

## Effects of chito-oligosaccharides and L-carnitine supplementation in diets for Japanese quails on performance, carcass traits and some blood parameters

[Efeitos da chito-oligosacarídeos e suplementação de L-carnitina em dietas para codornas Japonesas sobre o desempenho, características de carcaça e de alguns parâmetros sanguíneos]

T. Tufan<sup>1</sup>, C. Arslan<sup>1</sup>, Ö. Durna<sup>1</sup>, K. Önk<sup>2</sup>, M. Sari<sup>3</sup>, H. Erman<sup>4</sup>

<sup>1</sup>Faculty of Veterinary Medicine – University of Kafkas– Kars, Turkey

<sup>2</sup>Kars Vocational School – University of Kafka s– Kars, Turkey

<sup>3</sup>Faculty of Veterinary Medicine – University of Kafkas – Kars, Turkey

<sup>4</sup>Faculty of Medicine – University of Kafkas – Kars, Turkey

### ABSTRACT

The aim of this study was to determine effects of dietary supplementation with chitosan oligosaccharides (COS) and L-carnitine, individually or dually, on growth performance, carcass traits and some blood serum parameters in quails. A total of 192, four days old, Japanese quail chicks were allotted four groups, each of which included four replicates (12 birds per replicate). The groups received the same basal diet supplemented with 0 (Control), 150mg/kg chitosan oligosaccharides (COS), 150mg/kg L-carnitine (Carnitine), and 150 mg/kg chitosan oligosaccharides+150 mg/kg L-carnitine (COS+Car.) during the starter (1 to 21 days) and a grower (22 to 42 days) period. The feeding trial showed that COS, L-carnitine and COS+L-carnitine had no significant effect on live weight, live weight gain, feed consumption and feed conversion. Supplementation with COS+L-carnitine induced higher leg ratio from than that of the Control. There were no differences on serum albumin, total protein, glucose and total cholesterol concentrations. It is concluded that due to the obtained higher leg ratio from COS+Car. group, after analysis of the profit and loss, if is economically profitable, chitosan oligosaccharides+L-carnitine could be added quail diets.

Keywords: quail, chitosan oligosaccharides, L-carnitine, growth performance, carcass traits, blood parameters

### RESUMO

O estudo objetivou determinar os efeitos da suplementação com chito-oligosacarídeos (COS) e L-carnitina, individualmente ou em conjunto, sobre o desempenho, características de carcaça e alguns parâmetros sanguíneos em codornas. Um total de 192 codornas japonesas, com quatro dias de vida foi separado em quatro grupos, cada grupo com quatro repetições (12 aves por repetição). Os grupos receberam a mesma dieta basal suplementada com 0 (Controle), 150mg/kg chito-oligosacarídeos (COS), 150mg/kg L-carnitina (Carnitina), e 150mg/kg chito-oligosacarídeos +150 mg/kg L-carnitina (COS+Car.) durante o período inicial (1 a 21 dias) e de crescimento (22 a 42 dias). A fase de alimentação mostrou que COS, L-carnitina e COS+L-carnitina não tiveram efeito significativo no peso vivo, ganho de peso vivo, consumo de alimento e conversão de alimento. A suplementação com COS+L-carnitina induziu proporção de perna maior que o Controle. Não houve diferenças na concentração de albumina sérica, proteína total, glicose e colesterol total. Conclui-se que devido à proporção maior de perna obtida para o grupo COS+Car., após análise de perda e ganho, se for economicamente viável chito-oligosacarídeos+L-carnitina pode ser adicionado à dieta de codornas.

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E-mail: tuncay-tufan@hotmail.com

## INTRODUCTION

Antibiotics, as growth promoters and therapeutic medicines to decrease the sensitivity to infectious diseases, have been widely used in animal production for many years (Barton, 2000). However, in the development of antibiotic resistance and transfer of antibiotic resistance genes from animal to human the in-feed antibiotics have been banned in the European Union since January 1, 2006 (Castanon, 2007). After the banning of the use of antibiotics, further studies have increased on the use of natural feed additives instead of antibiotics. In this context, extensive studies have been done on chitosan and chitosan oligosaccharides (COS), which are accepted as a prebiotic. Chitosan and COS are obtained from chitin. Chitin, a N-acetylglucosamine ( $\beta$ -1,4 linked 2-acetamido-D-glucose) polymer, is a cellulose-like biopolymer richly present in the exoskeleton of crustaceans (Jeuniaux, 1982) and in the cell walls of most of the fungi, insects, and some algae (Ruiz-Herrera, 1978). Chitin is one of the most abundant, easily obtained and renewable natural fiber, second to cellulose on earth (Ruiz-Herrera, 1978). Chitosan is a deacetylated, demineralised and deproteinised product of chitin (Austine *et al.*, 1981). The COS is produced from chitin or chitosan by chemically or enzymatic decomposition methods (Li *et al.*, 2007). It has a far lower molecular weight, higher solubility and activity than chitosan (Jeon *et al.*, 2000; Kim and Rajapakse, 2005).

