

Strategies to combat the deleterious impacts of heat stress through feed restrictions and dietary supplementation (vitamins, minerals) in broilers

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ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi effect pembatasan pakan (FR) selama 3 jam dan/ atau suplementasi makanan dengan vitamin dan mineral terhadap pertumbuhan, tingkat mortalitas, beberapa sifat karkas, dan indeks biokimia serum dari anak ayam broiler di bawah tekanan panas di sistem kandang terbuka. Sebanyak 240 ayam pedaging berumur satu minggu ditimbang dan secara acak dikenai 4 jenis perlakuan eksperimental. Perlakuan 1 (T1): ayam diberi makan *ad libitum* pada diet basal, T2: ayam diberi pakan basal dengan FR, T3: ayam diberi makan *ad libitum* pada diet basal dengan vitamin C (200 mg) + vitamin E (200 mg) + Cr (1,5 mg) + Zn (100 mg) per kg pakan, dan T4: ayam diberi makan dengan diet basal dengan FR dan kombinasi suplemen vitamin dan mineral. Ditemukan bahwa parameter pertumbuhan meningkat pada T2, T3, dan T4, sementara tingkat kematian berkurang secara signifikan dibandingkan dengan kontrol. FR dan aditif pakan (T4) menunjukkan peningkatan total protein serum dan penurunan total kolesterol serum. Disimpulkan bahwa efek kombinasi FR dan aditif pakan dapat secara signifikan mengurangi efek stres panas untuk ayam pedaging dengan meningkatkan kinerja pertumbuhan dan menurunkan tingkat kematian.

Kata kunci: stres panas, aditif pakan, pembatasan pakan, pertumbuhan, mortalitas

ABSTRACT

The objective of the current study was to evaluate the impacts of feed restriction (FR) for 3 hours and/or dietary supplementation with vitamins and minerals on growth, mortality rate, some carcass traits, and serum biochemistry indices of broiler chicks under heat stress in the open housing system. A total of 240 one-week old broilers were weighed and randomly assigned into 4 equal experimental treatments. Treatment 1 (T1) was fed *ad libitum* on basal diets, T2 birds were fed on basal diets with FR, T3 birds were fed *ad libitum* on basal diets with vitamin C (200 mg) + vitamin E (200 mg) + Cr (1.5 mg) + Zn (100 mg) per kg diet, and T4 birds were fed on basal diets with FR and combined supplementation of vitamins and minerals. It was found that growth parameters improved in T2, T3, and T4, while the mortality rate was significantly reduced compared with control. FR and feed additives (T4) revealed marked increases in serum total protein and a decrease in total serum cholesterol. It was

concluded that combined effect of FR and feed additives could significantly alleviate heat stress effects for broilers by improving growth performance and decreasing mortality rate.

Keywords: heat stress, feed additives, feed restriction, growth, mortality

INTRODUCTION

Heat stress (HS) indicates an imbalance between body heat loss and heat production that connects to air temperature, relative humidity (RH), air speed, and radiant heat, which play the main roles in producing HS (Alagawany *et al.*, 2017). Currently, climate changes and temperatures increases have been recorded in numerous regions (Smith and Gregory, 2013). This will lead to increasing ambient temperature regularly in subtropical and tropical regions. During the summer season in the South of Russia, the temperature reaches 36-42°C (Fisinin and Kavtarashvili., 2015), and in Egypt, the temperatures reach 40°C and the humidity reaches 75% (Tawfeek *et al.*, 2014). Strains of broiler chicken are particularly sensitive to high environmental temperatures because of their feathers and lack of sweat glands on the skin. Furthermore, the reduction in heat tolerance of commercial broiler crosses results from the higher metabolism by intensive genetic selection for more rapid growth (Zhang *et al.*, 2017).

