



## Effect of Intensity and Duration of Quantitative Feed Restriction and Dietary Coenzyme Q10 on Growth Performance, Carcass Characteristics, Blood Constituents, Thyroid Hormones, Microbiota, Immunity, and Ascites Syndrome in Broiler Chickens

Jahanpour H<sup>1</sup>, Chamani M<sup>1</sup>, Seidavi AR<sup>2</sup>, Sadeghi AA<sup>1</sup> & Aminafschar M<sup>1</sup>

<sup>1</sup>Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

<sup>2</sup>Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran

Poultry Science Journal 2020, 8(2): 145-162

### Keywords

Ascites  
Growth  
Ubidecarenone  
Broiler chicken  
Feed restriction

### Corresponding author

Mohammad Chamani  
[m.chamani@srbiau.ac.ir](mailto:m.chamani@srbiau.ac.ir)

### Article history

Received: January 9, 2020  
Revised: May 7, 2020  
Accepted: June 25, 2020

### Abstract

This experiment was conducted to investigate the effects of feed restriction and dietary supplementation of coenzyme Q10 (CoQ10) on growth performance, carcass characteristics, blood parameters, hormonal, immune responses, and intestinal microbiota. The completely randomized design experiment used a 2×2×3 factorial arrangement of treatments to provide two dietary restriction levels (10 and 20% less than the standard guide for Ross strain 308 broilers), two restriction durations (7 and 14 days), and three levels of CoQ10 (0, 20, and 40 mg/kg). In addition to the above-mentioned treatments, 3 other treatments were provided without feed restriction for each of the 3 levels CoQ10. Each of the fifteen treatments was replicated 4 times with each replicate containing 10 male birds. No differences were observed in weight gain among treatments. Feed conversion ratio decreased significantly when chicks had the highest duration (14 days) and intensity (20%) of feed restriction and fed all 3 levels of coQ10 as well as when had the mild duration (7 days) and intensity (10%) of feed restriction without coQ10 supplementation ( $P < 0.05$ ). Heart weight and right ventricular to total ventricular ratio were not affected by feed restriction, but both total heart and right ventricular ratio decreased when CoQ10 was fed ( $P < 0.05$ ). Blood and hormonal parameters were relatively unaffected by treatments although cortisol decreased with CoQ10 supplementation and CoQ10 at 40 mg/kg increased immune globulins M and G ( $P < 0.05$ ). Under the conditions of this experiment, we conclude that supplementing CoQ10 can partially overcome the negative effects of feed restriction. Although the exact mechanism is unknown, CoQ10 appears to improve immune response and reduce subclinical ascites syndrome.

### Introduction

The economic efficiency of the broiler industry depends on achieving greater growth in a shorter time together with an improved feed conversion ratio. Poultry geneticists have greatly increased the selection for rapid growth breeds. However, the increase in rapid growth also increases the possibility of metabolic disorders. When the growth rate and metabolism increase, oxygen requirements for metabolic processes increase also. This may lead to a lack of oxygen in tissues, thus reducing blood oxygen levels and ultimately causing anoxia, which is considered the main factor for the onset of the ascites

syndrome in broilers (Huang *et al.*, 2011). The combination of improved feed intake, metabolic rate, growth rate, and high-density diets to achieve the broiler's genetic potential results in oxidative stress causing decreased performance (Shi-bin *et al.*, 2007). Oxidative stress occurs when concentrations of free radicals (oxygen radicals) are greater than antioxidants (Bautista-Ortega *et al.*, 2010). Reactive oxygen species (ROS) in the mitochondria and accumulation of reactive oxygen species (ROS) directly affect vascular regeneration and lead to pulmonary hypertension (Bautista-Ortega *et al.*, 2010).

Coenzyme Q (C59H90O4; ubiquinone, ubiquinone, CoQ10, CoQ, or Q10) is ubiquitous in most bacteria and animals. CoQ10 is found in all cell membranes, especially in the heart, liver, kidney, and pancreas. CoQ10 is formed from ring quinidine and a hydrophobic chain consisting of 10 isopropyl units (Ernster and Dallner, 1995). CoQ10 is located in the inner membrane of the cell as a fat-soluble moiety and is an essential substance for the conversion of cell energy into ATP. The CoQ10 also acts as a sink for free radicals to prevent oxidative damage.

Previous researchers have reported improved weight gain due to increased selection intensity for higher growth rates (Butzen *et al.*, 2013). One method for alleviating ascites syndrome is early feed restriction. Usually, followed by early feed restriction, a high-quality feeding program results in faster growth than that normally observed and is referred to as compensatory growth (Turkyilmaz, 2008). Feed restriction results in improved feed efficiency due to reduced energy required for maintenance. It also improves carcass quality due to reduced fat deposition (Plavnik and Hurwitz, 1988).

Previous studies reported positive effects of feed restrictions on broiler productivity (Jahanpour *et al.*, 2014a; Shabani *et al.*, 2015; Rahimi *et al.*, 2015), immunity (Shabani *et al.*, 2015; Rahimi *et al.*, 2015), blood constituents (Jahanpour *et al.*, 2013; Shabani *et al.*, 2015; Rahimi *et al.*, 2015), intestinal microbiota (Jahanpour *et al.*, 2014b; Shabani *et al.*, 2015), carcass characteristics (Jahanpour *et al.*, 2015; Shabani *et al.*, 2015; Rahimi *et al.*, 2015) and organ weight (Rahimi *et al.*, 2015). However, we are unaware of any research on the simultaneous use of CoQ10 and feed restriction program. Therefore, this study was conducted to determine the interactions between CoQ10 and restriction feeding.

## Materials and Methods

This experiment was conducted at the poultry husbandry unit of the Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Iran. Experimental protocols were approved by Islamic Azad University, Rasht Branch before beginning the trial. Diets were formulated to meet the nutritional requirements of broiler chickens based on catalog recommendations for the Ross 308 strain of broilers. All chickens were fed *ad libitum* before and after the restriction periods. Broilers were fed a starter from 1 to 12 days of age, a grower from 13 to 25 days of age, and a finisher diet from 26 to 42 days of age as presented in Table 1. As birds moved from starter to finisher diets, concentrations of metabolizable energy increased and crude protein decreased, resulting in widening energy to amino acid ratio. Six hundred Ross 308 male broiler chickens were purchased from a local provider (Rasht, Iran). Chickens were weighed on the first day and were randomly assigned to one of

60 individual pens (1m × 1m) with 10 birds per pen to provide 4 replicates for each treatment. The research was conducted as a completely randomized design with a 3×2×2 factorial arrangement of treatment to include 2 feed restriction levels (10 or 20% less than recommended), 2 restriction duration (7 or 14 days), and 3 concentrations of CoQ10 (0, 20 and 40 mg/kg of diet). In addition to these 12 treatments, there were 3 treatments with 4 replications each of 10 birds per pen that were not restricted fed but included 0, 20, or 40 mg/kg of CoQ10 (Table 2) to provide a negative control for feed restriction. Thus, there were 15 separate treatments with 40 birds per treatment. Litter for all treatments was shredded paper and cardboard. Feedrestriction was carried out either at days 7 to 14 or days 7 to 21 of age. The CoQ10 was fed in powder form (Webber Naturals, Ottawa, Canada) from 1 to 42 days of age. Temperature, humidity, light, and ventilation as well as vaccination programs were performed based on the Ross 308 guidelines. The birds were vaccinated against infectious bronchitis (1st and 17<sup>th</sup> days of age), Newcastle disease (10<sup>th</sup> and 20<sup>th</sup> days of age), avian influenza (1st day of age), and Gumboro disease (14<sup>th</sup> and 24<sup>th</sup> days of age).

Feed intake and body weight were measured weekly on a pen basis. The feed conversion ratio was calculated for each replicate. Blood samples were randomly collected from 3 chickens/pen, pooled, and transferred to the laboratory for biochemical analysis. Blood samples were collected into Eppendorf tubes without anticoagulant and immediately placed on ice. Whole blood samples were centrifuged at 11952 g for 10 minutes under room temperature after which serum was decanted. Blood was analyzed for concentrations of uric acid, cholesterol, triglycerides, total protein, albumin as described by Jahanpour *et al.* (2013). Thyroxin, triiodothyronine, insulin, cortisol, growth hormone, and insulin-like growth factor 1(IGF-1) concentrations were determined using commercial kits.

On the 42<sup>nd</sup> day of the experiment, 2 birds per pen were randomly chosen, weighed, and killed by cervical dislocation to evaluate the carcass characteristics. The weights of the carcass components and organs are measured according to Alimohammadi *et al.*, (2014).

Cecal microbiota counts were performed based on Jahanpour *et al.*, (2014b). *Escherichia coli*, *coliform* bacteria, *Lactobacillus*, and *Enterococcia* bacteria were determined by streaking agar plates with cecal contents.

Humoral immune competence was assessed on pooled blood from 2 birds per pen using a sheep red blood cell challenge (SRBC) on day 35 after a blood sample was drawn for determination of IgT, IgG, and IgM and hemagglutination microtitration method was used. For the SRBC challenge, 0.5 mL of a 10%

suspension of SRBC in sterile phosphate-buffered saline (PBS) solution (v/v) was inoculated under the skin of the breast, and a blood sample was collected in due course. Response to Newcastle and influenza vaccines was assessed in blood sampled twice at 7 days after initial and second vaccine inoculation. Response to influenza vaccine was assessed in blood samples twice at 21 and 28 days after initial vaccine

inoculation. Haemagglutination inhibition (HI) assays were used following the procedure described by Seidavi *et al* (2015) and Nosrati *et al* (2017).

