Impact of dietary energy density on the liver health of broilers exposed to heat stress and their performance during finisher period

M. Raghebian, A. A. Sadeghi* and M. Aminafshar

Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
*Corresponding author email: a.sadeghi@srbiau.ac.ir; aasdghi@gmail.com; Tel +989195579663

Journal of Livestock Science (ISSN online 2277-6214) 8: 122-130
Received on 27/1/2017; Accepted on 7/4/2017

Abstract

This study was aimed to assess the impact of dietary energy density on liver health parameters, carcass characteristics and performance of broilers exposed to heat stress. 600 Cobb male chicks 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 (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-hour daily from day 12 to 42 of age to induce heat stress. The highest level of ALP was for T3 and T4 and the lowest one was for T2. The highest levels of AST and ALP were for T4 and the lowest level was for T2. The lowest HSP70 gene expression was found in chicks fed T2 diet. The highest level of creatine kinase was for T4 at day 28 of age and for T3 and T4 at day 42 of age. The lowest level of creatine kinase was for T1 and T2 groups. The highest weight gain during finisher period found in T3 group andthe highest feed intake was for T2 group. The lowest feed conversion ratio was for T3 and T4 and the highest ones for C, T1 and T2 groups.Higher energy density provided with soybean oil inclusion could induce liver damages. Lower energy density in diet of broiler chickens under heat stress also resulted in liver damages and poor performance. It was concluded that soybean oil inclusion in diet of broiler chickens under heat stress caused better performance, when energy density of diet was satisfied based on strain recommendation.

Keywords: Heat stress; Finisher period; Energy density; Broiler

Introduction

High ambient temperature is one of the main stressors in broiler chickens, especially in tropical and subtropical areas coupled with high humidity. Following exposure to high ambient temperature, some possible toxic mechanisms maybe induced, of which the generation of high levels of reactive oxygen species. Hence, heat stress is known to impose severe oxidative stress on broiler chickens. The long summer months with humidity in tropical and subtropical regions adversely affect the survival rate (Geraert et al., 1996; Mashaly et al., 2004) and productivity of broiler chicken (Imik et al., 2012; Ghazi at al., 2012). Liver tissue is damage higher than other tissues due to heat stress(Lin et al., 2006), hence application of protective methods for liver health is appreciated, as Liver plays an important and unique role in energy homeostasis in the animal body.During the past two decades, various management techniques and dietary modifications have been examined to enhance heat tolerance or reduce the negative effects of heat stress in broiler chickens. There are evidences that a higher dietary fat content contributes to improved heat tolerance in broiler chickens (Daghir, 2008; Zulkifli et al., 2003) reported that providing diets containing high levels of palm oil enhanced growth performance and survivability of heat stressed broiler chickens. Information regarding the effect of energy density and source on liver health of broilers under heat stress are scarce. It was hypothesized that in the heat stress condition, lipid addition with higher energy level to diet could enhance liver health, HSP70 gene expression and performance compared to diet containing main energy source from carbohydrate. Therefore, this study was carried out to evaluate the effect of energy sources and levels on liver health parameters, HSP70 gene expression, performance, carcass parameters of broiler chickens under heat stress.

Materials and methods

Bird management and experimental design

This study was conducted in the poultry unit of AEOI located at Karaj (N, 50° 58′; E, 35° 49′) in summer of 2016. A total of 600 one-day-old Cobb male chicks with average weight of 39 g was purchased from a local hatchery. Chicks were randomly allocated to 20 floor pens covered with pine shaving.In a completely randomized design, chicks were assigned to five dietary treatments, four replicates with 30 chicks per each. Dietary treatments were: control group (C) which broilers fed diet with main energy from corn and energy level based on Cobb standard; T1: broilers fed diet with main energy from corn and 3% lesser energy than Cobb standard; T2: broilers fed diet with main energy from corn and 6% lesser energy than Cobb standard; T3: broilers fed diet with main energy from corn and soy oil and energy level based on Cobb standard and T4: broilers fed diet with main energy from corn and soy oil and 3% upper energy than Cobb standard. Diets were formulated to fortify the requirements of broilers based on the recommendations of strain as presented in Table 1. Diet were fed in there phases as starter (days 1 to 10 of age), grower (days 11 to 28 of age), and finisher (days 29 to 42 of age). Chicks were kept under controlled house based on Cobb broiler guides, except temperature. To induce heat stress, from day 12 to 42 of age, 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 declined to temperature controlled conditions based on Cobb 500 broiler guides. Feed and water were provided for ad libitum intake and throughout the heat challenge period. Filler (washed sand) was used to balance for dietary density of metabolizable energy. Weight of chicks was measured at days 28 and 42 of age. Average feed intake was measured the amount of feed eaten during days 28 to 42 of age. Feed conversion ratio for finisher period was calculated by dividing feed intake on weight gain. Dead chickens were collected and weighed for the correction of feed conversion ratio calculations.

