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EFFECTS OF FEEDING STRATEGIES AND SUPPLEMENTAL LIPOTROPIC FACTORS ON GROWTH PERFORMANCE, ASCITES-RELATED INDICES, SERUM METABOLITES AND MEAT QUALITY IN BROILER CHICKENS REARED AT HIGH ALTITUDE

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ABSTRACT

The aim of the study was to investigate the effects of feeding programs in combination with supplemental lipotropic agents on performance, ascites-related indices, blood metabolites and breast meat quality in broiler chickens reared at high altitude (2,200 m above sea). A total of 450 day-old Ross broiler chickens were randomly assigned to 6 treatments with 5 pens of 15 birds per each. The experiment was carried out in a completely randomized design with 2 × 3 factorial arrangement, including two feeding program (*ad libitum* or early feed restriction) and three status of lipotropic supplementations (a control diet, 150 mg/kg carnitine as L-carnitine, and 1,000 mg/kg choline as choline chloride). The results showed that dietary supplementation with carnitine improved body weight gain and feed conversion ratio (FCR) in broilers ($P \leq 0.05$). The relative weight of the heart was lower in broilers fed choline supplemented diet compared with control group ($P \leq 0.05$). Feeding broiler chickens with carnitine increased the serum concentration of glucose ($P \leq 0.05$). However, supplementation of the diet with carnitine reduced serum concentration of triglycerides ($P \leq 0.05$). Supplemental carnitine reduced the susceptibility of the meat (thiobarbituric acid number) to lipid peroxidation ($P \leq 0.05$). The ascites-related indices, including the weight of right ventricle (RV) and the ratio of right-to-total ventricular weight were lower in broilers which received carnitine and choline supplementation which reared under early feed restriction ($P \leq 0.05$). In conclusion, supplemental carnitine improved growth performance, ascites-related indices and meat quality of broilers. Besides, early feed restriction improved ascites-related indices in broiler chickens reared at high altitude.

Keywords: altitude; broilers; ascites; lipotropic agents; liver health.

INTRODUCTION

Ascites or pulmonary hypertension is a syndrome caused by several factors such as genetic, environment, nutrition, physiological problems and the interactions among these factors (Varmaghany *et al*., 2013). Broiler ascites syndrome used to be primarily observed in the birds raised at high altitude, which entails low partial oxygen pressure and thus diminished oxygen supply (Kalmar *et al*., 2013). Hypoxia triggers partial constriction of the pulmonary vasculature and hypoxemia leads to an increased cardiac output. These responses combine to increase the work load of the right ventricle, resulting in compensatory right ventricular hypertrophy (Özkan *et al*., 2010). Several alternative prevention strategies may be considered to overcome this problem. The most common prevention strategies are feed restriction and lighting regimes that slow down early growth in broiler chickens (Jia *et al*., 2014; Özkan *et al*., 2010). The cornerstone of these strategies is reduction of oxygen needs of broiler chickens via controlling growth rate, provided that the overall growth performance not impaired (Rajani *et al*., 2011). In addition, feed restriction could decrease fat content and increase protein deposition in carcasses, thus resulting in the improved carcass composition (Zhan *et al*., 2007). Feed restriction reduces growth rate at a critical time in the broiler lifecycle when it is the most susceptible to metabolic disease due to its high oxygen demands (Özkan *et al*., 2006). Previous experiments have revealed that early time feed restriction inhibited the pulmonary vascular remodeling (Pan *et al*., 2008; Özkan *et al*., 2010; Özkan *et al*., 2006). However, the underlying mechanisms are not fully understood.