The right and left ventricles were separated and weighed. The ascites heart index was calculated using the equation of Huchzermeyer and Ruyck (1986): Ascites Heart Index (AHI) = weight of the right ventricle/weight of total ventricle

**Table 1.** Ingredient composition and calculated nutrient analysis of experimental diets.

	Starter (1-12 d)	Grower (13-25 d)	Finisher (26-42 d)
Ingredient (%)			
Corn	53.75	58.88	61.55
Soybean meal (44% CP)	39.50	33.50	30.00
Soybean Oil	1.70	2.50	3.50
Di-Calcium phosphate	1.90	1.70	1.60
CaCO <sub>3</sub>	1.20	1.60	1.60
DL-Methionine	0.25	0.22	0.20
L-Lysine-Hydro-Chloride	0.05	0.05	0.05
L-Threonine	0.10	0.10	0.10
NaCl	0.20	0.20	0.20
Sodium bicarbonate (NaHCO <sub>3</sub> )	0.15	0.15	0.15
Vitamin- Mineral premix*	0.50	0.50	0.50
Vitamin A	0.10	0.10	0.10
Vitamin D <sub>3</sub>	0.15	0.10	0.10
Vitamin E	0.20	0.10	0.10
Vitamin K <sub>3</sub>	0.10	0.15	0.10
Vitamin B <sub>1</sub>	0.10	0.10	0.10
Coccidioacetate	0.05	0.05	0.05
Nutrient analysis			
Metabolizable Energy (ME) (kcal/kg)	3010	3150	3200
Crude Protein (%)	22.0	20.0	18.0
Lysine (%)	1.44	1.10	0.95
Methionine (%)	0.51	0.44	0.36
Met+Cys (%)	1.09	0.94	0.36
Threonine (%)	0.93	0.79	0.64
Tryptophan (%)	0.25	0.21	0.18
Arginine (%)	1.48	1.26	1.02
Iso-Leucine (%)	0.95	0.81	0.65
Calcium (%)	1	0.9	0.85
Available Phosphorus (%)	0.50	0.45	0.42
Sodium (%)	0.16	0.16	0.16
Potassium (%)	0.90	0.90	0.90
Chloride (%)	0.22	0.22	0.22

\*Amount per kg: vitamin A, 5,000 IU; vitamin D<sub>3</sub>, 500 IU; vitamin E, 3 mg; vitamin K<sub>3</sub>, 1.5 mg; vitamin B<sub>2</sub>, 1 mg; calcium pantothenate, 4 mg; niacin, 15 mg; vitamin B<sub>6</sub>, 13 mg; Cu, 3 mg; Zn, 15 mg; Mn, 20 mg; Fe, 10 mg; K, 0.3 mg.

**Table 2.** Arrangement of studied treatments

Treatment	Restriction duration (days)	Restriction intensity (%)	Coenzyme Q10 levels(mg/kg)
1	0	0	0
2	0	0	20
3	0	0	40
4	7	10	0
5	7	10	20
6	7	10	40
7	14	10	0
8	14	10	20
9	14	10	40
10	7	20	0
11	7	20	20
12	7	20	40
13	14	20	0
14	14	20	20
15	14	20	40

During the experiment, the number of dead chickens was recorded and autopsies were performed. Ascites was considered positive when fluid accumulation in the abdomen and/or pericardium and the enlargement of the right ventricle was detected. The ratio of the right ventricle to total ventricular was calculated after dissecting the large vessels, the corridors and adipocytes around the heart, the right and left ventricles from their attachment points in the wall, and were weighed once the two ventricles were completely separated. A right ventricle ratio of more than 25% was also used as an indicator of ascites syndrome.

The data were analyzed once in the form of  $2 \times 2 \times 3$  factorial arrangement with 12 treatments (4<sup>th</sup> to 15<sup>th</sup> treatments) and once based on a completely randomized design with all 15 treatments (SPSS, 1997). Significance was declared when  $P < 0.05$ . Duncan's test was used to compare the significance of the difference between the treatments.

## Results

### Growth performance

The average feed intake, body weight, and feed conversion ratio for the entire trial are presented in Table 3. Restriction duration and intensity as well as CoQ10 supplementation did not affect feed intake ( $P \geq 0.05$ ). Groups had the highest duration (14 days) and intensity (20%) of feed restriction supplemented with 20 mg/kg CoQ10 had the lowest feed intake ( $P < 0.05$ ), while the highest feed intake was for unrestricted groups ( $P < 0.05$ ). Feed intake significantly differed between treatments 0, 10, and 20% feed restriction ( $P < 0.05$ ), which indicates the effect of feed restriction on the reduction of feed intake.

Feed restriction until 21 days of age did not affect body weight gain ( $P \geq 0.05$ ). Over the entire trial, pens that received feed restriction from 7 through 21 days of age had a numerically higher body weight ( $P \geq 0.05$ ) (Table 3). Supplementation of CoQ10 also did not affect body weight gain ( $P \geq 0.05$ ).

**Table 3.** Effect of restriction intensity, restriction duration, and coenzyme Q10 levels on broiler performance at 1-42 days of age.

		Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio(g/g)
Restriction intensity (%)	10	111.36	60.52	1.83
	20	111.24	61.51	1.80
	SEM	0.58	1.15	0.02
	P-value	0.88	0.54	0.18
Restriction duration(day)	7	111.89	60.72	1.84
	14	110.71	61.31	1.80
	SEM	0.58	1.15	0.02
	P-value	0.15	0.72	0.46
Coenzyme Q (mg/kg)	0	110.86	62.47	1.77
	20	110.65	59.42	1.86
	40	112.39	61.16	1.83
	SEM	0.71	1.41	0.03
	P-value	0.18	0.32	0.96
Treatment 1		125.82 <sup>a</sup>	60.44	2.08 <sup>a</sup>
Treatment 2		126.79 <sup>a</sup>	63.74	1.98 <sup>ab</sup>
Treatment 3		126.27 <sup>a</sup>	64.70	1.95 <sup>abc</sup>
Treatment 4		110.72 <sup>bcd</sup>	63.82	1.73 <sup>c</sup>
Treatment 5		108.36 <sup>de</sup>	58.36	1.85 <sup>bc</sup>
Treatment 6		114.20 <sup>b</sup>	59.64	1.91 <sup>bc</sup>
Treatment 7		109.51 <sup>cde</sup>	59.50	1.84 <sup>bc</sup>
Treatment 8		112.73 <sup>bcd</sup>	60.70	1.85 <sup>bc</sup>
Treatment 9		112.65 <sup>bcd</sup>	61.11	1.84 <sup>bc</sup>
Treatment 10		112.57 <sup>bcd</sup>	61.94	1.81 <sup>bc</sup>
Treatment 11		113.36 <sup>bc</sup>	58.75	1.92 <sup>bc</sup>
Treatment 12		112.15 <sup>bcd</sup>	61.83	1.81 <sup>bc</sup>
Treatment 13		110.64 <sup>bcd</sup>	64.63	1.71 <sup>c</sup>
Treatment 14		108.16 <sup>e</sup>	59.85	1.80 <sup>c</sup>
Treatment 15		110.55 <sup>bcd</sup>	62.05	1.78 <sup>c</sup>
SEM		1.31	2.78	0.06
P-value		<0.0001	0.882	0.031

Means without superscript letters or with the same superscript letters within the same column do not differ ( $P \geq 0.05$ ); SEM: Standard Error of Means.

Duration and intensity of feed restriction as well as CoQ10 had no significant effect on feed conversion ratio ( $P \geq 0.05$ ). Feed restriction for 14 days resulted in a lower feed conversion ratio than those birds that received no feed restriction or

restriction for 7 days ( $P < 0.05$ ). Supplemental CoQ10 had no significant effect on feed conversion ratio ( $P \geq 0.05$ ). Feed conversion ratio decreased significantly when chicks had the highest duration (14 days) and intensity (20%) of feed restriction and

fed all 3 levels of CoQ10 as well as when had the mild duration (7 days) and intensity (10%) of feed restriction without CoQ10 supplementation ( $P < 0.05$ ).

**Economical indices**

Feed restrictions for 14 days of age resulted in the highest production index although this did not differ from 0 or 7-day restriction ( $P \geq 0.05$ ) (Table 4). However dietary supplementation of CoQ10 at 20 and 40 mg/kg values increased the numerical production index relative to the non-supplemented CoQ10 group ( $P \geq 0.05$ ).

**Ascites related parameters**

Feed restriction and supplemental CoQ10 reduced the right ventricle to the total ventricle (RV/ TV) ratio ( $P < 0.05$ ; Table 4). These parameters in groups that were fed CoQ10 was lower than groups that did not receive CoQ10 ( $P < 0.05$ ). The risk of ascites syndrome appears to be reduced in groups supplemented with either 20 or 40 mg/kg CoQ10. The feed restriction had no significant effect on heart weight or RV/TV ratio.

**Carcass characteristics**

Carcass analysis data are presented in Tables 5 and 6. The abdominal fat percentage was unaffected by CoQ10 or feed restriction (Table 6). Also, the weight percentage of breast and thighs in groups supplemented CoQ10 was higher than treatments without additives ( $P \geq 0.05$ ). Duration of feed restriction reduced breast weight and leg weight ( $P < 0.05$ ), although the percent of the total carcass for these cuts was unchanged.

The supplementation of 40 mg/kg of CoQ10 increased dressing weight, full carcass weight, and eviscerated carcass weight compared with 0 or 20 mg/kg ( $P < 0.05$ ; Table 5), although the live weight was not different between CoQ10 treatments.

The effect of various levels of CoQ10 supplementation on the length of the intestine was not significant ( $P \geq 0.05$ ). The length of the intestine in groups supplemented 20 or 40 mg/kg CoQ10 was significantly higher than non-supplemented groups ( $P < 0.05$ ) (Data not shown). The relative heart weight in groups supplemented 20 or 40 mg/kg CoQ10 was less than non-supplemented groups ( $P \geq 0.05$ ). The relative weight of abdominal fat in groups supplemented with 40 mg/kg CoQ10 was decreased ( $P < 0.05$ ).

**Table 4.** Effect of restriction intensity, restriction duration, and coenzyme Q10 levels on production index, final weight, heart weight, and right ventricle to total ventricle weight ratio in broiler at 6 weeks of age.

	Production index	Heart weight (gr)	Right ventricle to total ventricle weight ratio (RV/TV)
Restriction intensity (%)	10%	274.62	14.56
	20%	277.96	14.34
	SEM	29.68	0.35
	P-value	0.82	0.654
Restriction duration (day)	7	270.76	14.55
	14	281.82	14.35
	SEM	29.68	0.35
	P-value	0.38	0.69
Coenzyme Q (mg/kg)	0	253.82	15.06
	20	267.54	13.99
	40	277.51	12.31
	SEM	6.10	0.42
	P-value	0.17	0.062
Treatment 1	249.50	15.90 <sup>a</sup>	25.15 <sup>a</sup>
Treatment 2	262.16	11.54 <sup>c</sup>	22.92 <sup>bcd</sup>
Treatment 3	271.28	11.47 <sup>e</sup>	20.10 <sup>g</sup>
Treatment 4	283.71	15.05 <sup>a</sup>	24.60 <sup>ab</sup>
Treatment 5	263.74	13.23 <sup>cd</sup>	20.57 <sup>fg</sup>
Treatment 6	269.26	12.52 <sup>cd</sup>	20.27 <sup>g</sup>
Treatment 7	265.27	15.45 <sup>a</sup>	24.05 <sup>abc</sup>
Treatment 8	278.95	12.60 <sup>d</sup>	22.90 <sup>bcd</sup>
Treatment 9	286.78	12.75 <sup>d</sup>	22.22 <sup>def</sup>
Treatment 10	280.06	14.50 <sup>b</sup>	23.57 <sup>abcd</sup>
Treatment 11	256.99	13.50 <sup>c</sup>	21.64 <sup>efg</sup>
Treatment 12	270.77	12.72 <sup>cd</sup>	21.27 <sup>efg</sup>
Treatment 13	306.23	13.25 <sup>cd</sup>	21.52 <sup>efg</sup>
Treatment 14	270.49	13.85 <sup>bc</sup>	21.83 <sup>defg</sup>
Treatment 15	283.22	12.25 <sup>de</sup>	22.37 <sup>cdet</sup>
SEM	11.48	0.82	0.55
P-value	0.165	0.0018	<0.0001

<sup>abcdetfg</sup>Means without superscript letters or with the same superscript letters within the same column do not differ ( $P \geq 0.05$ ); SEM: Standard Error of Means.