Sample collection and analyses

On days 28 and 42 of age, the blood samples of four chicks per treatment were randomly collected from brachial vein in the vacuum tubes containing Heparin-gel. Plasma was separate using centrifuge (2000×*g* for 10 min) and stored at -20 °C for further analysis.

The concentration of plasma ALT was determined by using Alanine Aminotransferase Activity Assay Kit (Catalog Number MAK052. Sigma-Aldrich Co.). Plasma AST level was measured by using Aspartate Aminotransferase Activity Assay Kit (Catalog Number MAK055. Sigma-Aldrich Co.). Plasma ALT concentration was determined by using Alkaline Phosphatase Activity Colorimetric Assay Kit(Catalog# K412-500. BioVision, Inc).The activity of creatine kinase (CK) was assessed by using a commercial kit (Biotrol CK monoreactif, Alpha Laboratories, Hants, UK SO5 4NU).

On days 28 and 42 of age, immediately after blood sampling, four chicks per each treatment were sacrificed by cervical dislocation, then Breast, legs and abdominal fat were removed and weighed.

Results of carcass efficiency were expressed as percent per the live weight.

Table 1: Composition of the experimental diets for broiler chicks

	Starter					Grower					Finisher				
Ingredients (%)	C	T_1	T_2	T_3	T_4	C	T_1	T_2	T_3	T_4	C	T_1	T_2	T_3	T_4
Corn	63.35	63.25	62.13	58.17	54.17	69.22	68.80	67.70	65.31	59.31	70.18	71.47	71.50	65.10	62.40
Soybean meal	22.57	28.86	31.48	31.90	32.21	18.00	23.43	26.20	24.00	28.45	19.30	20.00	24.20	25.80	25.00
Soybean oil	-----	-----	-----	2.50	4.14	-----	-----	-----	2.00	5.00	-----	-----	-----	3.50	5.00
Corn gluten meal	9.10	3.00	-----	2.70	4.70	8.20	3.24	-----	4.20	2.90	6.23	4.20	-----	1.50	3.50
Di calcium phosphate	2.07	2.06	2.06	2.05	2.05	1.90	1.90	1.90	1.90	1.90	1.70	1.70	1.70	1.70	1.70
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	0.34	0.41	0.45	0.40	0.39	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]	-----	-----	1.51	-----	-----	-----	-----	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 (%)	0.67	0.67	0.67	0.67	0.67	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	0.78	0.78	0.78	0.78	0.78	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	0.19	0.19	0.19	0.19	0.19	0.16	0.16	0.16	0.16	0.16

1. per kg contains: Ca, 23% & P, 18.5%. **2.** Supplied by Razak Co., Tehran, Iran, and provided per kilogram of diet: vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg, vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, 6 mg; Choline chloride, 200000 mg, Manganese, 40000 mg, Iron, 20000 mg; Zinc, 40000 mg, copper, 4000mg; Iodine, 400 mg. **3.** Inert filler used to complete diet formulations to 100%.

