

1 **Effect of dietary energy source and level on the performance, antibody**  
2 **titers and the relative expression of *IL-2* and *IL-6* gene in broilers under**  
3 **heat stress**

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7  
8 **ABSTRACT**

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

25 **Keywords:** Chicken. Corticosterone. Inflammation. Interleukin. Ross strain.

26  
27 **INTRODUCTION**

28 The major broiler farms exist in subtropical and tropical regions of the world (Kpomasse *et al.*,  
29 2021). In these regions, farmers have to use various strategies to control the temperature of their  
30 houses, to reduce the negative effects of heat stress on the health and performance of broilers  
31 (Costantino *et al.*, 2018). After exposing broilers to high ambient temperatures, some toxic

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32 mechanisms may be induced in the body, including the generation of reactive oxygen species,  
33 which finally results in oxidative stress. Oxidative stress could affect the metabolic pathways  
34 liver and small intestine health, which reduce the nutrient digestion and absorption, and the  
35 merit of substrates for metabolism (Mancinelli *et al.*, 2023). Various management techniques,  
36 such as cooling systems, have been used to reduce the negative effects of heat stress on broiler  
37 chickens (Fisinin and Kavtarashvili, 2015). The cost of cooling broiler houses is high in many  
38 regions; hence, some researchers focused on nutritional management (Daghir, 2009). The  
39 manipulation of dietary energy level and source has been considered as a useful method in  
40 broiler farms to overcome the negative effects of heat stress (Daghir, 2009; Raghebian *et al.*,  
41 2016). Seifi *et al.* (2018) reported that feeding a high-fat diet could improve the heat tolerance  
42 in broiler chickens, and dietary inclusion of palm oil improved the growth performance and  
43 survivability of heat stressed broiler chickens (Zulkifli *et al.*, 2007).

44 Moreover, Kim *et al.* (2019) reported that fat supplementation had preventative effects on  
45 weight loss for hens raised under heat stress. In contrast, Rafiei-Tari *et al.* (2021) reported that  
46 feeding oils containing n-6 fatty acids had detrimental effects on the health of broilers exposed  
47 to heat stress. On the other hand, when chickens were fed with low energy diets, deviations  
48 from physiological homeostasis occurred, leading to impaired bird welfare (Cheng and  
49 Jefferson, 2008) and significant reductions in production capabilities (Jariyahathakij *et al.*,  
50 2018). In an interesting study, Raghebian *et al.* (2016) reported that high energy in a broiler  
51 diet could enhance heat resistance and improve performance parameters.

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

62 In the literature, the effects of energy source and level on the antibody titers and relative  
63 expression of pro-inflammatory cytokine genes (*IL-2* and *IL-6*) in chickens under heat stress  
64 have not been completely evaluated (Ndebe *et al.*, 2023). Taleb *et al.* (2017) reported that

65 antibody titers decreased in broiler chickens (Cobb 500 strain) raised under hot environmental  
66 conditions receiving soybean oil. In contrast, Sadeghi *et al.* (2013) reported that including  
67 soybean oil could enhance the immune response in broiler chickens (Ross 308). It is unclear  
68 what effects the soybean oil inclusion and the dietary energy concentration have on the  
69 expression of genes related to the immune system and the antibody titer.

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

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

78

## 79 MATERIALS AND METHODS

### 80 Chickens Management

81 A total of 465 one-day-old male Ross 308 broiler chickens (average weight of 40 g) were  
82 purchased from a local hatchery and allocated randomly to thirty one floor pens (200 cm × 180  
83 cm) covered with wood shaving. Chicks were randomly assigned to 6 dietary treatments with  
84 5 replicates and 15 chicks per each. Except ambient temperature, chicks were raised under  
85 controlled conditions, lighting program, and feed recommendations based on Ross 308 broiler  
86 guides. Chickens (n=450) were exposed to heat stress from day 12 to 42 of age in the relative  
87 humidity of 65%. During heat stress, temperatures were raised daily to 34±1 °C for 6 hours  
88 from 08:00 to 14:00 and then decreased to 24 ± 1 °C. Fifteen chicks were kept in a room at  
89 normal temperature to assess whether experimental chicks were exposed to heat stress. These  
90 chickens received corn grain and energy density based on Ross 308 recommendation (3100 and  
91 3200 kcal/kg during grower and finisher periods, respectively). Blood samples were taken from  
92 these chickens to measure corticosterone levels as a biological marker of heat stress. All  
93 chickens had access *ad libitum* to feed and fresh water, especially throughout the heat challenge  
94 period. Chickens were vaccinated with the Newcastle disease (ND) vaccine and infectious  
95 bursal disease (IBD) vaccine. In the experiment's initial and end, the amount of feed intake and  
96 body weight were measured, and the feed conversion ratio (FCR) was calculated. Dead chicken  
97 was weighed and the weight was included in the calculations of FCR.