**Table 5.** Effect of restriction intensity, restriction duration, and coenzyme Q10 levels on the carcass parameters at 42nd day of age

		Live body weight (g)	Dressing weight (g)	Full carcass weight (g)	Eviscerated carcass weight (g)	Eviscerated carcass (%)
Restriction intensity (%)	10	2832.08	2435.58	2213.16	1706.91	76.97
	20	2865.04	2433.41	2220.58	1716.41	77.21
	SEM	38.09	35.78	32.42	31.16	0.31
	P-value	0.54	0.96	0.87	0.83	0.60
Restriction duration (day)	7	2891.6	2474.95	2269.70 <sup>a</sup>	1759.29 <sup>a</sup>	77.40
	14	2805.45	2394.04	2164.04 <sup>b</sup>	1664.04 <sup>b</sup>	76.78
	SEM	38.09	35.78	32.42	31.16	0.31
	P-value	0.11	0.11	0.027	0.037	0.18
Coenzyme Q (mg/kg)	0	2706.00	2404.56 <sup>b</sup>	2160.81 <sup>b</sup>	1657.68 <sup>b</sup>	76.65
	20	2802.56	2374.00 <sup>ab</sup>	2171.12 <sup>b</sup>	1671.12 <sup>b</sup>	76.83
	40	2937.12	2524.93 <sup>a</sup>	2318.68 <sup>a</sup>	1806.18 <sup>a</sup>	77.78
	SEM	46.66	43.82	39.71	38.16	0.39
	P-value	0.08	0.047	0.012	0.016	0.10
Treatment 1		2885.75	2483.75	2206.25 <sup>bc</sup>	1706.25 <sup>bc</sup>	77.32
Treatment 2		2753.25	2410.00	2139.00 <sup>c</sup>	1639.00 <sup>bc</sup>	76.60
Treatment 3		2871.25	2437.50	2163.00 <sup>bc</sup>	1663.00 <sup>bc</sup>	76.73
Treatment 4		2832.00	2452.50	2186.00 <sup>bc</sup>	1686.00 <sup>bc</sup>	77.06
Treatment 5		2651.75	2231.75	2061.50 <sup>c</sup>	1561.50 <sup>c</sup>	75.65
Treatment 6		3095.00	2700.00	2491.25 <sup>a</sup>	1953.75 <sup>a</sup>	78.43
Treatment 7		2720.00	2337.00	2078.00 <sup>c</sup>	1578.00 <sup>c</sup>	75.90
Treatment 8		2806.25	2451.75	2239.25 <sup>bc</sup>	1739.25 <sup>abc</sup>	77.51
Treatment 9		2887.50	2440.50	2223.00 <sup>bc</sup>	1723.00 <sup>abc</sup>	77.28
Treatment 10		2817.75	2405.00	2232.50 <sup>bc</sup>	1720.00 <sup>abc</sup>	76.94
Treatment 11		2910.00	2464.50	2244.50 <sup>bc</sup>	1744.50 <sup>abc</sup>	77.65
Treatment 12		3043.50	2596.00	2402.50 <sup>ab</sup>	1890.00 <sup>ab</sup>	78.65
Treatment 13		2854.25	2423.75	2146.75 <sup>bc</sup>	1646.75 <sup>bc</sup>	76.70
Treatment 14		2842.25	2348.00	2139.25 <sup>c</sup>	1639.25 <sup>bc</sup>	76.52
Treatment 15		2722.50	2363.25	2158.00 <sup>bc</sup>	1658.00 <sup>bc</sup>	76.78
SEM		88.23	83.87	76.79	74.23	0.76
P-value		0.08	0.09	0.02	0.04	0.35

<sup>abc</sup>Means without superscript letters or with the same superscript letters within the same column do not differ ( $P \geq 0.05$ ); SEM: Standard Error of Means.

### Blood constituents

The results for blood biochemical parameters are shown in Table 7. Experimental treatments did not affect uric acid, or any measured lipid or protein serum metabolite. Cholesterol level was affected by experimental treatments ( $P \geq 0.05$ ) and 40 mg CoQ10 decreased serum cholesterol significantly ( $P \geq 0.05$ ).

### Thyroid hormones

The results for hormones are shown in Table 8. While feed restriction did not affect serum T3 concentrations, however, feed restriction from 7-14 days of age reduced T4 concentrations ( $P \geq 0.05$ ). The non-restricted group had a higher T3/T4 ratio compared with feed restriction ( $P < 0.05$ ). In general, measured hormone concentrations were not affected by treatments except for lower cortisol ( $P < 0.05$ ) for groups fed 20 or 40 mg/kg CoQ10.

### Immunity

Treatments did not affect the antibody production against the influenza virus at 28 days as well as the response to Newcastle disease at 7 days (Table 9).

However, a 20% feed restriction decreased the reaction to the influenza vaccine at 21 days compared to a 10% feed restriction. Supplementation of CoQ10 at 20 mg/kg increased antibody production against Newcastle disease at 7 days after the second injection while 40 mg/kg supplementation decreased this reaction (Table 9).

As shown in Table 10, feed restriction and supplementation of CoQ10 had no significant effect on the antibody titers against SRBC ( $P \geq 0.05$ ; Table 10) at 7 days. At 14 days post-injection, there was no significant effect of feed restriction on response to SRBC, and the only response to CoQ10 was that the 40 mg/kg dosage decreased ( $P < 0.05$ ) immunoglobulin M.

There were no significant differences in weight percentage of thymus, spleen, and bursa of Fabricius ( $P \geq 0.05$ ; Table 11) due to dietary treatment.

### Bacteria community

No differences were observed in bacterial populations among treatments. The results showed that with increasing levels of dietary CoQ10, the number of

colonies for harmful bacteria decreased, probably due to the effect of CoQ10 on the immune system and the health of the gastrointestinal tract ( $P \geq 0.05$ ) (Table 12).

**Table 6.** Effect of restriction intensity, restriction duration, and coenzyme Q10 levels on the carcass parameters at 42nd day of age

Restriction intensity (%)		Breast (g)	Relative weight of breast (%)	Legs (g)	Relative weight of legs (%)	Wings (g)	Relative weight of wings (%)	Abdominal fat (g)	Relative weight of abdominal fat (%)	Pancreas (g)	Relative weight of pancreas (%)
10		817.50	33.63	744.04	30.67	175.68	7.24	35.31	1.45	5.81	0.23
20		834.12	34.27	750.45	30.85	174.54	7.18	32.94	1.35	5.55	0.22
<i>SEM</i>		13.55	0.41	11.04	0.41	2.82	0.10	2.27	0.09	0.20	0.008
<i>P-value</i>		0.39	0.28	0.68	0.76	0.77	0.66	0.46	0.44	0.38	0.37
Restriction duration (day)		Breast (g)	Relative weight of breast (%)	Legs (g)	Relative weight of legs (%)	Wings (g)	Relative weight of wings (%)	Abdominal fat (g)	Relative weight of abdominal fat (%)	Pancreas (g)	Relative weight of pancreas (%)
7		851.41 <sup>a</sup>	34.42	763.33 <sup>a</sup>	30.92	178.68	7.25	35.13	1.42	5.97	0.24
14		800.20 <sup>b</sup>	33.47	731.16 <sup>b</sup>	30.60	171.54	7.17	33.11	1.38	5.38	0.22
<i>SEM</i>		13.55	0.41	11.04	0.41	2.82	0.10	2.27	0.09	0.20	0.008
<i>P-value</i>		0.011	0.28	0.04	0.59	0.08	0.56	0.53	0.74	0.05	0.13
Coenzyme Q (mg/kg)		Breast (g)	Relative weight of breast (%)	Legs (g)	Relative weight of legs (%)	Wings (g)	Relative weight of wings (%)	Abdominal fat (g)	Relative weight of abdominal fat (%)	Pancreas (g)	Relative weight of pancreas (%)
0		812.93	33.84	745.75	31.07	176.65	7.36	35.20	1.46	5.75	0.23 <sup>ab</sup>
20		820.56	33.19	739.18	31.21	173.43	7.31	30.64	1.44	5.90	0.24 <sup>a</sup>
40		852.93	33.82	756.81	30.00	175.25	6.96	28.52	1.30	5.38	0.21 <sup>b</sup>
<i>SEM</i>		16.59	0.51	13.53	0.50	3.46	0.12	2.78	0.11	0.25	0.01
<i>P-value</i>		0.14	0.84	0.65	0.19	0.80	0.074	0.77	0.52	0.33	0.04
Treatment 1		798.25 <sup>bc</sup>	32.14 <sup>bc</sup>	728.75	29.35	170.50	6.87 <sup>bc</sup>	30.23	1.21	5.07	0.20
Treatment 2		747.25 <sup>c</sup>	31.01 <sup>c</sup>	704.50	29.24	168.75	7.01 <sup>bc</sup>	27.47	1.13	5.96	0.24
Treatment 3		797.50 <sup>bc</sup>	32.79 <sup>bc</sup>	740.25	30.38	163.75	6.74 <sup>c</sup>	27.25	1.14	6.29	0.25
Treatment 4		848.75 <sup>bc</sup>	34.64 <sup>ab</sup>	761.00	31.07	176.87	7.23 <sup>abc</sup>	37.75	1.54	6.25	0.25
Treatment 5		763.75 <sup>c</sup>	34.18 <sup>abc</sup>	706.50	31.72	169.75	7.64 <sup>ab</sup>	32.87	1.45	5.56	0.24
Treatment 6		868.75 <sup>ab</sup>	32.22 <sup>bc</sup>	788.25	29.27	187.50	6.99 <sup>bc</sup>	35.17	1.31	5.88	0.21
Treatment 7		794.75 <sup>bc</sup>	34.00 <sup>abc</sup>	737.25	31.60	185.00	7.91 <sup>a</sup>	44.87	1.91	5.27	0.22
Treatment 8		830.75 <sup>bc</sup>	33.96 <sup>abc</sup>	746.75	30.59	171.25	6.94 <sup>bc</sup>	37.21	1.48	6.49	0.26
Treatment 9		798.25 <sup>bc</sup>	32.76 <sup>bc</sup>	724.50	29.78	163.75	6.74 <sup>c</sup>	23.97	1.00	5.41	0.22
Treatment 10		805.00 <sup>bc</sup>	33.56 <sup>abc</sup>	727.50	30.34	174.00	7.24 <sup>abc</sup>	32.93	1.35	6.29	0.26
Treatment 11		871.25 <sup>ab</sup>	35.35 <sup>ab</sup>	786.25	31.93	187.50	7.61 <sup>ab</sup>	40.25	1.64	6.42	0.26
Treatment 12		951.00 <sup>a</sup>	36.57 <sup>a</sup>	810.50	31.16	176.50	6.80 <sup>bc</sup>	31.83	1.23	5.43	0.21
Treatment 13		803.25 <sup>bc</sup>	33.14 <sup>bc</sup>	757.25	31.27	170.75	7.04 <sup>bc</sup>	25.25	1.04	5.21	0.21
Treatment 14		780.50 <sup>bc</sup>	33.25 <sup>bc</sup>	717.25	30.59	165.25	7.03 <sup>bc</sup>	28.25	1.21	5.15	0.22
Treatment 15		793.75 <sup>bc</sup>	33.71 <sup>abc</sup>	704.00	29.78	173.25	7.32 <sup>abc</sup>	39.12	1.63	4.80	0.20
<i>SEM</i>		30.56	0.94	26.27	0.95	6.36	0.25	5.18	0.20	0.49	0.02
<i>P-value</i>		0.0048	0.03	0.13	0.55	0.14	0.043	0.21	0.15	0.26	0.22