Table 2: Effect of dietary energy density on creatine kinase and weights of breast, legs and abdominal fat on days 28 and 42 of age (Mean ± standard error)

Item	C	T_1	T_2	T_3	T_4	P value
Day 28						
Creatine kinase (U/l)	259±7.93^{b}	169±7.76^{c}	186±6.55^{c}	291±14.79^{b}	346±14.57^{a}	0.0001
Breast (%)	12.96±1.120^{a}	16.98±1.195^{a}	15.39±2.495^{a}	17.92±3.030^{a}	16.77±1.085^{a}	0.5000
Legs (%)	21.75±0.620^{b}	24.03±0.175^{ab}	24.46±0.800^{a}	23.07±0.735^{ab}	24.26±0.735^{a}	0.0133
Abdominal fat (%)	2.06±0.141^{a}	1.68±0.273^{a}	2.60±0.467^{a}	2.23±0.226^{a}	1.92±0.207^{a}	0.2836
Day 42						
Creatine kinase (U/l)	238±8.73^{b}	211±9.29^{b}	209±7.57^{b}	319±16.92^{a}	326±11.34^{a}	0.0001
Breast (%)	14.77±1.135^{c}	17.08±0.845^{bc}	19.91±0.350^{ab}	20.16±0.705^{ab}	21.21±1.41^{a}	0.0238
Legs (%)	23.54±1.460^{b}	24.43±0.570^{ab}	28.09±0.345^{a}	26.83±0.130^{ab}	26.38±2.125^{ab}	0.0181
Abdominal fat (%)	1.99±0.291^{a}	1.88±0.154^{a}	2.09±0.205^{a}	2.16±0.075^{a}	1.89±0.288^{a}	0.8668

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

Table 3: Effect of dietary energy density on the plasmalevels of liver enzymes (Mean ± standard error)

Item	C	T_1	T_2	T_3	T_4	P value
Day 28						
AST (mg/dl)	213±13.5	231±75.5	200±15.5	207±26.5	226±56.0	0.9842
ALT (mg/dl)	4.0±0.0	5.0±2.0	2.5±0.5	1.5±0.5	2.5±1.5	0.3478
ALP (IU/l)	34.1±2.1^{b}	31.1±1.8^{bc}	27.7±1.0^{c}	44.1±2.4^{a}	46.9±1.7^{a}	0.0001
Day 42						
AST (mg/dl)	234±2.5^{b}	212±4.5^{b}	144±25.0^{c}	256±11.5^{ab}	301±23.0^{a}	0.0077
ALT (mg/dl)	4.5±2.5	5.5±3.5	3.0±0.0	3.0±1.0	3.5±1.5	0.8856
ALP (IU/l)	38.5±1.4^{b}	29.5±1.2^{c}	24.3±1.1^{c}	40.7±1.8^{b}	63.9±3.0^{a}	0.0001

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

At day 42 of age, 4 liver samples from each treatment were taken for gene expression of HSP70. These tissues were snap-frozen in liquid nitrogen and stored at -80 °C until RT-PCR analysis described by Aminoroaya et al. (2016). The frozen liver was crushed in a sterile mortar, and the powder was applied for total RNA extraction using a suitable kit (Bioneer Co., Seoul, South Korea). cDNA for each transporter gene was synthesized based on reverse transcription technique using kit (Bioneer Co., Seoul, South Korea). Quantitative PCR was performed with a specific primer pairs (145 bp; forward: 5'-CGTCAGTGCTGTGGACAAGAGTA-3'; reverse: 5'-CCTATCTCTGTTGGCTTCATCCT-3') using Quanti Fast SYBER Green PCR kit (QIAGEN, Cat. No.204052). GAPDH (128 bp; forward: 5'-CTTTGGCATTGTGGAGGGTC-3'; reverse: 5'-ACGCTGGGATGATGTTCTGG-3') was chosen as a reference gene. Amplification of liver HSP70 gene was performed for 45 cycles, which consisted of an initial activations step (95°C, 5 min), denaturation cycle (95°C, 10s) and combined annealing and extension (60°C, 30s). The relative expression ratio of HSP70 as a target gene was normalized to GAPDH gene using method as previously described by Livak and Schmittgen (2001). Quantification for each treatment group was performed in four replicates.

Liver samples (four samples from each treatment) were also collected for histology and fixed in 10% formalin solution. The liver sections were fixed and stabilized in paraffin and slices (six slices per section that stabilized on a microscope slide) with 6 µm thickness were prepared using microtome. Hematoxylin and eosin stain was used for staining of slices and then mounted by entellan. Slides were assessed under optic microscope by histologist. The changes in liver tissue and pathology observed were classified as not detected (ND), mild (+), moderate (++) and severe (+++).

