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Research Article

Effect of Energy Sources and Levels on Caecal Microbial Population, Jejunal Morphology, Gene Expression of Jejunal Transporters (SGLT1, FABP) and Performance of Broilers Under Heat Stress

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Abstract

The present study was conducted to evaluate the effects of energy sources and levels on microbial population, jejunal morphology, gene expression of glucose transporter (SGLT1), fatty acid binding protein (FABP) and performance in broilers under heat stress. In a completely randomized design, 600 one-day-old Cobb broiler chickens were assigned to five dietary treatments and four replicates. Chicks were fed diet based on corn as main energy source and energy level based on Cobb standard considered as control (C), corn based diet with 3% lesser energy than control (T1), corn based diet with 6% lesser energy than control (T2), corn and soybean oil based diet according to Cobb standard (T3), corn and soybean oil based diet with 3% upper energy than control (T4). Temperature was increased to 34°C for 8 hours daily from day 21 to 41 to induce heat stress. Chickens in T3 and T4 had higher *Lactobacillus* population and lower Escherichia coli population than C group (P<0.05). Chickens in T1 and T2 had shorter jejunal villi, deeper crypts, and lower villus height: crypt depth than those fed C, T3 and T4 diets (P<0.05). There were no significant differences among treatments for gene expression of both nutrient transporters (P>0.05). Chickens in T3 and T4 had higher weight gain compared to C, T1 and T2 (P<0.05). Feed intake in T3 was lower than C, consequently, feed conversion ratio of chicks fed T3 was better than C group (P<0.05). In conclusion, replacement a part of dietary energy source with soybean oil might improve intestinal parameters and performance of broilers under heat stress.

Keywords: *Energy, Lipid, Intestinal morphology, Transporter, Broiler*

Isı Stresi Altında Enerji Kaynakları ve Seviyelerinin Sekum Mikrobiyal Popülasyonuna, Jejunum Morfolojisine, Jejunal Transporterlerin (SGLT1, FABP) Gen Ekspresyonuna ve Broiler Performansına Etkileri

Özet

Bu çalışma; enerji kaynakları ve seviyelerinin ısı stresi altında mikrobiyal popülasyona, jejunum morfolojisine, glukoz transporteri (SGLT1) ve yağ asitleri bağlayıcı protein (FABP) gen ekspresyonlarına ve broiler performansına etkilerini araştırmak amacıyla yürütülmüştür. Rastgele örneklemeyle, 600 adet 1 günlük Cobb broiler civciv, 4 tekrar olmak üzere 5 farklı beslenme uygulanmasına alındı. Civcivler ana enerji kaynağı olarak mısıra dayalı diyetle beslendi. Kontrol grubuna (C) bazal seviyede Cobb standardına göre yem verilirken, T1 grubuna kontrole göre %3 daha düşük enerjili yem, T2 grubuna kontrole göre %6 daha düşük enerjili yem, T3 grubuna Cobb standardına göre mısır ve soya fasulyesi yağı tabanlı diyet ve T4 grubuna kontrole göre %3 daha fazla enerjili mısır ve soya fasulyesi yağı tabanlı diyet uygulandı. Isı stresi oluşturmak amacıyla 21. günden 41. güne kadar günde 8 saat boyunca sıcaklık 34°C'ye çıkarıldı. T3 ve T4 grubundaki civcivlerde kontrol grubuna oranla *Lactobacillus* popülasyonu daha yüksek iken Escherichia coli popülasyonu daha düşüktü (P<0.05). T1 ve T2 grubundaki civcivlerde C, T3 ve T4 grubundakilere kıyasla daha kısa jejunal villi, daha derin kriptler ve daha düşük villus yüksekliği:derinliği belirlendi (P<0.05). Her iki besin transporteri için de gen ekspresyonlarında farklı uygulamalar için herhangi bir fark gözlemlenmedi (P>0.05). T3 ve T4 grubundaki civcivlerde C, T1 ve T2 gruplarına kıyasla daha yüksek kilo kazanımı belirlendi (P<0.05). Kontrol grubuna oranla T3 grubundaki civcivlerin yem tüketimi daha düşük ve dolayısıyla yem konversiyon oranı daha iyiydi (P<0.05). Sonuç olarak, diyetteki enerji kaynağının bir bölümünün soya fasulyesi yağı ile değiştirilmesinin bağırsak parametrelerini ve ısı stresi altındaki broilerlerin performansını iyileştirebileceği belirlendi.

