Journal of Agricultural Science and Technology (2025) Vol. 27(6): 1371-1382

Effect of Dietary Energy Source and Level on the Performance, Antibody Titers and the Relative Expression of *IL-2* and *IL-6* Genes in Broilers under Heat Stress

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ABSTRACT

This study aimed to determine the effects of energy levels and sources on growth performance, antibody titers, and the gene expression of pro-inflammatory cytokines in broilers exposed to heat stress. A total of 450 one-day-old Ross chickens were assigned to six dietary treatments and five replicates in a completely randomized design. Chickens received diets differentiated by the main energy source (corn grain and soybean oil) and energy level (equal, 3% or 6% lower or higher than Ross 308 recommendation). Treatments were as follows: corn grain and equal as Control (CON), 3% lower corn grain (T1), 6% lower corn grain (T2), corn grain and soybean oil, equal (T3), 3% higher corn grain and soybean oil (T4), 6% higher corn grain and soybean oil (T5). The room temperature was increased to 34°C (6-h daily) from day 12 to 42 of age to induce heat stress. The highest corticosterone level was observed in T1, T2, and T5 groups. The lowest antibody titers were observed in T2 group and the highest expression levels of pro-inflammatory cytokines genes were in chickens receiving T5 diet. The highest Feed Conversion Ratio (FCR) during the grower and finisher periods was observed in T2, and the lowest in T3 and T4. It was recommended to feed Ross broiler with a diet containing oil instead of a part of grain based on energy recommended by the strain recommendation.

Keywords: Chicken, Corticosterone, Inflammation. Interleukin, Ross strain.

INTRODUCTION

The major broiler farms exist in subtropical and tropical regions of the world (Kpomasse *et al.,* 2021). In these regions, farmers have to use various strategies to control the temperature of their houses, to reduce the negative effects of heat stress on the health and performance of broilers (Costantino *et al.,* 2018). After exposing broilers to high ambient temperatures, some toxic mechanisms may be induced in the body, including the generation of reactive oxygen species, which finally results in oxidative stress. Oxidative stress could affect the metabolic pathways liver and small intestine health, which reduce the nutrient digestion and absorption, and the merit of substrates for metabolism (Mancinelli *et al.,* 2023). Various management techniques, such as cooling systems, have been used to reduce the negative effects of heat stress on broiler chickens (Fisinin and Kavtarashvili, 2015). The cost of cooling broiler houses is high in

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many regions; hence, some researchers focused on nutritional management (Daghir, 2009). The manipulation of dietary energy level and source has been considered as a useful method in broiler farms to overcome the negative effects of heat stress (Daghir, 2009; Raghebian *et al.,* 2016). Seifi *et al.* (2018) reported that feeding a high-fat diet could improve the heat tolerance in broiler chickens, and dietary inclusion of palm oil improved the growth performance and survivability of heat stressed broiler chickens (Zulkifli *et al.,* 2007).

Moreover, Kim *et al*. (2019) reported that fat supplementation had preventative effects on weight loss for hens raised under heat stress. In contrast, Rafiei-Tari *et al.* (2021) reported that feeding oils containing n-6 fatty acids had detrimental effects on the health of broilers exposed to heat stress. On the other hand, when chickens were fed with low energy diets, deviations from physiological homeostasis occurred, leading to impaired bird welfare (Cheng and Jefferson, 2008) and significant reductions in production capabilities (Jariyahatthakij *et al*., 2018). In an interesting study, Raghebian *et al*. (2016) reported that high energy in a broiler diet could enhance heat resistance and improve performance parameters.

Today, the effect of nutrition on gene expression is very important (Goel *et al*., 2021). The effect of energy level and source on the expression of genes related to heat resistance has been investigated (Raghebian *et al*., 2016), but its effect on gene expression of interleukins, as related to the immune response, was not evaluated. The Interleukin-2 (IL-2) and Interleukin-6 (IL-6) are pro-inflammatory cytokines that play an important role in the inflammatory response in the body of broiler chickens under heat stress (Goel *et al*., 2021) and prolong inflammation responses cause tissue damage, especially in the liver and immune system tissues (Helwig and Leon, 2011; Goel *et al*., 2021). Finding the relationship between the level and energy source of the diet with the relative expression of genes of these two cytokines helps to understand better the cause of the effects observed in the body of broiler chickens.

