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Effect of α-lipoic acid on thermoregulatory, haematological and gut microbiota populations of heat-stressed broiler chickens

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Target audience: *poultry producers; researchers; lecturers; veterinarians; medical practitioners.*

Abstract

A study was carried out to investigate the effect of alpha-lipoic acid (α-LA) on thermoregulatory, haematology and gut microbiota populations of heat-stressed broiler chickens. Two hundred and four (204) unsexed day-old Arbor acre chicks were randomly assigned to four treatment groups to receive α-LA at 0, 50, 100 and 200 mg/kg in a completely randomized design (CRD) for a period of 42 days after a one-week adjustment period. Average room morning and afternoon temperatures were 27.9oC and 35.1oc respectively, while the morning and afternoon humidities were 73.1% and 44.1% respectively. Feed and water were provided ad libitum. Average Temperature-Humidity-Index (THI) indicated the presence of severe heat stress in the afternoons (33.4). Thermoregulatory indices (respiratory rate and body temperature) were significantly ($p<0.05$) affected by α-LA with birds on 100 and 200mg α-LA having lower respiratory rates and body temperature. α-lipoic acid also had significant effect ($p<0.05$) on Mean Corpuscular Volume (MCV) and Mean Platelet Volume (MPV) with the highest levels observed in 100mg/kg α-LA group. Increasing levels of α-LA significantly ($p<0.05$) reduced white blood cells count and duodenal Salmonella population while Lactobacillus population increased ($p<0.05$). It was concluded that, α-LA had ameliorative effect on thermoregulation, haematological indices and gut microbiology of heat-stressed broiler chickens.

Keywords: α-lipoic acid; heat stress; thermoregulatory; haematology; bacteria

DESCRIPTION OF PROBLEM

Stress has been defined as the sum of all biologic reactions to physical, emotional, or mental stimuli that disturb an individual's homeostasis. Stressor can be defined as any internal or external stimuli or threat that disrupts homeostasis of the body, and elicits a coordinated physiological response within the body in an attempt to re-establish homeostasis (1). If birds are exposed to a stressor for an extended period, a disruption in physiological processes can be harmful

(2), and result in death. Therefore, to counteract the negative consequences of a stressor, various organs under the action of the neuroendocrine system are activated to regain homeostasis (3). With the increased severity of global warming (4), heat stress has become an increasingly prevalent environmental stressor that threatens human and animals' health (5), and it occurs when body heat production exceeds the heat lost to surrounding environment. Modern commercial broilers are especially vulnerable to heat stress under high ambient temperature, owing to their restrictive heat loss capacity, high metabolic rates, high heat production, hypoplasia of sweat gland, and intensive genetic selection (6). Heat stress impairs welfare and growth performance of broiler, and causes enormous economic losses to the commercial broiler industry (7).

Poultry develop and adopt certain morphological, physiological and behavioural traits under heat stress conditions to maintain their normal body temperature. Poultry are under heat stress conditions during an imbalance between body thermo-genesis and heat dissipation (8).

Despite these adjustments in behaviour, if the temperature rises to a point where the bird is unable to naturally cool down and an external method of heat dissipation is not used to reduce the ambient temperature, birds experience heat stress and often die from complications arising from this condition. Stress responses may include changes in feeding behaviour, hypertension, reproductive dysfunction, inefficient feed conversion, gastric and intestinal ulcers, electrolyte imbalance and immune deficiency (9).

Management of intestinal function has emerged as a research hotspot in poultry science. The small intestine plays an important role in digestion and subsequent absorption of nutrients from ingested feed, as well as in local defence against pathogenic bacteria and their harmful constituents such as lipopolysaccharide (LPS). However, heat stress disrupts intestinal function through pathogenic, metabolic, and endocrine stimuli, along with excessive production of oxidants. This results in increased permeability to potentially toxic luminal substances (10). The gastrointestinal compartments of chickens are densely populated with complex microbial communities (Bacteria, fungi, Archaea, protozoa, and virus) that are dominated by bacteria (11). A normal gut microbial community has benefits and costs to the host. The primary benefits that are provided by commensal microbiota are competitive exclusion of pathogens or non-indigenous microbes (12), immune stimulation and programming, and contributions to host nutrition.