As observed from previous studies, to overcome HS effects in broilers by nutritional strategies, one of the two main methods must be followed. Firstly, in feed restriction (FR), whether quantitative or qualitative, chicks have partial access to nutrients which are required for their optimal growth and development (Khetani *et al.*, 2009). However, previous studies have shown that the effect of FR methods on broiler performance is variable because many factors (timing, strain, age, sex, etc.) affect the responses of broilers to FR (Novele *et al.*, 2008). Various methods of FR have been used in stressed broilers can lead to a reduction in growth, and metabolic activity to some extent and reduce the occurrence of metabolic diseases which manage to an increase of feed efficiency in broilers (Mench., 2002). Secondly, feed additives like vitamins, minerals, and amino acids are used for alleviating HS impacts on broiler performance (Attia *et al.*, 2017; Hamidi and Pourreza., 2009). In high temperatures, chicks spend a short time in feeding, additional time in drinking, resting and panting as well as extra time in elevating their wings (Mack *et al.*, 2013). HS leads to feed intake

depression, resulting in the intake of fewer nutrients for healthy growth and development of broilers. As a result, high temperatures stress might cause a redirection in minerals and vitamins flow to meet metabolic requirements for immune responses in broilers (Niu, 2009 and Amizar *et al.*, 2017) as well as an increase in vitamins and minerals mobilization and excretion (Bartlett and Smith., 2003; Aengwanich., 2008).

Ascorbic acid can improve the immune system, which enhances resistance to infections (Khan *et al.* 2012). Supplemental vitamin C leads to an increase in growth parameters and immunity responses under various environmental stressors (Attia *et al.*, 2011; Khan *et al.* 2012). Vitamin E is a lipid-soluble antioxidant that is considered to be protective of lipid component in biological membranes that can protect them from free radical damage in stressed broilers. Additionally, dietary supplementation with vitamin E has been proven to improve the growth performance and immunity in broilers (El Dein *et al.*, 2013).

Trace minerals of Cr and Zn play a decisive role in broiler production through improvement in physiological and metabolic functions included enzyme secretion of the digestive system thereby increasing the digestion of nutrients and, consequently, growth performance (Sahin and Kucuk., 2003; Khan *et al.*, 2012). It has been reported that Cr increased weight gain of chicks and improved feed conversion as a result of the improved digestibility of nutrients and metabolism. Besides, adding Cr to diets can increase relative organs in meat-type chickens (Khan *et al.*, 2014; Sahin *et al.*, 2018). Zn is one of the essential trace elements in broiler physiology which has been found to enhance feed intake and improve growth rate and immune system function (Naz *et al.*, 2016).

Previous studies have evaluated the effect of FR and individual supplementation of vitamin C, vitamin E, Cr, and Zn in poultry production. However, there is still paucity of data on combined impact of the FR method and feed additives in broilers under heat stress. Therefore, this work aimed to investigate the influence of FR and/or vitamin C, vitamin E, mineral Cr and Zn supplementation on the performance of broilers under HS in open housing. This study was

dependently designed based on the results of own previous experiments (Mohamed *et al.*, 2019).

MATERIALS AND METHODS

Birds and Management

This study was conducted at Astrakhan State University (South Russia) during the summer months (July and August 2018). In an open housing system, 240 one-day-old, chicks (Ross 308) were purchased and kept in floor pens from day old to 35 days of age with initial body weight of 43.00 ± 0.65 g. Broilers were reared on a chopped mixture of wood shavings and wheat straw after the initial 7 days of brooding, while chicks were brooded together and fed a basal diet with feed and water *ad libitum*. An alighting period was 23 hours per day throughout the first week of brooding and throughout the experimental period (7-35 days). Chicks were weighed and grouped at random into 4 experimental groups consisting of three replicates. Chicks in each replicate (20 chicks) were allocated with floor space 2.5 m^2 (calculated based on 8 chicks/ m^2). Broiler chicks at all treatments were fed on commercial diets by starter and finisher according to National Research Council (NRC, 1994). The trial period was classified into two feeding periods: starter period from 1 to 21 days of age and finisher period from 22 to 35 days of age. Ingredients of diets of experiment and their nutrient composition are presented in Table 1. Nutrient composition was determined according to Association of Official Analytical Chemists (AOAC, 2005). The experimental treatments are detailed in Table 2.