In the literature, the effects of energy source and level on the antibody titers and relative expression of pro-inflammatory cytokine genes (IL-2 and IL-6) in chickens under heat stress have not been completely evaluated (Ndlebe *et al.,* 2023). Taleb *et al*. (2017) reported that antibody titers decreased in broiler chickens (Cobb 500 strain) raised under hot environmental conditions receiving soybean oil. In contrast, Sadeghi *et al*. (2013) reported that including soybean oil could enhance the immune response in broiler chickens (Ross 308). It is unclear what effects the soybean oil inclusion and the dietary energy concentration have on the expression of genes related to the immune system and the antibody titer.

It was hypothesized that in the heat stress condition, including soybean oil and formulation of high-energy diet could enhance health, immune responses, and performance compared to a diet containing the main energy source from a carbohydrate or low-energy diet. In the present study, low and high levels of dietary energy were considered factors that cause metabolic stress in the body to mimic the conditions chickens face in different breeding centers.

Therefore, the present study aimed to assess the effects of energy source and level on the growth performance, liver health, immune responses, and the relative expression of IL-2 and IL-6 genes in broiler chickens exposed to heat stress.

MATERIALS AND METHODS

Chickens Management

A total of 465 one-day-old male Ross 308 broiler chickens (average weight of 40 g) were purchased from a local hatchery and allocated randomly to thirty one floor pens (200×180 cm) covered with wood shaving. Chicks were randomly assigned to 6 dietary

treatments with 5 replicates and 15 chicks per each. Except ambient temperature, chicks were raised under controlled conditions, lighting program, and feed recommendations based on Ross 308 broiler guides. Chickens (n= 450) were exposed to heat stress from day 12 to 42 of age, with the relative humidity of 65%. During heat stress, temperatures were raised daily to 34±1°C for 6 hours from 08:00 to 14:00 and then decreased to 24±1°C. Fifteen chicks were kept in a room at normal temperature to assess whether experimental chicks were exposed to heat stress. These chickens received corn grain and energy density based on Ross 308 recommendation (3100 and 3200 kcal/kg during grower and finisher periods, respectively). Blood samples were taken from these chickens to measure corticosterone levels as a biological marker of heat stress. All chickens had access *ad libitum* to feed and fresh water, especially throughout the heat challenge period. Chickens were vaccinated with the Newcastle Disease (ND) vaccine and Infectious Bursal Disease (IBD) vaccine. In the experiment's initial and end, the amount of feed intake and body weight were measured, and the Feed Conversion Ratio (FCR) was calculated. Dead chicken was weighed and the weight was included in the calculations of FCR.

Experimental Design

Dietary treatments were included in the Control group (CON), chickens receiving the main energy source from corn grain and energy density based on Ross 308 recommendation. Treatments included the followings:

T1: Chickens receiving the main energy source from corn grain and 3% lower energy density than Ross 308 recommendation;

T2: Chickens receiving the main energy source from corn grain and 6% lower energy density than Ross 308 recommendation;

T3: Chickens receiving the main energy source from corn grain and soybean oil and energy density based on Ross 308 recommendation;

T4: Chickens receiving the main energy from corn grain and soybean oil and 3% higher energy density than Ross 308 recommendation,

T5: Chickens receiving the main energy from corn grain and soybean oil and 6% higher energy density than Ross 308 recommendation.

Metabolizable energy levels of diets were balanced using starch or washed sand. Chickens were raised at three feeding periods: starter (days 1 to 10), growers (days 11 to 24), and finishers (days 25-42) periods.

Sample Collection and Measurements

On days 24 and 42 of age, blood samples (6 mL) were collected using sterile Venoject directly from the heart of two chickens in each replicate. The serum of the blood sample was separated using a centrifuge (1,500×*g*, 15 minutes) and stored at -20 °C until further analysis. Two days after sampling, antibody titers against viruses of ND and IBD were determined in all serum samples. Biochemical measurements were done on samples taken on day 42 of age.

On day 24, immediately after blood sampling, chicks were sacrificed by cervical dislocation, then the spleen and liver were removed and sampled. Five spleen samples from each treatment were collected to analyze the relative expression of the *IL-2* and *IL-6* genes. Spleen tissues were transferred in a cry-protectant tube, snap-frozen in liquid nitrogen, and stored at -70°C until RT-PCR analysis.