Alpha lipoic acid (α-LA), especially in recent years, has been used as a food additive, in order to give beneficial effects in the management and treatment of various types of disorders, such as: endothelial dysfunction, atherosclerosis, diabetes mellitus, degenerative diseases and others (13). α-LA also known as thioctic acid (1,2-dithiolane-3-valeric acid) is an organic, sulfate-based compound produced by plants, humans, and animals. As a potent antioxidant and a natural dithiol compound, it performs a crucial role in mitochondrial bio-energetic reactions. A healthy human body, on the other hand, can synthesize enough α-lipoic acid to scavenge reactive oxygen species and

increase endogenous antioxidants; however, the amount of α-lipoic acid inside the body decreases significantly with age, resulting in endothelial dysfunction (14). α-lipoic acid is both fat and water-soluble and therefore, it can be easily absorbed and transported across cell membranes resulting in optimal nutrient availability. Lipoic acid described as metabolic antioxidant, as it is potent scavenger of reactive oxygen species like hydroxyl radicals, hypochlorous acid, and singlet oxygen, thereby reduces the oxidative stress in birds (15). It also acts as an essential cofactor for mitochondrial α-ketoacid dehydrogenase complexes, which is essential for normal oxidative metabolism (16). α-lipoic acid is a multifunction antioxidant that is produced in small amount by cells as well as its dietary provision facilitates fatty acid mobilization, energy expenditure as well as scavenge free radicals in poultry birds. α-LA scavenges reactive oxygen species and increase endogenous antioxidants. It is also a good chelating agent for metals in organisms and a reducing agent for the oxidized form of glutathione and vitamins C and E (14). α-Lipoic acid enantiomers and its reduced form have antioxidant, cognitive, cardiovascular, detoxifying, anti-aging, dietary supplement, anti-cancer, neuro-protective, anti-diabetic, antimicrobial, and anti-inflammatory properties (14). α-Lipoic acid has cytotoxic and anti-proliferative effects on several cancers, including polycystic ovarian syndrome. It also has usefulness in the context of female and male infertility. It exists in oxidized as well as reduced form, characterized by growth promoting, anti-inflammatory, antioxidative, immunostimulatory, and hypocholesterolemic properties when fed as dietary supplement to farm animals, particularly broiler chickens (16).

Aim and objective

The aim and objectives of study are detained the effect of α-Lipoic acid supplementation on:

1. Thermoregulatory indies of heat-stressed broiler chickens
2. Haematological indies of heat-stressed broiler chickens
3. Gut microbiota population of heat-stressed broiler chickens

MATERIALS AND METHODS

Experimental Design and Management of birds

Two hundred and four (204) unsexed day-old Abor acre broiler chicks were purchased from CHI farms Ibadan, Nigeria for the study. The birds were randomly allocated to four (4) dietary treatments with fifty-one (51) birds per treatment with each treatment replicated three (3) times with seventeen (17) birds per replicate in a Completely Randomized Design (CRD). The birds were raised on deep litter system in two phases; starter (week 0 – 4) and finisher (week 5 – 7). Indoor temperature and relative humidity readings were recorded daily using a digital thermos-hygrometer. Both readings were taken in the mornings (8.00 am) and afternoons (3.00 pm) throughout the experimental period and used to calculate the morning and afternoon Temperature-humidity index(THI) (17).

$$THI = 0.85T_{db} + 0.15T_{wb}$$

Where;
THI = temperature-humidity index in °C
T_{db} = dry-bulb or ambient temperature in °C
T_{wb} = wet-bulb temperature in °C
Wet bulb temperature was determined from ambient temperature and relative humidity using the empirical expression functions (18). Individual body temperatures and respiratory rates were also measured using an infrared digital thermometer and counting of respiration (breath/minute) with the aid of a stopwatch, respectively. Heat stress in birds is classified as: Thermoneutral zone (18-21oC), acute heat stress (27-38 oC for 1-2 hours), moderate heat stress (27-38 oC for aduration of 7 days) and chronic heat stress in high ambient temperatures of 38-50 oC for prolonged period of 7 days or more (19)
Four (4) experimental diets were formulated according to (20) specification for broiler starter and finisher. The basal diet (Table 1) was supplemented with α-LA at varying levels as follows:
0mg (Basal diet + 0g α-LA), 50mg (Basal diet + 50mg α-LA /kg of feed), 100mg (Basal diet + 100mg α-LA /kg of feed) and 200mg (Basal diet + 200mg α-LA /kg of feed). The diets were fed to the birds for 49 days. All standard management practices were strictly adhered to.

