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Research Article

# Heat Stress Impedance by Acidifiers in Broiler Chickens

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## Abstract

One day-old male Arbor Acres plus broiler chicks (n = 400) allotted into 4 equal treatments (groups1 - 4) were used in this study. At d 28-35 of age of male broiler chicks, groups 1 - 3 were maintained at an ambient temperature of 34-35°C and 50% relative humidity. Chicks of groups 1 and 2 were orally treated via drinking water with sodium butyrate liquid (NaB) contains 45% butyrate product (3 ml/L) and an acidifier blend liquid (AB) contains organic acids, nutritional substances and yeast cell walls (1 ml/L). Those of group 3 were kept untreated as positive control (PC). Chicks of group 4 were kept at a thermo-neutral circumstances (22°C) without treatment and served as blank control (BC) group. Heat stress in PC group resulted in detrimental effects on growth performance, carcass characteristics, hematological, clinical biochemical parameters, low grade inflammatory marker, oxidative stability markers and histological picture of liver, spleen and immune organs. Usage of NaB and AB already improved these detrimental effects provided that NaB was more effective than AB.

It is undisputable that the used organic acidifiers in the present investigation are of special interest in controlling heat stress.

**Keywords:** Acidifiers; Heat Stress; Broilers; Welfare; Production; Food Safety

## Abbreviations

PC: Positive Control; BC: Blank Control; NaB: Sodium Butyrate Liquid; AB: Acidifier Blend Liquid.

## Introduction

Due to the legislated limits on the use of antibacterial feed additives for poultry; earlier establishment of their immunity and intestinal integrity were focused to reach broiler maximum potential for growth and feed efficiency [1]. Eventually; poultry industry is exposing to stressful conditions, problems related to diseases and deterioration of environmental conditions that often occur and result in serious economic losses [2]. The issue of controlling stressful conditions and problems is becoming a big challenge. Feed additives such as enzymes, probiotic, prebiotic, synbiotic products and even nutrition has utilized in order to find better circumstances to enhance poultry gut health and prevent or limit production losses. Butyrate, which is a by-product of microbial fermentation of products (such as resistant starch) is considered to be important for normal development of epithelial cells [3,4]. It is taken-up by nonionic, passive absorption in the stomach [5]. In the field of animal nutrition, butyrate is used as a zootechnical ingredient and can be used as an unprotected salt or in the form of protected derivatives. Dietary protected butyrate significantly improved growth performance, immune response, intestinal morphology in experimentally induced necrotic enteritis (NE), and increased the expression level of insulin-like growth factor-1 (IGF-1) and decreased the DNA fragmentation induced by C. perfringens in chicken broilers [6]. Supplementation of unprotected butyrate leads to a signifi-

cant increase in butyrate concentration in the gastric region but not in the jejunal chyme of broilers [7]. Consequently; unprotected butyrate is supposed to exert mainly effects on epithelial cells and microbiota of the crop, proventriculus and gizzard of broiler chickens and it is unclear whether delivery at these segments of the gastrointestinal tract (GIT) is effective in eliciting effects on growth performance, gut morphology and immune system regulation [8]. They will be mainly present in a protonated form due to the low pH in the stomach of monogastric animals, due to its pKa of 4.82 [8].

It is already known that an increase in pro-oxidant molecules associated with a decrease in antioxidant defense produce free radicals that cause damage or death to the cell (oxidative stress) under stressful environments such as heat exposure [9]. This means that although oxidation reactions are crucial for life, they can also be damaging which considered a paradox in metabolism [10].

The potential advantage of organic acids in the feed of poultry has been proven and well documented for decades. However, new trials are still necessary to establish performance results under different production conditions.

As the importance and impact of heat stress in poultry production, focusing on broilers; the present investigation was dedicated to conduct a semi-field trial to elucidate the potential beneficial effects of sodium butyrate liquid containing 45% butyrate product (NaB) and a blend of acidifiers (AB) containing organic acids, nutritional substances and yeast cell walls supplementation on growth performance, carcass characteristics, hematological, clinical biochemical parameters, low grade inflammatory marker, oxidative stability markers and histological picture of liver and immune organs in broiler chickens under oxidative stress of increased heat.

## Materials and Methods

### Acidifiers

Sodium butyrate (NaB) and an acidifier blend (AB) were used in this investigation. NaB and AB are produced by NUTRI-AD international, Belgium under the names of ADIMIX® 45 Liquid (45% butyrate product) and REVITAL® PLUS liquid respectively. REVITAL® PLUS liquid contains citric acid, orthophosphoric acid, lactic acid and formic acid with fermented product of inactivated *Saccharomyces cerevisiea*. According to a preliminary testing of drinking water to guarantee a pH of water supply above 4.5 during the experiment; NaB and AB were added in a dose of 3 ml/L (pH 6.78) and 1 ml/L (pH 4.69) respectively.