Blood Sample Analysis

Serum corticosterone level was measured enzymatically using an enzyme-linked immunosorbent assay kit (Enzo Life Sciences, NY, USA). Serum concentrations of glucose, total protein, albumin, creatinine, and uric acid were measured using the photometric method

by auto-analyzer (BS-120 model, Minbray Co., USA) and commercial kits (Pars Azmon Co., Tehran, Iran).

Serology

The titers of antibodies against Newcastle disease virus were measured by hemagglutination-inhibition test (Allan and Gough, 1974) and against Infectious Bursal Disease virus by ELISA kit, IDEXX FlockChek standard (IDEXX Corporation, Westbrook, ME, USA). The value of antibody titers was transformed to log2(x) before statistical analysis.

Analysis of the Gene Expression of *IL-2* and *IL-6*

The relative abundances of *IL-2* and *IL-6* mRNA were determined by the RT-PCR technique described by Paraskeuas and Mountzouris (2019) and Long *et al.* (2019). The frozen spleen sample was crushed in a sterile mortar, and the powder was applied for total RNA extraction using a suitable kit (Bioneer Co., Seoul, South Korea). Then, each gene's cDNA was synthesized using a suitable kit using the reverse transcription technique (Bioneer Co., Seoul, South Korea). Quantitative PCR was performed with specific primer pairs for *IL-2* (Paraskeuas and Mountzouris, 2019) and *IL-6* (Long *et al.,* 2011) using Quanti Fast SYBER Green PCR kit (QIAGEN, Cat. No. 204052). GAPDH was chosen as a housekeeping gene. The relative gene expression of *IL-2* and *IL-6* as target genes was normalized to the GAPDH gene using the method previously described by Livak and Schmittgen (2001). Quantification for each treatment group was performed in triplicates.

Statistical Analysis

Statistical analyses were done using the General Linear Model procedure of the SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC) appropriate for a completely randomized design. To evaluate the normal distribution of data, the Kolmogorov-Smirnov test was done. Duncan multiple range tests were used to compare the means. Effects between the control and experimental groups were considered significant when $P< 0.05$.

RESULTS

Effect on Serum Corticosterone Level and Biochemical Measurements

Table 1 shows the serum corticosterone levels and biochemical parameters of broilers receiving different dietary energy levels and sources. The highest corticosterone level was observed in the T1, T2, and T5 groups, and no difference was observed among other treatments with the control group. Broilers in the T5 group had the highest serum glucose level, and those in the T2 group had the lowest. Broilers receiving the T4 diet had the highest albumin, globulin, and total protein, and broilers in the T2 group had the lowest protein sections. The highest concentration of creatinine and uric acid was observed in the T2 group, while broilers of CON, T3, and T4 had the lowest.

Effect on Antibody Titers and the Relative Expression Levels of Interleukins

Table 2 shows the effect of dietary energy level and source on antibody titers against Newcastle disease virus and infectious bursa disease virus determined on days 24 and 42 of age. There was no difference among treatments for ND titer on day 24, but differences were observed for ND titer on day 42. At day 42 of age, the lowest ND titer was observed in the T2 group and the highest in the T3 and T4 groups. On day 24, the IBD titer was the highest in the T3 and T4 groups and the lowest in the T2 group.

Table 1. Effect of dietary energy level and source on serum corticosterone level and biochemical parameters in broiler chickens under heat stress.

Item[a]	CON	T1	T2	T3	T4	T5	SEM	P value
Corticosterone (ng mL^{-1})	20.61^{b}	29.84^{a}	30.37^{a}	21.26^{b}	22.54^{b}	27.33^{a}	1.03	0.01
Glucose (mg dL^{-1})	200.09^{bc}	195.12^{cd}	189.45^{d}	204.72^{bc}	210.58^{ab}	219.21^{a}	3.50	0.01
Albumin (mg dL^{-1})	1.13^{b}	1.13^{b}	1.02^{c}	1.15^{ab}	1.23^{a}	1.07^{bc}	0.027	0.02
Globulin (mg dL^{-1})	1.35^{b}	1.27^{c}	1.17^{d}	1.33^{b}	1.51^{a}	1.17^{d}	0.021	0.03
Total protein (mg dL^{-1})	2.48^{b}	2.40^{b}	2.19^{c}	2.49^{b}	2.73^{a}	2.24^{c}	0.039	0.01
Creatinine (mg dL^{-1})	2.54^{c}	2.73^{bc}	3.74^{a}	2.53^{c}	2.51^{c}	3.01^{b}	0.103	0.02
Uric acid (mg dL^{-1})	4.22^{c}	4.58^{b}	4.94^{a}	4.20^{c}	4.24^{c}	4.83^{a}	0.023	0.01

