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

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8 ABSTRACT

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

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

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27 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

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

Moreover, Kim et al. (2019) reported that fat supplementation had preventative effects on 44 weight loss for hens raised under heat stress. In contrast, Rafiei-Tari et al. (2021) reported that 45 feeding oils containing n-6 fatty acids had detrimental effects on the health of broilers exposed 46 47 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 48 Jefferson, 2008) and significant reductions in production capabilities (Jariyahatthakij et al., 49 2018). In an interesting study, Raghebian et al. (2016) reported that high energy in a broiler 50 diet could enhance heat resistance and improve performance parameters. 51

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

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.

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

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

98 Experimental Design

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

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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 ($1500 \times g$, 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.

124 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 autoanalyzer (BS-120 model, Minbray Co., USA) and commercial kits (Pars Azmon Co.,
Tehran, Iran).

132 Serology

- 133 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
- 135 kit, IDEXX FlockChek standard (IDEXX Corporation, Westbrook, ME, USA). The value of
- antibody titers was transformed to log2(x) before statistical analysis.
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138 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 139 described by Paraskeuas and Mountzouris (2019) and Long et al. (2011). The frozen spleen 140 sample was crushed in a sterile mortar, and the powder was applied for total RNA extraction 141 using a suitable kit (Bioneer Co., Seoul, South Korea). Then, each gene's cDNA was 142 synthesized using a suitable kit using the reverse transcription technique (Bioneer Co., Seoul, 143 South Korea). Quantitative PCR was performed with specific primer pairs for IL-2 (Paraskeuas 144 and Mountzouris, 2019) and IL-6 (Long et al., 2011) using Quanti Fast SYBER Green PCR kit 145 (QIAGEN, Cat. No. 204052). GAPDH was chosen as a housekeeping gene. The relative gene 146 expression of *IL-2* and *IL-6* as target genes was normalized to the GAPDH gene using method 147 148 as previously described by Livak and Schmittgen (2001). Quantification for each treatment group was performed in triplicates. 149
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151 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.</p>

158 **RESULTS**

159 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, and broilers of CON, T3, and T4 hadthe lowest creatinine and uric acid concentrations.

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169 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 at day 24, but differences were observed for ND titer at 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. At day 24 of age, the IBD titer was the highest in the T3 and T4 groups and the lowest in the T2 group. At day 42 of age, the highest IBD titer was observed in the CON, T3, T4, and T5 groups, and the lowest titer was found in T1 and T2 groups.

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 and 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.
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185 Effect on Performance Parameters

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

197 DISCUSSION

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

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kept in normal temperature conditions (6.78 ng/ml). Consistent with our results, previous 200 201 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, 202 203 broilers' exposure to heat stress has not been shown to influence serum corticosterone levels (Mack et al., 2013; Xie et al., 2015). Possible reasons for the discrepancies among the results 204 205 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 hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis controls the adaptability of broilers 207 in response to various stressors (He et al., 2018). 208

In the present study, broilers receiving diets with energy restriction (T1 and T2) showed higher 209 corticosterone levels than the control group. In stressful conditions, a change in the energy 210 density of the diet causes additional metabolic stress in the body of chicks and may increase the 211 generation of free radicals (Raghebian et al., 2017; He et al., 2018). Based on reports (Emami 212 et al., 2021, Rafiei-Tari et al., 2021), chickens receiving a diet with restricted energy experience 213 higher protein turnover, and those receiving a diet with surplus energy experience higher 214 215 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 216 corticosterone concentration compared to the control group. Also, chicken in T3 and T4, which 217 received soybean oil instead of a part of starch from corn grain, showed lower corticosterone 218 levels than T1 and T2, which may be related to lower heat increment. In a previous study 219 (Sadeghi et al., 2013), a shift from starch to lipid during heat stress decreased heat increment. 220 Many researchers recommend replacing soybean oil with starch (Yaqoob, 2004; Cherian, 2015) 221 to reduce the heat increment and the negative effects of heat stress on the animal body. 222

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

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

The marked increase in the serum uric acid of broilers receiving low dietary energy (T2) may 235 be linked to an increase of protein turnover and, in those receiving surplus energy (T5), linked 236 to liver inflammation and oxidative stress. Previous studies reported increases (Ozbey et al., 237 238 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 239 among various studies may be related to the differences in metabolic rates and physiological 240 states and also signify protein catabolism for energy generation in energy-restricted birds 241 resulting from increased corticosterone levels (Vandana et al., 2021). 242