### Experimental birds

One day-old male Arbor Acres plus broiler chickens (n = 400) were used in this study. Duration of the trial extended from one day of age up to slaughter (35 days). These birds were allotted into 4 equal treatments (groups1 - 4) consisting of 100 birds each assigned into 4 equal replicates of 25 birds. All experimented chickens were floor reared in separate pens at a density of 10 birds/m2 with fresh wood shavings as bedding with a thickness of approximately 10 cm on a concrete floor and kept in environmentally controlled rooms. All birds vaccinated against different diseases according to the vaccination programs usually adopted in Egyp-

| Ingredients | Starter | Grower | Finisher |
|---|---|---|---|
| Yellow corn | 524.5 | 544.2 | 628.5 |
| Soybean meal 44% | 332.4 | 299.1 | 221.1 |
| Corn gluten meal 60% | 70 | 70 | 66.5 |
| Soya oil | 30 | 43.8 | 40 |
| Di-calcium phosphate | 18 | 18 | 18 |
| Lime stone | 13 | 13 | 13 |
| D.L. Methionine | 2.2 | 2.1 | 2.3 |
| Lysine hydrochloride | 2.9 | 2.8 | 3.6 |
| Sodium chloride | 4 | 4 | 4 |
| Premix* | 3 | 3 | 3 |
| **Calculated analysis:** | | | |
| Crude protein % | 23.0 | 21.0 | 19.0 |
| Metabolizable energy (kcal/kg) | 3000 | 3100 | 3200 |
| **Calculated nutritional values** | | | |
| Dry Matter | 88.5 | 88.7 | 88.3 |
| Fiber content | 3.7 | 3.6 | 3.2 |
| Ash content | 6.80 | 6.70 | 6.35 |
| Available Ca | 0.99% | 0.97% | 0.95% |
| Available P | 0.48% | 0.47% | 0.45% |

**Table 1:** Composition of the 3-phase diets (g/kg as fed) used and their calculated analysis and nutritional values.

*Each 3 gram of premix mixture contained: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopheryl acetate), 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron ($FeSO_4$ $H_2O$), 40 mg; copper ($CuSO_4$ $5H_2O$), 7 mg; iodine [$Ca(IO_3)_2$], 0.7 mg; selenium (Na2SeO3), 0.3 mg.

tian chicken broiler farms. All groups ran contemporaneously. The composition of the diets and their calculated analysis are shown in table 1. The diets used formulated to meet the nutrient requirements of the broiler chicks during starter, grower, and finisher periods. Semduramicin was added to rations at a concentration of 25 ppm as a coccidiostat. No antibiotics were administrated in water or feed for the whole experimental period. Birds had free access to feed and water.

### Experimental design

This experiment was carried out in Poultry Production Department, Faculty of Agriculture, Cairo University. At d 28 of age, chicken groups 1-3 were kept under heat stress (8 hours/day) where ambient temperature raised from 22°C to 34-35°C (at approximately 50% relative humidity till the end of the trial). While those of group 4 was kept at a thermo-neutral circumstances (22°C). Chickens of groups 1 and 2 supplemented with NaB and AB in drinking water respectively at d 28 - 35. Chickens of groups 3 and 4 fed on plain water without treatment and served as positive and blank controls (PC and BC) respectively.

### Measured parameters

### Health status and mortality assay

During the evaluation; the health status of the birds was checked daily. Experimented birds were clinically examined at the end of the experiment (d 35) for scoring of foot pododermatitis (FPD) after [11] (by using a 4-point scale from 0 to 3 in which 0 shows no sign of damage) as well as feathering scoring (1= little feathers 2= average feathering on the back or the thighs, zones of skin are visible 3 = animals with good feathering, except on the thighs 4 = really good feathering). Post mortem lesions of dead birds and total mortality/group were recorded.

### Productive Performance and carcass characteristics assay

Chicken performance response variables were determined according to [12-14]. Body weight (BW) measured on all birds. Feed consumption (g/d/bird), feed conversion ratio (FCR) (g feed/g live body wt.). For BW all birds weighed individually at 1st day of age and weekly. Feed consumption has been measured on the same days of birds weighting. An index of productivity (Production number) was also determined after Timmerman., *et al.* [15]. [which equals (kilograms of growth per day * (100 - mortality %) / FCR) * 100]. Carcass characteristics (dressing%, front part %, hind part %, breast meat %, thigh drumstick %, carcass meat %, heart wt. %, gizzard wt. %, liver wt.%, giblet wt.%, and intestinal length and diameter) were measured on randomly chosen 10 birds/group at the end of the experiment.