The temperature of brooding (1-7 days) was 33 to 35 °C with relative humidity (RH) ranged from 58.4 to 65.5 %. The ambient temperatures and relative humidity % were noted daily every 6 hours except for the period from 12:00 pm to 16:00 pm, when it was noted every hour. The temperature and humidity % throughout the experimental period (8-35 days of age) are detailed in Table 3.

Growth Performance Parameters and Mortality

Body weight (BW) and feed intake (FI) were recorded weekly, and total FI per week was divided by the number of chicks in each replicate to obtain the average weekly FI per bird. Body weight gain (BWG) was calculated weekly. And

feed conversion ratio (FCR) was calculated weekly and fixed for a number of chicks per replicate and calculated on the basis of a kilogram (kg) of FI per 1 kg of live BWG. Mortality rate was noticed daily.

Carcass Traits

At day 35 of age (the end of the trial period), after 12 hours of fasting, in each replicate, 9 chicks were randomly chosen in each treatment then weighted and slaughtered to determine broiler carcass traits. The feathers were picked, eviscerated carcass weights (g) without giblets and yield (%) were calculated, and liver, abdominal fat, and spleen were removed, separately weighted, and presented in percentage forms of live BW (AOAC, 2005).

Serum Biochemistry Indices

During slaughtering (day 35), 9 blood samples per treatment were collected (3/replicate) in clean centrifuge tubes for measurement of blood parameters. The samples were left for 20 minutes at room temperature to clot. Then, samples were centrifuged at 3,000 rpm for 15 minutes to separate serum from blood. Serum samples stored at -20°C for measurement of total protein, albumin, uric acid, creatinine, glucose, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL cholesterol) and low-density lipoprotein cholesterol (LDL cholesterol). These parameters were determined colorimetrically using commercial kits (produced by Olvex Diagnosticum, Russia) according to the manufacturer's recommendations.

Statistical Analysis

The obtained results of the experiment are shown as mean \pm SE. The statistical analysis processing of data was performed by one-way analysis of variance (ANOVA), using the general linear model (GLM) procedure of SAS software (Statistical analysis system, version 9.1.3, 2003). Duncan's multiple range test was applied to detect the significance degrees among means. Probability values less than 0.05 ($P < 0.05$) was considered significant.

RESULTS

Growth Performance and Mortality

Restricted feeding method (3 hours) and/or dietary supplementation with vitamin C (200 mg), vitamin E (200 mg), Cr (1.5 mg), and Zn (100

Table 1. Ingredient and Nutrient Composition of the Control Diet

Ingredient, %	Starter (1-21 days of age)	Finisher (22-35 days of age)
Yellow corn	53.90	56.35
Soybean meal	32.10	3.50
Corn gluten meal	5.80	30.87
Sun flower oil	4.20	5.55
Dicalcium phosphate	1.80	1.68
Sodium chloride	0.30	0.30
Limestone	1.30	1.20
DL-methionine	0.15	0.10
Lysine	0.15	0.15
Vitamin-mineral premix*	0.30	0.30
Analyzed chemical composition		
Dry matter	92.23	92.01
Crude protein	22.82	20.76
Crude fiber	3.05	3.22
Ether extract	6.45	7.92
Calculated chemical composition		
Metabolizable energy (MJ/kg)	12.98	13.40
Calcium	0.99	0.93
Available phosphorus	0.47	0.45
Methionine+ Cystine	0.90	0.79

