Effect of energy levels and sources on the blood attributes and immune response in broiler chickens exposed to heat stress

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Abstract

The purpose of study was to evaluate the impact of energy sources and levels on blood attributes and immune response in broiler chickens exposed to heat stress. Six hundreds 1-day-old Cobb chickens were assigned to five dietary treatments and four replicates at a completely randomized design. 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 the control (T1), corn based diet with 6% lesser energy than the control (T2), corn and soybean oil based diet according to Cobb standard (T3), corn and soybean oil based diet with 3% upper energy than the control (T4). Temperature was increased to 34 °C for 8-hour daily from day 12 to 42 of age to induce heat stress. Chicks in T3 and T4 had higher corticosterone levels than control group. Total blood cells in the chicks fed T3 and T4 was higher ($P<0.05$) as compared with the other treatments. Red blood cells count of chicks fed C, T3 and T4 diets was higher than T1 and T2. The average concentration of hemoglobin in red blood cells that the hemoglobin content and the average amount of hemoglobin inside a single red blood cell showed non-significant differences and the average volume of red cells in a specimen showed significant differences for those received higher energy level and especially in T4 that received soybean oil. There were no significant differences among treatments for WBC and lymphocyte counts, but significant differences exist among treatments for neutrophil, monocyte and platelet. There were significant differences among treatments for antibody titers against Newcastle disease virus (NDV) and Infectious Bursal Disease virus (IBD). Antibody titers of NDV and IBD decreased in chicks received soybean oil in ration. It was concluded that energy level of diet had higher impact on measured parameters than energy source. Energy level recommended by Cobb strain containing soybean oil had suitable red blood cells and immune factors in the condition of this study.

Key words: Broiler; Energy; Corticosterone; Immune response; Soybean oil

Introduction

Major poultry farms have been located in the subtropical and tropical zones (Windhorst, 2006; FAO, 2007). In these zones, farmers have to utilize facilities such as evaporative cooling systems for control of the temperature of housing, to decrease the negative effects of heat stress on the health and performance of broilers (Lin *et al*., 2006). The *cost* of cooling broiler houses is high in many regions and hence some researchers focused on the nutritional management (Konca *et al*., 2009). The methods mostly used for this reason included of antioxidant supplementation (Sayed Tawfeek *et al.*, 2014), dietary electrolyte balance (Ahmad *et al*., 2008), supplementation of mineral (Ebrahimzadeh *et al*., 2012) or vitamins (Ipek *et al*., 2007), high fat diet (Ghazalah *et al*., 2008) and energy density or sources (Leeson *et al*., 1996; Golian *et al*., 2010). The manipulation in dietary energy level and source has been considered a useful method in industrial broiler farms of Iran to overcome the consequences of heat stress. However, the energy density of diets is very variable, some producers decreased and others increased the energy level and the same case exits for using carbohydrate or lipid in the diet. The application of soybean oil in diet of broiler is common as it is available as a cheap dietary lipid source. As n-6 poly unsaturated fatty acid (n-6 PUFA) content of soybean oil is high, it may affect some immune parameters in animals. In this regard, researchers (van Heugten *et al*., 1996; Sijben *et al*., 2005) reported that high levels of oils containing n-6 PUFA resulted in a decrease antibody response against antigens or immunoglobulin production.

In the literature, there was no report concerning the effect of energy sources and levels on blood attributes and immune function, especially in the heat stress condition. It was hypothesized that in the heat stress condition, lipid addition to diet and higher energy level could enhance the health through increase the blood attributes and immune function compared with diet containing main energy source from carbohydrate or lower energy level. Therefore, the main objective was to evaluate the effects of energy sources and levels on blood red cells count, antibody titers and immune cells in broiler chickens under heat stress.

