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Original Article

Effect of Dietary Inclusion of Sodium Bicarbonate on Digestibility of Nutrients and Immune Response in Caged Layers During the Summer

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ABSTRACT

The aim of this project was to investigate the effect of dietary inclusion of sodium bicarbonate ($NaHCO_3$) on nutrient digestibility and immune response of caged layers during summer when the temperature exceeds 40 °C. For immune response trial, White Leghorn layers (n=160; 24 weeks old) were purchased from a poultry farm and were divided into five treatment/diets groups (4 replicate/treatment). Diet A, was without $NaHCO_3$ and served as control whereas, diets B, C, D, and E contained 0.5, 1.0, 1.5 and 2.0% $NaHCO_3$, respectively. All these birds were vaccinated against Newcastle disease (ND) virus at the start of the experiment and thereafter with one-month intervals. Blood samples were collected from two birds/replicate at 10 days post vaccination each time to check antibody titer against ND virus. For digestibility trial, fecal samples were collected (6 layers/treatment group) at the start of the 37th week of age for two days, at 3 hours interval. Results revealed that immune response against ND virus 10 days post vaccination after 1st, 2nd and 3rd month was significantly ($p<0.05$) higher in layer birds fed diets containing $NaHCO_3$. Digestibility of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and absorption of minerals were also found to be significantly ($p<0.05$) higher in groups treated with $NaHCO_3$ and the birds fed diet containing 1% $NaHCO_3$ showed the best results. In general, results revealed that 1% supplementation of $NaHCO_3$ in layers' diet have a beneficial impact in terms of immunity and diet digestibility.

INTRODUCTION

Birds are able to maintain their body temperature within narrow limits and an increase in body temperature due to higher ambient temperature or excessive metabolic activities may cause irreversible thermoregulatory events that could be harmful to the existence of the birds (Abbas *et al.*, 2017). Different researchers have found good result on the performance by the supplementation of AA in chickens (Saeed *et al.*, 2018a and Saeed *et al.*, 2018b) and heat stress has shown detrimental effects on feed intake, growth and feed conversion ratio of the birds (Al-Hassani *et al.*, 2001; Ekanayake *et al.*, 2004; Li *et al.*, 2015; Saeed *et al.*, 2017; Mohammed *et al.*, 2018). This reduction of performance might be explained by decreased digestibility of nutrients, increased heat production and reduced protein retention (Fouad *et al.*, 2016; Orhan *et al.*, 2018). Birds exposed to heat stress has shown reduced amylase and maltase activities, decreased protein and amino acid digestibility of complete diets and individual feed ingredients (Bayati *et al.*, 2017) and reduced minerals absorption (Belhadj *et al.*, 2016; Goff, 2018). The gastrointestinal size was also reported to decrease in heat-exposed chickens (Orhan *et al.*, 2018).

Sodium bicarbonate ($NaHCO_3$) is a white solid crystalline compound soluble in water, which is commonly used as an antacid to treat acid indigestion. It is commonly added as a simple solution for restoring the pH of water that has a high level of chlorine (Whiting *et al.,* 1991). Sodium bicarbonate in feed or water has shown potential benefits on production performance (Ahmad *et al.,* 2005; Khattak *et al.,* 2012; Peng *et al.*, 2013), egg characteristics (Kaya *et al.,* 2004; Jiang *et al.*, 2015) and blood profile (Kurtoglu *et al.,* 2007) in poultry birds exposed to heat stress.

Sodium bicarbonate in the diet of layers may improve nutrient digestibility by increasing sodium ions concentration (Fethiere *et al.,* 1994); improving electrolyte balance in poultry diets (Borges *et al.,* 2003); meeting the requirements for the HCO_3^- ions (Gorman & Balnave, 1994) and decrease the losses caused by heat stress (Abbas *et al.,* 2017). It is cheap, easily available and easy to handle, therefore, can be safely incorporated in poultry diets to ameliorate the adverse effects caused by heat stress.