**98 Experimental Design**

99 Dietary treatments were included in: the control group (CON), chickens receiving the main  
100 energy source from corn grain and energy density based on Ross 308 recommendation; T1:  
101 chickens receiving the main energy source from corn grain and 3% lower energy density than  
102 Ross 308 recommendation; T2: chickens receiving the main energy source from corn grain and  
103 6% lower energy density than Ross 308 recommendation; T3: chickens receiving the main  
104 energy source from corn grain and soybean oil and energy density based on Ross 308  
105 recommendation; T4: chickens receiving the main energy from corn grain and soybean oil and  
106 3% higher energy density than Ross 308 recommendation, and T5: chickens receiving the main  
107 energy from corn grain and soybean oil and 6% higher energy density than Ross 308  
108 recommendation. Metabolizable energy levels of diets were balanced using starch or washed  
109 sand. Chickens were raised at three feeding periods: starter (days 1 to 10), growers (days 11 to  
110 24), and finishers (days 25-42) periods.

111

**112 Sample Collection and Measurements**

113 On days 24 and 42 of age, blood samples (6 mL) were collected using sterile Venoject directly  
114 from the heart of two chickens in each replicate. The serum of the blood sample was separated  
115 using a centrifuge (1500 × g, 15 minutes) and stored at -20 °C until further analysis. Two days  
116 after sampling, antibody titers against viruses of ND and IBD were determined in all serum  
117 samples. Biochemical measurements were done on samples taken on day 42 of age.

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

123

**124 Blood Sample Analysis**

125 Serum corticosterone level was measured enzymatically using an enzyme-linked  
126 immunosorbent assay kit (Enzo Life Sciences, NY, USA). Serum concentrations of glucose,  
127 total protein, albumin, creatinine, and uric acid were measured using the photometric method  
128 by autoanalyzer (BS-120 model, Minbray Co., USA) and commercial kits (Pars Azmon Co.,  
129 Tehran, Iran).

130

131

**132 Serology**

133 The titers of antibodies against Newcastle disease virus were measured by hemagglutination-  
134 inhibition test (Allan and Gough, 1974) and against Infectious Bursal Disease virus by ELISA  
135 kit, IDEXX FlockChek standard (IDEXX Corporation, Westbrook, ME, USA). The value of  
136 antibody titers was transformed to  $\log_2(x)$  before statistical analysis.

137

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

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

150

**151 Statistical Analysis**

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

157

**158 RESULTS****159 Effect on Serum Corticosterone Level and Biochemical Measurements**

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

166 of creatinine and uric acid was observed in the T2 group, and broilers of CON, T3, and T4 had  
167 the lowest creatinine and uric acid concentrations.

168

### 169 **Effect on Antibody Titers and the Relative Expression Levels of Interleukins**

170 Table 2 shows the effect of dietary energy level and source on antibody titers against Newcastle  
171 disease virus and infectious bursa disease virus determined on days 24 and 42 of age. There  
172 was no difference among treatments for ND titer at day 24, but differences were observed for  
173 ND titer at day 42. At day 42 of age, the lowest ND titer was observed in the T2 group and the  
174 highest in the T3 and T4 groups. At day 24 of age, the IBD titer was the highest in the T3 and  
175 T4 groups and the lowest in the T2 group. At day 42 of age, the highest IBD titer was observed  
176 in the CON, T3, T4, and T5 groups, and the lowest titer was found in T1 and T2 groups.

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

184

### 185 **Effect on Performance Parameters**

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

196

## 197 **DISCUSSION**

198 In the present study, serum corticosterone levels were high in broilers receiving the control and  
199 experimental diets (heat stress condition) compared to the level of corticosterone in chickens



200 kept in normal temperature conditions (6.78 ng/ml). Consistent with our results, previous  
201 studies have reported that acute heat stress elevates corticosterone levels in the serum of broiler  
202 chickens (Quinteiro-Filho *et al.*, 2010; Soleimani *et al.*, 2011). In contrast to our findings,  
203 broilers' exposure to heat stress has not been shown to influence serum corticosterone levels  
204 (Mack *et al.*, 2013; Xie *et al.*, 2015). Possible reasons for the discrepancies among the results  
205 of various studies might be differences in temperature and humidity set, time of blood sampling,  
206 and chicken genotypes. Increases in corticosterone levels in broilers' serum are linked to the  
207 hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis controls the adaptability of broilers  
208 in response to various stressors (He *et al.*, 2018).