*Provided per kilogram of diet: vitamin A, 12,500 IU; vitamin D3, 4000 IU; vitamin E, 30 IU; vitamin K, 2.3 mg; thiamine, 2.2 mg; riboflavin, 8 mg; pantothenic acid, 24.3 mg; niacin, 65 mg; pyridoxine, 4 mg; folic acid, 1.2 mg; biotin, 0.25 mg; vitamin B12, 3 mg; choline, 600 mg; iron from ferrous sulfate, 60 mg; copper from copper sulfate, 7.5 mg; manganese from manganese oxide, 125.1 mg; zinc from zinc oxide, 110 mg; iodine from ethylene diamine dihydride, 1.8 mg; selenium from sodium selenite, 0.35.

mg/per 1 kg diet for broilers, in broilers improved growth performance parameters and decreased mortality rate in heat-stressed broiler chicks compared with the control treatment (Table 4). During all experimental periods, BW and BWG were higher in restricted broilers, ad libitum broilers with dietary supplementation of vitamin C, vitamin E, Cr, Zn, and their combination than those in the control treatment broilers.

No statistical difference in FI among treatments during the first two weeks (7-21) of the experimental period was detected. However, in the period from 22 to 35 days of age and throughout the 35-day period, FI was significantly decreased by applying FR method (T2) and dietary supplementation with vitamins and

minerals (T3) separated or combined (T4) as compared to control (T1). There were statistical differences in FCR between experimental treatments (T2, T3, and T4) and the control in all trial periods. As compared with broilers in the control, the T2, T3, and T4 groups had lower FCR 13.30, 14.78, and 18.72%, respectively than those fed the basal diet (T1) during the total period (7-35). The combined effect of FR and supplementation with vitamins + minerals exhibited the best FCR compared to the control and other treatments.

As shown in Table 4, mortality rate raised in the last two weeks of the trial period (22-35) in all treatments under HS, while the control (T1) showed the highest level (16.67%) compared to

Table 2. The Experimental Design

Treatments	Feed Restriction (FR)	Vitamins, mg/kg diet		Minerals, mg/ kg diet	
	Three hours without feeding (12:00-15:00pm)	Vitamin C (L-ascorbic acid, 99%)	Vitamin E (DL- α -Tocopherol acetate, 50 - 52.6%)	Cr (chromium picolinate, CrPic, 12,42%)	Zn (ZnSO ₄)
T1 (control)	-	-	-	-	-
T2	√	-	-	-	-
T3	-	200	200	1.5	100
T4	√	200	200	1.5	100

Table 3. Ambient Temperature and Relative Humidity during the Experimental Period (8-35 Days of Age) in Summer Season

Parameter	Period (days)			
	8-14	15-21	22-28	28-35
Ambient temperature, °C	32.20	31.30	31.50	31.30
Ambient temperature (12:00-16:00 pm), °C	32.90	32.60	32.70	32.30
Relative Humidity (RH), %	71.7	76.21	68.5	78.9

T2, T3, and T4 (16.67, 8.33, 6.67, and 5.00%, respectively) during the whole period (days 7-35).

Carcass Traits

The results of quantitative FR (3 hours) and/or dietary supplementation (vitamins and minerals) on carcass yield, liver, spleen and abdominal fat are presented in Table 5. The FR strategy and/or adding feed additives (vitamin C, vitamin E, Cr, Zn) improved carcass, spleen yields, and reduced abdominal fat but there was no effect on liver weight. The high values of carcass and spleen yields were noticed for broiler chicks in T4 compared to other treatments and the control. Moreover, abdominal fat deposition in T2, T3 and T4 decreased by 15.43%, 21.81%, and 32.98%, respectively, compared with broilers in the control at high environmental temperatures.