<sup>abc</sup>Means without superscript letters or with the same superscript letters within the same column do not differ ( $P \geq 0.05$ ); *SEM*: Standard Error of Means.

**Table 7.** Effect of restriction intensity, restriction duration, and coenzyme Q10 levels on broiler plasma constituents at 42nd day of age

Metabolite		Uric acid (mg/dL)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	Total protein (g/dL)	Total albumin (g/dL)
Restriction intensity (%)	10	4.71	152.41	126.95	3.67	1.22
	20	4.79	152.00	128.00	3.77	1.25
	<i>SEM</i>	0.27	3.17	5.94	0.06	0.02
	<i>P-value</i>	0.84	0.92	0.90	0.31	0.53
Restriction duration (day)	7	5.17 <sup>a</sup>	151.41	135.75	3.73	1.24
	14	4.32 <sup>b</sup>	153.00	119.20	3.72	1.24
	<i>SEM</i>	0.27	3.17	5.94	0.06	0.02
	<i>P-value</i>	0.037	0.72	135.75	0.86	1.00
Coenzyme Q (mg/kg)	0	4.75	157.25	123.37	3.85	1.28
	20	4.77	154.87	132.43	3.60	1.20
	40	4.73	152.50	126.62	3.73	1.23
	<i>SEM</i>	0.33	3.88	7.27	0.08	0.03
<i>P-value</i>		0.99	0.594	0.67	0.11	0.22

Treatment 1	3.97	157.00	127.75 <sup>abc</sup>	3.62	1.17
Treatment 2	3.20	168.50	116.25 <sup>abc</sup>	3.70	1.25
Treatment 3	2.95	157.00	87.50 <sup>c</sup>	3.47	1.17
Treatment 4	6.00	145.00	131.75 <sup>abc</sup>	3.67	1.25
Treatment 5	5.47	146.75	147.25 <sup>a</sup>	3.60	1.25
Treatment 6	4.25	153.25	122.00 <sup>abc</sup>	3.67	1.22
Treatment 7	4.32	153.75	147.50 <sup>a</sup>	3.70	1.20
Treatment 8	3.92	160.75	90.50 <sup>bc</sup>	3.55	1.17
Treatment 9	4.30	155.00	122.75 <sup>abc</sup>	3.87	1.27
Treatment 10	4.85	154.25	121.25 <sup>abc</sup>	4.17	1.37
Treatment 11	4.50	157.25	156.25 <sup>a</sup>	3.60	1.15
Treatment 12	5.97	152.00	136.00 <sup>ab</sup>	3.70	1.20
Treatment 13	3.82	144.00	93.00 <sup>bc</sup>	3.87	1.32
Treatment 14	5.20	154.75	135.75 <sup>ab</sup>	3.65	1.22
Treatment 15	4.40	149.75	125.75 <sup>abc</sup>	3.67	1.25
SEM	0.65	7.42	14.20	0.15	0.07
P-value	0.05	0.76	0.029	0.38	0.71

<sup>abc</sup>Means without superscript letters or with the same superscript letters within the same column do not differ ( $P \geq 0.05$ ); SEM: Standard Error of Means.

**Table 8.** Effect of restriction intensity, restriction duration and coenzyme Q10 levels on broiler plasma hormones at 42nd day of age

Hormones		Total Thyroxin (mg/dL)	Triiodothyronine (nmol/L)	Insulin (micIU/mL)	Cortisol (AM) (mcg/dL)	GH Serum (mL/ng)	(IGF-1) (mL/ng)
Restriction intensity (%)	10	0.04	0.92	0.22	0.86	0.05	3.65
	20	0.83	0.95	0.20	0.87	0.05	3.00
	SEM	0.03	0.03	0.01	0.25	0.00	0.38
	P-value	0.33	0.47	0.49	0.96	1.00	0.23
Restriction duration (day)	7	1.04	0.97	0.20	0.71	0.05	3.65
	14	1.01	0.90	0.22	1.03	0.05	3.00
	SEM	0.03	0.03	0.01	0.25	0.00	0.38
	P-value	0.62	0.18	0.11	0.37	1.00	0.23
Coenzyme Q (mg/kg)	0	0.03	0.91	0.20	1.52 <sup>a</sup>	0.05	3.00
	20	0.62	0.94	0.23	0.62 <sup>b</sup>	0.05	3.18
	40	0.03	0.95	0.21	0.46 <sup>b</sup>	0.05	3.79
	SEM	0.04	0.04	0.01	0.30	0.00	0.47
	P-value	0.14	0.83	0.37	0.042	1.00	0.46
Treatment 1		1.20	0.92 <sup>bc</sup>	0.20	0.69	0.05	3.00
Treatment 2		1.17	1.07 <sup>ab</sup>	0.20	0.83	0.05	3.00
Treatment 3		1.25	1.00 <sup>ab</sup>	0.20	0.38	0.05	3.00
Treatment 4		0.91	0.82 <sup>bc</sup>	0.20	0.61	0.05	3.00
Treatment 5		1.20	1.22 <sup>a</sup>	0.20	0.61	0.05	3.75
Treatment 6		1.12	0.87 <sup>bc</sup>	0.20	0.41	0.05	6.18
Treatment 7		0.91	0.85 <sup>bc</sup>	0.20	2.59	0.05	3.00
Treatment 8		1.05	0.65 <sup>c</sup>	0.30	0.47	0.05	3.00
Treatment 9		1.12	1.10 <sup>ab</sup>	0.22	0.47	0.05	3.00
Treatment 10		1.00	1.05 <sup>ab</sup>	0.20	1.56	0.05	3.00
Treatment 11		1.00	0.87 <sup>bc</sup>	0.20	0.66	0.05	3.00
Treatment 12		1.00	1.00 <sup>ab</sup>	0.20	0.38	0.05	3.00
Treatment 13		1.00	0.95 <sup>ab</sup>	0.20	1.33	0.05	3.00
Treatment 14		0.95	1.02 <sup>ab</sup>	0.22	0.73	0.05	3.00
Treatment 15		1.05	0.85 <sup>bc</sup>	0.22	0.58	0.05	3.00
SEM		0.10	0.08	0.02	0.55	0.00	0.84
P-value		0.45	0.006	0.57	0.35	1.00	0.49

<sup>abc</sup>Means without superscript letters or with the same superscript letters within the same column do not differ ( $P \geq 0.05$ ); SEM: Standard Error of Means.



**Table 9.** Effect of restriction intensity, restriction duration, and coenzyme Q10 levels on broiler antibody produced against influenza and Newcastle viruses ( $\log_{10}$ )

		The antibody produced against the influenza vaccine virus 21 days after injection	The antibody produced against the influenza vaccine virus 28 days after injection	The antibody produced against the Newcastle disease vaccine virus (HI) 7 days after the first injection	The antibody produced against the Newcastle disease vaccine virus (HI) 7 days after the second injection
Restriction intensity (%)	10	5.08 <sup>b</sup>	6.83	3.58	5.37
	20	5.58 <sup>a</sup>	6.75	3.37	5.58
	SEM	0.09	0.11	0.16	0.25
	P-value	0.0004	0.59	0.36	0.57
Restriction duration (day)	7	5.37	6.87	3.70	5.62
	14	5.29	6.70	3.25	5.33
	SEM	0.09	0.11	0.16	0.25
	P-value	0.51	0.29	0.05	0.42
Coenzyme Q (mg/kg)	0	5.25	6.81	3.25	5.25 <sup>b</sup>
	20	5.37	6.93	3.50	6.56 <sup>a</sup>
	40	5.37	6.62	3.68	4.62 <sup>b</sup>
	SEM	0.11	0.13	0.19	0.31
	P-value	0.65	0.27	0.29	0.0004
Treatment 1		4.75 <sup>c</sup>	6.75	2.50 <sup>c</sup>	6.50 <sup>ab</sup>
Treatment 2		5.50 <sup>abc</sup>	6.50	3.00 <sup>c</sup>	5.00 <sup>bcd</sup>
Treatment 3		5.25 <sup>abc</sup>	7.00	4.50 <sup>ab</sup>	3.50 <sup>e</sup>
Treatment 4		5.00 <sup>bc</sup>	7.25	3.50 <sup>abc</sup>	4.75 <sup>bcd</sup>
Treatment 5		5.50 <sup>abc</sup>	7.00	3.25 <sup>bc</sup>	7.50 <sup>a</sup>
Treatment 6		5.00 <sup>bc</sup>	6.50	4.75 <sup>a</sup>	4.25 <sup>cde</sup>
Treatment 7		4.75 <sup>c</sup>	6.25	3.00 <sup>c</sup>	4.75 <sup>bcd</sup>
Treatment 8		5.25 <sup>abc</sup>	7.25	3.50 <sup>abc</sup>	6.25 <sup>abc</sup>
Treatment 9		5.00 <sup>bc</sup>	6.75	3.50 <sup>abc</sup>	4.75 <sup>bcd</sup>
Treatment 10		5.75 <sup>ab</sup>	7.25	3.50 <sup>abc</sup>	5.75 <sup>abcd</sup>
Treatment 11		5.50 <sup>abc</sup>	6.50	3.75 <sup>abc</sup>	5.75 <sup>abcd</sup>
Treatment 12		5.50 <sup>abc</sup>	6.75	3.50 <sup>abc</sup>	5.75 <sup>abcd</sup>
Treatment 13		5.50 <sup>abc</sup>	6.50	3.00 <sup>c</sup>	5.75 <sup>abcd</sup>
Treatment 14		5.25 <sup>abc</sup>	7.00	3.50 <sup>abc</sup>	6.75 <sup>ab</sup>
Treatment 15		6.00 <sup>a</sup>	6.50	3.00 <sup>c</sup>	3.75 <sup>de</sup>
SEM		0.25	0.26	0.41	0.66
P-value		0.04	0.13	0.04	0.003

<sup>abcd</sup>Means without superscript letters or with the same superscript letters within the same column do not differ ( $P \geq 0.05$ ); SEM: Standard Error of Means.