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

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

233 inflammation. The result of a previous study (Ozbey *et al.*, 2004) is consistent with the  
234 reductions observed in our study after the heat challenge.

235 The marked increase in the serum uric acid of broilers receiving low dietary energy (T2) may  
236 be linked to an increase of protein turnover and, in those receiving surplus energy (T5), linked  
237 to liver inflammation and oxidative stress. Previous studies reported increases (Ozbey *et al.*,  
238 2004), reductions (Bogin *et al.*, 1996), and no alteration (Xie *et al.*, 2014) in the serum levels  
239 of uric acid after heat stress and energy restriction or surplus. The discrepancies in responses  
240 among various studies may be related to the differences in metabolic rates and physiological  
241 states and also signify protein catabolism for energy generation in energy-restricted birds  
242 resulting from increased corticosterone levels (Vandana *et al.*, 2021).

243 **Chicken receiving T1, T3, T4, and T5 had the same performance parameters**, but the chickens  
244 in the T2 group had higher feed intake and FCR than **the control group**. To compensate for the  
245 energy dilution of the diet, chickens receiving the T2 diet try to feed more. As feed intake  
246 increased, the activity of eating and the digestive tract increased, resulting in increased heat  
247 production (Herd and Arthur, 2009). In the heat stress condition, heat dissipating from the body  
248 decreases, and the animal body is exposed to oxidative stress (Teeter and Belay, 1996).  
249 Chickens exposed to oxidative stress could not grow perfectly and showed a lower feed  
250 conversion ratio than **CON**, T3 and T4 groups. In consistent with our finding, Classen (2017)  
251 and Azizi *et al.* (2021) reported that chickens increased feed intake in response to dietary energy  
252 dilution. However, Yuan *et al.* (2008) reported that the weight gain of chickens was not altered  
253 by dietary energy level, which contrasts with our results.

254 Antibody titers against ND and IBD were the highest in the T3 and T4 diet formulated with  
255 soybean oil and the lowest in the T1 and T2 diet formulated with low energy density, **which is**  
256 **inconsistent with the findings of Taleb *et al.* (2017). They reported that an increase in soybean**  
257 **oil level in the Cobb strain diet resulted in lower antibody titers against ND and IBD. In the**  
258 **current study, an increase in the level of soybean oil in the Ross broiler diet had no negative**  
259 **effect on the antibody titers against ND and IBD.**

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



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

283

## 284 CONCLUSIONS

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

293

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

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

431 <sup>a, b, c, d</sup> Means within a row with different superscripts are significantly different ( $P < 0.05$ ).

432 \*CON: control, energy based on Ross standard diet with main energy from corn; T1: chickens receiving 3% lesser  
433 energy than Ross standard diet with energy from corn; T2: chickens received 6% lesser energy than Ross standard diet  
434 with main energy from corn; T3: chicken receiving Ross standard diet with main energy from corn grain and soybean  
435 oil, T4: chicken receiving 3% upper energy than Ross standard diet with main energy from corn and soybean oil and  
436 T5: chicken receiving 6% upper energy than Ross standard diet with main energy from corn and soybean oil.

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438 **Table 2:** Effect of dietary energy level and source on antibody titers against viruses of Newcastle  
439 disease (ND) and infectious bursal disease (IBD) at days 24 and 42 of age.

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

440 \* ND: Newcastle disease virus; IBD: Infectious Bursal Disease virus

441 <sup>a, b, c</sup> Means within a row with different superscripts are significantly different ( $P < 0.05$ ).

442 \*CON: control, energy based on Ross standard diet with main energy from corn; T1: chickens receiving 3% lesser  
443 energy than Ross standard diet with energy from corn; T2: chickens received 6% lesser energy than Ross standard  
444 0 and soybean oil, T4: chicken receiving 3% upper energy than Ross standard diet with main energy from corn  
445 and soybean oil and T5: chicken receiving 6% upper energy than Ross standard diet with main energy from corn  
446 and soybean oil.