Serum Biochemistry Indices

The impacts of FR and/or dietary

supplementation under HS on serum concentrations of total protein, albumin, creatinine, uric acid, glucose, triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol are presented in Table 6. Feed restricted broilers (T2) had no significant difference in serum total protein, but in T3 and T4 significantly increased by 11.48% and 16.61%, respectively, as compared to the control group (T1). Serum creatinine, uric acid, and glucose were lower in T2, T3, and T4 than in the control group (T1). At high temperatures, FR and/or dietary supplementation (T2, T3, and T4) significantly decreased the serum of total lipids and LDL cholesterol in comparison with the control (T1). Moreover, the combination of FR (3 hours) and dietary supplementation with vitamin C (200 mg), vitamin E (200 mg), Cr (1.5 mg), and Zn (100 mg) per kg diet, had the highest levels in serum of total protein, albumin, and HDL cholesterol and the lowest levels in serum

Table 4. Growth Performance and Mortality Rate of Broilers as Affected by Feed Restriction (FR), Dietary Supplementation with Vitamins (C, E) and Minerals (Cr, Zn) under Heat Stress

Parameter	Treatments*				SEM	p-value
	T1 (control)	T2	T3	T4		
Body weight (BW), g						
7 day	155.43	149.82	155.87	155.70	2.75	0.39
21 day	754.90 ^c	775.32 ^{bc}	787.87 ^{ab}	806.46 ^a	6.45	0.003
35 day	1665.36 ^c	1792.38 ^b	1815.38 ^b	1874.02 ^a	7.78	0.0001
Body weight gain (BWG), g						
7-21 days	599.48 ^c	625.51 ^b	632.00 ^b	650.75 ^a	4.92	0.001
22-35 days	910.46 ^c	1017.05 ^b	1027.51 ^b	1067.57 ^a	7.52	0.0001
7-35 days	1509.93 ^c	1642.56 ^b	1659.51 ^b	1718.32 ^a	7.25	0.0001
Feed intake (FI), g						
7-21 days	986.00	946.96	935.78	933.73	15.03	0.12
22-35 days	2074.32 ^a	1950.01 ^b	1933.06 ^b	1900.18 ^b	15.94	0.0003
7-35 days	3060.33 ^a	2896.97 ^b	2868.85 ^{bc}	2833.91 ^c	12.41	0.0001
Feed conversion ratio (FCR)						
7-21 days	1.65 ^a	1.51 ^b	1.48 ^{bc}	1.43 ^c	0.02	0.001
22-35 days	2.28 ^a	1.92 ^b	1.88 ^b	1.78 ^c	0.02	0.0001
7-35 days	2.03 ^a	1.76 ^b	1.73 ^b	1.65 ^c	0.01	0.0001
Mortality rate						
7-21 days	6.67	1.67	0.00	0.00		
22-35 days	10.62	6.75	6.67	5		
7-35 days	16.67	8.33	6.67	5		

* See Table 2. ^{a,b,c} – means with different letters in the same row are significantly different at (P<0.05). SEM, standard error of the mean. BW and FI were measured weekly but the results of 7, 21 and 35 are presented in the table.

glucose, triglycerides, total cholesterol, and LDL cholesterol compared with T1, T2, and T3.

DISCUSSION

Our previous work (Mohamed *et al.*, 2019), we aimed to determine the best levels of FR (in timing) and feed additives (quantities). Thus, this study investigated to the combination of FR (3 hours) and feed additives (200 mg of vitamin C; 200 mg of vitamin E; 1.5 mg of Cr; 100 mg of

Zn / kg diet) to improve the productivity of broilers and decrease mortality rate under summer conditions (HS) in open housing. There is the question of whether combining both FR and dietary supplementation (vitamin C, vitamin E, Cr, Zn) can further alleviate the harmful effects of HS in broiler chicks. To our information, there is no data about experiments designed to study the impact of combining both procedures on the productivity in broilers under summer conditions.