Materials and methods

Bird management and experimental design

Six hundreds one-day-old Cobb male chickens (average weight of 39 g) was purchased from a local hatchery and allocated randomly to twenty pens covered with wood shaving. Chickens were assigned to five treatments, four replicates and thirty chickens per each replicate. Chickens were keep under controlled conditions based on Cobb broiler guides, except ambient temperature. Treatments were included of C: control which chickens received Cobb standard diet with main energy from corn; T1: chickens received 3% lesser energy than Cobb standard diet with energy from corn; T2: chickens received 6% lesser energy than Cobb standard diet with main energy from corn; T3: chicken received Cobb standard diet with main energy from corn and soybean oil and T4: chicken received 3% upper energy than Cobb standard diet with main energy from corn and soybean oil (Table 1). Three periods of raising were starter (day 1 to 10), grower (day 11 to 28) and finisher (day 29-42). Chickens had access *ad libitum* to feed and water at. Chickens were exposed to chronic heat stress from day 12 to 42 of age in relative humidity of 65%. During heat stress, temperature were raised daily to 34±1 °C for 8 hours from 08:00 to 16:00 and then decreased to 24 ± 1 °C. Each bird was vaccinated by commercial Newcastle disease virus (NDV) vaccine at day 12 (B1, eye drop) and day 20 (Lasota, drinking) and infectious bursal disease (IBD, days 10 and 22, eye drop).

Sample collection

At days 28 and 42 of age, blood samples were collected in the sterile tubes from wing vein of two birds in each replicate. Serum of blood sample taken at day 28 of age was used for measurement of antibody titers against Newcastle Disease and Infectious Bursa Disease viruses. Plasma of blood sample taken at day 42 of age was used for measurement of corticosterone, malondialdehyde and blood red cells and immune cells counts.

Sample analyses

An automated counter was used for counting blood red blood cells (RBC), white blood cells (WBC), lymphocyte, neutrophil, monocyte and platelets. Hemoglobin concentration was measured using the cyanmethemoglobin method. Thereafter, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated with the related formulas.

The titers of antibody against Newcastle disease were measured by hemagglutination-inhibition test (Allan and Gough, 1974) and against Infectious Bursal Disease by ELISA kit, IDEXX FlockChek standard (IDEXX Corporation, Westbrook, ME, USA). Value of antibody titers were transformed to log2(x) before statistical analysis.

Plasma level of malondialdehyde (MDA) was assayed by the thiobarbituric acid assay spectrophotometrically (Shimadzu UV-260, Shimadzu Corp, Tokyo, Japan) at 523 nm based on the method described by Ohkawa et al.

Table 1 : Composition (measured in %) of the experimental diets for broiler chicks

	Starter (0-10 days old)					Grower (11-28 days old)					Finisher (29-42 days old)				
Ingredients	T1*	T2	T3	T4	T5	T1*	T2	T3	T4	T5	T1*	T2	T3	T4	T5
Corn	63.35	63.25	62.13	58.17	54.17	69.22	68.8	67.7	65.31	59.31	70.18	71.47	71.5	65.1	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	9.10	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	0.34	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

*control

[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, 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%.

(1979). Plasma corticosterone level was measured enzymatically using photometric method by autoanalyser (BS-120 model, Minbray Co., USA) and commercial kits (Pars Azmon Co., Tehran, Iran).

Statistical analysis

The Statistical analysis was performed with ANOVA of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC). At first, the normality of data distribution was checked using the kolmogorov–smirnov test. ANOVA GLM (general linear model) procedure and Tukey test was used to compare the means. Statistical significance was accepted when $P<0.05$.

Results and Discussion

Dietary treatments significantly influenced corticosterone (Figure 1) and malondialdehyde levels (Figure 2).