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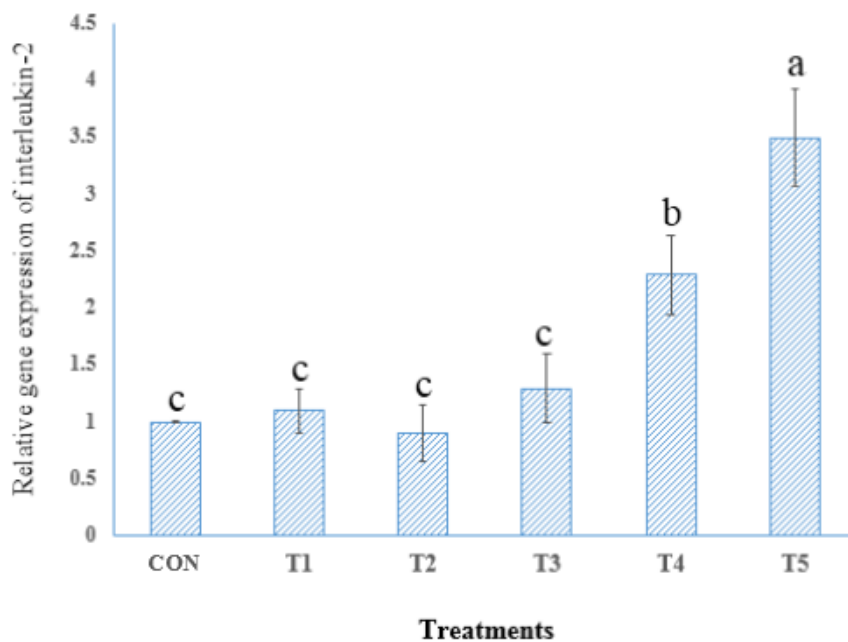
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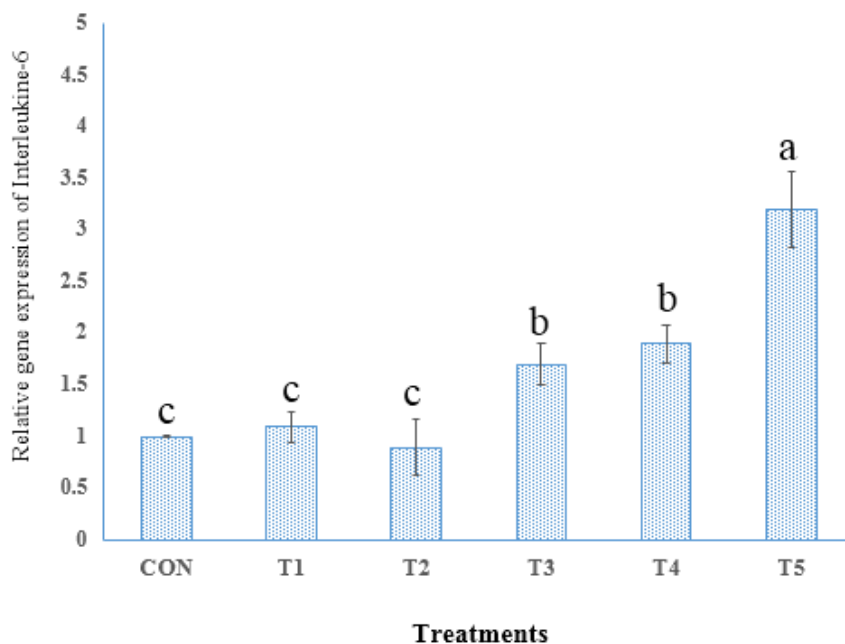
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**Figure 1.** The relative expression level of *IL-2* (A) and *IL-6* (B) genes in heat-stressed broilers receiving different energy level and source

\*CON: 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 standard diet with main energy from corn; 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 with main energy from corn and soybean oil and T5: chicken receiving 6% upper energy than Ross standard diet with main energy from corn and soybean oil.

