ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
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International Journal of Poultry Science 5 (10): 970-974, 2006
ISSN 1682-8356

Effect of L-Carnitine and Vegetable Fat on Broiler Breeder Fertility, Hatchability, Egg Yolk and Serum Cholesterol and Triglyceride

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Abstract: The effect of two dietary levels of L-carnitine and vegetable fat powder on broiler breeder fertility, hatchability, egg yolk and serum cholesterol and triglyceride was studied. Two hundred fifty female and twenty five male (Classic Hubbard parent stock) were distributed randomly in five groups of 50 with five replicate of 10 females and one male. Two levels of L-carnitine 0, 60 ppm (for females) and 0, 500 ppm (for males) and vegetable fat powder (0, 1.5%) and a diet with high lysine and methionine (0.3%) fed for both of male and female within one of treatment were used in a complete random design of treatments. The parameters as hatchability, fertility, egg weight, albumen height, Haugh unit, color of yolk, shell thickness, shell strength, yolk weights, egg yolk and serum cholesterol and triglyceride were measured. No significant differences were observed in external and internal egg quality. Supplemented diet with L-carnitine had effect on hatchability ($P<0.05$) and fertility ($P<0.01$). L-carnitine had no effect on egg production except on fifth and sixth weeks ($P<0.01$). None of experimental diet had no effect on male serum cholesterol, serum triglyceride in both sex and total yolk cholesterol but L- carnitine had effect on female serum cholesterol ($P<0.05$). L-carnitine had decreased egg yolk cholesterol (mg/gr) ($P<0.05$). Yolk weight increase in response to dietary supplementation of L-carnitine($P<0.05$) and L-carnitine content of egg yolk increase with L-carnitine supplementation ($P<0.05$).

Key word: Broiler breeder, fertility, L-carnitine, cholesterol, triglyceride

Introduction

L-carnitine is a chemical compound whose structure resembles that of an amino acid. L-carnitine can be formed in the animal's body. The amino acids lysine and methionine act as precursors. The vitamins B_6, B_{12}, C, folic acid and niacin and the trace element iron are also necessary as catalysis of the endogenous synthesis of L-carnitine. The highest synthesizing capacity is found in the liver. L-carinitine is a natural, vitamin like substance that acts in the cells as a receptor molecule for activated fatty acids. A shortage of this substance results primarily in impaired energy metabolism and membrane function (Harmeyer, 2002).

The effect of L-carnitine on reproductive parameters have been assessed in human and boars. Infertile men have significantly lower seminal carnitine concentrations than fertile men. When utilized as an epididymal marker and correlated with sperm concentration, L-carnitine levels are elevated in fertile vs. infertile men (Neuman *et al*., 2002). Free radicals or reactive oxygen species (ROS) are deleterious to cell membranes. Exposure of cell membranes to ROS induces lipid per oxidation causing membrane breakdown and loss of function. The major metabolic role of L-carnitine appears to be the transport of long-chain fatty acids into the mitochondria for B- oxidation (Coulter, 1995) thus dietary L-carnitine supplementation could improve fatty acid and energy utilization and therefore gain and feed efficient to meet endogenous requirements (Gropp *et al*., 1994). With laying hens, supplemental dietary L-carnitine resulted in an improvement in the albumen quality of eggs, measured as albumen height and Haugh unit score, during the early and late stages of laying period (Rabie *et al*., 1997a,b). Leibetseder (1995) has reported that egg hatchability increased from 83 to 87% and from 82.4 to 85.3% when broiler breeder were fed on diets supplemented with L- carnitine at levels of 50 and 100 mg/kg diet, respectively. In contrast, some researchers failed to observe any favourable responses to added dietary carnitine (Cartwright, 1986; Barker and Sell, 1994). Leibetseder (1995) recently produced evidence that the supplementation of a standard layer's ration with either 500 mg L-carnitine or 500 mg nicotinic acid, or a combination of the two compounds, had no effects on egg production, feed intake, body weight or concentrations of serum or yolk cholesterol during the early laying period. The objective of the present study was to investigate how far we can sustain normal laying performance, fertility and hatchability. The effect of L-carnitine and vegetable fat on broiler breeder fertility, hatchability, egg yolk and serum cholesterol and triglyceride and L-carnitine content of yolk were investigated.