Anahtar sözcükler: *Enerji, Yağ, Bağırsak morfolojisi, Transporter, Broiler*

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INTRODUCTION

Heat stress is considered as a major problem in poultry production on subtropical and tropical regions, where major broiler farms exists [1]. Heat stress occurs when a negative balance between the amount of heat energy produced by the animal and the amount of energy flowing from the broiler body to environment exists. This condition affects negatively the health and performance of broilers [2], In terms of changes in metabolic pathways [3], microbial population in the small intestine [4], hormonal levels [5], low feed consumption, body weight gain, high feed conversion ratio (FCR) [6] and damage to small intestine structure [7,8]. These changes not only could affect nutrient absorption, but also the merit of substrates for metabolism [9,10].

Intestinal bacterial flora are considered important for priming and maintaining an active immune system [11]. The microbiota composition can be affected by environmental factors, genetics and substrate availability within the gut [12]. Development of the intestinal villi in the early chicken`s life could increase efficiency of nutrient utilization and enhance the growth performance. Furthermore, an increase in villi height may increase the intestinal surface area and nutrient absorption [13]. Mitchell and Carlisle [14] reported that chronic heat stress decreased small intestinal villus height and wet and dried small intestinal weight of birds.

The process of absorption of carbohydrates into the enterocytes of the small intestine is mediated by sugar transporters, such as sodium-glucose transporter 1 (SGLT-1) [15], which diffuses monosaccharides into the extra-cellular fluid and then into the blood [16]. Triglycerides (TGs) are broken into glycerol and free fatty acids and then absorbed in the intestine and transported across the apical membrane of the enterocytes. Fatty-acid-binding proteins (FABPs) are intracellular lipid chaperones that transport lipids to a specific component in the cell [17]. Nutrient transporters in the small intestine are responsible for dietary nutrient assimilation; therefore, heat stress-related changes in the expression of these transporters affect the availability of nutrients and energy to the animal for growth and development. However, the effects of heat stress on the expression of nutrient transporters in the small intestine of broiler chickens are unclear. Some studies showed that starvation stress caused the increased expression of sodium glucose co-transporter 1 (SGLT1) mRNA in the small intestine of chickens and rats [18,19].

Wang et al.[20] showed that high apparent metabolizable energy in the diets fed to broilers improved their feed conversion ratio, emphasizing the potential role of nutrient density as an important factor that may affect animal intestine development. Chickens fed a higher nutrient density diet grow faster throughout all growing phases [21]. There are evidences that a higher dietary fat content contributes to improved heat tolerance in broiler chickens [22]. Zulkifli et al.[23] reported that providing diets containing high levels of palm oil enhanced growth performance and survivability of heat-stressed broiler chickens.

To our knowledge, there was no report concerning the effect of energy sources and levels on intestinal transporters gene expression, morphology and microbiology, especially in the context of heat stress condition. It was hypothesized that in the heat stress condition, lipid addition with higher energy level to diet could improves the small intestine morphology, gene expression of transporter and beneficiary bacteria population compared to diet containing main energy source from carbohydrate. Therefore, the main objective was to evaluate the effects of energy sources and levels on microbial population, small intestine morphology, gene expression of glucose transporter (SGLT1), fatty acid binding protein (FABP) and performance in broiler chickens under heat stress conditions.

MATERIAL And METHODS

Chickens Management

All animal procedures were approved by the Animal Care Committee of the Animal Sciences Research Institute of Iran. The use of broilers in this study was approved by the Animal Care Committee (Protocol 17-16-5-10938; 90-11-15).

A total of 600 one-day-old Cobb 500 male broiler chicks with an average weight of 39±0.50g was obtained from a local hatchery and randomly allocated to 20 floor pens (200 cm × 180 cm) covered with pine shaving. Chicks were randomly assigned to five dietary treatments with four replicates and 30 chicks per each. Chicks were raised under environmentally controlled conditions and lighting program based on Cobb 500 broiler guides (Cobb Broiler Management Guide, 2010), except temperature. Feed intake and live body weight were recorded in the beginning and at the end of experiment, and the feed conversion ratio was then calculated. Dead chicks were collected daily and weighed at the time of carcass removal; carcass weights were included in the feed conversion ratio calculations.