There is some evidence on the beneficial effect of $NaHCO_3$ on immune response (Fouad *et al.,* 2016) and nutrient digestibility (Lin *et al.,* 2006), in broilers exposed to high ambient temperature. However, information on the effect of $NaHCO_3$ on immune response and nutrient digestibility in caged layers during summer are scanty. Therefore, the present trial was carried out to study the effects of dietary inclusion of sodium bicarbonate on immune response against Newcastle disease virus and *in vivo* digestibility of DM, CP, CF, and EE. The effect of the addition of this compound in poultry diets was also studied on the absorption of some minerals *i.e.* calcium, phosphorus, sodium, potassium, and iron, in caged layers during the summer.

MATERIALS AND METHODS

Birds and housing

All the animal experimentation protocols were approved by the Directorate of Graduate Studies, University of Agriculture (UAF), Faisalabad (Pakistan). The experiment was conducted during the summer season when the temperature exceeds 40 ˚C. One hundred sixty commercial layers of 24 weeks of age having initial body weight as1328±14.3 of group A, 1310 ±7.0 of group B, 1318 ±16.4 of group C, 1324 ±11.0 of group D, 1312 ±12.1 of group E, were purchased from a commercial poultry farm. These layers were divided into 20 experimental units/replicates (8 layers/ replicate). These replicates were further allotted to five treatment groups (4 replicate/ treatment). Experimental birds were maintained in individual cages in a thoroughly cleaned and disinfected Poultry House of the Department of Parasitology, Faculty of Veterinary Sciences, UAF. These birds were maintained under similar managemental conditions like floor space, relative humidity, temperature and light in the open house.

Initially, these birds were reared in a group and were fed commercial layer ration during the 24 first weeks of age as an adaptation period. Thereafter, at the start of the 25th week, all the birds were individually weighed and transferred randomly to the individual cages using Completely Randomized Design. Each cage was supplied with a feeder and drinker line. The length, width and height of each cage were 41, 39 and 37 cm, respectively. Daily, 17 hours of light was provided to the birds throughout the experiment. A dry bulb thermometer was installed in the center of the house to record daily ambient temperature. Whereas, daily records of relative humidity inside the poultry house were maintained by using a digital hygrometer.

Experimental diets

Five experimental diets *i.e.* A (control, without Sodium bicarbonate), B (0.5% Sodium bicarbonate), C (1 % Sodium bicarbonate), D (1.5% Sodium bicarbonate) and E (2% Sodium bicarbonate) were used. Before the start of the experiment, all the diets were analyzed for their proximate chemical composition according to the technique described by AOAC (2010), in the Analytical Laboratory of the Institute of Animal Sciences, Faculty of Animal Husbandry, University of Agriculture, Faisalabad (Pakistan). Proportions of ingredients used in the experimental diets are shown in table 1. A weighed amount of the experimental diets was fed twice a day (morning and evening). All the diets were iso-nitrogenous (CP 16 %) and iso-caloric (ME 2700 Kcal/Kg diet) and were fed to the experimental birds (NRC, 1994), from 25-36 weeks of age (12 weeks).

Determination of antibody titer against Newcastle disease virus

At the end of the 36th week, five ml of blood was collected from healthy adult birds (wing-web) in a screw-top test tube having 1 mg/ml EDTA as an anticoagulant. The test tube was gently rotated for the mixing of blood and anticoagulant, but great care was taken to avoid hemolysis. Heamagglutination

Table 1 – Proportion of the ingredients used in the experimental diets and their proximate composition.