Table 1: Composition and nutrient content of experimental rations (%)

	Female					Male				
Ingredient	L_1F_1	L_1F_2	L_2F_1	L_2F_2	M	L_1F_1	L_1F_2	L_2F_1	L_2F_2	M
Corn	66	61.6	66	61.6	65.3	54.8	49.3	54.8	49.3	52
Bran	2	3.5	2	3.5	2.8	10.5	11	10.5	11	10
Barley	0	2	0	2	1.17	16.52	20	16.52	20	20.5
Soy bean meal	21.8	21.4	21.8	21.4	20.1	13.4	13.1	13.4	13.1	12
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.23
Di calcium phosphate	1.43	1.4	1.43	1.4	1.4	1.45	1.45	1.45	1.45	1.45
Min.+vit. Premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
L- carnitine(ppm)	0	0	60	60	0	0	0	500	500	0
DL-methionine	0.1	0.12	0.1	0.12	0.3	0.15	0.15	0.15	0.15	0.3
Lysine	0	0.02	0	0.02	0.3	0.1	0.1	0.1	0.1	0.3
Plant fat powder	0	1.5	0	1.5	0	0	1.5	0	1.5	0
Oister shell	7.2	7	7.2	7	7.15	1.9	1.7	1.9	1.7	1.9
Na_2CO_3	0.23	0.23	0.23	0.23	0.23	0.27	0.27	0.27	0.27	0.29
Calculated analyses	****	****	****	****	****	****	****	****	****	****
ME (Kcal/kg)	2726	2726	2726	2726	2726	2707	2707	2707	2707	2707
Crude protein%	15.5	15.5	15.5	15.5	15.5	14.1	14.1	14.1	14.1	14.1
Methionine+Cyste%	0.62	0.63	0.62	0.63	0.8	0.61	0.61	0.61	0.61	0.8
Lysine%	0.77	0.77	0.77	0.77	0.97	0.7	0.7	0.7	0.7	0.75
Ca%	3.12	3.12	3.12	3.12	3.1	1.1	1.1	1.1	1.1	1.1
P% (non phytate)	0.39	0.39	0.39	0.39	0.39	0.45	0.45	0.45	0.45	0.45
Linoleic acid%	1.57	2.25	1.57	2.25	1.57	1.57	2.23	1.57	2.23	1.57

L_1F_1 : control, L_1F_2 : without L-carnitine and with fat, L_2F_1 : without fat and with l-carnitine, L_2F_2 : with carnitine and fat, M : a diet with 0.3% L-carnitine precursors supplement (lysine, methionine)

Materials and Methods

This trail was conducted in a broiler parent stock using two hundred fifty female and twenty five male that were distributed randomly in five groups of 50 with five replicate of 10 females and one male. Two levels of L-carnitine 0, 60 ppm (for females) and 0, 500 ppm (for males) and vegetable fat powder (0 , 1.5%) and a diet with high lysine and methionine (0.3%) fed for both of male and female within one of treatment were used in a complete random design of treatments. The birds were kept in pens and managed according to standard practices. An ambient temperature was 18-20. The photoperiod was 8.5 hour dark and 15.5 hour light at 26 week of age at the Heidar Abad State of Animal Science Research Center. Composition and nutrient content of experimental rations in percent of original matter are presented in Table 1. For separate- sex feeding, males were given their food in an individual feeder that the female were unable to eat from them. The ration was fed in mash form. Water was provided *ad libitum* from belt drinker in each pen. The average males and females body weights in each pen were recorded weekly during the trail and food intake was adjusted weekly to achieve target body weight gains, egg production and proper hatchability published by the breeding company from 180 gram/day in peak of egg production. The males were given 160 gram/day during the experiments.

Daily records were made of egg production. The performance of hens was evaluated in terms of egg production rate. Laboratory evaluations of egg quality were performed once egg quality was based on determination of external and internal indices, and egg components. The external indices included egg weight, shell thickness, shell breaking strength and those of interior quality such as albumen height, Haugh unit score and yolk colour score (Haugh, 1937).

The egg yolk was separated from the albumen and then rolled on a damp paper towel to remove any adhering albumen. The chalazae were also removed of yolk before weighing the yolk. Shell thickness was measured using a special micrometer (Mintutoy, Tokyo, Japan). Two yolks from each bird were weighed, pooled, triturated and a sample drawn for cholesterol analysis. At the end of trail a blood sample (1 ml) from each bird was collected from the cutanea ulnaris vein into a syringe. Serum obtained centrifugated (4000 rpm) and used to determine cholesterol and triglyceride. The concentration of egg cholesterol was determined enzymatically (according to the manufacturer's directions), the yolk samples (0.25±0.01 gr) mixed with 0.05 M of NaOH, neutralized with 0.25 N HCl, and then assayed. For determining of egg yolk carnitine content, egg yolk samples were freeze-dried and sent to "Lohmann Animal Health Company".