Experimental Design

Dietary treatments were: control group (C) which broilers fed diet with main energy from corn and energy level; T1: broilers fed diet with main energy from corn and 3% lesser energy; T2: broilers fed diet with main energy from corn and 6% lesser energy; T3: broilers fed diet with main energy from corn and soy oil and energy level T4: broilers fed diet with main energy from corn and soy oil and 3% upper energy. The experimental diets were formulated based on Cobb standard (Cobb instruction manual, 2012) *(Table 1)*. Washed sand as filler was used to balance for dietary metabolizable energy levels. Chicks were fed a starter diet from day 1 to 10, grower diet from day 11 to 28, followed by a finisher diet from day 29 to 42 of age. Feed

Table 1. *Composition (measured in %) of the experimental diets for broiler chickens*

Ingredients	Starter (0-10 days old)					Grower (11-28 days old)					Finisher (29-42 days old)				
	C	T1	T2	T3	T4	C	T1	T2	T3	T4	C	T1	T2	T3	T4
Corn	63.35	63.33	62.13	58.24	54.26	69.22	68.8	67.7	65.31	59.31	70.18	71.47	71.5	65.07	62.4
Soybean meal	22.57	28.86	31.48	31.90	32.21	18	23.43	26.2	24	28.45	19.3	20	24.2	25.8	25
Soybean oil	--------	-------	------	2.5	4.14	------	------	------	2	5	------	----	-----	3.5	5
Corn gluten meal	9.17	3	-----	2.7	4.7	8.2	3.24	------	4.2	2.9	6.23	4.2	-----	1.5	3.5
Di-calcium phosphate	2.07	2.06	2.06	2.05	2.05	1.9	1.9	1.9	1.9	1.9	1.7	1.7	1.7	1.7	1.7
Calcium carbonate[1]	1.06	1.03	1.01	1.01	1.01	1.05	1.05	1.05	1.05	1.05	0.92	0.92	0.92	0.90	0.90
NaCl	0.38	0.38	0.38	0.38	0.38	0.37	0.37	0.37	0.37	0.37	0.32	0.32	0.32	0.32	0.32
DL - Methionine	0.27	0.33	0.38	0.33	0.30	0.22	0.27	0.30	0.25	0.25	0.18	0.22	0.27	0.22	0.20
L – Lys HCl	0.53	0.40	0.35	0.31	0.36	0.44	0.34	0.28	0.32	0.22	0.36	0.36	0.27	0.21	0.22
L - Threonine	0.10	0.11	0.13	0.08	0.09	0.10	0.10	0.12	0.10	0.05	0.09	0.09	0.10	0.06	0.04
Vitamin & Mineral Permix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	-----	------	-----	------	------	-----	-----	-----	-----	-----	0.22	0.22	0.22	0.22	0.22
Filler[3]	-----	-----	1.58	-----	-----	-----	-----	1.58	----	----	----	----	----	----	---
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Analyzed Nutrient content															
ME (kcal/kg)	3035	2934	2853	3035	3120	3108	3014	2921	3108	3201	3185	3085	2990	3185	3275
Digestible Methionine%	0.59	0.59	0.59	0.59	0.59	0.53	0.53	0.53	0.53	0.53	0.48	0.48	0.48	0.48	0.48
Digestible Lysine%	1.18	1.18	1.18	1.18	1.18	1.05	1.05	1.05	1.05	1.05	0.95	0.95	0.95	0.95	0.95
Digestible Threonine%	0.77	0.77	0.77	0.77	0.77	0.69	0.69	0.69	0.69	0.69	0.65	0.65	0.65	0.65	0.65
Calcium%	0.90	0.90	0.90	0.90	0.90	0.84	0.84	0.84	0.84	0.84	0.76	0.76	0.76	0.76	0.76
Available phosphorus%	0.45	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.42	0.42	0.38	0.38	0.38	0.38	0.38
Na%	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16

[1] per kg contains:Ca , 23% and P, 18.5%; [2] Supplied by Razak Co., Tehran, Iran, and provided per kilogram of diet: vitamin A, 360.000 IU; vitamin D3, 800.000 IU; vitamin E, 7.200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2.640 mg, vitamin B3, 4.000 mg; vitamin B5, 12.000 mg; vitamin B6, 1.200 mg; vitamin B12, 6 mg; Choline chloride, 200.000 mg, Manganese, 40.000 mg, Iron, 20000 mg; Zinc, 40.000 mg, copper, 4000mg; Iodine, 400 mg; [3] Inert filler used to complete diet formulations to 100%