**Table 10.** Effect of restriction intensity, restriction duration, and coenzyme Q10 levels on broiler immunity parameters within 7 and 14 days after injection of sheep red blood cell ( $\log_{10}$ )

Day		7			14		
		IgT	IgG	IgM	IgT	IgG	IgM
Restriction intensity (%)	10	2.58	0.70	1.87	3.708	1.41	2.29
	20	1.87	0.58	1.29	3.542	1.62	1.91
	SEM	0.41	0.17	0.28	0.283	0.29	0.17
	P-value	0.23	0.61	0.16	0.6791	0.62	0.12
Restriction duration (day)	7	2.16	0.83	1.33	3.833	1.87	1.95
	14	2.29	0.45	1.83	3.417	1.16	2.25
	SEM	0.41	0.17	0.28	0.283	0.29	0.17
	P-value	0.83	0.13	0.22	0.3041	0.10	0.23
Coenzyme Q (mg/kg)	0	1.75	0.37	1.37	4.188	1.81	2.37 <sup>a</sup>
	20	2.93	1.06	1.87	3.375	1.00	2.37 <sup>a</sup>
	40	2.00	0.50	1.50	3.313	1.75	1.56 <sup>b</sup>
	SEM	0.50	0.21	0.35	0.346	0.36	0.20
	P-value	0.23	0.06	0.58	0.15	0.22	0.01
Treatment 1		1.50	1.00	0.50	4.75	2.50	2.25 <sup>abcd</sup>
Treatment 2		1.75	0.00	1.75	5.00	2.75	2.25 <sup>abcd</sup>
Treatment 3		3.00	1.00	2.00	2.00	1.25	0.75 <sup>e</sup>
Treatment 4		2.00	0.25	1.75	4.25	0.75	3.50 <sup>a</sup>
Treatment 5		3.00	1.75	1.25	3.50	1.25	2.25 <sup>abcd</sup>

Treatment 6	2.00	0.75	1.25	4.75	3.25	1.50 <sup>cde</sup>
Treatment 7	1.75	0.50	1.25	4.00	2.00	2.00 <sup>bcd</sup>
Treatment 8	3.75	0.50	3.25	2.75	0.50	2.25 <sup>abcd</sup>
Treatment 9	3.00	0.50	2.50	3.00	0.75	2.25 <sup>abcd</sup>
Treatment 10	1.25	0.50	0.75	4.50	3.00	1.50 <sup>cde</sup>
Treatment 11	2.75	1.25	1.50	3.50	1.50	2.00 <sup>bcd</sup>
Treatment 12	2.00	0.50	1.50	2.50	1.50	1.00 <sup>de</sup>
Treatment 13	2.00	0.25	1.75	4.00	1.50	2.50 <sup>abc</sup>
Treatment 14	2.25	0.75	1.50	3.75	0.75	3.00 <sup>ab</sup>
Treatment 15	1.00	0.25	0.75	3.00	1.50	1.50 <sup>cde</sup>
SEM	1.04	0.49	0.72	0.74	0.76	0.40
P-value	0.90	0.63	0.53	0.16	0.24	0.002

<sup>abcde</sup> Means without superscript letters or with same superscript letters within the same column do not differ ( $P \geq 0.05$ ); SEM: Standard Error of Means.

IgT: Total immunoglobulin produced against the sheep red blood cell; IgG: Immunoglobulin G produced against the sheep red blood cell; IgM: Amount of immunoglobulin M produced against sheep red blood cells

**Table 11.** Effect of restriction intensity, restriction duration and coenzyme Q10 levels on lymphoid organs (gr or %) at 42nd day of age

		Thymus weight (g)	Relative weight of thymus (%)	Spleen (g)	Relative weight of spleen (%)	Bursa of Fabricius (g)	Relative weight of Bursa of Fabricius (%)
Restriction intensity (%)	10	14.42	0.59	3.24	0.13	6.02	0.24
	20	14.68	0.60	3.36	0.13	5.76	0.23
	SEM	0.54	0.02	0.14	0.006	0.24	0.009
	P-value	0.73	0.87	0.57	0.57	0.45	0.44
Restriction duration (day)	7	14.96	0.61	3.27	0.13	5.91	0.23
	14	14.14	0.59	3.33	0.13	5.87	0.24
	SEM	0.54	0.02	0.14	0.006	0.24	0.009
	P-value	0.29	0.60	0.77	0.48	0.91	0.61
Coenzyme Q (mg/kg)	0	13.69	0.57	3.29	0.13	6.14	0.25
	20	15.36	0.65	3.26	0.13	5.59	0.23
	40	14.60	0.58	3.36	0.13	5.93	0.23
	SEM	0.66	0.03	0.18	0.008	0.30	0.01
	P-value	0.22	0.14	0.92	0.88	0.43	0.35
Treatment 1	14.15	0.57	3.36	0.13	4.62	0.18	
Treatment 2	13.11	0.54	3.67	0.15	4.46	0.18	
Treatment 3	12.69	0.53	3.27	0.13	5.97	0.24	
Treatment 4	13.22	0.54	3.27	0.13	6.35	0.26	
Treatment 5	15.87	0.72	2.76	0.12	5.26	0.23	
Treatment 6	14.55	0.54	3.54	0.13	6.50	0.24	
Treatment 7	14.07	0.59	3.21	0.13	6.46	0.27	
Treatment 8	15.17	0.62	3.38	0.13	6.01	0.24	
Treatment 9	13.62	0.56	3.30	0.13	5.52	0.22	
Treatment 10	14.73	0.61	3.27	0.13	6.09	0.25	
Treatment 11	17.62	0.71	3.47	0.14	5.20	0.21	
Treatment 12	13.77	0.53	3.33	0.12	6.04	0.23	
Treatment 13	12.76	0.52	3.42	0.14	5.65	0.23	
Treatment 14	12.77	0.54	3.42	0.14	5.89	0.25	
Treatment 15	16.45	0.69	3.27	0.13	5.67	0.23	
SEM	1.38	0.06	0.37	0.01	0.58	0.02	
P-value	0.37	0.33	0.99	0.99	0.36	0.30	

<sup>ab</sup> Means without superscript letters or with same superscript letters within the same column do not differ ( $P \geq 0.05$ ); SEM: Standard Error of Means.

**Table 12.** Effect of restriction intensity, restriction duration and coenzyme Q10 levels on ceca microbiota [ $\log_{10}$  cfu/g] at 42nd day of age

		<i>Escherichia coli</i>	<i>Coliforms bacteria</i>	<i>Lactobacilli spp</i>	<i>Enterococcus spp</i>
Restriction intensity (%)	10	7.63	8.92	6.18	5.59
	20	7.75	8.57	6.24	5.57
	SEM	0.08	0.34	0.21	0.04
	P-value	0.32	0.48	0.84	0.78
Restriction duration (day)	7	7.62	8.96	6.01	5.60
	14	7.76	8.54	6.41	5.57
	SEM	0.08	0.34	0.21	0.04
	P-value	0.25	0.39	0.19	0.65
Coenzyme Q (mg/kg)	0	8.23	8.26	6.18	5.62
	20	7.83	8.49	6.27	5.54
	40	7.51	9.49	6.17	5.58
	SEM	0.10	0.42	0.26	0.05
	P-value	0.07	0.10	0.96	0.66
Treatment 1		8.00	8.52	5.68	5.50
Treatment 2		8.13	8.34	6.30	5.77
Treatment 3		8.58	8.20	6.56	5.88
Treatment 4		7.67	8.60	5.81	5.50
Treatment 5		7.50	8.81	5.85	5.50
Treatment 6		7.50	10.85	5.96	5.75
Treatment 7		7.50	7.81	6.88	5.76
Treatment 8		8.07	8.82	5.95	5.56
Treatment 9		7.57	8.63	6.62	5.50
Treatment 10		7.84	8.33	6.16	5.72
Treatment 11		7.74	8.28	5.65	5.63
Treatment 12		7.50	8.87	6.62	5.50
Treatment 13		7.91	8.31	5.87	5.50
Treatment 14		8.01	8.04	7.63	5.50
Treatment 15		7.50	9.61	5.50	5.60
SEM		0.23	0.79	0.48	0.13
P-value		0.064	0.593	0.20	0.56

<sup>ab</sup> Means without superscript letters or with same superscript letters within the same column do not differ ( $P \geq 0.05$ ); SEM: Standard Error of Means.

## Discussion

In this study, feed intake was highest for the unrestricted group and lowest for the 20% feed restricted group. Some researchers observed a decrease in feed intake by feed restriction, attributed it to declining the gastrointestinal tract volume (Zhan *et al.*, 2007) and reducing the chick's maintenance requirements (Zubair and Leeson, 1994). The feed restriction program did not harm body weight gain, indicating that birds were receiving nutritionally adequate nutrition for growth (Onbasilar *et al.*, 2009; Wijtten *et al.*, 2010; Yousefi, 2013). The feed restriction program did not have a negative effect on body weight gain, indicating that birds receive the nutrients requirements for growth during their access to feed (Onbasilar *et al.*, 2009; Wijtten *et al.*, 2010; Yousefi, 2013).

Chickens fed high-density diets are more susceptible to oxidative stress (Cardoso *et al.*, 2010), while feed restriction reduces oxidative damage (Özkan *et al.*, 2010). However, the early feed restriction has a profound effect on growth performance and lipid metabolism in broiler chickens (Demirci, 2014). Under the conditions where birds

are exposed to oxidative stress, it is expected that the CoQ10 supplementation acts as an antioxidant and increases energy consumption at the cellular level, which is expected to improve the broiler performance (Geng *et al.*, 2007).

The quinone ring in CoQ10 is responsible for receiving and transmitting electrons to oxygen in the mitochondrial respiratory chain. Hence, the produced concentration gradient for proton generates ATP (Geng *et al.*, 2007).

