these sows were subjected to feed restriction during the experimental period. During fasting the birds are reliant on oxidation of mobilized fatty acids to supply their energy demands. It is possible that L-carnitine supplementation benefits this process. In another study, Ramanau *et al.* (2004) found that 125 mg/d added L-carnitine did not improve growth of sows during lactation. Several authors (Weeden *et al.*, 1991; Owen *et al.*, 2001) working with pigs suggested that L-carnitine supplementation could reduce carcass fat and improve feed efficiency. Rabie *et al.* (1997a, b) suggests that L-carnitine reduces abdominal fat in chickens while others (Leibetseder, 1995; Buyse *et al.*, 2001; Lien and Horng, 2001) have reported little benefit of additional dietary L-carnitine in either growth efficiency or reduction in carcass fat.

The results of the N retention study showed that supplementary L-carnitine did not significantly improve N retention over a three week period. Several authors (Cho *et al.*, 2000; Heo *et al.*, 2000; Owen *et al.*, 2001)

have shown that L-carnitine can improve N utilization in pigs. Owen *et al.* (2001) demonstrated that dietary L-carnitine suppresses mitochondrial branched-chain alpha-keto acid dehydrogenase activity and enhances protein accretion in swine. In their work, flux through branched-chain alpha-keto acid dehydrogenase decreased in liver and muscle mitochondria with increasing dietary L-carnitine. They also found that flux through pyruvate carboxylase was increased in mitochondria from liver of pigs fed L-carnitine. They speculated that such changes would reduce oxidative loss of branched-chain amino acids and provide more carbons for amino acid biosynthesis. They concluded that pigs fed supplemental L-carnitine were more able to use fat for energy, divert carbon toward synthesis of amino acids and spare branched-chain amino acids for protein synthesis. While we found no benefit for N retention, the small positive effect of L-carnitine on FCR over 21 weeks in both experiments, suggests that three weeks may not have been long enough to clearly establish any differences in N utilization. It is clear however, that if any positive effects do exist, they are fairly small.

In this first experiment, using standard grower diets, we found that SK feeding improved flock uniformity (as measured by CV) over ED feeding. The improvement was similar to that reported by Bartov *et al.* (1988). Bennett and Leeson (1989) defined uniformity as the % of birds in a pen with BW within  $\pm 15\%$  of the pen mean. They found that SK pullets were consistently more uniform than ED pullets but that the differences were not significant. Using SK programs during periods of severe feed restriction does appear to improve flock uniformity. These results are in agreement with a previous report by de Beer and Coon (2007). In the second experiment however, when lower density grower diets were used, CV did not improve with SK feeding. The feed allocations were between 2 to 5 % higher during the grower period than in Experiment 1. Slightly higher feed allocations may have benefited uniformity in ED pullets by increasing cleanup time and allowing less aggressive breeders better access to feed. Supplemental L-carnitine did not affect CV of pullets in our experiments. Using SK feeding regimens during rearing delayed the onset of SM in spite of the fact that BW was not different from ED birds. The difference was 3 days in Experiment 1 and 5.3 days in Experiment 2, in favor of ED feeding. Hocking (2004) showed that as BW increased, age at SM decreased in a curvilinear fashion. However, in the experiments reported here, BW did not differ between ED and SK birds. Wilson *et al.* (1989) found that age at SM, which they defined as 50% production, was delayed in SK breeders compared to ED breeders. Katanbaf *et al.* (1989b) reported findings that SM was delayed up to 5 days in SK birds compared to ED birds when both were fed equal amounts. Although, their findings were not statistically significant they do agree with ours and

those of Wilson *et al.* (1989). Wilson *et al.* (1989) presented data that indicated that BW was not the only factor affecting SM. They found in two separate experiments that even though BW did not differ at 24 weeks of age, pullets restricted using ED programs from 2 weeks of age reached SM earlier than pullets restricted using SK from eight weeks of age. The amounts of body fat (Bornstein *et al.*, 1984) and lean body mass (Soller *et al.*, 1984) have also been shown to be critical in the initiation of reproductive development. In both experiments reported here, SK fed pullets had slightly less (none significant) total carcass protein at 22 weeks of age. This may partially explain differences in age at SM. Endocrine related differences between feeding regimens may also have played a role in delay of SM in SK birds.