Ingredients (%)	A Basal diet	B 0.5% $NaHCO_3$	C 1% $NaHCO_3$	D 1.5% $NaHCO_3$	E 2% $NaHCO_3$
Maize	31.50	28.00	29.00	30.60	30.60
Rice broken	30.20	30.00	30.00	30.00	30.00
Fish meal CP 48%	3.60	5.50	7.00	7.00	7.00
Soybean meal, CP 45%	17.00	1.50	0.00	2.00	4.40
Canola meal, CP 35%	4.50	14.00	13.60	11.60	8.40
Rapeseed meal, CP 34%	3.10	3.00	3.00	3.00	3.00
Guar meal, CP 38.5%	0.00	2.50	3.00	3.00	3.00
Corn gluten 60%	0.00	2.00	2.00	2.00	2.00
Rice polishing	0.00	2.20	2.00	0.00	0.00
Dicalcium phosphate	0.50	0.00	0.00	0.00	0.00
Limestone	9.00	9.00	8.70	8.70	8.70
Mineraland vitamin Premix	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.13	0.08	0.07	0.07	0.09
Lys. sulphate 65%	0.00	0.15	0.14	0.13	0.12
Salt	0.23	0.00	0.17	0.17	0.18
Sodium bicarbonate	0.00	0.50	1.00	1.50	2.00
Allzyme	0.015	0.015	0.015	0.015	0.015
Lincomix	0.02	0.02	0.02	0.02	0.02
Proximate composition					
ME Kcal/Kg	2700	2700	2700	2700	2700
Crude protein (%)	17.00	17.00	17.00	17.00	17.00
Crude fiber (%)	3.29	3.8	3.74	3.44	3.39
Crude fat (%)	3.27	3.9	3.9	3.67	3.65
Crude ash (%)	11.97	11.9	12.01	11.85	11.89

inhibitions (HI) test for determination of serum antibody titre was determined as described by Maff (1984).

Determination of nutrient digestibility and absorption of minerals

A digestibility trial was conducted during the experiment, at 36 weeks of age. For this purpose a separate group of 30 pullets was obtained from the same batch used for the performance trial. These layers were randomly allotted to five treatments (6 birds/ treatment) such that each bird served as a replicate. These pullets were fed rations mixed with cellite (acid insoluble ash; AIA) at the rate of 1% as a marker.

The birds of these groups were fed their respective diets for one week (week 37) to assure that the passage of marker (AIA) in the feces of the birds was stabilized (Sales & Janssens, 2003) and during this period feces were not collected. After stabilization of the marker in the feces (week 38), all the birds were offered the same amount of their respective diets. The feed offered to the birds was divided into two equal portions and half of the feed was given at 9:00 am, and the rest at 9:00 pm. The feed not eaten was removed from the feeders and weighed at the end of the digestibility period.

Excreta collections, which started at the 8th experimental day, were made for a period of 48 hours (2 consecutive days) at two hours interval. Excreta samples were immediately frozen after each collection. The samples (feed and excreta) were analyzed thus collected were dried, finely ground and then analyzed for the determination of digestibility of dry matter (DM), crude protein (CP), ether extract (EE) and crude fiber (CF) contents using the method described by AOAC (2010). The samples (feed and excreta) were also analyzed for their mineral contents (Ca, P, Na, K, Fe and Mg) using atomic absorption spectrophotometer (Perkin Elmer, Beaconsfield, UK).

Digestibility of the nutrient was calculated by the following formula (Sales & Janssens, 2003):

$$D(\%) = 100 - \frac{\text{Acid insoluble ash in feed}}{\text{Acid insoluble ash in feces}} \times \frac{\text{Nutrient in feces}}{\text{Nutiente in fedd}} \times 100$$

Statistical analysis

The data thus collected were subjected to statistical analysis for interpretation of results using completely randomized design (CRD). Treatment means were compared by the Least Significance Differences test (Steel *et al.,* 1997).

RESULTS

Antibody titer against Newcastle disease virus 10 days post vaccination during the 1st, 2nd and 3rd month is given in table 2. Findings of the study depicted that serum antibody titre against Newcastle disease virus of the birds 10 days post 1st vaccination was significantly influenced due to dietary inclusion of $NaHCO_3$ in their diets. Birds of group C, which were fed diet containing 1% $NaHCO_3$, showed maximum serum antibody titer against Newcastle disease virus when compared to the birds of other treated groups.