Table 5. Carcass Traits of Broilers as Affected by Feed Restriction (FR) and Dietary Supplementation with Vitamins (C, E) and Minerals (Cr, Zn) under Heat Stress

Parameter	Treatment*				SEM	p-value
	T1 (control)	T2	T3	T4		
Carcass yield (%)	70.34 ^d	71.45 ^c	72.05 ^b	72.82 ^a	0.09	0.0001
Liver (%)	2.42	2.48	2.47	2.51	0.06	0.78
Spleen (%)	0.10 ^c	0.13 ^b	0.15 ^{ab}	0.18 ^a	0.01	0.001
Abdominal fat yield (%)	1.88 ^a	1.59 ^b	1.47 ^b	1.26 ^c	0.06	0.001

* See Table 2. ^{a,b,c,d} – means with different letters in the same row are significantly different at (P<0.05).

Table 6. Blood Parameters as Affected by Feed Restriction (FR) and Dietary Supplementation with Vitamins (C, E) and Minerals (Cr, Zn) under Heat Stress

Parameter	Treatment*				SEM	p-value
	T1 (control)	T2	T3	T4		
Total protein (g/L)	42.33 ^c	44.43 ^c	47.16 ^b	49.36 ^a	0.35	0.0001
Albumin (g/L)	25.17 ^d	27.04 ^c	29.97 ^b	30.92 ^a	0.18	0.0001
Creatinine (µmol/L)	46.10 ^d	39.86 ^c	34.88 ^b	31.79 ^a	0.73	0.0001
Uric acid (µmol/L)	490.67 ^c	394.33 ^{cb}	355.67 ^b	313.67 ^a	13.36	0.0001
Glucose (mmol/L)	9.83 ^a	8.97 ^b	7.24 ^c	6.92 ^c	0.17	0.0001
Triglycerides (mmol/L)	1.31 ^a	1.22 ^a	0.96 ^b	0.79 ^c	0.04	0.0001
Total cholesterol (mmol/L)	3.31 ^a	3.03 ^b	2.89 ^b	2.59 ^c	0.06	0.0003
HDL cholesterol (mmol/L)	0.99 ^b	1.13 ^a	1.20 ^a	1.20 ^a	0.04	0.02
LDL cholesterol (mmol/L)	2.05 ^a	1.66 ^b	1.49 ^b	1.23 ^c	0.08	0.001

* See Table 2. ^{a,b,c,d} – means with different letters in the same row are significantly different at (P<0.05). SEM, standard error of the mean.

Growth Performance and Mortality

Restrictive feeding in broilers during the entire experimental period (7-35 days) in an open-sided environment improved broiler performance by increasing BW (7.63%) and BWG (8.78%) and decreasing FI, FCR, and mortality rate (5.34, 13.30, and 50.03%, respectively) during the summer season. This agreed with the findings of previous studies (Novele *et al.*, 2008; Butzen *et al.*, 2013; Ozkan *et al.*, 2010). The significant effect of FR in broilers may be associated with an increase in FI and the hypertrophy of the digestive tract that follows the FR period (Rincon and

Lesson., 2002). A reduction in body temperature of broilers as a result of a decline in FI leads to an increase in its survival ability (Basilio *et al.*, 2001). Moreover, applying the FR method in broilers can improve their thermal resistance (Lin *et al.*, 2006).

In the current study, a combined effect of vitamin C, vitamin E, Cr, and Zn improved growth performance under summer conditions. BWG significantly increased (13.80%), FCR improved (14.78%), and mortality decreased (59.99%). These results are in accordance with those presented by Tawfeek *et al.* (2014), who