[a-d] Means within a row with different superscripts are significantly different (P< 0.05). [a] CON: Control, energy based on Ross standard diet with main energy from corn; T1: Chickens receiving 3% lesser energy than Ross standard diet with energy from corn; T2: Chickens received 6% lesser energy than Ross, T3: Chicken receiving Ross standard diet with main energy from corn grain and soybean oil, T4: Chicken receiving 3% upper energy than Ross standard diet, and T5: Chicken receiving 6% upper energy than Ross standard diet.

Table 2. Effect of dietary energy level and source on antibody titers against viruses of Newcastle Disease (ND) ar Infectious Bursal Disease (IBD) on days 24 and 42.

Item[a]	CON	T1	T2	T3	T4	T5	SEM	P value
Day 24 of age								
ND (log 2)	4.67	4.35	4.00	3.65	3.34	4.00	0.45	0.369
IBD (log 2)	419.31^{ab}	414.25^{ab}	402.56^{b}	434.97^{a}	432.08^{a}	416.98^{ab}	9.20	0.016
Day 42 of age								
ND (log 2)	5.35^{abc}	5.09^{bc}	4.67^{c}	7.00^{a}	7.15^{a}	6.65^{ab}	0.55	0.021
IBD (log 2)	3177.30^{a}	3007.60^{b}	3017.09^{b}	3106.02^{a}	3187.72^{a}	3125.34^{a}	50.6	0.050

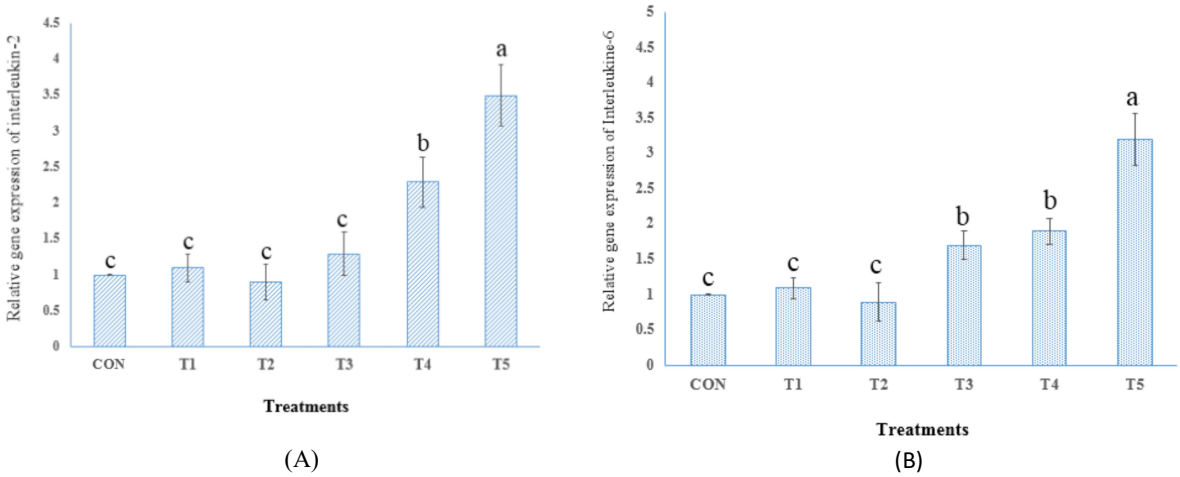
[a-c] Means within a row with different superscripts are significantly different (P< 0.05). [a] CON and T treatments as defined in the main text and Table 1.

On day 42, the highest IBD titer was observed in the CON, T3, T4, and T5 groups, and the lowest titer was found in T1 and T2.