Ingredients	Broilers starter	Broilers finisher
Maize corn	51.0	62.0
Soybean cake	41.0	30.0
Maize offal	3.0	3.0
Bone meal	4.0	4.0
Premix	0.3	0.3
Lysine	0.2	0.2
Methionine	0.2	0.2
Salt	0.3	0.3
Nutrient composition		
M.E (Kcal/kg)	2834.00	2952.00
CP	22.79	19.14
CF	3.42	4.07
EE	5.48	5.15
Ca	1.23	1.21
P	0.79	0.79
Lysine	1.08	0.93
Methionine	0.58	0.53

Haematological Analysis

At the end of the trial on day 49, two birds were randomly selected from each replicate for haematological and serum analyses. The sampled birds were bled by severing the jugular vein to obtain blood. Two (2) ml each were collected into bottles containing Ethylene Diamine Tetra Acetate (EDTA) for haematological assay. Haematological analysis was carried out using a Mindray BC3600 haematology analyser.

Duodenal Microbial Count

From the birds used for haematological analysis, samples of the duodenal portion of the small intestine were surgically removed into sterile sample bottles for microbial determination. *Lactobacillus*, *Campylobacter* and *Salmonella* identification and counts were determined using standard microbiological methods (21), and samples were analysed by applying the spreading technology. The colonies were counted at the end of the incubation periods and transformed to log values.

Data Analyses

The data collected from the experiment were subjected to analysis of variance (ANOVA) using the General Linear Model Procedure of (22). Where the result of ANOVA was statistically significant, Tukey post-hoc test for multiple comparisons was performed to compare means of all groups.

RESULTS

Temperature-Humidity-Index (Figure. 1) results indicate that mean THI was higher in the afternoon (33.4) than in the morning (27.8) during the period of the study. THI incorporates the effect of both temperature and relative humidity and is commonly used to quantify the degree of heat stress on animals. The birds experienced no heat stress in the mornings (23.8) and very severe heat stress in the afternoons (33.4) (19) during the period of the study. The morning and afternoon average room temperatures were 27.9oC and 35.1oc respectively, while the morning and afternoon average room humidity were 73.1% and 44.1% respectively. Effect of α-lipoic acid on respiratory rate and body temperatures of broiler chickens is shown in Figure 2 and 3 respectively. There were reductions in respiratory rates and body temperatures ($p<0.05$) with increasing levels of α-LA. Birds receiving 100 and 200 mg α -LA had similar respiratory rates and body temperatures and differed ($p<0.05$) from birds in the control group. Birds in the 50 mg group remained comparable ($p>0.05$) with birds in the control group. Result showing the effect of α-lipoic acid on serum haematological indices of broiler chickens is shown in Table 2. White blood cell count was significant($p<0.05$) affected, with birds in the control having higher WBC compared to the α-lipoic acid groups which were similar. No significant difference ($p>0.05$) was observed for granulocytes, lymphocytes, haemoglobin, haematocrit (PCV) and red blood cell counts. α-lipoic acid was observed to have a significant effect ($p<0.05$) on Mean Corpuscular Volume (MCV) and Mean Platelet Volume (MPV). Result showing the effect of α-lipoic acid on duodenal bacterial count of broiler chickens is shown in Table 3. Duodenal *Salmonella* count was significant ($p<0.05$) with birds receiving 100 and 200 mg α-LA being similar ($p>0.05$) and significantly ($p<0.05$) different from birds in the control group. *Lactobacillus* count was

significant ($p<0.05$) with birds receiving 50 and 100 mg α-LA being similar ($p>0.05$) and significantly ($p<0.05$) different from birds in the control. No significant difference ($p>0.05$) was observed for Campylobacter across all the treatment groups.