detected that dietary supplementation with vitamin C+ vitamin E, Zn + selenium, and Cr enhanced broiler performance under HS. Sahin *et al.* (2018) observed that supplemental Cr in hens overcomes the negative effects of HS on growth performance and metabolic profile. The combination of vitamin C and vitamin E in broiler diets markedly increased BWG and decreased FCR (Attia *et al.*, 2017). Ali *et al.* (2018) showed that dietary supplementation with vitamin E, Cr, and Zn either individually or in combination enhanced BWG and FCR in broilers. The harmful effects of HS in broiler chicks are a result of a decrease in FI, increase in body temperature, depressed immune response, declined energy bioavailability in cells, digestibility of nutrients, metabolism, and absorption, damage in endocrine functions, disorders in the role and structure of intestinal epithelium, and variations in microbiota of the gut (Lara and Rostagno., 2013; Zhang *et al.*, 2017; Wang *et al.*, 2018). Dietary supplementation with feed additives (vitamins and minerals) may improve broiler performance and combat these deleterious effects (Sahin *et al.*, 2018; Haq *et al.*, 2018; Zhu *et al.*, 2017; Attia *et al.*, 2017). In this study, the beneficial impacts of feed additives on broiler growth may be due to the individual effects of vitamin C, vitamin E, Cr, Zn or a combination. In contrast, previous studies have reported no significant effect on growth performance by supplementation with vitamin C (Abioja *et al.*, 2011), vitamin E (Pompeu *et al.*, 2016), Cr (Debski *et al.*, 2004), and Zn (Hamidi and Pourreza., 2009).

The combination of FR and feed additives (T4) reached the highest levels while improving growth performance and decreasing mortality rate when compared with other treatments and control. At the end of the experiment (35 days), the BW of restricted broilers supplemented with vitamin C, vitamin E, Cr, and Zn was increased to 1,874 g versus 1,665, 1,792, and 1,815 g in T1, T2, and T3, respectively. The combined effect in T4 represented a decrease in FI of approximately 7.40, 2.18, and 1.22% as compared with the control, T2, and T3, respectively. Moreover, FR with dietary supplementation recorded the lowest value in mortality rate by 5% as compared to 16.67, 8.33, and 6.67% in T1, T2, and T3, respectively. The noted improvement in growth and mortality rate may be due to synergism between FR and feed additives, while each procedure has many positive effects on performance and mortality rate as previously

explained. Our results are in agreement with those of Abdel-Hafeez *et al.* (2017), who reported that the biological feed additives with FR improved broiler performance.

Carcass traits

Results of the experiment confirmed that FR in broilers markedly enhanced carcass traits under summer conditions. However, liver yield was not significantly influenced. The significant effect of FR on carcass quality appeared in decreasing abdominal fat yield by 15.42% compared to the control (T1). This result is in agreements with those of Chen *et al.* (2012) who reported that there was no significant difference in liver weight between broilers in the control and FR broilers, while FR showed significantly increased spleen weight. In contrast, Shabani *et al.* (2015) indicated that FR was insufficient to improve carcass traits of broilers. As a result of FR, a decline in a fat deposition of FR broilers was mainly initiated by a reduction in feed fat (Chen *et al.*, 2012). A decrease of abdominal fat (%) by FR may contribute to the fasting period by more mobilization for energy and abdominal fat (Zhan *et al.*, 2007; Nielsen *et al.*, 2003). In this way, FR in broilers reared in summer conditions (HS) may be sufficient due to decreasing the activity of lipoprotein lipase enzymes in the chicken's adipose tissue. Lipoprotein lipase enzymes may control the release of fatty acids from lipoproteins and incorporation in adipocytes so that FR can reduce abdominal fat deposition (Faria *et al.*, 2005; Ghazanfari, *et al.*, 2010).

Dietary supplementation in stressed broilers markedly improved carcass and spleen yield and reduced abdominal fat. Similarly, Attia *et al.* (2017) found that supplementation of vitamin C and/or vitamin E slightly improved dressing percentage under heat stress. Khan *et al.* (2014) found that adding Cr to broiler diets increased lymphoid organ weight (spleen). The combined effects of Cr and vitamin C in broiler diets under HS improved quality and carcass traits (Haq *et al.*, 2016). Broilers fed supplemental Cr 500, 1000, and 1500 ppb under summer conditions showed significantly improved carcass yield and reduced abdominal fat (Toghyani *et al.*, 2006). In this way, Al-Masad. (2012) indicated that the relative weight of the spleen increased by the combination effect of vitamin C and Zn. The mechanism of additives in enhancing carcass traits might be due to protecting cells from the damaging effects of HS (Al-Masad., 2012). In addition, the

improvement of carcass traits can contribute to enhanced growth performance.