Statistical analysis: Data were subjected to statistical analysis using a completely randomized design, two levels of L-carnitine 0, 60 ppm (for females) and 0, 500 (for male) and fat powder (0, 1.5%) and a diet with 0.3% lysine and methionine were used. The data was analyzed using the SAS program (SAS Institute, 1986). The means of variables were compared using Duncan's multiple- range test (Duncan, 1955).

Table 2: The egg production in broiler breeder

Treatment	Egg production					
	Week 1	Week 2	Week 3	Week 4	Week 5	Total period
L_1F_1	79.65[a]	86.15[a]	83.65[a]	85.93[a]	84.09[b]	77.84[b]
L_1F_2	79.99[a]	81.42[a]	82.85[a]	86.57[a]	86[b]	84[b]
L_2F_1	82.31[a]	84.54[a]	85.65[a]	86.44[a]	93.20[a]	94.34[a]
L_2F_2	77.62[a]	80.95[a]	84.82[a]	83.52[a]	82.95[b]	83.04[b]
M	82.25[a]	84.72[a]	83.50[a]	84.89[a]	86.83[b]	84.79[b]
SEM	1.24	1.20	1.21	1.25	1.23	1.22

a,b Mean values in the same column with different superscript letters were significantly different($p<0.01$).

Results

Data for laying performance, as well as external and internal indices of egg quality are presented in The tables 2 and 3 respectively.

SEM there weren't any significant differences in the external and internal egg quality but the highest values for L-carnitine content of egg yolk were achieved by birds fed on the L_2F_1 diet (Table 3) ($P<0.05$).

L-carnitine had no effect on egg production except on fifth and sixth weeks (Table 2) ($P<0.01$).

Hatchability and fertility of treatments are presented in the Tables 4 and 5, respectively.

Supplemented diet with L-carnitine had effect on hatching rate ($P<0.05$) and fertility ($P<0.01$). The hatching rates and fertility in the groups fed with L_2F_1 and L_2F_2 diet during the trail period were higher by an average of 4% and in the groups fed with high lysine and methionine (groups M) were the least.

The effect of trail diets on relative yolk weights, cholesterol and triglycerides of serum (mg/100ml) and egg yolk cholesterol (mg/gr) and total yolk cholesterol are presented in Table 6 and 7. The relative weights of yolk differed significantly ($P<0.05$) in response to L-carnitine supplementation. L-carnitine had significantly decreased egg yolk cholesterol (mg/gr) ($P<0.05$), but no significant differences were observed in total egg yolk cholesterol. Dietary L_2F_1 and L_2F_2 reduced the serum cholesterol level in females ($P<0.05$) and the highest mean values for female serum cholesterol were achieved by the control group (L_1F_1). None of trail dietary had no effect on male cholesterol, male and female serum triglyceride.

Discussion

Some researches suggest that dietary L-carnitine did not influence laying performance (egg production rate, egg weight, daily feed intake, daily egg mass and feed conversion rate) (Rabie *et al.*, 1997a,b). But in this trail L- carnitin had significant effect on egg production on fifth and sxith weeks of trail. L- carnitine plays a well-established role in lipid metabolism, so it may induce some favorable modifications in poultry products, particularly eggs and meat. Rabie (1997a,b) found that L-carnitine had no effect on the external egg quality (egg weight, egg shell index, shell breaking strength, shell weight and shell thickness) but albumen quality (albumen height and Haugh unit score) was improved, while the yolk index and yolk colour score were not affected by dietary L-carnitine. It seems evident that these different expressions of response of animals to dietary carnitine are mainly related to species differences, age, sex, nutritional and physiological status of the animal and the nutrient composition of their diets (Rabie *et al.*, 1997a,b). Lettner *et al.* (1992) indicated that feeding diets supplemented with L-carnitine up to 60 mg/kg significantly affected the fatty acid composition of abdominal fat and result to improve fattening performance of broiler chickens. Leibetseder (1995) found that L-carnitine concentration increased significantly in the egg yolks of birds fed on L-carnitine supplemented diets (500 mg l-carnitine) compared with the control groups, L-carnitine concentrations in tissues (liver, kidney, heart, specific skeletal muscles) were found to increase significantly in response to dietary L-carnitine supplementation too and our results indicate that dietary L- carnitine supplementation (L_2F_1) resulted in an increase the content of L-carnitine in the yolk. Leibetseder (1995) indicated that the L-carnitine concentrations in the yolk and egg rose significantly in both groups receiving L-carnitine supplementation, the increase being approximately double that noted in the groups without L-carnitine supplementation.