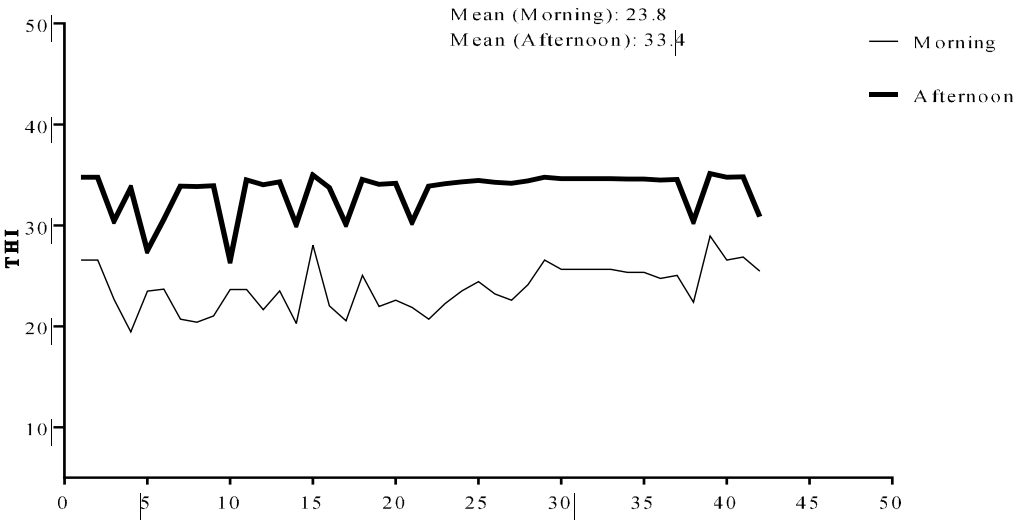

Figure1: *Daily Temperature-humidity index inside the poultry house during the experimental period*