Figure 1 shows the relative expression level of *IL-2* and *IL-6* genes in the spleen of broiler chickens under heat stress receiving different energy densities and sources. Significant differences were observed among treatments for the relative expression of *IL-2* and *IL-6* genes. The highest relative expression of the *IL-2* gene was observed in chickens receiving T5, then, in T4 diets, and the lowest expression was observed in chickens receiving CON, T1, T2, and T3 diets. Chickens receiving the T5 diet had the highest relative gene expression of IL-6, and the lowest expression was observed in chickens receiving CON, T1, and T2 diets.

Effect on Performance Parameters

The performance parameters of broiler chickens are presented in Table 3. There were no differences among treatments for performance parameters during the starter period. These differences appeared at grower and finisher periods. The daily gain of broilers in the T3 and T4 was higher than in T1 and T2. The lowest daily gain during grower and finisher periods was observed in T2 and the highest daily gain was observed in broilers receiving T3 diet. Feed intake of broilers during grower and finisher periods was the highest in the T2, and there was no difference in feed intake among other treatments. The highest FCR during the grower and finisher periods was observed in T2, and the lowest FCR was observed in the T3 and T4. Broilers in the T5 group had the same FCR as T3 and T4.



(A) (B)

Figure 1. The relative expression level of *IL-2* (A) and *IL-6* (B) genes in heat-stressed broilers receiving different energy level and source. CON and T treatments as defined in the main text and Table 1.

Table 3. Effect of dietary energy level and source on performance parameters of broiler chickens under heat stress.

Item[a]	CON	T1	T2	T3	T4	T5	SEM	P value
			Starter phase					
Gain (g d^{-1})	17.21	17.42	17.15	17.02	17.00	17.06	0.209	0.70
Feed intake (g d^{-1})	24.10	24.30	24.20	24.00	23.90	23.90	0.161	0.29
FCR	1.40	1.41	1.41	1.41	1.40	1.40	0.023	0.98
			Grower phase					
Gain (g d^{-1})	51.05^{ab}	48.22^{bc}	46.41^{c}	52.05^{a}	53.12^{a}	50.72^{ab}	0.681	0.01
Feed intake g d^{-1})	87.95^{ab}	89.67^{ab}	90.45^{a}	85.47^{b}	85.32^{b}	86.32^{ab}	1.301	0.01
FCR	1.72^{bc}	1.86^{ab}	1.95^{a}	1.64^{bc}	1.60^{c}	1.70^{bc}	0.044	0.01
			Finisher					
Gain (g/d)	80.41^{bc}	77.52^{cd}	73.67^{d}	87.37^{a}	85.75^{ab}	83.87^{ab}	0.985	0. 01
Feed intake (g/d)	160.21^{b}	163.63^{b}	172.12^{a}	161.71^{b}	160.80^{b}	160.3^{b}	1.53	0.03
FCR	1.99^{bc}	2.11^{b}	2.33^{a}	1.85^{d}	1.87^{cd}	1.91^{cd}	0.033	0.01

[a-c] Means within a row with different superscripts are significantly different (P< 0.05). [a] CON and T treatments as defined in the main text and Table 1.

DISCUSSION

In the present study, serum corticosterone levels were high in broilers receiving the control and experimental diets (heat stress condition) compared to the level of corticosterone in chickens kept in normal temperature conditions (6.78 ng mL^{-1}). Consistent with our results, previous studies have reported that acute heat stress elevates corticosterone levels in the serum of broiler chickens (Quinteiro-Filho *et al.,* 2010; Soleimani *et al.,* 2011). In contrast to our findings, broilers' exposure to heat did not show to influence serum corticosterone levels (Mack *et al.,* 2013; Xie *et al.,* 2015). Possible reasons for the discrepancies among the results of various studies might be differences in temperature and humidity set, time of blood sampling, and chicken genotypes. Increases in corticosterone levels in broilers' serum are linked to the Hypothalamic-Pituitary-Adrenal (HPA) axis. The HPA axis controls the adaptability of broilers in response to various stressors (He *et al.,* 2018).