FR with additives significantly increased carcass and spleen yields and decreased abdominal fat compared with control (T1) and other treatments in summer conditions. However, the combined effect did not affect liver yield. These results match the observations of Abdel-Hafeez *et al.* (2017), revealing that the biological supplementation of feed additives could be more effective, especially when FR method is followed. In general, these results may be due to the actual effect of feed additives and FR, which can improve the rate of metabolism and increase carcass characteristics.

Serum biochemistry indices

The blood components are sensitive to FR under high temperatures (HS). There was a notable increase in the albumin and HDL cholesterol, but a significant decline in glucose, creatinine, triglycerides, total cholesterol, and LDL cholesterol in serum stressed broilers in T2 compared with broilers in the control group. Nevertheless, total protein and uric acid were not significantly influenced. The results of this study are in line with those of Boostani *et al.* (2010), who reported that FR did not change the values of serum total protein but decreased glucose and serum cholesterol. Furthermore, FR did not affect total serum protein, but decreased serum total cholesterol and glucose (Abdel-Hafeez *et al.*, 2017). On the other hand, Chen *et al.* (2012) revealed that serum glucose and triacylglycerols were not overtly influenced, but serum albumin, uric acid increased and total cholesterol, HDL cholesterol, LDL cholesterol were decreased by FR. In the present study, the significant differences in total lipids, triglycerides, HDL cholesterol, and LDL cholesterol may contribute to FR effects on lipid metabolism of broiler chicks through the changes in mitochondria function and morphology (Yang *et al.*, 2010).

The combined effects of vitamin C, vitamin E, Cr, and Zn markedly increased serum total protein, albumin, and HDL cholesterol but significantly reduced serum glucose, triglycerides, total cholesterol, LDL cholesterol, creatinine, and uric acid. These findings, except for glucose concentration, are similar to those of Tawfeek *et al.* (2014), who indicated that supplementation with vitamin C, vitamin E, Zn + selenium, and Cr recorded high values in total protein and low values in total cholesterol. Hag *et al.* (2018)

showed that Cr (0.5 mg/kg) with ascorbic acid (250 mg/kg) or Cr (0.5 mg/kg) with vitamin E (250 mg/kg) reduced total lipids, triglycerides, LDL cholesterol, and glucose; however, HDL cholesterol was not statically influenced. Under HS effects (35 °C), dietary supplementation with Zn and vitamin E (50 and 100 mg/kg diet, respectively) significantly lowered cholesterol, triglycerides, and glucose (Hosseini-Mansoub *et al.*, 2010). Improvement of serum total protein in the present study can be explained via the increased time of digestibility in the digestive tract by slower passage time of feed as a result of a fundamental role of Zn that activates digestive enzymes, metabolism, and growth and promotes protein synthesis (Zakaria *et al.*, 2017). The combined effect of FR with supplemental vitamins and minerals significantly improved total serum protein and decreased total cholesterol, uric acid, and creatinine as compared with control and other treatments (FR and feed additives for each study individually). In this study, the improvement of growth performance and a reduction in abdominal fat might reflect the high values of total protein and low values of total cholesterol and glycerides under HS by the combined effect of FR and feed additives. Furthermore, the significant improvement of growth and serum total protein in broilers (T4) leads to a decrease in protein catabolism in broilers (T4) as compared with broilers in control treatment under HS, which explained the increase in serum uric acid content in control compared with T4 (Xue *et al.*, 2017).

CONCLUSION

Feed restriction for 3 hours in hot times and dietary supplementation with 200 mg of vitamin C, 200 mg of vitamin E, 1.5 mg of Cr, and 100 mg of Zn per kg diet improved growth and carcass characteristics and decreased mortality rate in heat stressed broilers. The combination of FR and feed additives was more effective ($P < 0.05$) than the individual effect of each of them under summer conditions.

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