Despite this finding, there are different observations due to the variability in the duration and severity of feed restriction. The quantitative/qualitative feed restriction is one of the primary management tools used today to reduce the incidence of ascites in broiler chickens (Saber *et al.*, 2011). Singh *et al.*, (2011) recommended feed restriction as a management strategy for reducing the ascites syndrome. Feed restriction mainly reduces body growth and as a result, metabolic requirements decrease during the early critical period along with an improvement in oxygen arterial involvement (Özkan *et al.*, 2006). However, feed restriction can have a negative effect on the final body weight and relative

weight of the breast (Acar *et al.*, 1995). It also has been reported that feed restriction can negatively affect thyroid gland activity and lower concentrations of plasma T3 (Luger *et al.*, 2001). It was been reported that withdrawal of daily feed as much as four hours has no significant effect on body weight, feed intake, and carcass characteristics (Onbasilar *et al.*, 2009). It has also been reported that feed restriction reduced ascites related mortality, but there was decreased body weight and eviscerated carcass in restricted birds versus full-fed birds (Acar *et al.*, 1995). Feed restriction had no significant effect on the feed conversion ratio and carcass composition although it reduces feed intake (Dastar *et al.*, 2013).

Camacho *et al.*, (2004) reported that an 8 hours feed restriction program at an early age has no significant effect on the feed intake of broilers. The effect of different types of feed restriction on broiler chicken's performance and feed intake was not significant (Butzen *et al.*, 2013). The strain of the bird is one of the major factors affecting feed intake (Wijten *et al.*, 2010). One possible reason for no effect of feed restriction on feed intake is the bird's digestive tract can adapt to feed restriction conditions. Long-term feed restriction in older broilers enlarges the digestive system and so when the broilers had access to feed, there was more storage for feed in the crop, gizzard, proventriculus, etc (Özkan *et al.*, 2010). Feed restriction and CoQ10 supplementation could affect the feed conversion ratio positively. It seems that reducing the feed intake and improving the feed conversion ratio is due to the temporary decrease of the basal metabolic rate in feed-restricted birds (Zubair and Leeson, 1994) and correlates with lower body weight in the early growth period which reduces the maintenance requirement (Marks, 1991).

Acar *et al.*, (1995) concluded that feed restriction improves the feed conversion ratio. Fanooci and Torki (2010) observed no difference in feed conversion ratio between restricted and non-restricted broilers. Our results for carcass characteristics revealed there are no differences in the weight of breast, thighs, wings, abdominal fat, liver, kidneys, spleen, and bursa of Fabricius. This may be due to the effective use of feed-in restricted fed chickens after removing the restriction and within the rehabilitation period. The weight percentage of breast, thighs, wings, abdominal fat, thymus, liver, spleen, and bursa of Fabricius in groups fed supplemental CoQ10 was higher than non-restricted groups. Wijten *et al.*, (2010) reported that the type of feed restriction and bird genetics affects the weight of the breast and abdominal fat. Since body fat, especially abdominal fat, is affected by many factors such as strain, ration, sex, temperature, and rearing conditions, opposite conflicting have been presented about the effects of different feed restriction programs on abdominal fat. This difference may be due to genetic differences,

severity, and duration of feed restriction, rearing duration, and diet type (Dastar *et al.*, 2013). Fontana *et al.*, (1992), Scheideler and Baughman (1993), and Özkan *et al.*, (2006) observed feed restriction programs do not affect carcass fat content.

Feed restriction reduced feed intake whereas groups supplemented with 40 mg/kg CoQ10 had the highest feed intake. The highest feed intake was for non-restricted groups and the lowest was for 20% feed restriction plus 40 mg/kg CoQ10. Some researchers observed decreased feed intake with feed restriction, reported declining gastrointestinal tract (Alimohammadi *et al.*, 2014), and reduced maintenance requirements (Zubair and Leeson, 1994). Feed intake in groups supplemented CoQ10 was lower than in groups not supplement with CoQ10. Since CoQ10 plays a vital role in supplying energy for tissues, it can provide part of the energy needed and save feed intake for energy supply.

Feed restriction and CoQ10 supplementation improved feed conversion ratio which could be due to a temporary reduction of basal metabolic rate in restricted birds (Zubair and Leeson, 1994) and/or lower body weight in early periods which reduces the maintenance requirement (Marks, 1991). Since the feed restriction program had not negatively affect body weight, we concluded that birds received adequate nutrients during the feed restriction period (Onbaşlar *et al.*, 2009; Wijten *et al.*, 2010; Yousefi, 2013).

Camacho *et al.*, (2004) reported that an 8 hours feed restriction at an early age does not affect feed intake. In other reports, the effect of different types of feed restriction on broiler chickens performance was investigated and no significant differences were observed for feed intake (Saber *et al.*, 2011; Butzen *et al.*, 2013). Wijten *et al.*, (2010) reported that strain is one of the factors affecting feed intake.

Schmelzer *et al.*, (2011) attributed the reduction in the serum LDL-c level to the action of the reduced form of CoQ10 which induces gene expression patterns, which are translated into reduced LDL-c level. As our obtained results, Gopi *et al.*, (2014) concluded the serum HDL-c and triglycerides were not influenced by the CoQ10 supplementation.

Feed restriction resulted in increased concentrations of the T3 hormone and the use of antioxidants such as CoQ10 could reduce this increase somewhat. The trend for T4, unlike T3, was converted to its more active form, i.e. T3, under conditions of feed restriction due to the need for more energy which is consistent with the Yahav (2002) and Hangalaputa *et al.*, (2003). Gonzales *et al.*, (1998) reported that feed restriction and CoQ10 supplementation can reduce T3 concentrations, and T3/T4 ratio and increase T4 concentration. In our study, plasma T3 was increased by feed restriction, which may be due to the severity of the feed

restriction resulting in a very fast response of thyroid hormones to feed restriction. Özkan *et al.*, (2006) reported that limited growth is effective for increasing the recovery of thyroid gland function. It is reported that these two hormones increase in broiler chickens susceptible to ascites (Gonzales *et al.*, 1998; Shahir *et al.*, 2012). Buys *et al.*, (1998) noted that low T3 concentrations could be promoted storing the body's energy and induce higher growth rates. As a result, reducing the metabolic activity and the need for oxygen may be a factor in reducing mortality, especially when the metabolic activity is maximal. There are some positive reports on the effect of feed additives supplementation on cortisol in the broiler (Samanta *et al.*, 2008; Sohail *et al.*, 2010). While we did not find a significant effect of feed restriction on cortisol, however, there is a significantly decreased cortisol level after CoQ10 supplementation.

Feed restriction resulted in a reduction in the relative weight of the spleen and bursa of Fabricius. Bursa cells have a high priority for glucose, isoleucine, and lysine utilization. Moreover, when there is nutrient deficiency, the bursa cells increase their ability to obtain glucose and lysine (Julian, 1993). The increase of relative weight of the bursa of Fabricius and the decrease of the relative weight of the spleen under stress conditions is probably due to the priority of these issues in supplying nutrients. The increased relative weight of the spleen in chicks supplemented CoQ10 was been reported by Geng *et al.*, (2004) which is consistent with our results. Our results on the immune response to influenza and Newcastle viruses indicated that there was no difference between the experimental groups which is consistent with Petek (2000). Supplementation of CoQ10 leads to improved serum lysosomal activity and improved immune system. Chicken's requirement for CoQ10 is high under stress conditions due to feed restriction and its supplementation can help and increase the body's defensive capacity and reduce the incidence of anomalies such as ascites. Compared to other antioxidants like vitamin E, it has been observed that the enhanced immunity properties created by CoQ10 are specific to themselves and due to an increase in the amount of immunoglobulin G, increased phagocytosis activity of macrophages, and increased granulocyte proliferation. Other antioxidants like vitamin E do not have the same activity (Gopi *et al.*, 2014). Little research has been reported regarding the effect of CoQ10 in broiler chickens. Geng *et al.*, (2007) observed increased weight gain when 40 mg/kg of CoQ10 was added to broiler diets. Huang *et al.*, (2011) achieved greater weight gain in broilers after 21 days with 20 and 40 mg/kg of CoQ10. The CoQ10 is preventing lipid peroxidation and is also involved in the regeneration of other endogenous antioxidants (Navas *et al.*, 2007).

The right: total ventricular weight (RV: TV) ratio calculated as an index of pulmonary hypertension. The studies that show the RV/TV is called as the ascites index and suggest it as a reliable indicator of ascites (Balog *et al.*, 2000). In the present experiment, although there was no difference in the ascites index between treatments, this index was higher for non-restricted treatments compared with restricted treatments. Previous research showed that the feed restriction reduces ascites symptoms such as heart space, right ventricle space, right ventricular weight, total heart weight, and RV/TV ratio (Julian, 1993). It is believed that a reduced growth rate in birds exposed to feed restriction allows the heart to reach weight proportional to body weight. Hence, Özkan *et al.*, (2006) did not find any difference in RV/TV ratio by restricting the feed intake. In this study, heart indices in chicks not supplemented with CoQ10 were lower than chicks supplemented with CoQ10 (Geng *et al.*, 2004). They also reported decreased ascites rate using supplemental CoQ10, although they suggest 40 mg/kg CoQ10 was more effective than 20 mg/kg CoQ10. The reason for this decrease was the improvement in the achievement of low function potential for chicken heart muscle cells, previously described by Azuma *et al.*, (1985).

It has been reported that increasing genetic selection intensity, appetite has increased in modern broiler chickens, and hence they cannot adjust the voluntary consumption optimally according to the broiler energy requirements (Onbasilar *et al.*, 2009). When these broilers have *ad libitum* access to feed, they consume as much as 2-3 times more than their maintenance requirements. Also, the increased selection intensity for higher growth rate resulted in problems such as increased fat tissue accumulation above the physiologic requirement (Fanooci and Torki, 2010), increased cardiovascular system disorders, increased metabolic rate, skeletal disorders, high sensitivity to metabolic diseases such as sudden death syndrome (Dastar *et al.*, 2013), ascites (Özkan *et al.*, 2010), decreased immunity and resistance to diseases (Yousefi, 2013) that eventually increase mortalities. Such a situation is highly correlated with increased growth rate, enhanced metabolic rate, and increased feed intake in modern strains (Wijtten *et al.*, 2010). Dastar *et al.*, (2013) believe *ad libitum* feeding in broiler chickens is not efficient due to the problems outlined above. They provide alternative nutritional-management strategies to maximize productivity, minimize fat body accumulation and mortality and reduce other problems.