Since hypertension seems to be involved in the development of ascites, any factor that reduces the blood pressure, especially pulmonary vascular pressure, may help control ascites (Wideman *et al*., 2010). Several experiments have been conducted to the effects of the nutritional factors such as L-arginine (Khajali *et al*., 2014), antioxidants (Rajani *et al*., 2011), or plant oils (Varmaghany *et al*., 2013) on the incidence of ascites in broiler chickens. It is well documented that the type of the dietary fat such as monounsaturated fatty acids reduced the concentration of the plasma lipids and prevented the incidence of cardiovascular disease (Varmaghany *et al*., 2013). One of the best strategies to handle the fat metabolism for poultry is the application of lipotropic factors such as choline and carnitine. These molecules can reduce fat deposition in the liver, due to the liberation of them from the liver. Choline as a main lipotropic agent, has an important role in the metabolism of fatty acids in poultry and increases the biodegradation of fatty acids to prevent the storage of fat in tissues (Azadmanesh and Jahanian 2014). In addition, choline plays an important role in synthesis of the membrane phospholipids. It is well demonstrated that the dietary choline prevents excessive lipid accumulation and the incidence of fatty liver (Wen *et al*., 2014).

Carnitine is known for its potential to transport long chain fatty acids from the cytoplasm into the mitochondrial matrix for beta-oxidation and may have an effect on serum triglyceride and hypertension syndrome (Azadmanesh and Jahanian, 2014). Although the effects of dietary choline and carnitine on growth performance and fat deposition in poultry were reported by many authors (Azadmanesh and Jahanian 2014; Wen *et al*., 2014; Xu *et al*., 2003; Keralapurath *et al*., 2010), the data regarding the effect of these lipotropic agents on hypertensive response were not given in their studies.

Therefore, the objective of this trial was to investigate the effects of dietary lipotropic agents in combination with early feed restriction on the ascites-related indices, growth performance, blood metabolites and meat quality of broiler chickens reared at high altitude.

MATERIALS AND METHODS

All animal care and use procedures were approved by the Department of Animal Science, Islamic Azad University (Qaemshahr branch, Qaemshahr, Iran).

The study was conducted at a commercial poultry farm in a highland region (Semirom, Isfahan province, Iran). This region is located in the southwest of the Isfahan province and has 2,200 m height above the sea level. Four hundred and fifty day-old broiler chickens (mixed sex) were obtained from a commercial hatchery and randomly assigned into 6 groups with 5 replicate pens of 15 birds per each. The experiment was carried out in a completely randomized design with a 2 × 3 factorial arrangement, including two feeding programs (*ad libitum* or early feed restriction) and three states of lipotropic supplementation (a control diet, 150 mg/kg carnitine as L-carnitine and 1,000 mg/kg choline as choline chloride). A quantitative feed restriction program was applied 6 h per day from 4 to 14 days of age (starter phase). The second group received its feed *ad libitum*. All experimental diets (pellet form) were formulated to meet or exceed the nutrient requirement for broiler chickens recommended by the Ross-308 broiler chickens requirements. The ingredients and chemical composition of the experimental diets are presented in Table 1. The birds were kept in the pens for the experimental period of 42 days. In this experiment, floor pens with dimensions of 1.5 m × 1.5 m were used. Each pen was equipped with a separate feeder and a manual drinker. The house temperature was maintained at 35° C in the first week, and a weekly reduction of 2° C was practiced until a temperature of 23° C was attained.

Feed intake and body weight gain of each pen was measured at the end of each phase. Feed conversion ratio (FCR) for each pen was calculated by dividing feed intake by body weight gain. At the end of the experiment (42 days of age), one broiler chick from each pen, which was closed to the mean of the pen, was selected and euthanized by cervical dislocation. Then, the viscera were removed manually. The carcass characteristics including the weight of the breast, thigh, liver, heart, pancreas, spleen and the length of the intestine were recorded. All carcass data are presented based on percent of live weight of each broiler chicken.

The blood enzyme activities were evaluated as indicators of liver health. Briefly, At 35 days of age in fasting state, the blood samples were collected from the wing vein of one bird per pen, and rapidly were centrifuged at 5,000×*g* during 5 min at 23º C. Then, sera were analyzed by using commercial kits for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in auto analyzer. The remaining part of serum sample was used to measure serum concentration of glucose, cholesterol, triglycerides and high-density lipoprotein (HDL) by spectrophotometer (Shimadzu, Japan) using Pars azmoon kits (Pars azmoon company, Iran).