The increase in L-carnitine content of eggs has been suggested to be desirable effect for the egg as food, but it may also be beneficial to the development of the chick embryo. L- carnitine synthesis is limited in the chick embryo and the chick, as it is in the human infant, although the latter is supplied with L- carnitine pre- and post- natally via the placenta and milk respectively. The possible effects of a higher L- carnitine content of the hatching egg not only on hatchability but also with regard to chick vitality and a possible reduction in mortality in the first few days of life are the subject of on going investigations (Leibetseder, 1995). Generally, under normal nutritional conditions, the proportion of the yolk in the edible part of the egg has been reported to increase with hen age (Rabie *et al.*, 1997a,b). Yolk cholesterol contents were subject to a certain degree of fluctuation during the laying period which, after the first phase of laying, was related to egg yield. In fact, the

Table 3: The external and internal indices of egg quality in broiler breeder.

Treatment	Traits						
	Egg weight (gr)	Albumen height (mm)	Yolk color (Roche color)	Shell strength (kg)	Shell thickness (mm)	Haugh unit	Yolk L-carnitine (mg/kg)
L_1F_1	61.344	5.241	5.976	3.774	0.302	68.156	6^{c}
L_1F_2	60.446	5.492	5.706	3.768	0.298	69.176	8^{bc}
L_2F_1	60.192	6.502	5.732	3.42	0.295	71.686	10^{a}
L_2F_2	60.916	5.985	5.592	3.636	0.301	70.75	9^{b}
M	60.83	5.28	5.76	3.27	0.29	69.622	8^{bc}
SEM	1.29	0.17	0.09	0.05	0.007	2.54	0.008

a,b,c Mean values in the same column with different superscript letters were significantly different ($p<0.05$).

Table 4: The htchability in broiler breeder

Treatment	Hatchability rate(%)						
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Total period
L_1F_1	77.992^{ab}	79.872^{bc}	80.80^{b}	80.25^{b}	82.604^{b}	85.89^{b}	81.118^{c}
L_1F_2	76.624^{b}	79.666^{c}	80.15^{b}	81.09^{b}	83.12^{b}	85.98^{b}	81.11^{c}
L_2F_1	82.032^{a}	83.1^{a}	84.16^{a}	84.77^{a}	87.40^{a}	90.97^{a}	85.40^{a}
L_2F_2	79.612^{ab}	81.172^{b}	81.73^{ab}	83.05^{ab}	85.172^{ab}	89.27^{ab}	83.33^{b}
M	70.384^{c}	74.448^{d}	73.224^{c}	69.32^{c}	77.076^{c}	79.36^{c}	73.972^{d}
SEM	1.025	1.045	1.015	1.021	1.033	1.089	1.0147

a,b,c Mean values in the same column with different superscript letters were significantly different ($p<0.05$).

higher the egg yield the lower the yolk cholesterol content. Since L-carnitine has been shown to produce a pronounced drop in the serum cholesterol levels in rats, of course the same effect was achieved by L- carnitine in this trail.

It is well known that one of the major effects of epididymal transit is the stabilization of sperm head and tail structures, in particular nuclear protamine, mitochondrial capsule and the coarse outer fibres of flagella thought to be related to the formation of intra- and inter-molecular disulfides. This process is essential for acquiring motility, ultrastructural stability and fertilizing ability; thus, an improvement in epididymal micro environment is likely to lead to an increase in sperm quality. Most of this stabilization process is due to oxidation of protein thiols (-SH) to form disulfides(S-S) and an increase in the(–SH) and (-SH+S-S) ratio has been reported in asthenozoospermic patients, suggesting that this "over oxidation" reflects an abnormal maturation process of the epididymis. In this context, it is intriguing to note, that carnitine also acts as a secondary antioxidant that repairs damage occurring after oxidative noxae and its administration to aging rats improves to glutathione and over all thiols status, perhaps by exerting a sparing activity on thiol and methionine (Stradaioli *et al*., 2004). Therefore a direct effect of carnitine on the functionality of sertoli cells is also plausible as observed by Palmero *et al*. (1990) who reported an increase in both lipid oxidation and glucose utilization by *in vitro* cultured sertoli cells in response to carnitine, and concluded that the improvement in semen quality reported after *in vivo* treatments could be related to its interactions with sertoli cell functions. Carnitine's protective role is further sustained by reducing toxicity and accelerating repair processes following physical and chemical damages on the testicular parenchyma.