Table 2. *Primers sequences used in RT-PCR*

Primers	Sequence
SGLT1 F	5-GATGTGCGGATACCTGAAGC-3
SGLT1 R	5-AGGGATGCCAACATGACTGA-3
FABP F	5-AGAAAGTTAGGAGGAGCCCACG-3
FABP R	5-TCGGTCACGGATTTCAGC-3
β-actin F	5-CCACCGCAAATGCTTCTAAAC-3
β-actinR	5-AAGACTGCTGCTGACACCTTC-3

and water were provided for *ad libitum* intake. From day 21 to 41, all the chickens were exposed to 34±1°C and 60-70% relative humidity for 8 hours per day from 08:00 to 16:00, and then raised at 24±1°C. Feed and water were provided throughout the heat challenge period.

Sample Collection

On day 28 of age, five chicks from each pen were weighed and euthanized by cervical dislocation. After excising jejunum, as described by Uni et al.[24] segments were washed with cold phosphate buffer saline, sectioned and immediately frozen in liquid nitrogen, and stored at -80°C. 2 cm sections were selected for histo-morphology examination. The sections were flushed with phosphate buffer solution and then fixed in buffered formalin solution (10%). The entry of ceca of five chicks from each pen was sealed, removed and placed in ice and the contents used immediately for microbial assays.

Total RNA Extraction and Reverse Transcription

The frozen jejunum was crushed in a sterile mortar, and the powder was applied for total RNA extraction using a suitable kit (Bioneer Co., Seoul, South Korea). The integrity of the RNA was verified by optical density (OD) absorption ratio 1.97>OD260 nm/OD280 nm>1.9 Ribonucleic acid integrity was determined by gel electrophoresis on a 1% agarose gel. Extracted RNA was stored at -80°C. Then, cDNA for each transporter gene was synthesized based on reverse transcription technique using kit (Bioneer Co., Seoul, South Korea) and stored at -20°C.

Quantitative Real-Time PCR

The relative abundance of SGLT1 and FABP mRNA was determined by quantitative real-time PCR. Quantitative real-time PCR was conducted using a Real-time PCR systems (Applied Biosystems). Each reaction contained the followings: 2 µL of cDNA, 10 µL of 2X SYBR Green Master Mix (Applied Biosystems), 0.4 µL each of the forward primer (4 µM) and reverse primer (4 µM), and 7.2 µL of nuclease-free water. Primers were designed by using Primer software (primer-BLAST) at website (www.ncbi.nlm.nih.gov). PCR reactions were performed with primers designed and synthesized for both transporters and the house-keeping gene β-actin *(Table 2)*. Amplification for transporter in the jejunum (SGLT1 and FABP) was performed for 45 cycles, which consisted of an initial activations step (95°C, 5 min), denaturation cycle (95°C, 30s) and annealing at 54°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 5 min. The PCR products were electrophoresed on a 1% agarose gel. Average gene expression relative to the endogenous control for each sample was calculated using the $2^{-\Delta\Delta CT}$ method [25].

Intestinal Microbial Populations

Immediately, samples of ileum and ceca were collected into glass containers under CO_2 sealed and put on ice until enumeration of microbial populations. Ten grams of mixed contents were blended under CO_2 in 90 mL of anaerobic dilution solution. Further serial dilutions were made in anaerobic dilution solution for anaerobic bacterial enumeration. The initial dilution in anaerobic dilution solution was also used as a source for serial dilutions in phosphate buffered saline for enumeration of aerobic bacterial populations. Triplicate plates were then inoculated with 0.1mL samples and incubated at 37°C aerobically or anaerobically as appropriate. Three dilutions were plated for each medium. Bacteria were enumerated on MRS agar (Merck, Germany) for *Lactobacillus* and MacConkey's (Merck, Germany) for *Escherichia coli*. Colony forming units (cfu) were defined as being distinct colonies measuring at least 1 mm in diameter [26].