Statistical analysis

Statistical analyses were carried out using the General Linear Model procedure of the Statistical Analysis System software (SAS) for Windows version 9.1 (SAS Institute Inc., Cary, NC) appropriate for completely randomized design. To evaluate the normal distribution of data, Kolmogorov-Smirnov test was done. Duncanmultiple range tests was used to compare the means. Effects between the control and experimental groups were considered significant when $P<0.05$ and finally results were presented as means with standard error (Mean±SE).

Results

The effects of treatments on the plasma creatine kinase concentration and relative weights of thighs, breast and abdominal fat at days 28 and 42 of age are presented in Table 2. The highest level of creatine kinase was for T4 at day 28 of age and for T3 and T4 at day 42 of age. The lowest level of creatine kinase was for T1 and T2 groups. The relative weight of breast was not significant at day 28 of age, but differences appeared at day 42 of age. The highest relative weight of breast was for T4 and the lowest one was for T2. Significant difference exists between T2 and other treatments for relative weight of legs. The lowest relative weight of legs was for T2 and no significant difference found among the control group and T1, T3 and T4. There was no difference among treatments for relative weight of abdominal fat.

The effects of treatments on the plasma levels of AST, ALT and ALP are presented in Table 3. There was no difference among treatments for AST and ALT at day 28 of age, but differences exist for ALP. The highest level of ALP was for T3 and T4 and the lowest one was for T2.There were significant differences for AST and ALP at days 42 of age, but no significant difference was found for ALT level. The highest levels of AST and ALP were for T4 and the lowest level was for T2.

As shown in in Table 4, the chickens fed T1 and T2 diets revealed microvesicular steatosis, macrovesicular steatosis, venous congestion and sinusoid dilation at day 28 of age, as well as, focal necrosis and perivascular cell at day 42 of age. Intense microvesicular steatosis was detected in broilers fed T4 at days 28 and 42 of age. Chickens in T3 had the lowest pathological signs in the liver compared with other treatments. Images of dietary treatments effect on hepatic histopathology of broiler chickens are shown in Figure 1.

Effect of energy density on HSP70 gene expression in broiler chickens at day 42 of age is presented in Figure 2. There was a significant difference between T2 and other treatments for HSP70 gene expression. The lowest gene expression was found in chicks fed T2 diet.

The effect of energy density and source on body weight gain, feed intake and feed conversion ratio during finisher period is presented in Table 5. There was significant difference among treatments for weight gain during finisher period. The highest weight gain found in T3 and T4 groups. Significant difference found among treatments for feed intake. The highest feed intake was for T2 group. There was

difference among treatments for feed conversion ratio. The lowest feed conversion ratio was for T3 and T4 and the highest ones for C, T1 and T2 groups.

Table 4. Hepatic histopathology of broiler chickens fed diets containing different energy density and source.

Item*	A	B	C	D	E	F	G	H	I	J	K
Day 28											
C	+	++	++	++	++	++	ND	ND	ND	ND	ND
T1	+++	+++	+	+	++	+	+	+++	+++	ND	ND
T2	+++	+++	+	++	+	ND	+	+++	+++	ND	ND
T3	+	++	+	ND	+	+	+	ND	ND	ND	ND
T4	+++	++	++	+	+	+	++	ND	ND	ND	ND
Day 42											
C	++	++	+++	+	++	+	++	+	ND	ND	ND
T1	++	+++	+++	++	++	++	++	ND	ND	+	++
T2	+++	+++	+++	++	++	++	++	ND	ND	+	++
T3	+	+	+	+	ND	+	++	ND	ND	ND	+
T4	+++	++	++	++	+	+	++	ND	ND	+	++

* A: Microvesicular steatosis– Steatosis; B: venous congestion in liver; C: perivascular cell; D: Dilation of the portal vein; E: bile duct proliferation; F: connective tissue in artery wall; G: inflammatory foci; H: Sinusoidal dilatation and congestion; I: Macrovesicular steatosis; J: liver haemorrhage; K: focal necrosis

Table 5: Effects of dietary energy density on performance of broiler chickens in the finisher period (Mean ± standard error)