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

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

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284 CONCLUSIONS

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

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,0	bioenennear pa	i ameters m	biolici cilici	cens under n	ical stress.				
Item		CON	<i>T1</i>	<i>T2</i>	<i>T3</i>	<i>T4</i>	Т5	SEM	Р
									value
	Corticosterone (ng/ml)	20.61 ^b	29.84 ^a	30.37ª	21.26 ^b	22.54 ^b	27.33ª	1.03	0.01
	Glucose (mg/dl)	200.09 ^{bc}	195.12 ^{cd}	189.45 ^d	204.72 ^{bc}	210.58 ^{ab}	219.21ª	3.50	0.01
	Albumin (mg/dl)	1.13 ^b	1.13 ^b	1.02 ^c	1.15^{ab}	1.23ª	1.07 ^{bc}	0.027	0.02
	Globulin (mg/dl)	1.35 ^b	1.27°	1.17 ^d	1.33 ^b	1.51ª	1.17 ^d	0.021	0.03
	Total protein (mg/dl)	2.48 ^b	2.40 ^b	2.19°	2.49 ^b	2.73ª	2.24°	0.039	0.01
	Creatinine (mg/dl)	2.54°	2.73 ^{bc}	3.74 ^a	2.53°	2.51°	3.01 ^b	0.103	0.02
_	Uric acid (mg/dl)	4.22°	4.58 ^b	4.94 ^a	4.20 ^c	4.24 ^c	4.83 ^a	0.023	0.01

429	Table 1:	Effect	of	dietary	energy	level	and	source	on	serum	corticosterone	level	and
430	biochemic	al para	met	ers in br	oiler chi	ckens	unde	r heat st	ress				

431 ^{a, b, c,d} Means within a row with different superscripts are significantly different (P<0.05).

*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

oil, T4: chicken receiving 3% upper energy than Ross standard diet with main energy from corn and soybea
 T5: chicken receiving 6% upper energy than Ross standard diet with main energy from corn and soybean oil.

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

Item*	CON	T1	T2	Т3	T4	<i>T5</i>	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°	7.00 ^a	7.15 ^a	6.65 ^{ab}	0.55	0.021
IBD (log	3177.30ª	3007.60 ^b	3017.09 ^b	3106.02 ^a	3187.72 ^a	3125.34ª	50.6	0.050

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

^{a, b, c} Means within a row with different superscripts are significantly different (P<0.05).

*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 0 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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*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.

under neat stress.								
Item	CON	T1	<i>T2</i>	<i>T3</i>	<i>T4</i>	<i>T5</i>	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	87.37ª	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	2.33ª	1.85 ^d	1.87 ^{cd}	1.91 ^{cd}	0.033	0.01

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

 $\overline{a, b, c}$ Means within a row with different superscripts are significantly different (P<0.05). 492

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 diet with main energy from corn; T3: chicken receiving Ross standard diet with main energy from corn grain and 495 496 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.

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

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504 این مطالعه با هدف تعیین تأثیر سطوح و نوع منبع انرژی بر عملکرد رشد، تیتر آنتیبادی و بیان ژن های سیتوکین های 505 بیشالتهابی در جوجههای گوشتی در معرض تنش گرمایی انجام شد. 450 قطعه جوجه راس یک روزه در قالب طرح 506 کاملا تصادفی در شش جیره آزمایشی و پنج تکرار قرار گرفتند. جوجه ها جیره های متمایز شده بر اساس منبع اصلی 507 انرژی (دانه ذرت و روغن سویا) و سطح انرژی) بر ابر، 3 یا 6 درصد کمتر یا بالاتر از توصیه (Ross 308 دریافت 508 کرده اند. تیمار ها به شرح زیر بود: دانه ذرت و بر ابر با شاهد (CON) ، دانه ذرت، 3 درصد کمتر (T1) ، دانه ذرت، 6 509 درصد کمتر (T2) ، دانه ذرت و روغن سویا، بر ابر (T3) ، دانه ذرت و روغن سویا، 3 درصد بیشتر (T4) ، دانه ذرت و 510 روغن سویا، 6 درصد بیشتر .(T5) دمای سالن از روز 12 تا 42 دره پرورش به 34 درجه سلسیوس (6 ساعت در روز) 511 افز ایش یافت تا تنش گرمایی ایجاد شود. بالاترین سطح کور تیکوسترون در گروه هایT1، T2 و T5 مشاهده شد. کمترین 512 تیتر آنتی بادی در گروه T2 و بالاترین سطح بیان ژن های سیتوکین های پیش التهابی در جوجه های دریافت کننده جیره 513 T5مشاهده شد. بیشترین ضریب تبدیل خور اک (FCR) در طول دوره رشد و پایانی در T2 و کمترین FCR در گروه 514

515	T3و 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	