Intestinal Morphological Analysis

Formalin fixed tissue sections were processed by dehydration through a series of graded alcohol solutions (50, 70, 80, 90, 95 and 100%), cleared with xylene, and embedded in paraffin. For each segment, a 5-µm cross-section was sectioned using a microtome and placed on a glass slide. The slides were stained using routine procedures for Mayer´s hematoxylin and eosin. Villus height, villus length and crypt depth were measured using a light microscope (Olympus attached camera) using the method presented by Wang et al.[27]. Double-stained of samples with Periodic Acid-Schiff and hematoxylin was done to determine the goblet cell count according to the method of Wang et al.[27]. The goblet cells were counted in the scale of 300 µm of epithelium length.

Statistical Analysis

Statistical analyses were carried out using ANOVA of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC) [28]. The Kolmogorov-Smirnov test was used to test the normality of the data before ANOVA was performed. Tukey test was used to compare the means. Statistical differences were declared at $P<0.05$.

RESULTS

Effect on Performance

The effect of energy sources and levels on daily body weight gain (WG), daily feed intake (FI) and feed conversion ratio (FCR) is presented in *Table 3*. Chickens fed T1 and T2 diets as compared to C group had lower WG, higher FI and higher FCR ($P<0.05$). Chickens in T3 and T4 that received soybean oil and energy level equal to or greater than guideline of Cobb had higher WG compare to those received equal to or lower energy with main energy source of corn grain ($P<0.05$). Chicks in C and T3 group received the same amount of energy with different sources, had difference in FI and FCR, but had no significant differences in weight gain ($P>0.05$). Feed intake in T3 was lower than C, consequently, FCR of chicks fed T3 was better than C group. Although chicks in T3 and T4 groups received diets containing soybean oil, chicks in T4 received 3% higher energy than T3. Chicks in T3 also had lower FI than, same FCR and weight gain as group T4. The highest BW was for T4 and the lowest for T2 group. No difference on BW was observed between C and T3 groups consuming the same energy level with different energy source ($P>0.05$).

Effect on Small Intestine Morphology

Dietary treatments significantly influenced jejunal villus height, crypt depth and villus height: crypt depth ratio *(Table 4)*, but had no effect ($P>0.05$) on villus width and goblet cell count. The smallest villus height, deeper crypt and lowest their ratio was related to chicks in T2 group, and in contrast respectively the largest, shallowest and highest of these parameters was related to T4 group. Chickens received diets with lower energy level had poorer jejunal morphological parameters than those fed with diet in sufficient or over energy level. There were no differences for villus height and crypt depth between T3 and T4, but their ratio was higher ($P<0.05$) in T4 than T3. Chicks in C and T3 group received the same amount of energy with different sources, had difference in villus height and villus height: crypt depth ratio, but difference for crypt depth was not significant ($P>0.05$).

Effect on Microbial Population

Effect of energy sources and levels on microbial population of broiler chicks is shown in *Table 5*. There

were no significant differences among C, T1 and T2 for *Lactobacillus* population (P>0.05), whereas addition of soybean oil to diet in T3 and T4 groups increased (P<0.05) *Lactobacillus* population. The highest population of *Escherichia coli was* seen in C group and the lowest in chickens received soybean oil. Chickens in T2 group that received the lowest energy level had less *Escherichia coli* population than T1 and C group (P<0.05). Chicks in T3 group that received soybean oil in comparison with C group that received the same amount of energy main from corn grain had higher (P<0.05) *Lactobacillus* population and lower *Escherichia coli* population.

Effect on SGLT1 and I-FABP Gene Expression

Effect of dietary treatment on jejunal gene expression of SGLT1 and FABP in broiler chickens is shown in *Table 6*. There were no significant differences (P>0.05) among treatments for gene expression of both nutrient transporters.