### Hematological, clinical biochemical and oxidative stability assays

Serum samples for stress index "Heterophil/Lymphocyte (H/L) ratio" [16] were collected 6 hours post heat challenge at d 28 of age from 8 randomly chosen birds (2 birds/replicate) of different studied groups.

At d 31; blood samples were also collected by heart puncture of randomly chosen 8 birds/group (2 birds/replicate) and a part of the collected blood was received on dipotassium EDTA (for hematological studies) while another part was collected in plain tubes, allowed to clot and centrifuged at 3000 rpm for 10 minutes and the clear non-haemolysed supernatant sera were harvested for biochemical examination.

### I-Hematological assay

Total leukocyte counts (TLC) were done by using an improved Neubauer haemocytometer. Blood was diluted with Natt-Herrick,s solution according to Harrison and Harrison [17]. Moderately thin blood films were fixed with methyl alcohol, stained with Field's stain according to Feldman., *et al*. [18].

### II-Clinical biochemical assay

By using commercial diagnostic kits supplied by Spectrum Company, serum total proteins [19] (Weichselbaum,1946), serum albumin [20], serum globulins (determined by subtracting value of the serum albumin from the value of serum total proteins concentration) [21], A/G ratio (obtained by subdividing values of the serum albumin by those of serum globulins). The entire biochemical test used for evaluation of enzymatic activity of liver including alanine aminotransferase (ALT), aspartate aminotransferase (AST) [22], alkaline phosphatase (ALP) [23], lactate dehydrogenase (LDH) and creatine kinase (CK) [24] were determined by an autoanalyzer, Vital Scientific-Netherland, using commercial diagnostic kits supplied from ELITech company. Vitamin C and E were also determined using spectrophotometer (British Pharmacopia, 2012). Plasma samples were analyzed for markers of lipids including total cholesterol (TL), low density lipid (LDL), high density lipid (HDL) and triglycerides (TG) calorimetrically using commercial

kits (Diamond Diagnostics, Egypt). Serum samples collected from 10 randomly chosen broilers at the end of the trial were analysed for Hemagglutination inhibition test (HI) against Newcastle disease (ND) vaccination as described by [25].

### III-Oxidative stability assay

At d 35, blood samples were collected from 8 randomly chosen birds out of each group (2 bird/replicate), immediately placed on ice in heparinized tubes, centrifuged at 1000 rpm for 20 minutes and plasma stored at -20°C for further analysis. Plasma samples were assayed for superoxide dismutase (SOD) total antioxidant capacity (TAC), malnodialdehyde (MDA) and glutathione peroxidase (GSH-PX) (Diamond Biodiagnostic, Egypt).

### Low-Grade inflammation parameter

Determination of C reactive protein (CRP) was assayed by CRP Turbilatex according to Young [26]. The registered values for CRP were expressed as mg/l.

### Histopathological assay

At the end of the trial (d 35); specimens of liver and major immune organs (spleen, thymus glands, bursa of Fabricius and caecal tonsils) were collected from 4 sacrificed birds/group (one bird/replicate), fixed in 15% buffered formalin and paraffin-embedded sections stained with Hematoxylin and Eosin were made [27] and scored for histpathological changes according to the method described by Rosales *et al*. [28].

### Statistical analysis

Data were analyzed using the SAS statistical package [29]. General liner model procedure with a one way ANOVA model using NaB and AB as main effect. Mean values were compared using multiple rang test [30]. The significant level set at 5%. All percentage values were transferred to arc-sine before the analysis [31].

## Results and Discussion

Heat stress is one of the most important environmental stressors challenging poultry production worldwide [32]. In poultry industry; it results in estimated total annual economic loss to the U.S. $128 to $165 million [33]. In the present study; no clinical signs of foot pododermatitis (FPD) was detected in all experimented groups and all birds showed really good feathering. However; post mortem examination of dead birds in heat challenged groups showed areas of bruises in subcutaneous tissues (Figure 1) that accords with those reported by Lara and Rostagno [32] who stated that bruises are associated with ambient temperature. At d 35 of age; numerical increase in mortality was recorded in PC group vs. BC, NaB and AB treated groups.