Antibody response to inactivated Newcastle disease virus (NDV) vaccine was determined in the broiler chickens as immunity status. The broiler chickens were immunized against NDV at 9 d of age. The non-heparinized blood samples were collected from the wing vein of each bird at 7 and 14 day after vaccination to determine the primary and secondary antibody responses. The samples were centrifuged at 3,000×*g* for 15 min at 23 ℃ to separate serum. Serum samples were used for the hemagglutination inhibition test to determine antibody titer against NDV.

At the end of the experiment (42 days of age), the breast meat of one bird per each pen were removed and sampled for meat quality. The extent of lipid oxidation in breast meat was determined by measuring the thiobarbituric acid reactive substances (TBARS) at 14 days after freezer storage and was expressed as mg of malondialdehyde per kg of breast meat using the procedure described by (Jung *et al*., 2010). The acidity (pH) was measured in the breast meat of broiler chickens using a pH meter (pH55, Italy) with an insertion glass electrode after calibration at room temperature. Also, one gram of breast meat was used to measuring the water holding capacity (WHC). Briefly, the sample of minced breast meat was placed on a round filter paper (No. 4, Whatman). This round paper was centrifuged at 6,000×*g* for 10 min. The released water absorbed into the filter paper was weighed and calculated as a percentage of the initial moisture of the breast meat.

At the end of the experiment, one bird per replicate pen was euthanized, and the heart was removed gently. Then, the pericardium, peripheral adipose tissues and atriums were removed. The individual weights of right (RV) and total (TV) ventricles, as ascites-related indices, were measured. In addition, the right-to-total ventricular weight ratio (RV/TV) was calculated. The RV/TV values higher than 0.25 are considered to be indicative of sustained pulmonary hypertension (Khajali *et al*., 2014).

Statistical analysis was conducted using general linear model procedure (SAS, 1999) to evaluate the effects of treatments on the growth performance, carcass traits, ascites-related indices, blood metabolites, and breast meat quality of broiler chickens. The model included the main effects of feeding program (*ad libitum* or feed restriction) and lipotropic factors (control, carnitine or choline) and their interactions. Statistical significance of differences among treatments was done using the Duncan multiple range test.

RESULTS

The effects of treatments on the growth performance of broiler chickens are presented in Table 2. The early feed restriction decreased feed intake (1 to 14 days of age) in broilers ($P \leq 0.05$). In contrast, significantly enhanced feed intake was observed in broiler chickens reared under early feed restriction compared with *ad libitum* group in grower (15 to 28 days of age) phase ($P \leq 0.05$). The results indicated that body weight gain of broilers fed supplemental carnitine was higher in starter phase ($P \leq 0.05$). Similarly, the dietary supplementation with carnitine or choline decreased feed intake at grower (15 to 28 days of age) and total period (1 to 42 days of age) in broilers ($P \leq 0.05$). The results also showed that carnitine supplementation improved feed conversion ratio (FCR) at starter (1 to 14 days of age) and total period (1 to 42 days of age) in broilers ($P \leq 0.05$).

The results of carcass characteristics and the relative weight of internal organs of broiler chickens are shown in Table 3. The results showed that, except for the heart weight, carcass traits were not influenced by the experimental treatments. The relative weight of the heart was decreased in broilers which received choline feed additive ($P \leq 0.05$).

Effects of treatments on serum biochemical metabolites, liver enzymes activity and antibody titres against Newcastle disease virus (NDV) are presented in Table 4. The liver enzymes concentrations including alanine amino transferase (ALT) and aspartate amino transferase (AST), and antibody titre against Newcastle disease virus (NDV) were not affected by treatments. The serum concentration levels of glucose and triglycerides were altered by the experimental treatments. In this regard, the birds received carnitine supplementation had a higher level of glucose concentration ($P \leq 0.05$). In contrast, the serum concentration of triglycerides was lower in birds that received carnitine supplementation ($P \leq 0.05$).