In our experiment, supplementation of 40 mg/kg CoQ10 decreased the RT/TV ratio and ascites related deaths. Probably CoQ10 was able to improve cardiovascularly and reduce cardiovascular problems (as one of the symptoms of ascites' deaths) by improving blood flow in the lungs vessels, reducing

the blood pressure, reducing the pressure on the right ventricle, and reducing the hypertrophy. Supplementation of 40 mg/kg CoQ10 reduces systolic blood pressure but did not affect right ventricular pressure. Moreover, CoQ10 improved blood flow in the lungs vesicles (Geng *et al.*, 2004). It has also been reported that CoQ10 supplementation in humans can reduce the viscosity of the blood and reduce vascular resistance into the blood flow, which can reduce blood pressure and its problems (Kato *et al.*, 1990).

Gian and Luca, (2007) showed that ubiquinol is the most important inhibitor of peroxidation of plasma lipid contents. Therefore, by decreasing lipid peroxidation with CoQ10, it is likely that the blood vessels and heart cells will maintain the health of the blood vessels and improve the function of the cardiovascular system. There are many studies where the RV/TV ratio has been identified as a reliable cardiac index ascites indicator (Ernster and Dallner, 1995; Balog *et al.*, 2000). The ascites index difference between different treatments in our experiment, but this index was higher in non-restricted groups than restricted groups. Feed restriction and CoQ10 reduced ascites symptoms, including heart space, right ventricular space, total heart rate, and RV/TV ratio (Julian, 1993). It is believed that reducing the growth rate in birds exposed to feed restriction will allow their hearts to reach a weigh proportional to body weight. Özkan *et al.*, (2006) did not find any difference in the RV/TV ratio following feed restriction. In our study, the heart ascites index in groups fed CoQ10 was lower than groups not supplemented CoQ10. Geng *et al.*, (2004) also reported a decrease in ascites' heart rate using supplementation of CoQ10, although they reported 40 mg/kg CoQ10 was more effective than 20 mg/kg CoQ10. They reported that the decrease was due to improvements in low function potential in broiler heart muscle cells, previously described by Azuma *et al.*, (1985).

Based on our results, the RV/TV ratio was not affected by the feed restriction program. Yousefi (2013) stated continuous/non-continuous feed restriction programs did not affect RV/TV ratio. In our study, feed restriction severity was higher in the groups 7-21 days of age which also had the least amount of ascites related deaths. Based on available reports, the effectiveness of feed restriction programs is affected by various factors such as the strain (Wijtten *et al.*, 2010), the time of onset, and severity of feed restriction (Saffar and Khajali, 2010). Given our results, the method of feed restriction can be effective in reducing the incidence of ascites. Other research has shown such results for the effect of feed restriction on death, argued that the effect of feed restriction can reduce the broiler growth rate during the growth period when the bird was under most susceptible period to metabolic abnormalities due to

the high requirements for oxygen demand (Azuma *et al.*, 1985). Therefore, reducing mortality due to feed restriction can be due to the decrease in the incidence of diseases caused by the rapid growth of broilers. Similar to our results, Geng *et al.*, (2004) observed decreased mortality with CoQ10 supplementation. These researchers stated that the increased survival in birds was due to the decrease in the incidence of ascites. CoQ10 may protect the cell membrane as well as the cell's structure against peroxidation and thus stables the heart's muscle cells and red blood cells against metabolic stresses.

Feed restriction and CoQ10 supplementation did not affect antibody titer (IgG and IgM) against SRBC. Feed restriction due to deprivation and hungry leads to stress in the bird; however, some researchers observed decreased immunity in feed restricted birds (Daneshyar *et al.*, 2009). Knight and Dibner (1998) argued that stress due to feed restriction has a stimulatory effect on the secretion of corticosteroids, and these hormones are potent inhibitors against the production of antibodies. However, in this experiment and others, the ineffectiveness of feed restriction has been reported on the production of antibodies and immunoglobulin G (Liew *et al.*, 2003). Research about the effect of CoQ10 supplementation on IgG, IgM, and total antibody titers against sheep red blood cells showed that CoQ10 supplementation increased the concentrations of phagocytes and antibodies. Moreover, studies in humans have shown that CoQ10, along with vitamin B6, increased lymphocyte production, and immunoglobulin G level (Dibner *et al.*, 1996). There was no increase in IgG level in our study, but CoQ10 increased total immunoglobulin, whereas Mohsane (2011) reported a positive effect of the feed restriction program on antibody against sheep red blood cells and immunoglobulin M, but no effect on the immunoglobulin G.

### Conclusion

The highest weight at 42 days of age was for non-restricted chicks supplemented with CoQ10. There was a difference between treatments with and without CoQ10. At 42 days of age, the restricted group as much as 20% had more body weight than the restricted group as much as 10% significantly. Feed restriction reduced feed intake. Groups supplemented with 40 mg/kg CoQ10 had the lowest feed intake which indicates feed restriction reduces feed intake. The highest feed intake was for non-restricted and the lowest feed intake was for groups restricted 20% and supplemented 20 mg/kg CoQ10. Finally, considering the positive effects of feed restriction on reducing the mortality and improving the production index, We can conclude feed restriction can be considered as a management tool to improve performance in broiler chickens. Also, CoQ10 improves performance and

can also be effective in the improvement of the production index.

### Acknowledgments

This manuscript is prepared based on the Ph.D. thesis of the first author at the Science and Research Branch, Islamic Azad University, Tehran, Iran. We

are grateful to the Science and Research Branch, Islamic Azad University, Tehran, Iran for support.

### Conflict of interest

The authors stated no conflict of interest relating to the financial and substance of the study.

### References

- Acar N, Sizemore F, Leach G, Wideman R, Owen R & Barbato G. 1995. Growth of broiler chickens in response to feed restriction regimens to reduce ascites. *Poultry Science*, 74: 833-843. DOI: 10.3382/ps.0740833
- Alimohammadi Saraei MH, Seidavi AR, Dadashbeiki M, Laudadio V & Tufarelli V. 2014. Effect of dietary supplementation with different levels of green tea powder and fish oil or their combination on carcass characteristics in broiler chickens. *Pakistan Journal of Zoology*, 46: 1767-1773. DOI: 10.4067/S0301-732X2016000100008
- Azuma J, Harada H, Sawamura A, Ohta H, Awata N, Yamauchi K, Kishimoto S & Sperelakis N. 1985. Beneficial effect of coenzyme Q on myocardial low action potentials in hearts subjected to decreased perfusion pressure-hypoxia -substrate-free perfusion. *Basic Research of Cardiology*, 80: 147-155. DOI: 10.1007/BF01910462
- Balog MJ, Anthony NB, Cooper MA, Kidd BD, Huff GR, Huff WE & Rath NC. 2000. Ascites syndrome and related pathologies in feed restricted broilers raised in a hypobaric chamber. *Poultry Science*, 79: 318-323. DOI: 10.1093/ps/82.9.1383
- Bautista-Ortega J, Ruiz-Feria CA, Ellis A. 2010. Cytochemical and immunocytochemical localization of oxidative and nitrosative stress in pulmonary hypertension in lungs of broiler chickens, *Gallus domesticus*. *Microscopy Microanalysis*, 16: 964-96. DOI: 10.1017/S1431927610056497
- Butzen F, Ribeiro A, Vieira M, Kessler A, Dadalt J & Della M. 2013. Early feed restriction in broilers. I- Performance, body fraction weights, and meat quality. *The Journal of Applied Poultry Research*, 22: 251-259. DOI: 10.3382/japr.2012-00639
- Buys N, Dewil E, Gonzales E & Decuypere E. 1998. Different CO<sub>2</sub> levels during incubation interact with hatching time and ascites susceptibility in two broiler lines selected for different growth rate. *Avian Pathology*, 27: 605-612.
- Camacho M, Suarez M, Herrera J, Cuca J & Garcia-Bojalil C. 2004. Effect of age of feed restriction and microelement supplementation to control ascites on production and carcass characteristics of broilers. *Poultry Science*, 83: 526-532. DOI: 10.1093/ps/83.4.526
- Cardoso AR, Cabral-Costa JV, Kowaltowski AJ. 2010. Effects of a high fat diet on liver mitochondria: increased ATP-sensitive K channel activity and reactive oxygen species generation. *Journal of Bioenergetics Biomembranes*, 42: 245-253. DOI: 10.1007/s10863-010-9284-9
- Daneshyar M, Kermanshahi H & Golian A. 2009. Changes of biochemical parameters and enzyme activities in broiler chickens with cold-induced ascites. *Poultry Science*, 88: 106-110. DOI: 10.3382/ps.2008-00170
- Dastar B, Shams Shargh M, Zerehdaran S & Mohebedini H. 2013. Evaluate function and incidence of ascites in broiler chickens in response to feed restriction and nutritional programs. *Iranian Journal of Animal Science*, 5: 268-274.
- Demirci N. 2014. Effects of daily added coQ10 on certain hematological and biochemical parameters in elite endurance skiing athletes. *Journal of Biology*, 2: 18-24.
- Dibner JJ, Kitehell ML, Atwell CA & Ivey FYL. 1996. The effect of dietary ingredient and age on the microscopic structure of gastrointestinal tract in poultry. *Journal of Applied Poultry Research*, 5: 70-77. DOI: 10.1093/japr/5.1.70
- Ernster, L. And G. Dallner. 1995. Biochemical, physiological and medical aspects of ubiquinone function. *Biochemistry and Biophysics Acta*, 1271: 195-204. DOI: 10.1016/0925-4439(95)00028-3
- Fanooci, M & Toriki, M. 2010. Effects of qualitative dietary restriction on performance, carcass characteristics, white blood cell count and humoral immune response of broiler chicks. *Global Veterinaria*, 4: 277-282.
- Fontana E, Weaver W, Watkins B & Denbow D. 1992. Effect of early feed restriction on growth, feed conversion, and mortality in broiler chickens. *Poultry Science*, 71: 1296-1305. DOI: 10.3382/ps.0711296
- Geng A, Li B & Guo Y. 2007. Effects of dietary L-carnitine and coq10 at different supplemental ages on growth performance and some immune response in ascites-susceptible broilers. *Archives of Animal Nutrition*, 61: 50-60. DOI: 10.1080/17450390601117041
- Geng AL, Guo YM & Yang Y. 2004. Reduction of ascites mortality in broilers by CoeQ10. *Poultry*