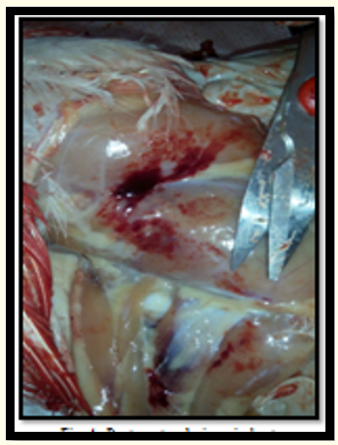

**Figure 1:** Post mortem lesions in heat challenged groups showing areas of bruises.

Productive performance and carcass characteristics revealed -51 and -7 units of FCR in NaB and AB treated groups vs. PC group respectively. In NaB treated group there was a significant increase in BW, BW gain, production number, dressing %, front parts %, breast meat % as well as intestinal length and diameter vs. PC group ($P \leq 0.05$). While in AB treated group there was significant increase in dressing %, front parts %, breast meat % and intestinal diameter ($P \leq 0.05$) together with a numerical increase in BW, BW gain, production number and intestinal length vs. PC group. Both treated groups showed numerical increase in cumulative feed intake (Table 2). The impaired production and growth performance in broilers subjected to heat stress in the present investigation accords with findings of other investigators [34,35]. It could be attributed to reduced dietary digestibility [36]. and decreased feed intake which is very likely the starting point leading to decreased body weight and feed efficiency [37,38]. Broilers subjected to chronic heat stress had higher FCR [39]. Additionally; in broilers; heat stress has been associated with undesirable meat characteristics and quality loss [40]. Table 2 showed that C reactive protein (CRP) as a low grade inflammation marker revealed significant decrease in NaB treated group vs. PC group ($P \leq 0.05$) that reflects the positive effect of this acidifier in reduction of inflammation resulted from heat stress.

| Group | Final Body Wt.(g) | Cumulative feed intake (g/bird) | Final FCR | Wt. gain (1-35 days of age) (g) | Mortality % | Production Number | Dressing % | Breast-meat % | Front parts % | Intestinal length (Cm) | Intestinal diameter (Cm) | C reactive protein (CRP) (mg/l) | Total leukocyte count (TLC) x $10^3$ | Heterophil /Lympho-cyte ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-NaB Treated | 1997.50 ± 23.14$^{ab*}$ | 3243.28 ± 26.77 | 1.625 ± 0.037 | 1957.34 ± 23.14$^{ab}$ | 5.00 ± 1.65 | 328.55 ± 12.20$^{a*}$ | 66.83 ± 0.21$^{a*}$ | 20.14 ± 0.26$^{a}$ | 36.50 ± 0.18$^{a}$ | 193.50 ± 3.12$^{a*}$ | 0.750 ± 0.023$^{a}$ | 0.613 ± 0.095$^{c}$ | 12.50 ± 0.72 | 0.53 ± 0.028$^{a}$ |
| 2-AB Treated | 1940.85 ± 25.86$^{bc}$ | 3234.25 ± 35.61 | 1.669 ± 0.052 | 1901.22 ± 25.84$^{bc}$ | 6.00 ± 1.69 | 307.30 ± 11.19$^{ab}$ | 66.55 ± 0.38$^{a}$ | 20.25 ± 0.19$^{a}$ | 36.16 ± 0.38$^{a}$ | 188.92 ± 3.14$^{ab}$ | 0.750 ± 0.019$^{a}$ | 1.625 ± 0.282$^{a}$ | 12.54 ± 0.56 | 0.55 ± 0.028$^{a}$ |
| 3-Positive Ctrl. | 1915.87 ± 19.73$^{c}$ | 3206.00 ± 13.37 | 1.676 ± 0.028 | 1876.17 ± 19.73$^{c}$ | 8.00 ± 1.51 | 295.46 ± 11.27$^{b}$ | 65.47 ± 0.21$^{b}$ | 18.98 ± 0.11$^{b}$ | 34.99 ± 0.18$^{b}$ | 183.83± 2.26$^{b}$ | 0.608 ± 0.023$^{b}$ | 1.500 ± 0.252$^{ab}$ | 10.68 ± 1.04 | 0.61 ± 0.038$^{a}$ |
| 4-Blank Ctrl. | 1967.14 ± 21.16$^{abc}$ | 3230.25 ± 51.62 | 1.642 ± 0.024 | 1927.55 ± 21.16$^{abc}$ | 5.00 ± 1.25 | 318.75 ± 5.41$^{ab}$ | 66.61 ± 0.28$^{a}$ | 20.08 ± 0.21$^{a}$ | 36.47 ± 0.15$^{a}$ | 189.75 ± 2.98$^{ab}$ | 0.725 ± 0.037$^{a}$ | 0.775 ± 0.140$^{c}$ | 10.88 ± 0.32 | 0.40 ± 0.011$^{b}$ |
| *Probability* | 0.0224 | 0.8875 | 0.7472 | 0.0234 | 0.7805 | 0.0194 | 0.0053 | 0.0001 | 0.0001 | 0.0146 | 0.0010 | 0.0027 | 0.1180 | 0.0002 |

**Table 2:** Productive performance, carcass characteristics, low grade inflammation marker, total leucocytic count, heterophil/lymphocyte ratio of broiler chickens under challenging model of increased heat stress.