The results of antioxidative potential of breast meat and hypertensive response of broiler chickens are shown in Table 5. The birds fed diets supplemented with carnitine had a lower Thiobarbituric acid (TBA) number as mg malondialdehyde per kg of breast meat ($P \leq 0.05$). The water holding capacity (WHC) and pH of breast meat were not affected by the experimental treatments. According to the results of Table 5, the weight of the right ventricle (RV) and the ratio of RV/TV (as ascites-related indices) were lower in broilers that received carnitine and choline supplementation ($P \leq 0.05$). Also, the birds reared under early feed restriction showed a decrease in the weight of the RV and the ratio of RV/TV.

Table 1. Composition of basal diets (as-fed basis).

Item	Starter d 1 to 14	Grower d 15 to 28	Finisher d 29 to 42
Ingredient(g/kg unless stated otherwise)			
Corn grain	588.5	606.4	634.5
Soybean meal (440 g CP/kg)	350.0	328.0	290.2
Soybean oil	12.0	25.0	37.2
Oyster shell	13.9	11.2	11.3
Dicalcium phosphate	18.4	16.3	14.9
Common salt	4.6	4.1	3.6
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
DL-Met	3.7	2.6	2.4
L-Lys	2.9	1.1	0.8
L-Thr	1.0	0.2	0.1
Chemical composition (g/kg)			
ME (kcal/kg)	2950	3050	3150
CP	224.5	213.0	197.0
Ca	10.3	8.7	8.4
Available P	4.9	4.4	4.0
Na	2.0	1.8	1.6
Lys	14.0	12.0	10.8
Met + Cys	10.6	9.3	8.7
Thr	9.2	8.0	7.5

[1] Provides per kilogram of diet: 9,000 IU vitamin A; 2,000 IU vitamin D_3; 18 IU vitamin E; 2 mg menadion; 1.8 mg thiamine; 6.6 mg riboflavin; 30 mg niacin; 3 mg pyridoxine; 15 µg vitamin B_{12}; 100 mg D-pantothenic acid; 1 mg folic acid; 0.1 mg biotin; 500 mg choline chloride; and 100 mg antioxidant.
[2] Provides per kilogram of diet: 100 mg Mn; 84.7 mg Zn; 50 mg Fe; 10 mg Cu; 1 mg I; and 0.2 mg Se.

Table 2. Effects of treatments on live weight gain, feed intake and feed conversion ratio (FCR) of broiler chickens.

Item	Weight gain (g/d)				Feed intake (g/d)				FCR			
	d 1 to 14	d 15 to 28	d 29 to 42	d 1 to 42	d 1 to 14	d 15 to 28	d 29 to 42	d 1 to 42	d 1 to 14	d 15 to 28	d 29 to 42	d 1 to 42
FS												
Restricted	32.47[b]	49.61	72.17	51.42	37.88[b]	83.68[a]	160.8	94.15	1.17	1.69	2.23	1.83
Ad libitum	35.31[a]	46.53	74.97	52.27	43.17[a]	78.67[b]	159.9	93.88	1.23	1.70	2.13	1.79
SEM	0.61	1.32	1.14	0.43	0.57	1.47	1.23	0.69	0.02	0.03	0.03	0.01
LF												
Control	32.73[b]	50.12	71.78	51.54	40.64	86.13[a]	160.9	95.91[a]	1.24[a]	1.73	2.24	1.86[a]
Carnitine	35.82[a]	48.63	74.02	52.82	40.30	79.94[b]	160.3	93.53[ab]	1.13[b]	1.64	2.17	1.77[b]
Choline	33.12[b]	45.45	74.93	51.16	40.65	77.46[b]	159.7	92.62[b]	1.23[a]	1.71	2.14	1.81[ab]
SEM	0.74	1.61	1.40	0.52	0.70	1.80	1.50	0.85	0.03	0.04	0.04	0.02
P-value												
FS	0.004	0.11	0.10	0.18	0.001	0.03	0.54	0.79	0.15	0.96	0.06	0.13
LF	0.01	0.14	0.28	0.09	0.92	0.009	0.83	0.03	0.04	0.31	0.18	0.02
FS ×LF	0.003	0.81	0.68	0.22	0.06	0.49	0.32	0.11	0.08	0.23	0.87	0.84

FS: feeding strategies; LF: lipotropic factors; SEM: standard error of the mean.
[a,b]Means in the same column with different superscripts differ significantly ($P \leq 0.05$).