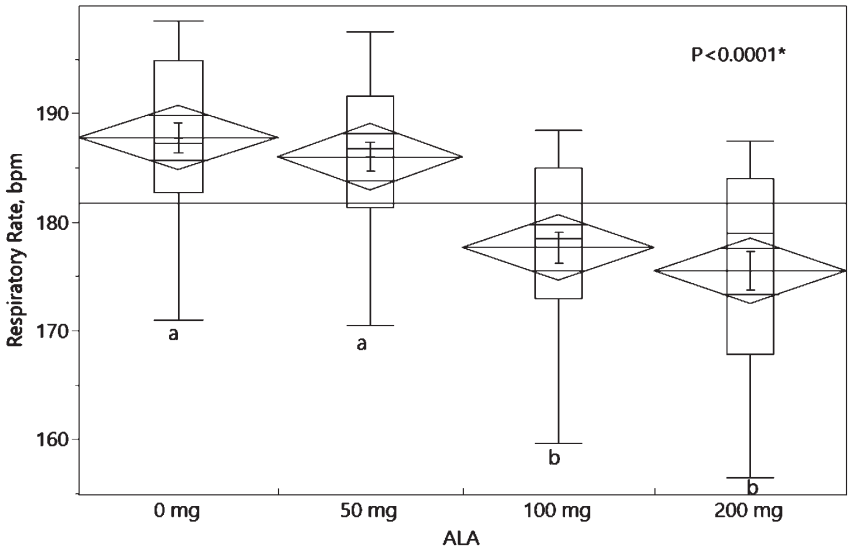

Figure 2: *Respiratory rate of heat-stressed broiler chickens fed α-LA*

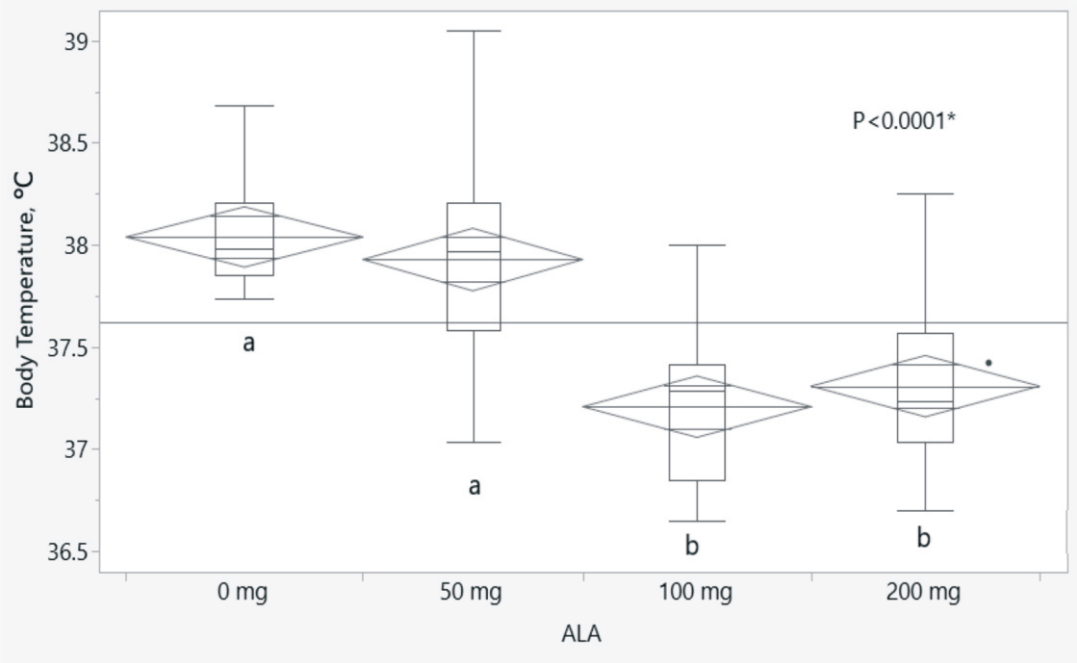

Figure 3: *Body temperature of heat-stressed broiler chickens fed α-LA*

Table 2: Haematological indices of heat-stressed broiler chickens fed α-lipoic acid

	α-Lipoic acid (mg/kg)					
Parameters	0	50	100	200	SEM	p value
White Blood Cell (x109/L)	50.14^{a}	43.05^{b}	46.48^{b}	42.91^{b}	2.05	0.0221
Lymphocytes (%)	91.28	91.86	91.10	92.48	0.73	0.5462
Granulocytes (%)	2.38	2.12	2.77	2.45	0.52	0.8668
Red Blood Cell (x1012/L)	2.52	2.47	2.52	2.55	0.11	0.9687
Haemoglobin (g/Dl)	2.02	11.78	12.27	10.72	0.93	0.6593
Haematocrit (%)	28.77	26.03	30.25	25.25	2.39	0.4348
MCV (fL)	114.50^{ab}	105.25^{b}	120.32^{a}	115.63^{ab}	3.54	0.0468
MPV (fL)	5.80^{ab}	5.83^{ab}	6.23^{a}	5.72^{b}	0.13	0.0473

a,b Means with difference on the same row differ significantly (P<0.05), SEM: Standard error of the mean, MPV: Mean platelet volume, MCV: Mean Corpuscular Volume, g/Dl: gram per decilitre fL: fluid ounce, 109/L and 1012/L: microliter.

Table 3: Duodenal microbial count of heat-stressed broiler chickens fed α-lipoic acid

	α-Lipoic acid (mg/kg)					
Indices	0	50	100	200	SEM	p value
Salmonella (CFU)	19.00 [a]	19.00[a]	15.00[ab]	0.00 [b]	1.15	0.0325
Lactobacillus (CFU)	5.00[b]	44.50[a]	44.50[a]	5.00 [b]	3.75	0.0235
Campylobacter (CFU)	0.00	0.00	0.00	0.00	0.00	0.00

a,b, Means with difference on the same row differ significantly (P<0.05), SEM: Standard error of the mean, CFU: colony forming unit.

DISCUSSION

Heat stress is influenced by air temperature, humidity, air movement, solar radiation, and precipitation. However, temperature-humidity index (THI) is a single value depicting the integrated effects of air temperature and humidity associated with the level of heat stress (23). The THI incorporates the effects of both temperature and relative humidity and is commonly used to quantify the degree of heat stress on animals. Heat stress was classified as: absence of heat stress (<27.8), moderate heat stress (27.8 -28.8), severe heat stress (28.9 - 29.9) and very severe heat stress (>30.0) (19). The average THI of the experimental environment indicates very severe heat stress. The tolerance of animals to high air temperatures depends on the amount of water vapour in the air as this affects the rate of heat loss by evaporation. For the study of heat stress in animal husbandry, the THI is a commonly used bioclimatic index (24).

Respiratory rate and body temperature are the most important physiological markers to look out for animals under heat stress. Higher respiratory rates were observed in the control and 50 mg/kg α-LA, indicative of heat stress in the group. Poultry under heat stress conditions develop and adopt certain morphological, physiological and behavioural traits such as panting, increasing respiration rate and slowed heart rate to maintain their normal body temperature (24)(8). Panting is the key procedure acquired by poultry birds to undergo heat loss during heat stress conditions, which brought about the increase in respiratory rate and body temperature in the control. Evaporative cooling is accomplished by increasing the respiratory rate (25)(26) also known as panting and this can lead to respiratory alkalosis (27). Unfavourable temperatures such as high temperature leads to increased heat production by the animal, that is, there is more loss of energy, and in consequence less energy remains for production at the same level of energy intake, and the efficiency of energy utilization deteriorate. Broiler chickens exposed to high temperature and relative humidity find it difficult to maintain their core body temperature (27). Hot humid environments reduce the opportunity for heat loss through sensible and evaporative means (28).