Means with different, lower case, superscripts, within age, are significantly different (P ≤ 0.05).

The hematological assay revealed numerical increase of total leucocytic count (TLC) in treated groups vs. PC group. Heterophil/lymphocyte ratio (H/L) revealed significant increase in PC group vs. BC group (Table 2). Greater H/L ratios are a dependable biomarker of stress in chickens [41]. Administration of the used acidifiers numerically ameliorated this increase.

Liver function enzymes and metabolites in the present investigation elucidated that PC group had lowered serum total protein than those treated with NaB or AB (provided that the effect of the first was more evident). Blank control (BC) chicken group showed lower serum total protein than PC group which is contrary to those reported by Zhang., *et al.* [40] who showed that protein content was lower in birds subjected to heat stress. Daneshyar., *et al.* [42] in cold-induced ascitic and healthy broiler chicks found that serum total protein of cold temperature treatment was lower than normal temperature treated birds. Significant increase in Aspartate Aminotransferase (AST) has been detected in NaB treated group with numerical increase in AB treated group vs. PC group (P ≤ 0.05). Numerical increase in Alanine Aminotransferase (ALT) and Lactate Dehydro-genase (LDH) with numerical decrease in Alkaline phosphatase (AKP) and Kinetic Creatine Kinase (CK) in NaB and AB treated groups vs. PC group. Kraljevic., *et al.* [43] reported that determination of a so-called enzyme profile in blood plasma activities may serve as an additional test for functional liver damages in chickens. Investigation of markers of plasma lipids showed a numerical increase (+28 ml/dl) in total cholesterol (TL) in the present investigation in PC group than BC one that could be attributed to the endocrinological changes caused by heat stress that stimulate lipid accumulation through increased lipogenesis, reduced lipolysis, and enhanced amino acid catabolism [44]. Supplementation of the used acidifiers significantly reduced this increase in total cholesterol (TL) and in high density lipid (HDL) (P ≤ 0.05). Low density lipids (LDL) and triglycerides (TG) showed numerical decrease in NaB and AB treated groups vs. PC group. Ascorbic acid showed numerical increase in NaB treated group and numerical decrease in AB treated group vs. PC group. Vitamin E showed numerical increase in NaB and AB treated groups vs. PC group. Ascorbic acid is the most important water-soluble antioxidant provided with feed and synthesised within the animal/chicken body [45]. Vitamin E is considered to be a main chain breaking antioxidant in biological systems and its roles in poultry production are greatly appreciated [46]. A significant decrease in HI titers against ND vaccination in PC group vs. BC group was detected that indicates the immunosuppressive effect of heat stress on broiler chickens (P ≤ 0.05). Numerical increase in HI titers reported in treated groups vs. PC group might elucidate their immunostimulant effect.