Table 3. Effects of treatments on carcass characteristics and internal organs of broiler chickens (g/100 g body weight of bird).

Item	Breast	Thigh	Liver	Heart	Pancreas	Spleen
				(%)		
FS						
Restricted	21.88	18.92	2.90	0.52	0.22	0.12
Ad libitum	22.44	18.46	2.81	0.56	0.23	0.13
SEM	0.47	0.30	0.10	0.01	0.009	0.011
LF						
Control	22.07	18.07	2.84	0.57[a]	0.23	0.13
Carnitine	22.40	19.04	2.90	0.54[ab]	0.23	0.13
Choline	22.01	18.96	2.80	0.50[b]	0.21	0.12
SEM	0.57	0.35	0.12	0.02	0.01	0.013
P-value						
FS	0.44	0.28	0.50	0.09	0.33	0.91
LF	0.88	0.13	0.84	0.02	0.50	0.31
FS ×LF	0.08	0.73	0.32	0.01	0.18	0.55

FS: feeding strategies; LF: lipotropic factors; SEM: standard error of the mean.
[a,b]Means in the same column with different superscripts differ significantly ($P \leq 0.05$).

Table 4. Effects of treatments on blood metabolites, liver enzymes activity and antibody titres against Newcastle disease virus (NDV).

Item	Glucose, mg/dl	Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	ALT U/L	AST U/L	Anti-NDV titer (log_2)	
							7 dpi	14 dpi
FS								
Restricted	224.0	134.5	86.91	101.1	3.91	330.0	3.08	3.66
Ad libitum	228.8	128.6	87.66	108.3	3.86	329.3	2.96	3.75
SEM	3.32	3.14	4.50	3.47	0.03	2.80	0.22	0.27
LF								
Control	216.1[b]	135.6	98.75[a]	100.3	3.90	332.0	3.01	3.37
Carnitine	232.6[a]	134.1	75.50[b]	109.2	3.86	328.4	3.12	3.50

Choline	230.5[a]	124.8	87.62[ab]	104.6	3.88	328.5	2.87	4.25
SEM	4.06	3.84	5.53	4.25	0.04	3.40	0.27	0.33
P-value								
FS	0.31	0.19	0.89	0.16	0.32	0.88	0.60	0.83
LF	0.02	0.13	0.01	0.35	0.77	0.69	0.81	0.16
FS ×LF	0.57	0.005	0.27	0.26	0.97	0.65	0.62	0.20

FS: feeding strategies; LF: lipotropic factors; SEM: standard error of the mean.
[a,b]Means in the same column with different superscripts differ significantly ($P \leq 0.05$).

Table 5. Effects of treatments on TBA number (mg malondialdehyde/kg meat), pH, water holding capacity (WHC) and ascites-related indices.

Item	TBA	pH	WHC %	RV gr	TV gr	RV/TV
FS						
Restricted	0.68	5.47	61.15	1.95[b]	9.74	0.204[b]
Ad libitum	0.69	5.46	60.45	2.18[a]	9.92	0.221[a]
SEM	0.01	0.03	0.81	0.03	0.12	0.003
LF						
Control	0.70[a]	5.43	59.62	2.17[a]	9.84	0.221[a]
Carnitine	0.64[b]	5.51	62.48	1.98[b]	9.83	0.202[b]
Choline	0.71[a]	5.45	60.30	2.02[b]	9.82	0.205[b]
SEM	0.02	0.04	0.98	0.04	0.15	0.004
P-value						
FS	0.93	0.92	0.55	0.001	0.29	0.001
LF	0.02	0.47	0.13	0.01	0.99	0.02
FS ×LF	0.94	0.83	0.32	0.19	0.68	0.31

TBA: thiobarbituric acid; RV: right ventricle; TV: total ventricle; FS: feeding strategies; LF: lipotropic factors; SEM: standard error of the mean.
[a,b]Means in the same column with different superscripts differ significantly ($P \leq 0.05$).