From the studies conducted on different animal species regarding the chitosan and COS it was determined that they have an increased effect on the nutritional value of feed (Zhou *et al.*, 2009), antioxidant (Sun *et al.*, 2008), antitumoral (Jeon and Kim, 2002), hypocholesterolemic (Razdan and Petterson, 1994), reducing the absorption of fat (Kobayashi and Itoh, 1991; Razdan and Petterson, 1994), antibacterial (Choi *et al.*, 2001; No *et al.*, 2002), antifungal (Roller and Covill, 1999), free radical scavenging (Je *et al.*, 2004.), enhanced immune function (Huang *et al.*, 2005), protection against infectious diseases, and reducing pathogens in the small intestines (No *et al.*, 2002; Zhou *et al.*, 2009). It is also established that COS supplementation to the broiler chicken diets improved live weight (Huang *et al.*, 2005), live weight gain (Li *et al.*, 2007), feed intake (Li *et al.*, 2007; Zhou *et al.*, 2009), and feed

conversion (Huang *et al.*, 2005; Li *et al.*, 2007). However, Keser *et al.* (2011) reported that COS supplementation to the diets did not change the growth performance of broiler chickens. Furthermore, Zhou *et al.* (2009) reported that COS supplementation to the broiler diets did not change breast meat and relative organ weight and reduced the concentration of triglycerides in blood serum, but Keser *et al.* (2011) found no effect on the concentration of triglycerides.

In the recent years, another study in feed additive was carried out using L-carnitine in the feeding of poultry (Arslan, 2006). The major metabolic role of L-carnitine appears to be the transport of long-chain fatty acids from cytoplasm into the mitochondria for  $\beta$ -oxidation (Borum, 1983). Cakir and Yalcin (2007) offered low (2800kcal/kg ME) and normal energy level (3000kcal/kg ME) diets supplemented with 100mg/kg L-carnitine in broiler chickens. They established that L-carnitine supplementation did not provide significant effects on growth performance, but increased the liver weight of birds, which consumed a lower energy diet. In the same study, blood parameters, carcass yield, heart, gizzard, stomach and abdominal fat weights revealed no significant differences among the groups. Some previous studies established that L-carnitine supplementation to the broiler chicken diets reduced the amount of abdominal fat (Rabie *et al.*, 1997; Xu *et al.*, 2003). There are indications that L-carnitine supplementation to the broiler diet with 25, 50, 75 and 100 mg/kg has not affected body weight gain and feed conversion (Xu *et al.*, 2003). Arslan *et al.* (2004) established that 100 mg/l L-carnitine administration via drinking water of quails did not change growth performance, increased the breast meat ratio and concentration of serum glucose.

The aim of the present study was determine the effects of COS and L-carnitine supplementation individually or dually to the Japanese quail diets on growth performance, carcass yield traits and some blood parameters.

## MATERIALS AND METHODS

One hundred and ninety two unsexed Japanese quail chicks (*Coturnix coturnix japonica*), four days old, were allotted to 4 treatment groups, each of which included four replicates of 12

birds. Quail were offered the same basal diet that was supplemented with 0 (Control), 150 mg COS (COS), 150 mg L-carnitine (Carnitine), and 150 mg COS+150 mg L-carnitine (COS+Car.)/kg diet. Diets were fed from 1 to 42 d and included a starter (1 to 21 d) and a grower (22 to 42 d). The nutrient level of the diets was based on the National Research Council (1994) recommended nutrient requirements of quails (Table 1). The COS used in this study was provided by the GlycoBio Company, (Dalian, China) and L-carnitine from a pharmacy (Carnitine (L-carnitine), Sigma-Tau, Italy). Diets were offered *ad libitum* in mash form and water was available at all times during the experimental period. The study was conducted in wire cages.

