

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**

4
5 **Nematollah Dayani¹, Mohammad Chamani^{1*}, Parvin Shawrang², Asa Ebrahimi³, and Ali**
6 **Asghar Sadeghi¹**

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

¹ Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Islamic Republic of Iran.

² Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, Karaj, Islamic Republic of Iran.

³ Department of Biotechnology, Science and Research Branch, Islamic Azad University, Tehran, Islamic Republic of Iran.

* Corresponding author; e-mail: , poultsai@gmail.com or m.chamani@srbiau.ac.ir

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

294 References

295 Aami Azghadi, M., Kermanshahi, H. and Golian, A. 2014. The effect of dietary energy
296 and protein levels on growth performance and antibody responses of offspring of laying
297 Japanese quails. *Iran. J. Appl. Anim. Sci.*, **4(1)**: 185-190.

- 298 Azizi, B., Sadeghi, G., Karimi, A., and Abed, F. 2011. Effects of dietary energy and protein
299 dilution and time of feed replacement from starter to grower on broiler chickens
300 performance. *J. Central Europ. Agri.*, **12(1)**: 44-52.
- 301 Bogin, E., Avidar, Y., Pech-Waffenschmidt, V., Doron, Y., Israeli, B. A., and Kevkhayev, E.
302 1996. The relationship between heat stress, survivability and blood composition of the
303 domestic chicken. *European J. Clinical Chem. Clinical Biochem.*, **34(6)**: 463-469.
- 304 Cheng, H. W., and Jefferson, L. 2008. Different behavioral and physiological responses in two
305 genetic lines of laying hens after transportation. *Poult. Sci.*, **87(5)**: 885-892.
- 306 Cherian, G. 2015. Nutrition and metabolism in poultry: role of lipids in early diet. *J. Anim.*
307 *Sci. Biotechnol.*, **6(1)**: 1-9.
- 308 Classen, H. L. 2017. Diet energy and feed intake in chickens. *Anim. Feed Sci. Technol.*, **233**:
309 13-21.
- 310 Costantino, A., Fabrizio, E., Ghiggini, A., and Bariani, M. 2018. Climate control in broiler
311 houses: A thermal model for the calculation of the energy use and indoor environmental
312 conditions. *Energy and Buildings*, **169**: 110-126.
- 313 Dagher, N. J. 2009. Nutritional strategies to reduce heat stress in broilers and broiler breeders.
314 *Lohmann Inform.*, **44(1)**: 6-15.
- 315 Emami, N. K., Greene, E. S., Kogut, M. H., and Dridi, S. 2021. Heat stress and feed restriction
316 distinctly affect performance, carcass and meat yield, intestinal integrity, and inflammatory
317 (chemo) cytokines in broiler chickens. *Front. Physiol.*, **12**:707757.
- 318 Fisinin, V. I., and Kavtarashvili, A. S. 2015. Heat stress in poultry. II. Methods and techniques
319 for prevention and alleviation. *Agri. Biol.*, **50(4)**: 431-443.
- 320 Goel, A., Ncho, C. M., and Choi, Y. H. 2021. Regulation of gene expression in chickens by
321 heat stress. *J. Anim. Sci. Biotechnol.*, **12(1)**: 1-13.
- 322 He, S.P., Arowolo, M.A., Medrano, R.F., Li, S., Yu, Q.F., Chen, J.Y. and He, J.H. 2018.
323 Impact of heat stress and nutritional interventions on poultry production. *World's Poult. Sci.*
324 *J.*, **74(4)**: 647-664.
- 325 Helwig, B.G., and Leon, L.R. 2011. Tissue and circulating expression of IL-1 family members
326 following heat stroke. *Physiol. Genom.*, **43(19)**: 1096-1104.
- 327 Herd, R.M., and Arthur, P.F. 2009. Physiological basis for residual feed intake. *J. Anim. Sci.*,
328 **87(suppl_14)**: E64-E71.
- 329 Higami, Y., Barger, J. L., Page, G. P., Allison, D. B., Smith, S. R., Prolla, T. A., and
330 Weindruch, R. 2006. Energy restriction lowers the expression of genes linked to