- Science, 83: 1587-1602. DOI: 10.1093/ps/83.9.1587
- Gian PL & Luca T. 2007. Bioenergetic and Antioxidant Properties of coq10. *Recent Developments. Molecular Biotechnology*, 37: 31-37. DOI: 10.1007/s12033-007-0052-y
- Gonzales E, Buyse J, Loddi MM, Takita TS, Buys N & Decuyper E. 1998. Performance, incidence of metabolic disturbances and endocrine variables of food restricted male broiler chickens. *British Poultry Science*, 39: 671-678. DOI: 10.1080/00071669888557
- Gopi M, Purushothaman MR & Chandrasekaran D. 2014. Effect of dietary Coq10 supplementation on serum and bone minerals and leg weakness mortality in broilers. *Veterinary World*, 7: 347-350. DOI: 10.1080/713654926
- Hangalaputa BN, Nieuwland MGB, De Vries Reilingh G, Heetkamp MJW, Van den Brand H, Kemp B & Parmentier HK. 2003. Effect of cold stress on immune responses and body weight of chicken's lines divergently selected for antibody responses to sheep red blood cells. *Poultry Science*, 82: 1692-1700. DOI: 10.1093/ps/82.11.1692
- Huang B, Guo Y, Hu X & Song Y. 2011. Effects of coq10 on growth performance and heart mitochondrial function of broilers under high altitude induced hypoxia. *Journal of Poultry Science*, 48: 40-46. DOI: 10.2141/jpsa.010084
- Huchzermeyer FW & Ruyck AMW. 1986. Pulmonary hypertension syndrome associated with ascites in broilers. *Veterinary Records*, 119: 94. DOI: 10.1136/vr.119.4.94
- Jahanpour H, Seidavi AR. & Qotbi AA A. 2014a. Effects of intensity and duration of quantitative restriction of feed on broiler performance. *Journal of Hellenic Veterinary Medicine Society*, 65: 83-98. DOI: 10.12681/jhvms.15519
- Jahanpour H, Seidavi AR, Qotbi, A AA & Payan-Carreira R. 2013. Effects of two levels of quantitative feed restriction for a 7- or 14- days period on broilers blood parameters. *Acta Scientiae Veterinariae*, 41: 1-11. DOI: 10.5194/aab-58-99-2015
- Jahanpour H, Seidavi AR, Qotbi AAA, Delgado F & Gamboa S. 2014b. Effect of intensity and duration of quantitative feed restriction on broiler caecum microbiota. *Indian Journal of Animal Science*, 84: 554-558.
- Jahanpour H, Seidavi A, Qotbi A AA, Van Den Hoven R, Rocha e Silva S, Laudadio V & Tufarelli V. 2015. Effects of the level and duration of feeding restriction on carcass components of broilers. *Archives Animal Breeding*, 58: 99-105. DOI: 10.5194/aab-58-99-2015
- Julian JR. 1993. Ascites in poultry. *Avian Pathology*, 22: 419-454. DOI: 10.1080/03079459308418934
- Kato T, Yoneda S, Kako T, Koketsu M, Hayano I & Fujinami T. 1990. Reduction in blood viscosity by treatment with coq10 in patients with ischemic heart disease. *International Journal of Clinical Pharmacolther toxicology*, 28: 123-126.
- Knight CD & Dibner JJ. 1998. Nutritional programming in hatchling poultry: Why a good start is important. *Poultry Dig. (Aug/ Sept)*: 20-26.
- Liew PK, Zulkifli I, Hair-Bejo M, Omar AR & Israf DA. 2003. Effects of early age feed restriction and heat conditioning on heat shock protein 70 expression, resistance to infectious bursal disease, and growth in male broiler chickens subjected to heat stress. *Poultry Science*, 82: 1879-1885. DOI: 10.1093/ps/82.12.1879
- Luger D, Shinder DR, Zepakovsky V, Rusal M & Yahav S. 2001. Association between weight gain, blood parameters, and thyroid hormones and the development of ascites syndrome in broiler chickens. *Poultry Science*, 80: 965-971. DOI: 10.1093/ps/80.7.965
- Marks HL. 1991. Feed efficiency changes accompanying selection for body weight in chickens and quails. *World's Poultry Science Journal*, 47: 197-212. DOI: 10.1079/WPS19910017
- Mohsane S. 2011. The effect of feeding program and diet containing different vitamin-mineral additives on performance, immune response and ascites syndrome in broiler chickens. Master of science thesis, Karaj Azad University.
- Navas P, Villalba JM & Cabo RD. 2007. The importance of plasma membrane coenzyme Q in aging and stress responses. *Mitochondrion*, 7S: 34-40. DOI: 10.1016/j.mito.2007.02.010
- Nosrati M, Javandel F, Camacho LM, Khusro A, Cipriano M, Seidavi AR & Salem AZM. 2017. The effects of antibiotic, probiotic, organic acid, vitamin C, and Echinacea purpurea extract on performance, carcass characteristics, blood chemistry, microbiota, and immunity of broiler chickens. *Journal of Applied Poultry Research*, 26: 295-306. DOI: 10.1080/09712119.2017.1377079
- Onbaşılar E, Yalçın S, Torlak E & Özdemir P. 2009. Effects of early feed restriction on live performance, carcass characteristics, meat and liver composition, some blood parameters, heterophil lymphocyte ratio, antibody production and tonic immobility duration. *Tropical Animal Health and Production*, 41: 1513-1519. DOI: 10.1007/s11250-009-9341-8
- Özkan S, Plavnik I & Yahav S. 2006. Effects of early feed restriction on performance and ascites development in broiler chickens subsequently



- raised at low ambient temperature. *Journal of Applied Poultry Research*, 15: 9-19. DOI: 10.1093/japr/15.1.9
- Özkan S, Takma C, Yahav S, Sögüt B, Türkmüt L, Erturun H & Cahaner A. 2010. The effects of feed restriction and ambient temperature on growth and ascites mortality of broilers reared at high altitude. *Poultry Science*, 89: 974-985. DOI: 10.3382/ps.2009-00293
- Petek M. 2000. The effects of feed removal during the day on some production traits and blood parameters of broilers. *Turkish Journal of Veterinary and Animal Science*, 24: 447-452.
- Plavnik I & Hurwitz S. 1988. Early feed restriction in chicks: Effect of age duration and sex. *Poultry Science*, 67: 348-390. DOI: 10.3382/ps.0670384
- Rahimi S, Seidavi AR, Sahraei M, Blanco FP, Schiavone A & Martínez Marin, AL. 2015. Effects of feed restriction and diet nutrient density during re-alimentation on growth performance, carcass traits, organ weight, blood parameters and the immune response of broilers. *Italian Journal of Animal Science*, 14: 583-590. DOI: org/10.4081/ijas.2015.4037
- Saber N, Maheri-Sis N, Shaddel-Telli A, Hatefinezhad K, Gorbani A & Yousefi J. 2011. Effect of feed restriction on growth performance of broiler chickens. *Annals of Biological Research*, 2: 247-252.
- Saffar A & Khajali F. 2010. Application of meal feeding and skip-a-day feeding with or without probiotics for broiler chickens grown at high-altitude to prevent ascites mortality. *American Journal of Animal and Veterinary Sciences*, 5: 13-19. DOI: 10.3844/ajavsp.2010.13.19
- Samanta S, Haldar S, Bahadur V & Ghosh TK. 2008. Chromium picolinate can ameliorate the negative effects of heat stress and enhance performance, carcass and meat traits in broiler chickens by reducing the circulatory cortisol level. *Journal of the Science of Food and Agriculture*, 88: 787-796. DOI: 10.1002/jsfa.3146
- Scheideler SE & Baughman GR. 1993. Computerized early feed restriction programs for various strains of broilers. *Poultry Science*, 72: 236-242. DOI: 10.3382/ps.0720236
- Schmelzer C, Niklowitz P, Okun JG, Haas D, Menke T & Doring F. 2011. Ubiquinol-induced gene expression signatures are translated into altered parameters of erythropoiesis and reduced low density lipoprotein cholesterol levels in humans. *IUBMB Life*, 63: 42-48. DOI: 10.1002/iub.413
- Seidavi AR, Ebrahimi A, Qotbi AAA & Nieto JAG. 2015. Effect of different levels of *Citrus sinensis* peel extract on broiler performance, blood parameters, thyroid gland activity and bone ash. *Indian Journal of Animal Sciences*, 85: 1225-1228. DOI: 10.1111/asj.12250
- Shabani S, Seidavi AR, Asadpour L & Corazzin M. 2015. Effects of physical form of diet and intensity and duration of feed restriction on the growth performance, blood variables, microbial flora, immunity, and carcass and organ characteristics of broiler chickens. *Livestock Science*, 180: 150-157. DOI: 10.1016/j.livsci.2015.07.006
- Shahir M, Dilmagani S & Tzschentke B. 2012. Early-age cold conditioning of broilers: effects of timing and temperature. *British Poultry Science*, 53: 538-544. DOI: 10.1080/00071668.2012.719604
- Shi-bin Y, Dai-wen C, Keying Z, Bing Z. 2007. Effects of oxidative stress on growth performance, nutrient digestibilities and activities of antioxidant enzymes of weanling pigs. *Asian-Australian Journal of Animal Science*, 20: 1600-1605. DOI: 10.5713/ajas.2007.1600
- Singh PK, Shekhar P & Kumar K. 2011. Nutritional and manage mental control of ascites syndrome in poultry. *International Journal of Livestock Production*, 2: 117-123. DOI: 10.5897/IJLP.9000020
- Sohail MU, Ijaz A, Yousaf MS, Ashraf K, Zaneb H, Aleem M & Rehman H. 2010. Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: Dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. *Poultry Science*, 89: 1934-1938. DOI: 10.3382/ps.2010-00751
- SPSS. 1997. SPSS Base 7.5 for Windows. SPSS, Chicago, IL.
- Turkyilmaz MK. 2008. Effect of stocking density on stress reaction in broiler chickens during summer. *Turkish Journal of Veterinary and Animal Science*, 32: 31-36.
- Wijten P, Hangoor E, Sparla J & Versteegen M. 2010. Dietary amino acid levels and feed restriction affect small intestinal development, mortality, and weight gain of male broilers. *Poultry Science*, 89: 1424-1439. DOI: 10.3382/ps.2009-00626
- Yahav S. 2002. Limitations in energy intake affect the ability of young turkeys to cope with low ambient temperatures. *Journal of Thermal Biology*, 27: 103-108. DOI: 10.1016/S0306-4565(01)00021-3
- Yousefi K. 2013. The effect of amino acids levels and methods of feeding on performance, development of ascites, immune response and intestinal structure of Arian broilers. Ph.D. Thesis Poultry Nutrition, College of Agriculture and Natural Resources Karaj. (In Persian).
- Zubair AK & Lesson S. 1994. Effect of varying periods of early nutrient restriction on growth compensation and carcass characteristics of male broilers. *Poultry Science*, 73: 129-136. DOI: 10.3382/ps.0730129

Zhan XA, Wang M, Ren H, Zhao RQ, Li JX & Tan ZL. 2007. Effect of early feed restriction on metabolic programming and compensatory

growth in broiler chickens. *Poultry Science*, 86: 654-660. DOI: 10.1093/ps/86.4.654