| Trait Treatment | Liver function enzymes and metabolites | | | | | | | | | Lipid Markers | | | | Vitamins | | Antibody Titer Against ND vaccination ($Log_2$) (HI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Serum Total Pro-tein (g/dl) | Serum Albumin (g/dl) | Serum Globulin (g/dl) | A/G Ratio | AKP (U/l) | ALT (U/ml) | AST (U/ml) * | CK (U/l) | LDH (U/l) | TG ml/dl | TL ml/dl | LDL ml/dl | HDL ml/dl | C mg/ml | E mg/ml | |
| 1-NaB Treated | 3.866 ± 0.208 | 2.446 ± 0.084 | 1.420 ± 0.245 | 2.109 ± 0.346 | 2147.5 ± 136.6 | 7.75 ± 1.01 | 222.1 ± 25.95$^{a}$ | 3564.4 ± 713.6 | 2161.0 ± 234.8 | 190.08 ± 16.62 | 147.50 ± 11.63$^{b*}$ | 91.04 ± 10.85$^{b}$ | 56.50 ± 7.08$^{b}$ | 47.13 ± 2.74 | 29.31 ± 6.78 | 5.50 ± 0.42$^{ab}$ |
| 2-AB Treated | 3.640 ± 0.150 | 2.460 ± 0.061 | 1.180 ± 0.155 | 2.433 ± 0.396 | 2580.0 ± 129.9 | 6.63 ± 1.67 | 152.1 ± 23.64$^{b}$ | 2691.1 ± 441.2 | 2447.6 ± 363.2 | 184.30 ± 18.31 | 153.90 ± 8.8$^{8}$b | 101.44 ± 9.63$^{ab}$ | 52.63 ± 5.93$^{b}$ | 37.13 ± 5.97 | 27.78 ± 5.64 | 5.92 ± 0.53$^{ab}$ |
| 3-Positive Ctrl. | 3.615 ± 0.187 | 2.408 ± 0.062 | 1.207 ± 0.160 | 2.294 ± 0.348 | 3379.5 ± 810.6 | 4.63 ± 1.45 | 118.9 ± 21.87$^{b}$ | 3890.8 ± 1009.6 | 1969.1 ± 412.1 | 194.15 ± 18.93 | 206.68 ± 21.17$^{a}$ | 126.88 ± 18.02$^{ab}$ | 80.00 ± 11.36$^{a}$ | 46.60 ± 3.31 | 26.36 ± 3.08 | 4.75 ± 0.22$^{b}$ |
| 4-Blank Ctrl. | 3.559 ± 0.077 | 2.406 ± 0.032 | 1.153 ± 0.089 | 2.175 ± 0.165 | 2460.0 ± 233.0 | 7.00 ± 1.13 | 128.5 ± 18.62$^{b}$ | 3555.5 ± 413.2 | 1531.9 ± 384.9 | 231.99 ± 12.01 | 178.68 ± 14.11$^{ab}$ | 142.39 ± 18.88$^{a}$ | 36.50 ± 5.63$^{b}$ | 41.24 ± 3.66 | 24.74 ± 4.71 | 6.50 ± 0.34$^{a}$ |
| *Probability* | 0.5672 | 0.9019 | 0.6818 | 0.9017 | 0.2430 | 0.412 | 0.0139 | 0.6474 | 0.3386 | 0.2194 | 0.0243 | 0.0409 | 0.0028 | 0.2708 | 0.9346 | 0.0225 |

**Table 3:** Results of clinical biochemical assay (Liver function enzymes and metabolites), Lipid markers, Vitamins (C and E), Hemagglutination inhibition titers (HI) of broiler chickens under challenging model of increased heat stress.

A/G ratio=Albumin/Globulin Ratio. AKP: Alkaline phosphatase; ALT: Alanine Aminotrans-ferase; AST: Aspartate Aminotransferase; CK: Kinetic Creatine Kinase; LDH: Lactate Dehydro-genase; TG: Triglycerides; TL: Total cholesterol; LDL: Low density lipid; HDL: High density lipid;

* Means with different, superscripts, within trait, are significantly different (P ≤ 0.05).

Oxidative stress has been regarded as one of the major factors negatively affecting performance of birds in condensed poultry industry [47]. Heat stress increases lipid oxidative stress peroxidation and depresses growth of birds [48]. Oxidative stability biomarkers carried out in the present study revealed numerical and significant decrease in GSH-Px and MDA respectively in heat stressed broiler chickens in PC group as compared with BC group. These findings are in complete accordance with those reported by other investigators [49,50]. Since the superoxide radical is the main free radical produced in physiological conditions in the cell [51] SOD is considered to be the main element of the first level of antioxidant defense in the cell [52]. In the present study; NaB treated group showed significant increase in SOD ($P \leq 0.05$), numerical increase in MDA and GSH-PX with numerical decrease in TAC vs. PC group. While AB treated group showed significant increase in MDA ($P \leq 0.05$), numerical increase in GSH-PX and numerical decrease in TAC vs. PC group (Table 4). Yang., *et al.* [53] mentioned that acute heat stress (35°C) in chickens was shown to induce a significant production of reactive oxygen species (ROS), which ultimately results in lipid peroxidation and oxidative stress. Regarding our results; it could be concluded that treatment with the acidifiers (NaB and AB) improved the oxidative stability responses in total antioxidant capacity (TAC), malnodialdehyde (MDA) and glutathione peroxidase (GSH-PX).

| Trait Treatment | Total antioxidant capacity (TAC) (mM/L) | Malnodialdehyde (MDA)(nmol//mL) | Superoxide dismutase (SOD) (U/mL) | Glutathione peroxidase (GSH-PX) (nmol//mL) |
|---|---|---|---|---|
| 1-NaB Treated Challenged | 1.442 ± 0.130 | 7.00 ± 0.84$^{ab*}$ | 92.31 ± 0.26$^{a}$ | 14.28 ± 1.46 |
| 2-AB Treated Challenged | 1.440 ± 0.117 | 9.32 ± 0.93$^{a}$ | 91.39 ± 0.19$^{b}$ | 13.20 ± 1.15 |
| 3-Positive Ctrl. | 1.516 ± 0.182 | 5.68 ± 0.89$^{b}$ | 91.48 ± 0.18$^{b}$ | 12.87 ± 1.33 |
| 4-Blank Ctrl. | 1.412 ± 2.15 | 9.22 ± 1.27$^{a}$ | 90.89 ± 0.16$^{b}$ | 14.83 ± 1.56 |
| Probability | 0.4007 | 0.0312 | 0.0001 | 0.7284 |