The highly significant correlations among carnitine and spermatozoa concentrations could only be due to an increase in the intracellular pool of carnitine, although too slight to induce an increase of seminal levels (Stradaioli *et al*., 2004). In agreement of the acetyl carnitine with a reduction of the acetyl carnitine/L-carnitine ratio and seminal plasma carnitines levels observed in several forms of human infertility on seminal deficit (Lewin *et al*., 1981 and Golan *et al*., 1984).

L-carnitine administration improves pyruvate utilization, an elective energetic substratum for sperm motility. The correct Acetyl coA/coA ratio is fundamental in order to maintain the proper functionality of the Kreb's cycle for a sufficient production of Adenosine Three Phosphate (ATP) and the high levels of Acetyl coA inhibit pyruvic dehydrogenase enzyme activity; consequently, the metabolic flow of pyruvate into the Kreb's cycle is showed down. Through Carnitine Acyl Transferase (CAT), carnitine is transformed into acetylcarnitine (buffering effect), which reduces the AcetylcoA/coA ratio and improves the metabolic flux to Kreb's cycle, with an increased production of ATP, preserving a high motility of the spermatozoa (Stradaioli *et al*., 2004).

In conclusion, oral L-carnitine administration to the Hubbard broiler breeder flock could be benefit to increase semen volume, sperm quality and quantity consequently increase fertility and hatching rate, egg production specially in peak of production until the flock show the higher own genetic potential. However L-carnitine administration seems to be ineffective on egg

Table 5: The fertility in broiler breeder

Treatment	Fertility rate(%)				
	Week 3	Week 4	Week 5	Week 6	Total period
L_1F_1	83^{bc}	84.6^{b}	88.2^{abc}	88.4^{b}	86b
L_1F_2	82^{bc}	84.8^{b}	86.4^{bc}	88.8^{b}	85.5^{bc}
L_2F_1	92.6^{a}	93.8^{a}	93.4^{a}	99.2^{a}	94.7^{a}
L_2F_2	88.2^{ab}	87.4^{b}	89.6^{ab}	95.2^{ab}	90.1^{b}
M	79^{c}	77.6^{c}	83^{c}	86^{c}	81.4^{c}
SEM	0.035	0.012	0.026	0.014	0.031

Table 6: The serum cholesterol and triglyceride in broiler breeder

Treatment	serum cholesterol and triglyceride (mg/100ml)			
	Cholesterol		Triglyceride	
	Female	Male	Female	Male
L_1F_1	1.084^{a}	0.14	9.55	0.072
L_1F_2	0.97^{ab}	0.081	9.45	0.046
L_2F_1	79^{bc}	0.088	10.72	0.7
L_2F_2	0.65^{c}	0.12	7.77	0.062
M	0.93^{ab}	0.098	9.11	0.057
SEM	0.002	0.0003	0.015	0.041

a,b,c Mean values in the same column with different superscript letters were significantly different ($p<0.05$).

Table 7: The egg yolk cholesterol, total yolk cholesterol and yolk weights in broiler breeder

Treatment	Traits		
	yolk cholesterol (mg/gr)	total yolk cholesterol	yolk weights (gr)
L_1F_1	14.85^{a}	271.18	18.31^{b}
L_1F_2	14.06^{bc}	262.39	18.66^{ab}
L_2F_1	13.68^{c}	264.79	19.35^{a}
L_2F_2	14.39^{b}	271.88	18.51^{ab}
M	14.14^{b}	264.46	18.7^{ab}
SEM	0.016	3.8	0.13

a,b,c Mean values in the same column with different superscript letters were significantly different ($p<0.01$).

characteristics. A direct effect of carnitine supplementation on egg yolk carnitine content and decrease of yolk cholesterol (mg/gr) also seems plausible, in the other hand dietary L-carnitine may benefit to diet fat and lipid utilization and improve energy utilization but a longer treatment period should be tested in order to obtain definitive conclusions.

Acknowledgements

This study supported by funds "Lohmann Animal Health Company". The authors would like to thank Dr Ashraffpour for friendly cooperation and preparation L-carnitine and birds.

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