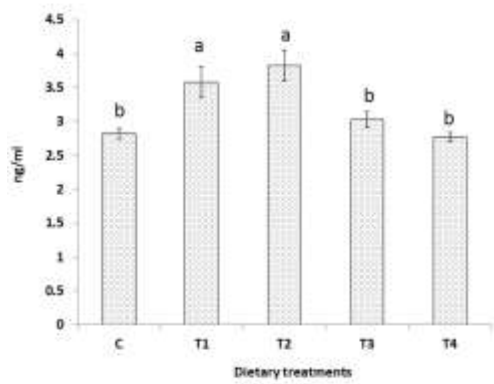

Fig 1

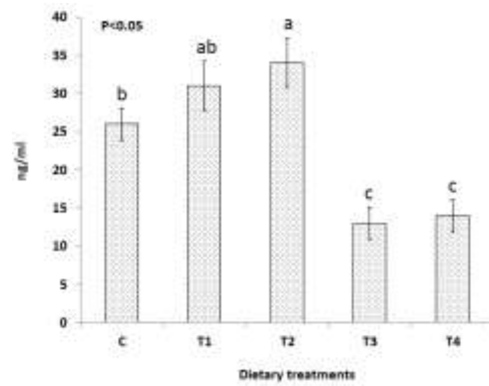

Fig 2

Figure 1: The effect of dietary treatments on plasma corticosterone levels of broiler chicks.
Figure 2: The effect of dietary treatments on plasma malondialdehyde levels of broiler chicks.
C: control, energy based on Cobb standard diet with main energy from corn; T1: chickens received 3% lesser energy than Cobb standard diet with energy from corn; T2: chickens received 6% lesser energy than Cobb standard diet with main energy from corn; T3: chicken received Cobb standard diet with main energy from corn and soybean oil and T4: chicken received 3% upper energy than Cobb standard diet with main energy from corn and soybean oil.

There were no differences ($P>0.05$) among C, T3 and T4 groups for plasma corticosterone level, but differences appeared among these treatments and T1 and T2. In this study application of diet with lower energy level increased the plasma corticosterone level and also malondialdehyde level. It was clearly evidenced that under stressful conditions, plasma corticosterone level and malondialdehyde level increase and could negatively influence on the health status (Geraert *et al*., 1996; Rastad *et al*., 2016).

The effects of energy source and level on total blood cells count, RBC, MCHC, MCV and MCH in broilers are shown in Table 2. Total blood cells in the chicks fed T3 and T4 was higher ($P<0.05$) as compared with other treatments. Difference in T3 and T4 diet with other treatments is substitution a part of energy from corn with soybean oil and for T4 application of higher soybean oil. The increase in total blood cells is related to RBC count, as WBC count had no differences among treatments.

As shown in Table 2, RBC count of chicks fed C, T3 and T4 diets was higher ($P<0.05$) than T1 and T2. T3 and T4 diets had soybean oil and the same and higher energy level compared with C diet, respectively. The energy level of these three diets was higher than T1 and T2 diets. This means that an increase in RBC count related to the level of energy. It was shown that a correct dietary energy level exists for erythropoiesis or increase of RBC counts (Karadeniz *et al*., 2008). In their study, the dietary energy level has also directly affected the hematopoiesis rate in broiler chicks; as chicks received normal energy diets had higher the RBC counts and their load in hemoglobin (MCHC) compared with those received lower energy level (Karadeniz *et al*., 2008). Satisfying the energy for chicks in C, T1 and T4 diets may be resulted in the acceleration of the erythrocyte differentiation which leads to higher count of red blood cells compared to T1 and T2 diets.

Table 2: Effects of energy sources and levels on red blood cells counts and hemoglobin contents

Item*	Red blood cells $\times 10^6$/mm^3	MCHC %	MCV fl	MCH pg
C	4.95[ab]	35.8	47.8[abc]	35.0
T1	4.16[bc]	32.6	41.4[bc]	30.1
T2	3.96[c]	32.6	32.6[c]	31.2
T3	4.88[ab]	35.7	39.8[ab]	34.4
T4	5.41[a]	36.9	52.7[a]	36.6
SEM	0.184	1.75	1.93	1.64

[a, b, c] Means within a column with different superscripts are significantly different (P<0.05).
*C: control, energy based on Cobb standard diet with main energy from corn; T1: chickens received 3% lesser energy than Cobb standard diet with energy from corn; T2: chickens received 6% lesser energy than Cobb standard diet with main energy from corn; T3: chicken received Cobb standard diet with main energy from corn and soybean oil and T4: chicken received 3% upper energy than Cobb standard diet with main energy from corn and soybean oil.