Item	C	T_1	T_2	T_3	T_4	P value
Weight gain (g)	997.6± 16.41^{c}	984.6± 33.62^{c}	1058.1± 66.90^{bc}	1195.9± 22.27^{a}	1123.9± 18.91^{ab}	0.0003
Feed intake (g)	1891.5± 22.73^{b}	1905.8± 12.88^{b}	2055.2± 37.49^{a}	2036.0± 19.92^{ab}	1934.6± 17.74^{b}	0.0005
Feed conversion ratio	1.89± 0.011^{a}	1.94± 0.060^{a}	1.94± 0.052^{a}	1.70± 0.008^{b}	1.72± 0.005^{b}	0.0005

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

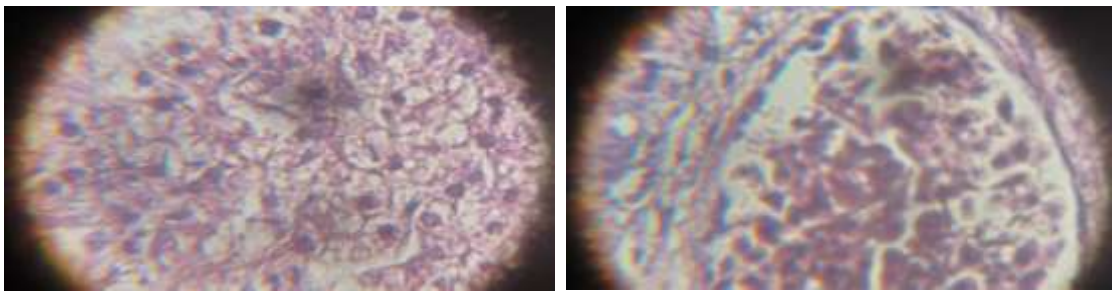

Figure 1: Effect of dietary treatments on the hepatic histopathology (left: control group and right T4 group)

Discussion

The nutrient density and availability of diet influence on the performance parameters and health of broilers (Geyra et al., 2001). Liver health and function is influence by nutrient quantity and quality, especially energy restriction or surplus. Any change in energy density cause a stressful condition in the body of chicks and may be increase generation of free radicals. Replacement of soybean oil with starch recommended by many researchers (Yaqoob, 2004; Cherian, 2015) to reduce the negative effects of heat stress on animal body. Soybean meal contains poly unsaturated fatty acids that have beneficial impact on the health and production. To decrease the heat increment, it was recommended to shift from starch to lipid in heat stress condition (Sadeghi et al., 2013). The effect of change in energy density and source on liver and muscle health and also performance of chickens is not clear. This work aimed to assess the effect of energy density and source on liver health in broiler chickens under heat stress.

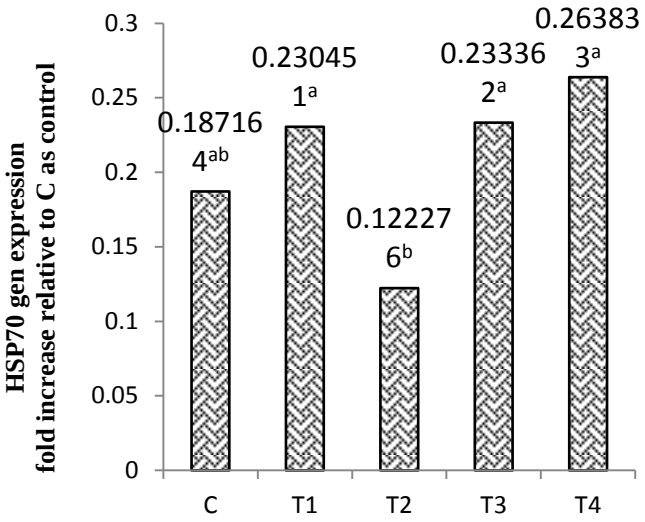

Figure 2: Effect of dietary treatments on hepatic HSP70 gene expression on day 42 of age

In this study the plasma level of creatine kinase was higher in chickens received diet containing soybean oil. Increasing plasma level of creatine kinase, reflecting muscle damage (Mitchell and Sandercock, 1997). The changes in muscle membrane permeability, changes in muscle metabolism and loss of membrane integrity may be occur due to stress (Sandercock et al., 2001). Kubíková et al. (2001) found elevation of creatinine level in restricted broiler breeders. There is some evidence that creatinine excretion in birds increases in response to reduced feed intake (Work et al., 1999). The finding of this study is consistent with the study of Hada et al. (2013) who showed that feeding the low-lipid diet caused higher levels of this metabolites compared with the control and the low-carbohydrate diets. In contrast to our finding, Malheiros et al. (2004) who fed iso-energetic diets and found no differences in the levels of creatine kinase between broilers fed low carbohydrate-high lipid or low-lipid-high carbohydrate levels.