Table 3. *Effect of energy sources and levels on performance parameters of broiler chickens at total period [1]*

Treatments	Daily Weight Gain (g/bird)	Daily Feed Intake (g/bird)	Feed Conversion Ratio	BW (g)
C	51.42^{b}	93.07^{c}	1.81^{c}	2160^{b}
T1	49.83^{bc}	96.67^{b}	1.94^{b}	2093^{c}
T2	48.33^{c}	99.55^{a}	2.06^{a}	2030^{d}
T3	52.09^{ab}	89.59^{d}	1.72^{d}	2188^{b}
T4	53.80^{a}	93.61^{c}	1.74^{d}	2260^{a}
SEM	0.490	0.579	0.029	18.690
P-value	0.002	0.004	0.001	0.001

^{a,b,c} Means within a column with different superscripts are significantly different (P<0.05); [1] Data are means of 20 pens of 30 broilers each

Table 4. *Effect of energy sources and levels on jejunal morphological parameters of broiler chickens at d 28 of age [1]*

Treatments	Villus Height[2] (mm)	Villus Width[2] (mm)	Crypt Depth[2] (mm)	Height:Crypt Depth[1]	Goblet Cell (count/mm)[2]
C	1.01^{b}	0.74	0.40^{ab}	2.52^{c}	140
T1	0.90^{bc}	0.75	0.43^{ab}	2.09^{d}	138
T2	0.70^{c}	0.75	0.50^{a}	1.4^{e}	137
T3	1.13^{a}	0.74	0.38^{b}	2.97^{b}	147
T4	1.19^{a}	0.72	0.36^{b}	3.30^{a}	146
SEM	0.23	0.651	0.09	0.08	0.606
P-value	0.001	0.07	0.001	0.04	0.08

^{a,b,c} Means within a column with different superscripts are significantly different (P<0.05); [1] Data are means of 100 birds (5 birds from each pen); [2] Data were obtained from transmission electron microscopy

Table 5. *Effect of energy sources and levels on viable counts of Lactobacilli and Escherichia coli (log_{10}CFU/g of digesta) in cecal digesta of broiler chickens at d 28 of age [1]*

Microflora	Experimental Treatments					SEM	P-value
	C	T1	T2	T3	T4		
Lactobacillus	3.68^{c}	3.72^{c}	3.50^{c}	4.67^{b}	5.83^{a}	0.539	0.008
Escherichia coli	5.00^{a}	4.82^{a}	2.20^{b}	1.30^{c}	1.10^{c}	0.500	0.008

^{a,b,c} Means within a row with different superscripts are significantly different (P<0.05); [1] Data are means of 100 birds (5 birds from each pen)

Table 6. *Effects of energy sources and levels on SGLT1 and FABP gene expression in jejunal of broiler chickens at 28 d of age*

Relative Gene Expression[1]	Experimental Treatments					SEM	P-value
	C	T1	T2	T3	T4		
SGLT1	1.000	1.074	1.264	1.255	1.187	0.22	0.06
FABP	1.000	1.112	1.127	1.181	1.413	0.33	0.06

Means within a row without superscripts are not different (P>0.05); [1] Relative gene expression ($2^{-\Delta\Delta C_T}$) ±SEM was calculated using the $\Delta\Delta^{CT}$ method

DISCUSSION

Chicken health and performance are greatly influenced by diet quality and nutrient availability [29]. These traits are more dependent on the intestine health to assimilate nutrients efficiently as the bird shifts from the lipid-rich yolk as the main source of energy to a carbohydrate-based diet [30]. To improve the health and performance, addition of lipids to diet in the early life of chicks and later was recommended by many researchers [31-33]. In stress condition, plasma corticosterone level tends to increase and this hormone has negative effect on intestinal morphology and consequently performance [34]. A recent study revealed that birds exposed to chronic heat stress had poor growth performance and increased corticosterone concentrations [6]. Deteriorated performance resulted from heat stress in broilers can be attributed to a greater expenditure of energy for physiological adaptation to the stressful situation instead of growth enhancement [35]. Alternatively, it is believed that less weight gain in the heat stress groups is due to a smaller appetite and lower feed intake, as it maybe seemingly a defense mechanism to help reduce heat production.

The main objective of this study was to evaluate the effect of energy sources and levels on microbial population, SGLT1 and FABP gene expression and small intestinal parameters in broiler chickens under heat stress. In the literature, study about this subject under heat stress was not found; hence comparison of our findings with others was done on non-stressed condition.