Table 1. Ingredient composition and analyzed content of nutrient of the diets used in the study, %

Ingredients	Starter diet	Grower diet
Corn	47.21	52.21
Soy bean meal	33.00	27.80
Sunflower meal	5.18	5.00
Wheat	6.50	5.60
Vegetable oil	3.35	5.12
DCP	2.33	2.06
Lime stone	0.86	0.81
Vit. Min. prem.*	0.35	0.35
Salt	0.30	0.30
D-L Methionine	0.38	0.30
L- Lysine	0.34	0.25
Sodium bicarbonate	0.20	0.20
<i>Nutritional content, DM basis</i>		
Dry matter	92.20	93.10
Metabolic energy, MJ/kg**	12.48	13.08
Crude protein	22.12	20.10
Crude fat	6.88	7.21
Crude fibre	4.43	4.62
Crude ash	5.63	6.97

\*Per 2.5kg containing the following nutrients: vit. A: 6000000 IU, vit. D3: 32000000 IU, vit. E: 40000mg, vit. K<sub>3</sub>: 1600mg, vit. B<sub>1</sub>: 1200mg, vit. B<sub>2</sub>: 3200mg, Niacin: 24000mg, Cal.D-Pantothenate: 7200mg, vit. B<sub>6</sub>: 2000mg, vit. B<sub>12</sub>: 6,4mg, D-Biotin: 80mg, Folic acid: 800mg, vit. C: 40000mg, Manganese: 42000mg, Iron: 33600mg, Zinc: 33600mg, Copper: 3600mg, Cobalt: 80mg, Iodine: 400mg, Selenium: 72mg, Molybdenum: 416mg.

\*\* : Provided by calculation (National..., 1994).

Quails were weighed at weekly intervals, feed intake and feed conversions were also determined at the same time. At the 42 d of the

age, 20 quails per treatment group (five birds per replicate) were slaughtered for carcass analyses; 80 quails were slaughtered in total.

During the slaughtering process blood samples were also taken from the quails. Blood samples were allowed to cool at room temperature for 6 hours and then centrifuged at 1500g for 10 minutes. Serum albumin, total protein, glucose and total cholesterol concentrations were analysed by an auto analyser (Roche Cobas C-6000), using the commercial kits belonging to the same firm.

Dry matter, crude protein, crude fiber, ether extract, and ash content of the experimental diets were determined according to the AOAC Association... (1995) procedure.

Data were analysed by ANOVA using the SPSS program (2003). Significant differences among the groups were determined using Duncan's multiple range tests, with a 5% level probability. The results are given as mean  $\pm$  standard error, ( $X \pm Sx$ ).

## RESULTS

No difference (except for the higher live weight in the COS group than the other groups at four weeks) in live weight, live weight gain, average feed intake and feed conversion were observed in quails fed with COS or L-carnitine, individually or dually supplemented diets at the starter period and at the grower period, as well as the overall experiment (Table 2).

Supplementation with COS, L-carnitine and COS+L-carnitine did not affect live weight, carcass weight and carcass ratio, or breast, wing, heart, liver and abdominal fat percentage to the carcass weight (Table 3). The COS and L-carnitine supplementations on the diet showed no significant increase trend for the leg ratio and the carcass weight. However, leg percentage in the COS+Car. group was significantly higher than that of the Control group ( $P < 0.05$ ). Gizzard percentage for the carcass weights in the trial groups were significantly higher than that of the Control group ( $P < 0.05$ ).

Supplementation with COS, L-carnitine and COS+L-carnitine did not affect the serum albumin, total protein, glucose and total cholesterol concentrations (Table 4).

Table 2. Effect of COS, L-carnitine and COS+L-carnitine on growth performance of quails