In the normal status, AST, ALT and ALP are found mostly inside the liver cells, but if the liver is inflamed or injured, liver cells corrupted and cytoplasm content especially these enzymes are released into the blood stream. Hence, the levels of ALT, AST and ALP were considered as useful biomarkers for detection of liver damages (Racicot et al., 1975). In this study, higher energy density and especially replacement of starch with soybean oil cause liver and muscle damages. As seen in Table 3, the plasma levels of AST and ALP increased in T3 and T4 groups. This impact of soybean oil may be related to n-6 fatty acids that induce inflammation processes. Inflammation could increase the tissue sensitivity to damages and elevation of hepatic enzymes in the plasma (Zhang et al., 2005). Chickens in T1 and T2 received lower dietary energy density and had the lowest levels of AST and ALP. The level of ALT was not influenced by dietary treatments that is in line with finding of Rajman et al. (2006) who observed no difference in ALT levels in the blood of chickens due to feed restriction. These authors also found no difference for the level of AST. In contrast, Jang et al. (2009) found a higher AST content in the plasma of feed restricted broilers. It seems that lower energy density in heat stress condition decrease the metabolic loads and generation of free radicals, hence the damage to liver cells decreased.

The liver of the chickens in T1, T2 and T4 groups showed severe macrovesicular and microvesicular steatosis. Macrovesicular steatosis is a form of fatty degeneration in the liver and caused by oversupply of energy especially from lipids or by the entrance of lipids to liver with low output. Microvesicular steatosis is small fat vacuoles accumulated in the cytoplasm of liver cells, which caused by fatty liver. This result was in line with finding of McLean and Dutton (1995), who reported that steatosis or fatty degeneration caused by impair in lipid transport from the liver rather than an increase in lipid biosynthesis.Venous congestion observed in the liver of chickens in T1 and T2 groups. When hepatic arterial inflow is higher than venous outflow due to inflammation or obstruction, venous congestion occurs in the liver. The cause of most pathologies in the liver of fast growing broiler chickens is a prolonged state of hypoxia (Olkowski et al., 2005). Under this condition especially limiting energy and heat stress, the liver of chickens may respond with regressive lesions such as fatty degeneration, inflammation and venous congestion. Chickens in T1 and T2 had pale livers with inflammation in 9 out of 16 (56%) lower energy density in diet, hence the load on the liver may be high. The hepatic sinusoidal

dilatation and congestion is associated with venous outflow impairment or inflammation. In the present study, hepatic lesions and pathological signs in the liver of chickens at day 28 of age were much more than day 42 of age, that may be related to hypoxia.

Increases in mRNA stability and gene transcription rate are two mechanisms of dietary substrate for gene expression regulation (Adibi, 2003). The impact of energy density on HSP70 gene expression is poorly studied. In contrast to our finding, Heydari and Richardson (1995) found an increase in the level of HSP70 gene expression in rats received food restriction. In chickens received T2 a decrease and in T4, an increase in the HSP70 gene expression was seen. Enhancement in the expression of HSP70 protect cells from damages as it help them to recover after and during stressors (Li et al., 1995). When organisms are exposed to heat stress, the synthesis of many proteins retarded, but the synthesis of heat shock proteins rapidly increased. In this condition, the heat shock proteins bind to important proteins for body that are heat sensitive. Heat shock proteins protect these important sensitive proteins from degradation (Etches et al., 2008). Chickens in T1 and T2 had a lowest HSP70 gene expression. In contrast, Lee et al. (2016) reported that feed restriction increased HSP70 levels in the gill tissue of green sturgeon. These authors concluded that green sturgeon may be more susceptible to temperature stress under food-limited conditions. Also, Zulkifli et al. (2003) reported that feed restriction increased HSP70 expression in the brain. We could not find study concerning impact of energy density on the HSP70 gene expression.