Improvements in egg production through 45 weeks for breeders reared with ED feeding regimen has also been reported by de Beer and Coon (2007). In Experiment 1 ED fed breeders produced 5.0 more settable eggs and in Experiment 2 they produced 4.4 more settable eggs than SK fed breeders. Wilson *et al.* (1989) reported lower egg production in birds fed using SK programs from eight weeks of age compared to birds fed restricted amounts ED from two weeks of age. In their study, BW did not differ between the two groups. The improved total egg production in ED breeders at 45 weeks of age is explained in some part by earlier onset of lay in these birds. Peak (not shown) did not differ between the two feeding regimens, but was slightly delayed in SK birds. Feeding regimens did not affect abnormal egg production. This result is similar to previous reports from Katanbaf *et al.* (1989c) and de beer and Coon (2007). L-carnitine also had no effect on abnormal egg production. Consistent with previous findings (de Beer and Coon, 2007), early EW was higher (Experiment 2) in SK than in ED birds. Relative EW was also higher (experiment 1) in SK birds. Wilson, *et al.* (1989) reported that breeders fed SK from eight weeks of age produced significantly larger eggs than breeders fed ED from two weeks of age. They also found a non-significant increase of 0.3 g in EW in breeders fed skip-a-day from two weeks of age compared to everyday fed breeders. This increase was found in spite of the fact that ED hens weighed 125 g more at housing and 97 g more at SM than SK hens. Leeson and Summers (1985) reported that EW was 0.3 g greater in SK birds than in ED, even though 20 week BW was 100 g greater in ED birds. This phenomenon may be a result of delayed SM and lower total egg numbers in SK hens. Chick weights were highly correlated to egg weights but did not differ between feeding regimens.

De Beer and Coon (2007) also reported that feeding regimen had no affect on breeder hen fertility. These findings are in agreement with other previously published literature (Leeson and Summers, 1985; Katanbaf *et al.*, 1989c; Wilson, *et al.*, 1989).

Few reports have been published on the effects of L-carnitine on breeder performance. Rabie *et al.* (1997c) found no improvement in laying hen performance when adding 50-500 mg/ kg L-carnitine to the diets of laying hens. Yalcin *et al.* (2006) also found no benefit to feeding L-carnitine to laying hens. L-carnitine did not affect age at SM, total or settable egg production. While the effect of L-carnitine on total egg production was not significant, L-carnitine supplemented birds produced 3.9 and 2.7 more total eggs at 45 weeks than non-supplemented birds in Experiments 1 and 2, respectively. The consistency of the results and the associated P values ( $p = 0.12$ ;  $p = 0.13$ ) for total egg production in the two experiments suggest that L-carnitine may have some beneficial effects on egg production. Baumgartner (2003) reported that L-carnitine supplementation at 20 mg/kg from 26 to 65 weeks of age resulted in approximately 8 more eggs per hen compared to non-supplemented controls in laying breeder hens. In the same report data was presented to show that 25 mg/kg added L-carnitine resulted in an increase of 4.5 chicks per hen. In another 40 week trial, number of fertile eggs per bird and egg weight was increased by L-carnitine addition. No mechanisms for improved performance were suggested. It was also noted in the same report that 50 mg/ kg L-carnitine did not affect breeder body weight during the production period.

In Experiment 1, EW was increased by addition of L-carnitine. The increase in EW for supplemented birds was not significant in Experiment 2 ( $p = 0.13$ ). The findings in Experiment 1 are in contrast with those of Rabie *et al.* (1997c), who found no differences in EW for laying hens supplemented with different levels of L-carnitine, but their study only covered the period from 65 to 73 weeks of age. There was no L-carnitine supplementation prior to that age in their trial. L-carnitine supplementation occurred over the entire lifespan of the bird in our studies. They did find that yolk weight was reduced and albumen weight was increased in response to supplemental L-carnitine. Analysis of yolk, albumen and shell weights (not shown) of eggs from our birds showed no alteration in the proportion of each component. Previous research by de Beer and Coon (2009) and Roncero and Goodridge (1992) show that L-carnitine supplementation increases *de novo* fatty acid synthesis in the liver of supplemented birds. This increase in lipogenesis occurred both during the rearing period and during production. It is possible that increased fatty acid production and subsequent packaging into yolk targeted very low density lipoprotein (VLDL<sub>y</sub>) for export from the liver may benefit the processes of yolk formation.