In the present study, broilers receiving diets with energy restriction (T1 and T2) showed higher corticosterone levels than the control group. In stressful conditions, a change in the energy density of the diet

causes additional metabolic stress in the body of chicks and may increase the generation of free radicals (Raghebian *et al.,* 2017; He *et al.,* 2018). Based on reports (Emami *et al.,* 2021, Rafiei-Tari *et al.,* 2021), chickens receiving a diet with restricted energy experience higher protein turnover, and those receiving a diet with surplus energy experience higher metabolic rate, which both processes increase the heat production and expose body to intense heat stress. Chicken receiving diets containing soybean oil (T4 and T5) had no difference in corticosterone concentration compared to the control group. Also, chicken in T3 and T4, which received soybean oil instead of a part of starch from corn grain, showed lower corticosterone levels than T1 and T2, which may be related to lower heat increment. In a previous study (Sadeghi *et al*., 2013), a shift from starch to lipid during heat stress decreased heat increment. Many researchers recommend replacing soybean oil with starch (Yaqoob, 2004; Cherian, 2015) to reduce the heat increment and the negative effects of heat stress on the animal body.

The serum glucose level of chickens in heat stress was higher than those raised in normal conditions (185 mg/mL), which might be an adaptation for survivability and tolerance. In agreement with our finding, Bogin *et al.* (1996) reported that chickens that survived under intense heat stress had higher blood glucose levels than the non-surviving chickens. The reductions in serum albumin, globulin, and total protein levels in the chicken receiving low dietary energy (T1 and T2) and high dietary energy (T5) compared to the control group can be linked to elevation of serum corticosterone levels. Corticosterone can change metabolic pathways, reduce protein synthesis (Sadeghi *et al.,* 2013), and increases the catabolism of proteins to use as fuel in broilers receiving low dietary energy (Kitaysky *et al.,* 1999). In broilers receiving high dietary energy (T5), reduced total protein in the serum may be linked to liver inflammation. The result of a previous study (Özbey *et al.,* 2004) is consistent with the reductions observed in our study after the heat challenge.

The marked increase in the serum uric acid of broilers receiving low dietary energy (T2) may be linked to an increase of protein turnover and, in those receiving surplus energy (T5), linked to liver inflammation and oxidative stress. Previous studies reported increases (Özbey *et al.,* 2004), reductions (Bogin *et al.,* 1996), and no alteration (Xie *et al.,* 2014) in the serum levels of uric acid after heat stress and energy restriction or surplus. The discrepancies in responses among various studies may be related to the differences in metabolic rates and physiological states and also signify protein catabolism for energy generation in energy-restricted birds resulting from increased corticosterone levels (Vandana *et al.,* 2021).

Chicken receiving T1, T3, T4, and T5 had the same performance parameters, but the chickens in the T2 group had higher feed intake and FCR than the control group. To compensate for the energy dilution of the diet, chickens receiving the T2 diet try to feed more. As feed intake increased, the activity of eating and the digestive tract increased, resulting in increased heat production (Herd and Arthur, 2009). In the heat stress condition, heat dissipating from the body decreases, and the animal body is exposed to oxidative stress (Teeter and Belay, 1996). Chickens exposed to oxidative stress could not grow perfectly and showed a lower feed conversion ratio than CON, T3 and T4 groups. Consistent with our finding, Classen (2017) and Azizi *et al.* (2011) reported that chickens increased feed intake in response to dietary energy dilution. However, Yuan *et al*. (2008) reported that the weight gain of chickens was not altered by dietary energy level, in contrast with our results.

Antibody titers against ND and IBD were the highest in the T3 and T4 diet formulated with soybean oil, and the lowest in the T1 and T2 diet formulated with low energy density, which is inconsistent with the findings of Taleb *et al*. (2017). They

reported that an increase in soybean oil level in the Cobb strain diet resulted in lower antibody titers against ND and IBD. In the current study, an increase in the level of soybean oil in the Ross broiler diet had no negative effect on the antibody titers against ND and IBD.

In broilers receiving low-energy diets, the effect of metabolic stress caused by energy level on the high corticosterone level and low blood glucose level may play an important role in reducing the immune response and antibody production (Yang *et al*., 2015; Aami Azghady *et al.,* 2014). The factors above cause disturbances in the process of growth and maturation of T and B cells in primary and secondary lymphoid tissues, which ultimately causes numerous immune abnormalities in broiler chickens (Hirakawa *et al.,* 2020).