- 331 inflammation, the cytoskeleton, the extracellular matrix, and angiogenesis in mouse adipose
332 tissue. *J. Nut.*, **136(2)**: 343-352.
- 333 Hirakawa, R., Nurjanah, S., Furukawa, K., Murai, A., Kikusato, M., Nochi, T., and Toyomizu,
334 M. 2020. Heat stress causes immune abnormalities via massive damage to effect
335 proliferation and differentiation of lymphocytes in broiler chickens. *Front. Vet. Sci.*, **7**: 46.
- 336 Jariyahatthakij, P., Chomtee, B., Poeikhampha, T., Loongyai, W., and Bunchasak, C. 2018.
337 Effects of adding methionine in low-protein diet and subsequently fed low-energy diet on
338 productive performance, blood chemical profile, and lipid metabolism-related gene
339 expression of broiler chickens. *Poult. Sci.*, **97(6)**: 2021-2033.
- 340 Kim, J.H., Lee, H.K., Yang, T.S., Kang, H.K., and Kil, D.Y. 2019. Effect of different sources
341 and inclusion levels of dietary fat on productive performance and egg quality in laying hens
342 raised under hot environmental conditions. *Asian-Austral. J. Anim. Sci.*, **32(9)**:1407-1412.
- 343 Kitaysky, A.S., Piatt, J. F., Wingfield, J. C., and Romano, M. 1999. The adrenocortical stress-
344 response of black-legged kittiwake chicks in relation to dietary restrictions. *J. Comp.*
345 *Physiol. B*, **169**: 303-310.
- 346 Kochumon, S., Al Madhoun, A., Al-Rashed, F., Thomas, R., Sindhu, S., Al-Ozairi, E. and
347 Ahmad, R. (2020). Elevated adipose tissue associated *IL-2* expression in obesity correlates
348 with metabolic inflammation and insulin resistance. *Scientific Reports*, **10(1)**: 16364.
- 349 Kpomasse, C. C., Oke, O. E., Houndonougbo, F. M., and Tona, K. 2021. Broiler production
350 challenges in the tropics: A review. *Vet. Med. Sci.*, **7(3)**: 831-842.
- 351 Long, G. L., Hao, W. X., Bao, L. F., Li, J. H., Zhang, Y., and Li, G. H. 2019. Effects of dietary
352 inclusion levels of palm oil on growth performance, antioxidative status and serum
353 cytokines of broiler chickens. *J. Anim. Physiol. Anim. Nut.*, **103(4)**: 1116-1124.
- 354 Mack, L. A., Felver-Gant, J. N., Dennis, R. L., and Cheng, H. W. 2013. Genetic variations
355 alter production and behavioral responses following heat stress in 2 strains of laying hens.
356 *Poult. Sci.*, **92(2)**: 285-294.
- 357 Mancinelli, A. C., Baldi, G., Soglia, F., Mattioli, S., Sirri, F., Petracchi, M., and Zampiga, M.
358 2023. Impact of chronic heat stress on behavior, oxidative status and meat quality traits of
359 fast-growing broiler chickens. *Front. Physiol.*, **14**: 1-8.
- 360 Mu, H., Shen, H., Liu, J., Xie, F., Zhang, W., and Mai, K. 2018. High level of dietary soybean
361 oil depresses the growth and anti-oxidative capacity and induces inflammatory response in
362 large yellow croaker *Larimichthys crocea*. *Fish Shellfish Immunol.*, **77**: 465-473.

- 363 Ndlebe, L., Tyler, N. C., and Ciacciariello, M. 2023. Effect of varying levels of dietary energy
364 and protein on broiler performance: a review. *World's Poult. Sci. J.*, **79(3)**: 449-465.
- 365 Nikravesht-Masouleh, T., Seidavi, A. R., Kawka, M. and Dadashbeiki, M. 2018. The effect of
366 dietary energy and protein levels on body weight, size, and microflora of ostrich chicks.
367 *Trop. Anim. Health Product.*, **50(3)**: 635-641.
- 368 Özbey, O., Yıldız, N., Aysöndü, M. H., and Özmen, Ö. 2004. The effects of high temperature
369 on blood serum parameters and the egg productivity characteristics of Japanese quails
370 (*Coturnix coturnix japonica*). *Inter. J. Poult. Sci.*, **3 (7)**: 485-489.
- 371 Paraskeuas, V.V., and Mountzouris, K.C. 2019. Modulation of broiler gut microbiota and gene
372 expression of Toll-like receptors and tight junction proteins by diet type and inclusion of
373 phytogenics. *Poult. Sci.*, **98(5)**: 2220-2230.
- 374 Rafiei-Tari, A., Sadeghi, A. A., and Mousavi, S. N. 2021. Inclusion of vegetable oils in diets
375 of broiler chicken raised in hot weather and effects on antioxidant capacity, lipid
376 components in the blood and immune responses. *Acta Scient.. Anim. Sci.*, **43**: 1-6.
- 377 Raghebian, M., Sadeghi, A. A., and Aminafshar, M. 2017. Impact of dietary energy density
378 on the liver health of broilers exposed to heat stress and their performance during finisher
379 period. *J. Livestock Sci.*, **8**: 122-130.
- 380 Raghebian, M., Sadeghi, A. A., and Aminafshar, M. 2016. Energy sources and levels
381 influenced on performance parameters, thyroid hormones, and HSP70 gene expression of
382 broiler chickens under heat stress. *Trop. Anim. Health Product.*, **48**: 1697-1702.
- 383 Sadeghi, A. A., Mirmohseni, M., Shawrang, P., and Aminafshar, M. 2013. The effect of soy
384 oil addition to the diet of broiler chicks on the immune response. *Turk. J. Vet. Anim. Sci.*,
385 **37(3)**: 264-270.
- 386 Seifi, K., Rezaei, M., Yansari, A. T., Riazi, G. H., Zamiri, M. J., and Heidari, R. 2018.
387 Saturated fatty acids may ameliorate environmental heat stress in broiler birds by affecting
388 mitochondrial energetics and related genes. *J. Thermal Biol.*, **78**: 1-9.
- 389 Soleimani, A. F., Zulkifli, I., Omar, A. R., and Raha, A. R. 2011. Physiological responses of
390 3 chicken breeds to acute heat stress. *Poult. Sci.*, **90(7)**: 1435-1440.
- 391 Taleb, Z., Sadeghi, A.A., Shawrang, P., Chamani, M. and Aminafshar, M. 2017. Effect of
392 energy levels and sources on the blood attributes and immune response in broiler chickens
393 exposed to heat stress. *J. Livestock Sci.*, **8**: 123-144.
- 394 Trayhurn, P., and Wood, I. S. 2004. Adipokines: inflammation and the pleiotropic role of white
395 adipose tissue. *Brit. J. Nut.*, **92(3)**: 347-355.