DISCUSSION

Under the experimental conditions of this study, feed restriction increased feed intake of broilers in grower phase and permitted a compensatory growth response of the restricted birds great enough to equal the production of the *ad libitum* group at 42 days of age. These results are in accordance with findings of Özkan *et al*. (2006), Özkan *et al*. (2010), and Camacho *et al*. (2004) who reported that early feed restriction had no negatives effect on the final efficiency of broiler production. It is widely accepted that feed restriction causes to increase of enzyme activity in broilers digestive tract and this mechanism is one of the important factors which improve the compensatory growth in chicks (Zhan *et al*., 2007). In this regard, it is demonstrated that the birds subjected to early feed restriction used energy the most efficient via improving nutrient digestibility and enzyme activity in the digestive tract (Leeson and Zubair, 1997).

Our results indicated that the addition of carnitine as a lipotropic agent improved FCR of broiler chickens. In contrast with this finding, it was found that no differences in weight, feed intake and FCR were observed in male broilers fed the carnitine supplemented diets (Xu *et al*., 2003). The beneficial impacts of carnitine on the growth performance of broiler chickens are in agreement with the results of Rabie and Szilágyi (1998) who observed the positive effect of carnitine on feed efficiency in the broiler chickens. Reduction of feed intake and improvement in FCR value by dietary supplementation of choline or carnitine suggest that lipotropic agents may improve the utilization of diet energy to achieve a better performance (Azadmanesh and Jahanian, 2014).

The results of the present experiment showed that the relative weight of the heart was lower in broilers that received lipotropic factors (especially choline). In a study conducted by Buyse *et al*. (2001), the carnitine supplementation induced marked increases of the heart weight in the broiler chickens. A dearth of information exists in terms of relative weight of the heart in broiler chickens. Thus, further study needed thereby clarify the mechanism of lipotropes actions on the weight of the heart in broiler chickens.

As shown in Table 4, supplemental lipotropic agents and early feed restriction had no significant effects on the antibody titer against NDV. According to these results, Deng *et al*. (2006) observed no effect of dietary lipotropic agents on humoral immunity in broiler chickens. Azadmanesh and Jahanian (2014) also indicated that addition of dietary lipotropic agents had no significant effect on antibody titer against NDV in broiler chickens. In contrast, it is reported that specific antibody response against bovine serum albumin was improved in pigeons that received carnitine supplementation (Janssens *et al*., 2000). In feeding program, the results of the present experiment did not support the findings of Zhan *et al*. (2007) who observed that feed restriction program decreased the concentration of serum triglycerides and glucose at 21 days of age in broiler chickens, whereas serum concentration of glucose and very low density lipoproteins (VLDL) increased in the broilers reared under feed restricted program at 63 days of age. These researchers indicated that the higher level of glucose and triglyceride might be due to the enhanced insulin resistance and reduced glucose tolerance caused by the metabolic programming for early malnutrition.

The results of Table 4 also showed that serum ALT and AST did not influence by feeding programs. Liver as a main organ in avian metabolism is sensitive to nutritional modifications. The activities of enzymes related to this organ in serum are usually considered as an important index for understanding the liver health. It is cleared that when liver works healthy, the activity enzymes such as AST and ALT in serum will reduce in broiler chickens (Corduk *et al*., 2007). In the present experiment, serum concentrations of ALT and AST did not affected by the dietary lipotropes in broilers. In contrast with the present results, Azadmanesh and Jahanian (2014) found that the serum ALT and AST were lower in broiler chickens that received dietary choline and carnitine. Khosravinia *et al*. (2015) reported that serum AST was lower in broiler chickens fed dietary bio-choline and lecitine. In accordance with our results, Kazemi-Fard *et al*. (2015) indicated that serum ALT and AST did not alter by the dietary carnitine in layers. According to these results, carnitine can be considered as a protecting agent, particularly in the liver parenchyma.