**Table 4.** Oxidative stress parameters of plasma of broiler chickens under challenging model of increased heat stress.

* Means with different, superscripts, within trait, are significantly different ($P \leq 0.05$)

Heat stressed untreated PC group showed massive hepatocellular necrosis associated with diffuse vacuolization of hepatocytes, sinusoidal congestion and massive mononuclear cell and heterophiles infiltration in hepatic portal areas together with severe lymphoid depletion and lymphocytolysis of lymphoid elements of spleen, severe lymphocytic depletion of cortex and medulla with heterophiles infiltration of Bursa of Fabricius (BF), moderate lymphoid depletion with lymphocytolysis of thymic cortex and cecal tonsil. These severe detrimental effects markedly ameliorated in acidifiers treated groups (Figure 2 and 3) where there were few heterophiles infiltrating the portal area of liver associated with individual cell necrosis and mild vacuolization of hepatocytes, mild lymphoid depletion of lymphoid elements comprising the splenic lymphoid follicles, cortex and medulla of BF, thymus gland cortex accompanied with mild lymphocytolysis of cecal tonsils. Microscopic lesion scoring of liver showed significant reduction in hepatocellular vacuolization and necrosis in NaB and AB treated groups vs. PC group ($P \leq 0.05$). Caecal tonsils showed significant lymphoid depletion in both treated groups vs. PC group ($P \leq 0.05$). Bursa of Fabricius (BF) showed significant reduction in heterophiles infiltration in both treated groups vs. PC group with significant and numerical decrease in lymphoid depletion in BF of NaB and AB vs. PC group respectively ($P \leq 0.05$) (Table 5). The obtained deleterious findings of heat stress on immune organs (thymus, BF and cecal tonsils) are on line with those reported by Aengwanich [54] who found reduction of lymphocytes number involving the cortex and medulla of BF. Lin., *et al.* [55] reported that high ambient temperature is one of the most important stressors causing economic losses to the poultry industry, including immunosuppression. Heat stress is known to limit the immunocompetence of a hen through decreasing antibody production [38]. The numerical increase in HI titers reported in treated groups vs. PC group together with the mitosis recorded in lymphoid elements of cecal tonsils with the increase in tonsilar size of broilers treated with NaB confirm its

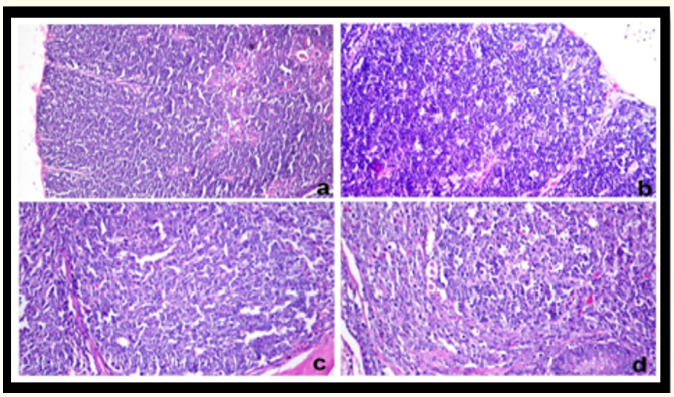


**Figure 2:** a) Thymus gland of NaB treated group showing mild lymphocytic depletion of thymic cortex. b) Thymus gland of positive control group showing moderate lymphocytic depletion involving thymic cortex. c) Cecal tonsils of AB treated group showing individual lymphocytolsis with increased mitosis of lymphoid elements with increased tonsilar size. d) Cecal tonsils of positive control group showing moderate depletion of lymphoid elements with lymphocytolysis (H and E).

immunomodulatory effects that can oppose this immunosuppression.