In both of these experiments, fertility was not improved by the addition of L-carnitine. Rinaudo *et al.* (1991) suggested that increased L-carnitine levels in the embryo would benefit chick development. This notion

was supported by the work of Leibetseder (1995) who showed that hatchability was increased from 83 to 87% and 82.4 to 85.3% in groups of broiler breeders supplemented with 50 and 100 mg L-carnitine respectively. Our experiments also revealed no improvement in hatchability as a result of L-carnitine. This is in contrast with the findings of Thiemel and Jelšnek (2004), who reported an increase in hatchability of 8.89% after addition of 30 mg/kg L-carnitine to the diet of breeding layers.

Some researchers have found that adding L-carnitine to the diet results in decreased abdominal fat in broilers (Rabie *et al.*, 1997a, b) while others (Barker and Sell, 1994; Leibetseder, 1995; Lien and Horng, 2001) have found no effects on abdominal fat. Reports with respect to broiler growth have also been contradictory. In Experiment 1 carcass fat was unaffected by L-carnitine supplementation. In Experiment 2, however, carcass fat% was significantly lower in L-carnitine supplemented birds at 22 weeks of age. After SM was reached body fat content was no longer affected by L-carnitine supplementation. Whether, the differences between the two experiments are a reflection of the different nutrient densities in the grower diets is unclear. Carcass protein content was generally unaffected by L-carnitine supplementation in both of our experiments.

In Experiment 1, L-carnitine did not affect carcass ash% or total ash. In Experiment 2, L-carnitine supplemented birds consistently had higher carcass ash% and total carcass ash. The effect of L-carnitine on carcass ash% was significant at 22 and 40 weeks of age. Cho *et al.* (2000) found that carcass ash was increased by the inclusion of L-carnitine to diets of weanling pigs. Benvenga *et al.* (2001) showed that L-carnitine increased bone mineral density in humans with hyperthyroidism like symptoms. Treatment of hyperthyroid patients with carnitine resulted in an improvement in symptoms without decreasing serum thyroid hormone levels, by inhibiting entry of thyroid hormone into the cell nucleus. The mechanism by which L-carnitine increased carcass ash in Experiment 2 is not clear. The two experiments conducted here show that the effects of L-carnitine on carcass composition are not consistent. The contradictory reports in the literature suggest as much. It is likely that other factors, such as environmental temperature, dietary nutrient (lysine) levels and level of supplementation influence the effects of L-carnitine.

Mortality was not affected by L-carnitine addition in either of the trials reported here. Supplemented at 50 mg/ kg over a total of 45 weeks, L-carnitine displayed no toxic effects on broiler breeders.

The results of these studies highlight the reduced efficiency of feed utilization for growth and egg production in SK compared to ED fed hens. The inefficiency is associated with cycles of repeated

mobilization of stored nutrients during the fasting periods associated with SK programs. Total and settable egg production was also benefited by ED rather than SK feeding even though BW did not differ between feeding regimens in either of our experiments. It was hypothesized that L-carnitine supplementation may be uniquely beneficial to breeders on SK feeding programs, due to their obvious need to mobilize and oxidize large amounts of fatty acids during the fasting periods. The general lack of interactions between feeding programs and L-carnitine suggested that this was not the case. Feeding additional L-carnitine did not attenuate the effects of SK feeding in any way. L-carnitine supplementation did, however, improve certain measures of efficiency, independent of feeding regimen. The use of a low density grower diet did not alter the effects of feeding regimens or L-carnitine on breeder performance, but some alterations in body composition were noted. The benefits of improved uniformity associated with SK programs may be outweighed by the potential savings in feed cost and performance improvements in ED fed breeders. Lifelong L-carnitine supplementation may provide marginal benefits to efficiency and performance of broiler breeder hens.

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