In the present study, performance of chicks fed diets containing soybean oil in the finisher period was higher than other treatments. Soybean oil had an extra caloric effect than starch (Nitsan et al., 1997) and this replacement reflected in higher body weight gain than other treatments. Lipids could increase the energy efficiency via increases in density of energy, lower heat increment and consequently higher net energy. Our results is in agreement with the finding of Nitsan et al. (1997) who demonstrated that feed conversion ratio was improved with dietary supplementation of 3% soybean oil. Feeding ration with lower energy exposed chicks to higher heat production and consequently thermo-sensitive. In addition, as seen in Table 1, chicks in T1 and T2 consume higher feed to compensate the energy dilution of diet. Higher feed intake results in higher activity of chicks for eating and digestive tract activity and heat production. Heat loss in these chicks resulted in lower feed conversion ratio compared to C, T3 and T4 group.

Based on the results of this study, higher energy density provided with soybean oil inclusion could induce liver damages. Lower energy density in diet of broiler chickens under heat stress also resulted in liver damages and poor performance. As was seen in the results of T3 group, soybean oil inclusion in diet of broiler chickens under heat stress caused better performance, when energy density of diet was satisfied based on strain recommendation.

Acknowledgments

The authors are grateful to the Islamic Azad University for research funding support. We also thank all staffs in the poultry unit, for the assistance in the care and feeding of chicks used in this research.

REFERENCES

1) Adibi, S.A. 2003. Regulation of expression of the intestinal oligopeptide transporter (Pept-1) in health and disease. American Journal of Physiology-Gastrointestinal and Liver Physiology, 285: 779-788.
2) Aminoroaya, K., Sadeghi, A.A., Ansari-pirsaraei, Z. and Kashan, N. 2016. Effect of cyclical cold stress during embryonic development on aspects of physiological responses and HSP70 gene expression of chicks. Journal of Thermal Biology 61: 50-54.
3) Cherian, G., 2015. Nutrition and metabolism in poultry: role of lipids in early diet. Journal of Animal Science and Biotechnology 6: 28-33.
4) Daghir, N.J. 2008. Broiler feeding and management in hot climates. Poultry production in hot climate, 4th edition, CABI international, London, UK. pages 227-260.
5) Etches, R.J., John, T.M. and Gibbins, A.V., 2008. Behavioural, physiological, neuroendocrine and molecular responses to heat stress. Poultry production in hot climates, 4th edition, CABI international, London, UK. pages 31-66.
6) Geraert, P.A., Padilha, J.C.F. and Guillaumin, S. 1996. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. British Journal of Nutrition 75: 195-204.
7) Geyra, A., Uni, Z. and Sklan, D. 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poultry Science 80: 776-782.