- 396 Teeter, R. G., and Belay, T. 1996. Broiler management during acute heat stress. *Anim. Feed S.*
397 *Technol.*, **58(1-2)**: 127-142.
- 398 Quinteiro-Filho, W. M., Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M. L., Sakai, M., Sá, L. R.
399 M. D. and Palermo-Neto, J. 2010. Heat stress impairs performance parameters, induces
400 intestinal injury, and decreases macrophage activity in broiler chickens. *Poult. Sci.*, **89(9)**:
401 1905-1914.
- 402 Vandana, G. D., Sejian, V., Lees, A. M., Pragna, P., Silpa, M. V., and Maloney, S. K. 2021.
403 Heat stress and poultry production: impact and amelioration. *Inter. J. Biometeorol.*, **65**: 163-
404 179.
- 405 Xie, J., Tang, L., Lu, L., Zhang, L., Lin, X., Liu, H. C. and Luo, X. 2015. Effects of acute and
406 chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed
407 broiler breeders. *Poult. Sci.*, **94(7)**: 1635-1644.
- 408 Yang, J., Liu, L., Sheikahmadi, A., Wang, Y., Li, C., Jiao, H. and Song, Z. 2015. Effects of
409 corticosterone and dietary energy on immune function of broiler chickens. *PLoS One*, **10(3)**:
410 e0119750.
- 411 Yaqoob, P. 2004. Fatty acids and the immune system: from basic science to clinical
412 applications. *Proceed. Nut. Society*, **63(1)**: 89-105.
- 413 Yuan, L., Lin, H., Jiang, K. J., Jiao, H. C., and Song, Z. G. 2008. Corticosterone administration
414 and high-energy feed results in enhanced fat accumulation and insulin resistance in broiler
415 chickens. *Brit. Poult. Sci.*, **49(4)**: 487-495.
- 416 Zulkifli, I., Siegel, H. S., Mashaly, M. M., Dunnington, E. A., and Siegel, P. B. 1995.
417 Inhibition of adrenal steroidogenesis, neonatal feed restriction, and pituitary-adrenal axis
418 response to subsequent fasting in chickens. *General Comparat. Endocrinol.*, **97(1)**: 49-56.
- 419 Zulkifli, I., Htin, N. N., Alimon, A. R., Loh, T. C., and Hair-Bejo, M. 2007. Dietary selection
420 of fat by heat-stressed broiler chickens. *Asian-Austral. J. Anim. Sci.*, **20(2)**: 245-251.

421
422
423
424
425
426
427
428

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 ^b | 29.84 ^a | 30.37 ^a | 21.26 ^b | 22.54 ^b | 27.33 ^a | 1.03 | 0.01 |
| Glucose (mg/dl) | 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) | 1.13 ^b | 1.13 ^b | 1.02 ^c | 1.15 ^{ab} | 1.23 ^a | 1.07 ^{bc} | 0.027 | 0.02 |
| Globulin (mg/dl) | 1.35 ^b | 1.27 ^c | 1.17 ^d | 1.33 ^b | 1.51 ^a | 1.17 ^d | 0.021 | 0.03 |
| Total protein (mg/dl) | 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) | 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) | 4.22 ^c | 4.58 ^b | 4.94 ^a | 4.20 ^c | 4.24 ^c | 4.83 ^a | 0.023 | 0.01 |

431 ^{a, b, c, d} 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.