As noted in Table 4, dietary carnitine caused an increase in serum glucose, whereas serum concentration of triglycerides was lower in broilers fed diatary carnitine. In accordance with these results, Lien and Horng (2001) and Xu *et al*. (2003) observed a decrease of serum triglyceride in broilers fed dietary carnitine. Xu *et al*. (2003) indicated that feeding carnitine increased activity of hormone-sensitive lipase (HSL) and decreased activity of lipoprotein lipase (LPL), thereby leading to a higher concentration of fatty acid in serum by accelerating hydrolysis of triglyceride to glycerol and fatty acid, while reducing the concentration of triglyceride in serum. In consistent with these findings, Azadmanesh and Jahanian (2014) reported that the addition of lipotropic agent (carnitine) increased the serum concentration level of triglyceride in broilers. They noted that the incremental effect of carnitine on serum triglyceride may be due to its function to mobilize the stored lipid into the blood stream to be metabolized and oxidized in tissues. Buyse *et al*. (2001) also observed an increase in serum glucose and triglyceride in broilers received carnitine supplementation. Jia *et al*. (2014) reported that differences in physiological status of the animals and feeding environment may be responsible for the discrepancies between these studies.

In the present trial, the addition of supplemental carnitine to broilers diet improved antioxidative potential of breast meat. Azadmanesh and Jahanian (2014) indicated that dietary supplementation with carnitine or choline decreased TBARS in the serum of broiler chickens. These authors implied that since TBARS are formed as the lipid peroxidation byproducts, carnitine may reduce the availability of lipids for peroxidation via facilitating their entrance into the mitochondrial matrix, causing reduction of serum and tissue TBARS. A small inhibition of TBARS production was observed in the samples treated with carnitine with respect to the control (Djenane *et al*., 2004). It is demonstrated that carnitine might have a beneficial effect on lipids oxidation in which it is possible to recognize free radicals as potential mediators of cellular damage (Arduini, 1992). According to these authors, in vitro evidence would support the concept that carnitine might posses a direct antioxidant activity.

The results of the present experiment showed that early feed restriction decreased the values of RV and RV/TV as indices for ascites in broilers. Among several indicators for ascites assessment, the RV/TV proved the strongest relationship to ascites incidence (Rajani *et al*. 2011). According to our results, several authors Özkan *et al*. (2006), Özkan *et al*. (2010) and Camacho *et al*. (2004) found that early feed restriction as a feeding strategy decreased the ascites incidence in broiler chickens. Özkan *et al*. (2010) demonstrated that the reduction of ascites incidence to feed restriction probably due to the reduced level of metabolism per metabolic body weight as reflected by the lower growth rate of the feed restricted birds at early ages and the consequent reduction in the demand for oxygen. On the other hand, Pan *et al*. (2008) reported that the inhibition of pulmonary vascular remodeling might be a result of alleviated systemic hypoxia, but the underlying mechanisms are not well understood.

Feeding lipotropes significantly decreased the values of RV and RV/TV in broiler chickens. Yousefi *et al*. (2013) indicated that carnitine supplement (100mg/kg) increased circulatory level of nitric oxide (NO), an important cellular signaling molecule involved in many physiological processes. This finding is in agreement with the results of Sharifi *et al*. (2015) who reported that the addition of carnitine supplemental in broiler diets decreased the indices of ascites including RV and RV/TV and circulatory level of nitric oxide as a potent vasodilator that oppose the onset of pulmonary hypertension in broiler chickens. Recent research revealed that carnitine could increase NO production through activation of phosphatidyl inositol 3- kinase and subsequent stimulation of endothelial nitric oxide synthase (Ning and Zhao, 2013).

Conclusions: In conclusion, the early feed restriction had no negative effect on the growth performance of broilers reared at high altitude. In addition, this feeding strategy improved the indices of ascites in broiler chickens. In addition, feeding lipotropic agents (particularly carnitine) improved growth performance, ascites-related indices and breast meat quality of broilers reared at high altitude.

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