The average concentration of hemoglobin in red blood cells that the hemoglobin content (MCHC) and the average amount of hemoglobin inside a single red blood cell (MCH) showed non-significant differences (P>0.05) and the average volume of red cells in a specimen (MCV) showed significant difference (P<0.05) for those received higher energy level and especially in T4 that received soybean oil. This finding support this hypothesis that increase in red blood cells related to acceleration of differentiation.

The effect of different treatments on WBC, lymphocyte, neutrophil, monocytes and platelet are shown in Table 3. There were no significant differences (P>0.05) among treatments for WBC and lymphocyte counts, but significant differences exist among treatments for neutrophil, monocyte and platelet. High neutrophil content in T4 related to inclusion of soybean oil and may be related to inflammatory effect of n-6 fatty acids (Kelley *et al*., 2005). Soybean has high content of linoleic acid and could promote the formation of arachidonic acid-derived eicosanoids and consequently induce the inflammatory response (Kearns *et al*., 1999). During the early phase of inflammation the count of these cells increase in the blood.

Table 3: Effects of energy sources and levels on the total and differential of white cells counts

Item*	White cells count ($\times 10^3$/mm^3)	Lymphocyte $\times 10^3$/mm^3	Neutrophil $\times 10^3$/mm^3	Monocytes $\times 10^3$/mm^3	Platelets $\times 10^3$/mm^3
C	8.30	5.71	2.66[b]	0.030[ab]	315[a]
T1	7.58	5.37	2.17[b]	0.009[c]	188[bc]
T2	7.61	5.32	3.09[b]	0.011[c]	162[c]
T3	8.27	5.78	2.71[b]	0.026[b]	249[b]
T4	8.83	6.11	3.40[a]	0.038[a]	351[a]
SEM	0.31	0.306	0.136	0.002	13.4

[a, b, c] Means within a column with different superscripts are significantly different (P<0.05).
*C: control, energy based on Cobb standard diet with main energy from corn; T1: chickens received 3% lesser energy than Cobb standard diet with energy from corn; T2: chickens received 6% lesser energy than Cobb standard diet with main energy from corn; T3: chicken received Cobb standard diet with main energy from corn and soybean oil and T4: chicken received 3% upper energy than Cobb standard diet with main energy from corn and soybean oil.

There were significant differences among treatments for antibody titers against Newcastle disease virus and infectious bursal disease virus (Table 4). Antibody titers of NDV and IBD decreased in chicks received soybean oil in ration (P<0.05). It was speculated that inclusion of soybean oil in the diet could increase the level serum corticosterone (Song and Horrobin, 2004; Sadeghi *et al*., 2013). Many studies revealed that corticosterone has immunosuppressive effect (Gross, 1992; El-Lethey *et al*., 2003), resulted in decrease of immune organ weight (Sadeghi *et al*., 2013) and finally could inhibit the production of antibodies (Siegel, 1995; Post *et al*., 2003).

Table 4: Effects of energy sources and levels on antibody titers (log 2) against NDV and IBD

Item*	C	T1	T2	T3	T4	P value
NDV	5.67[a]	4.00[ab]	2.35[c]	5.35[b]	3.71[b]	0.650
IBD	4.65[a]	3.70[ab]	2.35[c]	5.00[b]	3.00[b]	0.516

[a, b, c] Means within a row with different superscripts are significantly different (P<0.05).
*C: control, energy based on Cobb standard diet with main energy from corn; T1: chickens received 3% lesser energy than Cobb standard diet with energy from corn; T2: chickens received 6% lesser energy than Cobb standard diet with main energy from corn; T3: chicken received Cobb standard diet with main energy from corn and soybean oil and T4: chicken received 3% upper energy than Cobb standard diet with main energy from corn and soybean oil.

It was concluded that energy level of diet had higher impact on measured parameters than energy source. Energy level recommended by Cobb strain containing soybean oil had suitable red blood cells and immune factors in the condition of this study.

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