The results of this study indicate that the source and level of metabolizable energy in broilers under heat stress had a statistically significant influence on intestinal morphology and microbiology and consequently performance ($P<0.05$), but it had no effect on gene expression of nutrient transporters of glucose and fatty acid binding protein. Maintenance of normal microarchitecture of the small intestine is very important for proper growth and development. Quite a few studies have reported that stress hampered the development of intestinal morphology and function [14]. Sohail et al.[6] observed that heat stress decreased villus height and width, crypt depth, and villus surface area. Stressors such as fasting and nutrient deficiency or corticosterone injections have noxious effects on the intestinal microarchitecture, resulting in reduction in the absorptive surface area [36,37]. Feeding ration with lower energy level to chickens resulted in short villus height and poor performance. The villi play a crucial role in the digestion and absorption processes of the small intestine, as is the first to make contact with nutrients in the lumen [38]. A shortening of the villus height may lead to poor nutrient absorption along with lower performance [26].

In contrast, substitution of a part of corn energy with soybean oil in the present study improved the intestinal parameters. The positive effect of lipids on intestinal health status and growth performance of broilers is well documented [39]. The extra caloric effect of added soybean oil resulted in an improved body weight gain [40]. Inclusion of soybean oil and delivery of higher energy than Cobb standard (T4) resulted in the highest villus height, the shallowest crypt depth, lowest *E. coli* and highest lactic acid bacteria population and finally higher body weight and the highest feed efficiency. It was reported that dietary soybean oil supplementation significantly increased villus height of the jejunum [41].

In this experiment, addition of soybean oil in diet T3 and T4 enhanced the growth of *Lactobacillus*, but inhibited that of *E. coli* in the small intestine. Freitas et al.[42] reported that addition of soybean oil could maximize the growth of the bacterial population. Intestinal microflora plays an important role in digestive health. Microbial population is dependent on food rations as the ultimate source of the organic substrate metabolism [43]. It is known that dietary fats cause changes in the intestinal microflora composition with a direct effect on digestion and absorption of nutrients by the birds. Innis et al.[44] reported that canola oil supplementation increased lactic acid bacteria population in the gut. The effect of fat on the microbial flora of the gastrointestinal tract may be due to its effect on digestion, viscosity, pH level and the transport of nutrients in the gastrointestinal tract [45].

No differences were found among treatments for gene expression of SGLT1 and FABP in this study. It was demonstrated that regulation of nutrient transporters by dietary substrate appears to occur by increasing mRNA stability or by increasing gene transcription rate [46]. It seems that metabolizable substrates are not necessary for its regulation. Sun et al.[47] reported that the expression levels of FABP were significantly decreased by heat exposure, but heat stress had no significant effect on gene expression of SGLT1. In Shepherd et al.[48] mentioned study has suggested that environmental stress decreases GLUT-2 expression at the brush border membrane level but does not alter SGLT-1 expression. Moreover, corticosterone-induced stress does not alter the expression of SGLT-1 in the jejunum of broiler chickens [49].

Moreover, an increased body temperature during heat stress conditions can lead to increased maintenance energy requirements [50] to keep body temperature around a normal level. Different levels of energy significantly affected body weight, weight gain, feed intake and feed conversion ratio [51]. The final live weight was significantly highest in broiler chickens fed dietary treatment with normal energy and was lowest in those fed dietary treatment with low energy [52]. Increased live weight was mostly due to higher metabolizable energy consumption in the same unit of diets by chickens, similarly supplementation of oil caused a positive trend in cumulative live weight gain (g/bird) of broilers at different ages [53]. Fats and vegetable oils

has been frequently included in broiler diets to increase the energy density of the diet, to improve efficiency and to increase nutrient digestibility in broilers [54]. Lipid supplementation increases the energetic efficiency in two ways. It increases density of energy and it has a lower heat increment or greater net energy. Increased energy and nutrient density of the diet and replacing carbohydrate calories reduced feed intake almost proportionally but increased live weight gains in hot weather. Nitsan et al.[40] stated that feed conversion ratio were significantly improved with addition of 3% soybean oil in the diet.

The present study has demonstrated that the levels and sources of dietary energy have significant effects on intestinal populations of *Lactobacillus* and *E. coli* in broiler chickens. It can be concluded that providing high levels of dietary energy with oil in broiler nutrition, increases beneficial intestinal flora and reduction of noxious microbes and simultaneously cause an increase intestinal villus height and decrease crypt depth and intestinal absorption that finally can ensure the intestinal health of broilers. The results showed that the higher energy level than nutritional needs based on Cobb broiler chickens requirements as specified in the manual was affective on microbial population and morphological parameters that can cause increasing weight gain and final body weight under heat stress.

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