Haematology is an important part of clinical pathology as well as diagnostic process (29). The higher the value of leukocyte count, the better the ability of the

animal to fight diseases (30). It could also be indicative of a heat stress response. Elevated WBC count observed for the control group is as a result of heat stress which is also proven on several studies with different animals (31)(32)(33). α-LA supplementation was reported (34) to show a statistically significant ($p<0.05$) association with the reduction of total leukocytes, increase in the number of neutrophils and reductions in the serum levels of iron and TSI of diabetic patients (human). Heat stress affects the physiology, metabolism, and immune response of chickens, causing electrolyte imbalance, oxidative stress, endocrine disorders, inflammation, and immune-suppression. These changes do not occur independently, pointing to a systemic mechanism (10). No disorder or imbalance was observed in the α-LA supplemented groups which indicate that α-LA had no negative effect on haematological indices of broiler chickens. The mean corpuscular volume or mean cell volume (MCV) which is a measure of the average volume of a red blood corpuscle (or red blood cells). MCV is a measurement that allows classification as either microcytic anemia, normocytic anemia or macrocytic anemia (35). Higher and normal MCV levels were found in the α-LA supplemented groups indicative of normocytic anemia. α-LA supplemented at 50, 100 and 200 mg/kg prevented microcytic and macrocytic anemia. Mean platelet volume (MPV) is a machine calculated measurement of the average size of platelets found in blood and is typically included in blood tests as part of the complete blood count (CBC). Since the average platelet size is larger when the body is producing increased numbers of platelets, the MPV test results can be used to make inferences about platelet production in bone marrow or platelet destruction problems (36). Higher levels of MPV are indicatives of higher blood clotting potentials of an organism. α-LA supplementation improves the clotting potentials of the blood by increasing the MPV levels. The platelet (thrombocytes) are blood cells made in the spongy tissues in the bone marrow, they help in blood clotting. When injured, platelets clump together at the site of wound; they slow and stop the flow of blood. α-LA supplemented at various levels (50, 100 and 200 mg/kg) had no negative effect on haematological indices of heat-stressed broiler chickens.

Broiler chickens' gastrointestinal tract (GIT) plays a pivotal role in growth and health during their lifespan. Alteration of bacterial microbiota may adversely affect feed efficiency, productivity, and chicken health status (10)(37). The gut microbiota can form a protective barrier by attaching to the epithelial walls of the enterocyte and thus reduce the opportunity for the colonization of pathogenic bacteria (38). α-LA was able to prevent *Salmonella* colonization in the 200mg group. *Salmonella* species are intracellular pathogens, of which certain serotypes cause illness. Most infections are due to the ingestion of food contaminated by faeces (39).

Higher population of *Lactobacillus* was observed in the 50mg/kg α-LA and 100mg/kg α-LA groups. *Lactobacillus* species constitute a significant component of the human and animal microbiota at a number of body sites, such as the digestive system, and the female genital system. Lactobacillus exhibits a mutualistic relationship with the animal body, as it protects the host against potential invasions by pathogens, and in turn, the host provides a

source of nutrients (40). There was no presence of Campylobacter observed in all the treatment groups. Campylobacters generally colonize avian spp as a commensal organism. It is generally assumed that Campylobacters contaminate poultry meat during processing, surviving throughout the food chain supply to constitute a risk to human health (41).

CONCLUSION AND RECOMMENDATION

The findings of the present study indicated that supplementation of α-lipoic acid had beneficial effect on thermoregulation (respiratory rate and body temperature) of heat stressed broiler chickens at 100 and 200 mg/kg. Haematological indices of heat-stressed broiler chickens at various levels of inclusion were also influenced. α-lipoic acid also had a positive effect on duodenal *Salmonella*, *Lactobacillus* and *Campylobacter* populations of heat-stressed broiler chickens. 100mg/kg α-LA is recommended for a better thermoregulation, haematological indices and gut microbial population of heat stressed broiler chickens.

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