Weeks/Periods/Item	Control	COS	Carnitine	COS+Car.	Significance
----- Live weights, g -----					
Initial	12.6±0.2	12.4±0.1	12.6±0.2	12.7±0.3	NS
1	44.0±1.2	42.5±1.1	43.6±1.6	44.8±1.3	NS
2	88.4±1.8	78.7±4.4	89.0±2.7	86.4±1.7	NS
3	135.2±1.3	129.2±2.7	134.6±1.5	134.1±1.6	NS
4	166.0±1.8a	160.3±1.2b	168.5±0.5a	168.2±1.9a	**
5	193.4±2.4	187.4±3.3	195.8±1.0	193.6±3.5	NS
6	215.2±4.3	209.8±4.7	222.2±1.8	213.6±3.4	NS
<i>Starter period (1 to 21 d)</i>					
Average daily gain, g	5.8±0.7	5.6±0.9	5.7±0.8	5.8±0.7	NS
Average feed intake, g	11.7±2.7	10.8±2.9	11.6±2.6	11.4±2.8	NS
Feed conversion, g/g	1.94±0.3	1.88±0.3	1.94±0.3	1.93±0.3	NS
<i>Grower period (22 to 42 d)</i>					
Average daily gain, g	3.8±0.4	3.8±0.4	4.2±0.3	3.8±0.6	NS
Average feed intake, g	22.0±3.1	21.0±2.9	22.2±3.2	20.9±2.4	NS
Feed conversion, g/g	6.38±1.8	5.93±1.4	5.52±1.1	6.07±1.5	NS
<i>Overall experiment (1 to 42 d)</i>					
Average daily gain, g	4.8±0.6	4.7±0.6	4.9±0.5	4.8±0.6	NS
Average feed intake, g	16.7±2.8	15.9±2.9	16.9±3.0	16.2±2.7	NS
Feed conversion, g/g	4.16±1.3	3.91±1.1	3.73±1.0	4.00±1.2	NS

a, b: Values in the same row with a different letter are significantly different (\*\*: P<0.01). NS: Not significant.

Table 3. Effect of COS, L-carnitine and COS+L-carnitine on carcass composition of quails

Item	Control	COS	Carnitine	COS+Car.	Significance
Live weight, g	199.1±2.8	202.3±3.5	199.9±4.5	200.6±4.1	NS
Carcass weight, g	130.3±2.3	130.7±2.5	132.3±2.8	125.9±1.2	NS
Carcass ratio, %	65.5±0.8	64.8±0.8	66.3±0.8	63.0±1.1	NS
<i>The percentage of carcass weight, %</i>					
Leg	22.7±0.4b	23.6±0.3ab	23.5±0.5ab	24.3±0.3a	*
Breast	39.6±0.5	39.2±0.6	38.7±0.5	38.9±0.5	NS
Wing	8.5±0.4	8.6±0.4	8.6±0.1	8.7±0.1	NS
Heart	1.3±0.1	1.4±0.1	1.4±0.0	1.5±0.1	NS
Liver	2.8±0.2	3.1±0.0	2.8±0.2	3.3±0.3	NS
Gizzard	2.7±0.1b	3.1±0.10a	3.1±0.1a	3.2±0.1a	*
Abdominal fat	1.6±0.1	1.5±0.2	1.5±0.2	2.1±0.3	NS

a, b: Values in the same row with a different letter are significantly different (\*: P<0.05). NS: Not significant.

Table 4. Effect of COS, L-carnitine and COS+L-carnitine on albumin, total protein, glucose and total cholesterol levels in quails

Item	Control	COS	Carnitine	COS+Car.	Significance
Albumin, g/dL	1.20±0.0	1.30±0.1	1.40±0.1	1.40±0.0	NS
Total protein, g/dL	2.50±0.1	2.80±0.2	3.00±0.2	2.90±0.2	NS
Glucose, mg/dL	257±9.6	246±14.9	258±10.6	260±13.3	NS
Total cholesterol, mg/dL	223±11.1	247±15.0	212±28.6	204±21.9	NS

NS: Not significant.

## DISCUSSION

In the study presented here no differences in live weight, weight gain, feed intake, and feed conversion were observed in quails both in the starter and grower periods as well as over the entire experimental period (Table 2). Our results reflected that COS and L-carnitine additions to the quail diets, individually or dually, did not change the growth performance of quails. In this study, additions of COS did not affect the growth performance of quails, our results are in accordance with other results in broiler chicks (Huang *et al.*, 2005; Keser *et al.*, 2011). However, there are reports about the positive effect of COS supplementation to the diet on the growth performance of broiler chicks (Li *et al.*, 2007; Zhou *et al.*, 2009). Similar to this study, it has been reported that L-carnitine supplementation via drinking water to the quails (Arslan *et al.*, 2004), or diets does not affect the growth performance of broiler chicks (Buyse *et al.*, 2001; Lien and Horng, 2001; Cakir and Yalcin, 2007). However, in other studies beneficial effects of L-carnitine supplementation were observed on performance in broiler chickens (Lettner, 1992; Rabie *et al.*, 1997; Kita *et al.*, 2002). In the study, the addition of L-carnitine to the diets did not alter the performance of quails, this situation suggests that the concentration of L-carnitine or its precursors, such as methionine and lysine, in the used diets may be sufficient for the quail's requirement. Schumacher *et al.* (1993) concluded that carnitine supplementation to the broiler diets improved growth performance, mainly in diets marginally deficient in lysine and methionine plus cysteine. Discrepancies on the growth performance between this study and other studies may be related to the dose and species of COS

and L-carnitine, and also differences in used diets and animal species.