8) Ghazi, S.H., Habibian, M., Moeini, M.M. and Abdolmohammadi, A.R. 2012. Effects of different levels of organic and inorganic chromium on growth performance and immunocompetence of broilers under heat stress. Biological Trace Element Research 146: 309-317.
9) Hada, F.H., Malheiros, R.D., Silva, J.D.T., Marques, R.H., Gravena, R.A., Silva, V.K. and Moraes, V.M.B. 2013. Effect of protein, carbohydrate, lipid, and selenium levels on the performance, carcass yield, and blood changes in broilers. Revista Brasileira de Ciência Avícola 15: 385-394.
10) Heydari, A.R., Conrad, C.C. and Richardson, A., 1995. Expression of heat shock genes in hepatocytes is affected by age and food restriction in rats. The Journal of Nutrition 125 410-418.
11) Imik, H., Atasever, M.A., Urcar, S., Ozlu, H., Gumus, R. and Atasever, M. 2012. Meat quality of heat stress exposed broilers and effect of protein and vitamin E. British Poultry Science 53: 689-698.
12) Jang, I.S., Kang, S.Y., Ko, Y.H., Moon, Y.S. and Sohn, S.H. 2009. Effect of qualitative and quantitative feed restriction on growth performance and immune function in broiler chickens. Asian-Australasian Journal of Animal Science 22: 388-395.
13) Kubíková, Ľ., Výboh, P. and Košťál, Ľ. 2001. Behavioural, Endocrine, and Metabolic Effects of Food Restriction in Broiler Breeder Hens. Acta Veterinaria Brno 70*:* 247-257.
14) Lee, S., Hung, S.S., Fangue, N.A., Haller, L., Verhille, C.E., Zhao, J. and Todgham, A.E. 2016. Effects of feed restriction on the upper temperature tolerance and heat shock response in juvenile green and white sturgeon. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 198: 87-95.
15) Li, G.C., Mivechi, N.F. and Weitzel, G. 1995. Heat shock proteins, thermotolerance, and their relevance to clinical hyperthermia. International Journal of Hyperthermia 11: 459-488.
16) Lin, H., Decuypere, E. and Buyse, J. 2006. Acute heat stress induces oxidative stress in broiler chickens. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 144: 11-17.
17) Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25: 402-408.
18) Malheiros, R.D., Moraes, V.M.B., Collin, A., Janssens, G.P.J., Decuypere, E. and Buyse, J. 2004. Dietary macronutrients and performance and plasma hormone and metabolite levels of broiler chickens fat by carbohydrate substitution. Archiv fur geflugelkunde 68: 87-93.
19) Mashaly, M.M., Hendricks, G.L., Kalama, M.A., Gehad, A.E., Abbas, A.O. and Patterson, P.H. 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poultry Science 83: 889-894.
20) McLean, M. and Dutton, M.F. 1995. Cellular interactions and metabolism of aflatoxin: an update. Pharmacology & therapeutics 65: 163-192.
21) Mitchell, M.A. and Sandercock, D.A., 1997. Possible mechanisms of heat stress induced myopathy in the domestic fowl. Journal of Physiology and Biochemistry 53: 75-79.
22) Nitsan, Z., Dvorin, A., Zoref, Z. and Mokady, S. 1997. Effect of added soyabean oil and dietary energy on metabolisable and net energy of broiler diets. British poultry science 38: 101-106.
23) Olkowski, A.A., Duke, T. and Wojnarowicz, C. 2005. The aetiology of hypoxaemia in chickens selected for rapid growth. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 141: 122-131.
24) Racicot, J.G., Gaudet, M. and Leray, C. 1975. Blood and liver enzymes in rainbow trout (Salmo gairdneri Rich.) with emphasis on their diagnostic use: Study of CCl4 toxicity and a case of Aeromonas infection. Journal of fish Biology 7: 825-835.
25) Rajman, M., Juráni, M., Lamošová, D., Máčajová, M., Sedlačková, M., Košťál, Ľ., Ježová, D. and Výboh, P. 2006. The effects of feed restriction on plasma biochemistry in growing meat type chickens (Gallus gallus). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 145: 363-371.
26) Sadeghi, A.A., Mirmohseni, M., Shawrang, P. and Aminafshar, M. 2013. The effect of soy oil addition to the diet of broiler chicks on the immune response. Turkish Journal of Veterinary and Animal Sciences, 37: 264-270.
27) Sandercock, D.A., Hunter, R.R., Nute, G.R., Mitchell, M.A. and Hocking, P.M. 2001. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. Poultry science 80: 418-425.
28) Work, T.M., Massey, J.G., Johnson, L., Dougill, S. and Banko, P.C., 1999. Survival and physiologic response of Common Amakihi and Japanese White-eyes during simulated translocation. Condor 101: 21-27.

29) Yaqoob, P. 2004. Fatty acids and the immune system: from basic science to clinical applications. Proceedings of the Nutrition Society 63: 89-105.
30) Zhang, A.W., Lee, B.D., Lee, S.K., Lee, K.W., An, G.H., Song, K.B. and Lee, C.H. 2005. Effects of yeast (Saccharomyces cerevisiae) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry science 84: 1015-1021.
31) Zulkifli, I., Liew, P.K., Israf, D.A., Omar, A.R. and Hair-Bejo, M. 2003. Effects of early age feed restriction and heat conditioning on heterophil/lymphocyte ratios, heat shock protein 70 expression and body temperature of heat-stressed broiler chickens. Journal of thermal biology **28**: 217-222.