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

Item	CON	T1	T2	T3	T4	T5	SEM	P value
Starter phase								
Gain (g/d)	17.21	17.42	17.15	17.02	17.00	17.06	0.209	0.70
Feed intake (g/d)	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)	51.05 <sup>ab</sup>	48.22 <sup>bc</sup>	46.41 <sup>c</sup>	52.05 <sup>a</sup>	53.12 <sup>a</sup>	50.72 <sup>ab</sup>	0.681	0.01
Feed intake (g/d)	87.95 <sup>ab</sup>	89.67 <sup>ab</sup>	90.45 <sup>a</sup>	85.47 <sup>b</sup>	85.32 <sup>b</sup>	86.32 <sup>ab</sup>	1.301	0.01
FCR	1.72 <sup>bc</sup>	1.86 <sup>ab</sup>	1.95 <sup>a</sup>	1.64 <sup>bc</sup>	1.60 <sup>c</sup>	1.70 <sup>bc</sup>	0.044	0.01
Finisher								
Gain (g/d)	80.41 <sup>bc</sup>	77.52 <sup>cd</sup>	73.67 <sup>d</sup>	87.37 <sup>a</sup>	85.75 <sup>ab</sup>	83.87 <sup>ab</sup>	0.985	0.01
Feed intake (g/d)	160.21 <sup>b</sup>	163.63 <sup>b</sup>	172.12 <sup>a</sup>	161.71 <sup>b</sup>	160.80 <sup>b</sup>	160.3 <sup>b</sup>	1.53	0.03
FCR	1.99 <sup>bc</sup>	2.11 <sup>b</sup>	2.33 <sup>a</sup>	1.85 <sup>d</sup>	1.87 <sup>cd</sup>	1.91 <sup>cd</sup>	0.033	0.01

492 <sup>a, b, c</sup> Means within a row with different superscripts are significantly different (P<0.05).

493 \*CON: control, energy based on Ross standard diet with main energy from corn; T1: chickens receiving 3% lesser  
494 energy than Ross standard diet with energy from corn; T2: chickens received 6% lesser energy than Ross standard  
495 diet with main energy from corn; T3: chicken receiving Ross standard diet with main energy from corn grain and  
496 soybean oil, T4: chicken receiving 3% upper energy than Ross standard diet with main energy from corn and  
497 soybean oil and T5: chicken receiving 6% upper energy than Ross standard diet with main energy from corn and  
498 soybean oil.

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500 اثر منبع و سطح انرژی جیره بر عملکرد، تیتز آنتی بادی و بیان نسبی ژن های استرلوکین 2 و 6 در جوجه های  
501 گوشتی تحت تنش گرمایی

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503 نعمت الله دینانی، محمد چمنی، پروین شورنگ، آسا ابراهیمی، و علی اصغر صادقی

504 **چکیده**

505 این مطالعه با هدف تعیین تأثیر سطوح و نوع منبع انرژی بر عملکرد رشد، تیتز آنتی بادی و بیان ژن های سیتوکین های  
506 پیش التهابی در جوجه های گوشتی در معرض تنش گرمایی انجام شد. 450 قطعه جوجه راس یک روزه در قالب طرح  
507 کاملاً تصادفی در شش جیره آزمایشی و پنج تکرار قرار گرفتند. جوجه ها جیره های متمایز شده بر اساس منبع اصلی  
508 انرژی (دانه ذرت و روغن سویا) و سطح انرژی (سطح انرژی) برابر، 3 یا 6 درصد کمتر یا بالاتر از توصیه Ross 308 دریافت  
509 کرده اند. تیمارها به شرح زیر بود: دانه ذرت و برابر با شاهد (CON)، دانه ذرت، 3 درصد کمتر (T1)، دانه ذرت، 6  
510 درصد کمتر (T2)، دانه ذرت و روغن سویا، برابر (T3)، دانه ذرت و روغن سویا، 3 درصد بیشتر (T4)، دانه ذرت و  
511 روغن سویا، 6 درصد بیشتر (T5). دمای سالن از روز 12 تا 42 درجه پرورش به 34 درجه سلسیوس (6 ساعت در روز)  
512 افزایش یافت تا تنش گرمایی ایجاد شود. بالاترین سطح کورتیکوسترون در گروه های T1، T2 و T5 مشاهده شد. کمترین  
513 تیتز آنتی بادی در گروه T2 و بالاترین سطح بیان ژن های سیتوکین های پیش التهابی در جوجه های دریافت کننده جیره  
514 T5 مشاهده شد. بیشترین ضریب تبدیل خوراک (FCR) در طول دوره رشد و پایانی در T2 و کمترین FCR در گروه

515 T3 و T4 مشاهده شد. تغذیه جوجه های گوشتی راس با جیره غذایی حاوی روغن به جای بخشی از غلات بر اساس  
516 انرژی پیشنهاد شده در کاتالوگ سویه توصیه می شود.  
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