The COS, L-carnitine and COS+L-carnitine supplementation to the diet did not affect live weight, carcass weight and carcass ratio, and also breast, wing, heart, liver and abdominal fat percentage to the carcass weight (Table 3). In this study, COS supplementation to the diet did not affect carcass yield traits, except for the higher gizzard ratio in the COS group than that of the Control group. A study conducted on the broiler chickens established that COS supplementation to the diet did not change breast and gizzard ratio, but decreased abdominal fat ratio (Zhou *et al.*, 2009). Interestingly COS+L-carnitine supplementation to the diet induced higher leg ratio than the Control group. The reason for this is not understood, but this is a positive situation regarding the economic condition. Similarly, the higher ratio of gizzard was obtained in the COS, Carnitine and COS+Car. group than the Control group. Arslan *et al.* (2004) found no changes on the carcass yield traits, but higher breast ratio in the quail supplemented L-carnitine via drinking water. Lien and Horng (2001) found no positive or negative effect of L-carnitine supplementation to the broiler chicken diet on carcass yield traits. Discrepancies between this study and other studies on carcass yield traits may be related to differences of COS and L-carnitine and their doses.

As seen in Table 4, serum albumin, total protein, glucose and total cholesterol concentrations did not differ in all groups. In accordance with this study, it is reported that COS supplementation to the broiler diet did not change serum albumin and total protein concentration (Zhou *et al.*, 2009; Keser *et al.*, 2011; Tufan and Arslan,

2012). In contrast to these reports, Li *et al.* (2007) found a significant increase in the total protein concentration fed with COS diet in broiler chickens. In the study, serum glucose concentration did not change with the addition of COS, this result is in accordance with the result of Keser *et al.* (2011). Similar to our result, Li *et al.* (2007), and Keser *et al.* (2011) determined no changes in the concentration of total cholesterol in the broiler chicken fed with COS supplemented diets. However, Tufan and Arslan (2012) found a significant decrease in the concentration of total cholesterol fed with COS supplemented diets in the broiler chickens. L-carnitine supplementation to the quail diet did not change the albumin concentration in this study. Many studies conducted on different poultry species established that L-carnitine supplementation did not change the serum albumin concentration (Uysal *et al.*, 1999; Arslan *et al.*, 2004). Contrary to these reports, Cakir and Yalcin (2007) found a significant increase in concentration of serum albumin supplemented L-carnitine in broiler chickens. In this study, serum total protein concentration did not change in the Carnitine group; our result is in accordance with results from other studies (Arslan *et al.*, 2004; Cakir and Yalcin, 2007). However, Uysal *et al.* (1999) reported that L-carnitine supplementation to the quail diet significantly decreased total protein concentration. In this study, L-carnitine supplementation did not change glucose concentration; this finding is in accordance with the other researchers' results (Uysal *et al.*, 1999; Arslan *et al.*, 2003). However, Arslan *et al.* (2004) found that L-carnitine supplementation via drinking water to quail increased serum glucose concentration. Similar to our result, different researchers reported that L-carnitine supplementation to the poultry diet did not change the total cholesterol concentration (Uysal *et al.*, 1999; Arslan *et al.*, 2003). Discrepancies between this study and other studies on serum parameters may be related to differences of COS and L-carnitine, and their doses, and the animal species used. It is worth noting that we could not come across any research that deals with dually using of COS and L-carnitine in animal feed as feed additives. Therefore, we could not compare all our results in the COS+Car. group to other research.

## CONCLUSION

COS and L-carnitine individual or dual supplementation did not affect growth performance, serum albumin, total protein, glucose and total cholesterol, but COS+L-carnitine supplementation enhanced leg ratio when compared to the Control. Furthermore, COS, Carnitine and COS+L-carnitine supplementation enhanced the gizzard ratio. It is concluded that COS and L-carnitine supplementation individually or dually induced similar growth performance results with the Control, due to the higher leg ratio obtained from the dual combination of COS and L-carnitine, they might be added to quail diet, after profit and loss analysis.

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