Pro-inflammatory cytokines such as *IL-2* and *IL-6* have been found to play an active role in the inflammatory response under stressful conditions (Helwig and Leon, 2011). In the literature, limited information exists concerning energy level's effect on the gene expression of pro-inflammatory cytokines. A striking finding in the present study was the low expression of genes involved in inflammation in broiler chickens' diets with low energy density (T1 and T2). This finding agrees with some studies (Trayhurn and Wood, 2004; Higami *et al.,* 2006) that reported low energy diet resulted in low inflammation and gene expression of pro-inflammatory cytokines in laboratory animals. In contrast, high-energy diets (T4 and T5) increased the relative gene expression of *IL-2* and *IL-6*. In T4 and T5 groups, high corticosterone levels may be influenced by the expression of pro-inflammatory cytokines as it could increase the proliferation of lymphocytes and macrophages (Hirakawa *et al.,* 2020; Goel *et al.,* 2021). In energy-dense diets, soybean oil is included, and higher expression of these genes may be related to oil inclusion. Previous studies (Mu *et al.,* 2018) revealed that dietary soybean oil significantly increased the gene expression of pro-inflammatory cytokines. The results observed for fasting glucose level and *IL-2* gene expression in the present study are consistence with the finding of Kochumon *et al*. (2020), who reported that the level of *IL-2* expression was associated positively with fasting blood glucose.

CONCLUSIONS

The results of the present study indicate that energy restriction and surplus negatively affect the immune response and performance of chickens raised under heat stress. Surplus energy negatively affects the relative expression levels of pro-inflammatory cytokines genes (IL-2 and IL-6), and energy restriction results in higher protein catabolism (higher uric acid and creatinine), which reduces broiler performance and immune responses. The inclusion of soybean oil in the diet positively affected immune response and performance. It was recommended to feed Ross broiler chickens under heat stress with a diet containing oil instead of a part of grain based on the energy recommended by the strain recommendation.

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اثر منبع و سطح انرژی جیره بر عملکرد، تیتر آنتی بادی و بیان نسبی ژن های اینترلوکین ۲ و ۶ در جوجه های گوشتی تحت تنش گرمایی

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چکیده

این مطالعه با هدف تعیین تأثیر سطوح و نوع منبع انرژی بر عملکرد رشد، تیتر آنتی‌بادی و بیان ژن های سیتوکین‌های پیش‌التهابی در جوجه‌های گوشتی در معرض تنش گرمایی انجام شد. ۴۵۰ قطعه جوجه راس یک روزه در قالب طرح کاملا تصادفی در شش جیره آزمایشی و پنج تکرار قرار گرفتند. جوجه ها جیره های متمایز شده بر اساس منبع اصلی انرژی (دانه ذرت و روغن سویا) و سطح انرژی (برابر، ۳ یا ۶ درصد کمتر یا بالاتر از توصیه (Ross 308 دریافت کردند. تیمارها به شرح زیر بود: دانه ذرت و انرژی برابر با شاهد (CON)، دانه ذرت، ۳ درصد کمتر(T1) ، دانه ذرت، ۶ درصد کمتر(T2) ، دانه ذرت و روغن سویا، برابر (T3)، دانه ذرت و روغن سویا، ۳ درصد بیشتر(T4) ، دانه ذرت و روغن سویا، ۶ درصد بیشتر .(T5) دمای سالن از روز ۱۲ تا ۴۲ دره پرورش به ۳۴ درجه سلسیوس (۶ ساعت در روز) افزایش یافت تا تنش گرمایی ایجاد شود. بالاترین سطح کورتیکوسترون در گروه های T1 ، T2و T5 مشاهده شد. کمترین تیتر آنتی بادی در گروه T2 و بالاترین سطح بیان ژن های سیتوکین های پیش التهابی در جوجه های دریافت کننده جیره T5 مشاهده شد. بیشترین ضریب تبدیل خوراک (FCR) در طول دوره رشد و پایانی در T2 و کمترین FCR در گروه T3 و T4 مشاهده شد. تغذیه جوجه های گوشتی راس با جیره غذایی حاوی روغن به جای بخشی از غلات بر اساس انرژی پیشنهاد شده در کاتالوگ سویه توصیه می شود.