437

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> |
|-------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|------------|----------------|
| 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 |

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

441 ^{a, b, c} 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.

447

448

449

450

451

452

453

454

455

456

457

458

459

460

461

462

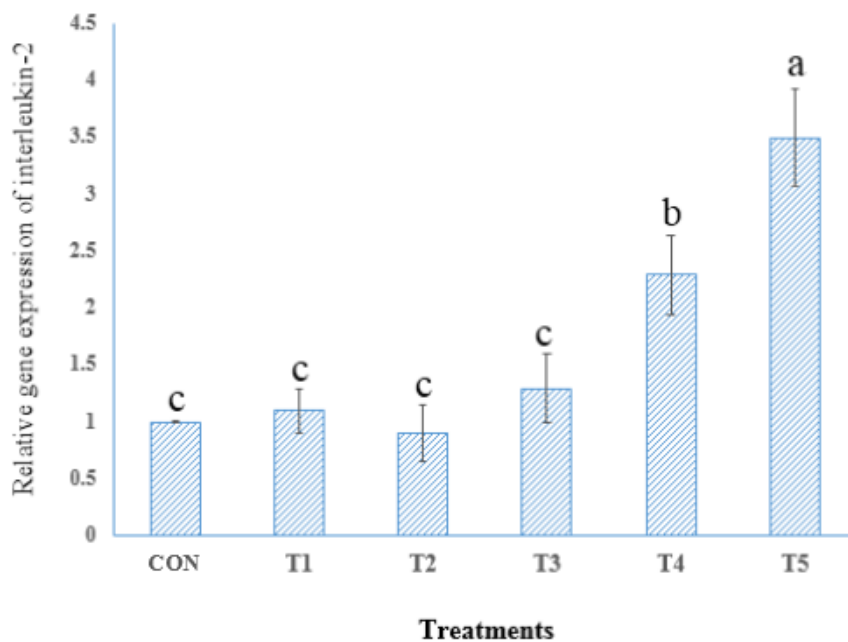
463

464

465

466

467 A



468

469

470

471

472

473

474

475

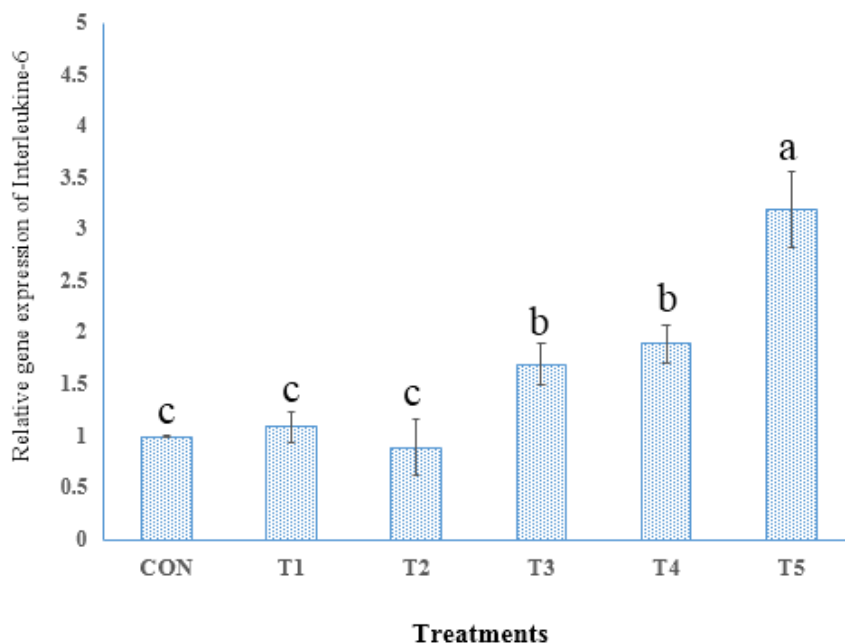
476

477

478

479

480 B



481

482

483

484

485

486

487

488

489

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.

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 ^{ab} | 48.22 ^{bc} | 46.41 ^c | 52.05 ^a | 53.12 ^a | 50.72 ^{ab} | 0.681 | 0.01 |
| Feed intake (g/d) | 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 |

492 ^{a, b, c} 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.

499

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

502

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 دره 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.
517