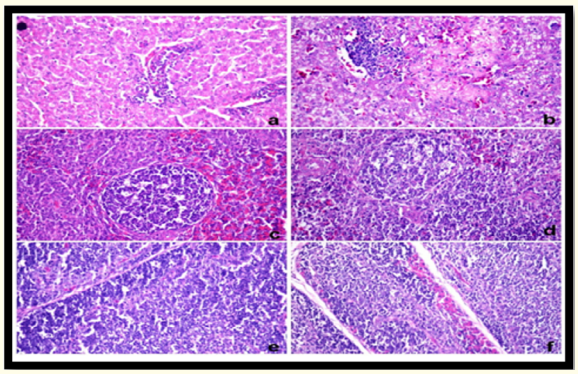


**Figure 3:** a) Liver of NaB treated group showing mild heterophiles infiltrating the portal area with individual hepatocellular vacuolation and necrosis. b) Liver of positive control group showing large foci of hepatocellular necrosis with diffuse vacuolization of surrounding hepatocytes associated with sinusoidal congestion and mononuclear cell infiltration. c) Spleen of NaB treated group showing lymphocytolysis and depletion of individual lymphoid elements. d) Spleen of positive control group showing moderate lymphoid depletion comprising the lymphoid follicle. e) Bursa Fabricius of AB treated group showing mild lymphoid depletion of cortical and medullary area with mitosis of lymphocytes. f) Bursa Fabricius of positive control group showing severe cortical and medullary lymphocytic depletion with heterophiles infiltration. (H and E).

| Trait Treatment | Organs | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Liver | | | Spleen | Caecal tonsils | BF | | Thymus glands | |
| | Portal heterophiles infiltration | Hepato-cecellular vaculization | Necrosis | Lymphoid depletion | Lym-phocytic depletion | Lymphoid depletion | Hetero-philes infiltration | Lymphoid depletion | Heterophiles infiltration |
| 1-NaB Treated Challenged | 1.000 ± 0.167$^{a*}$ | 1.571 ± 0.300$^{b}$ | 0.833 ± 0.167$^{b}$ | 1.714 ± 0.286$^{a}$ | 1.000 ± 0.000$^{b}$ | 1.222 ± 0.222$^{b*}$ | 0.000 ± 0.000$^{b}$ | 1.818 ± 0.352$^{a}$ | 1.250 ± 0.313$^{a}$ |
| 2-AB Treated Challenged | 1.556 ± 0.176$^{a}$ | 1.714 ± 0.286$^{b}$ | 1.286 ± 0.184$^{b}$ | 1.571 ± 0.202$^{a}$ | 1.250 ± 0.295$^{b}$ | 1.500 ± 0.167$^{ab}$ | 0.000 ± 0.000$^{b}$ | 1.222 ± 0.147$^{a}$ | 0.286 ± 0.286$^{b}$ |
| 3-Positive Ctrl. | 1.143 ± 0.404$^{a}$ | 3.86 ± 0.421$^{a}$ | 2.143 ± 0.340$^{a}$ | 1.125 ± 0.125$^{a}$ | 2.222 ± 0.222$^{a}$ | 1.917 ± 0.260$^{a}$ | 0.800 ± 0.291$^{a}$ | 1.364 ± 0.152$^{a}$ | 0.625 ± 0.183$^{ab}$ |
| 4-Blank Ctrl. | 0.167 ± 0.167$^{b}$ | 1.000 ± 0.218$^{b}$ | 1.400 ± 0.245$^{ab}$ | 0.444 ± 0.176$^{b}$ | 0.700 ± 0.213$^{b}$ | 0.333 ± 0.167$^{c}$ | 0.000 ± 0.000$^{b}$ | 0.444 ± 0.176$^{b}$ | 0.000 ± 0.000$^{b}$ |
| ***Probability*** | 0.0051 | 0.0002 | 0.0097 | 0.0003 | 0.0001 | 0.0001 | 0.0026 | 0.0026 | 0.0057 |

**Table 5.** Histopathological (microscopic)lesion scoring of broiler chickens under challenging model of increased heat stress.

* Means with different, superscripts, within trait, are significantly different (P ≤ 0.05).

## Conclusion

Our overall results suggest that heat stress resulted in detrimental effects on growth performance, carcass characteristics, hematological, clinical biochemical parameters, low grade inflammatory marker, oxidative stability markers and histology of liver and immune organs. As food safety is increasingly being considered an important part of the modern food quality concept [34], it is of great importance to be aware that environmental stresses, such as heat stress, can potentially alter the host-pathogen interaction which negatively affects fat deposition and meat quality in broilers [56]. It is also associated with undesirable meat characteristics and quality loss [41]. The potential advantage of the used NaB and AB in drinking water has been proven and elucidated their positive role in ameliorating these detrimental effects in broiler chickens. Moreover; NaB and AB could be considered antioxidants that prevents oxygen radical from damaging cells that can develop from cell injury and inflammation due to heat stress.

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## Conflict of Interest

The authors declare that they have no conflict of interests.

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