Statistical analysis of the data revealed that birds using diets containing $NaHCO_3$ exhibited significantly (p<0.05) higher serum antibody titer against Newcastle disease virus,10 days post the 2nd vaccination, when compared to those of the control group. Birds of group D, which were fed diet containing 1.5% $NaHCO_3$, showed maximum serum antibody titer against Newcastle disease virus when compared to the birds of other treated groups. Findings of the study depicted that serum antibody titer against Newcastle disease virus of the birds 10 days post the 3rd vaccination was significantly influenced due to the dietary inclusion of $NaHCO_3$ in their diets. Birds of group C, which were fed diet containing 1% $NaHCO_3$, showed maximum serum antibody titer against Newcastle disease virus when compared to the birds of other treated groups.

Table 2 – Effect of dietary inclusion of sodium bicarbonate on immune response against Newcastle disease of caged layers.

Variables	Treatment				
	A Control	B 0.5%$NaHCO_3$	C 1%$NaHCO_3$	D 1.5%$NaHCO_3$	E 2%$NaHCO_3$
1st Vaccination	43 ^{d}	86 ^{cd}	167^{a}	145 ^{ab}	118 ^{bc}
2nd Vaccination	135 ^{b}	154 ^{b}	237 ^{a}	263 ^{a}	205 ^{ab}
3rd Vaccination	208 ^{b}	272 ^{ab}	368 ^{a}	288 ^{ab}	272 ^{ab}

Values within the same row which have different superscripts are significantly different (p<0.05).

Mean values regarding digestibility of DM, CP, CF and EE in birds fed diets with or without dietary inclusion of $NaHCO_3$ are shown in table 3. The results revealed a significant (p<0.05) effect on DM digestibility due to the inclusion of $NaHCO_3$ in the diets of layer when compared to those of the control group. The differences in DM digestibility values were also found to be significant among the treated groups. Birds using diet containing 1% $NaHCO_3$ exhibited maximum digestibility followed by those of group D, B and E, whereas, the lowest DM digestibility was recorded in the control group.

The results showed a significant effect on CP digestibility due to the inclusion of $NaHCO_3$ in the diets of layer when compared to those of the control group. Birds using diet containing 1% $NaHCO_3$ exhibited maximum digestibility followed by those of group B, D and E, whereas, the lowest CP digestibility was recorded in the control group. Statistical analysis of the data depicted that the birds of treated groups, using diets containing $NaHCO_3$ showed significantly (p<0.05) higher CF digestibility as compared to those of the control group. Birds using diet containing 1% $NaHCO_3$ exhibited maximum digestibility followed by those of group B, D and E, whereas, the lowest CF digestibility was recorded in the control group. The results of the present study revealed a significant effect on EE digestibility due to the inclusion of $NaHCO_3$ in the diets of the layer when compared to those of the control group. Birds using diet containing 1% $NaHCO_3$ exhibited maximum digestibility followed by those of group B, D, and E, whereas, the lowest EE digestibility was recorded in the control group.

Mean values pertaining to the absorption of minerals *i.e.* calcium, phosphorous, iron, sodium and potassium in birds fed diets with or without the dietary inclusion of $NaHCO_3$ are shown in table 4. The results revealed a significant effect on absorption of Ca due

Table 3 – Effects of dietary inclusion of sodium bicarbonate on nutrient digestibility in layers.

Variables	Treatment				
	A Control	B 0.5%$NaHCO_3$	C 1%$NaHCO_3$	D 1.5%$NaHCO_3$	E 2%$NaHCO_3$
Dry matter (%)	70.4± 6.09^{c}	73.4±5.89^{b}	77.1 ±5.79^{a}	74.1± 6.34^{b}	71.6±6.36^{c}
Crude protein (%)	68.6±5.72^{c}	72.7±4.65^{a}	75±3.27^{a}	72.2±3.38^{ab}	69.2±5.44^{bc}
Crude fibers (%)	29.0±2.81^{b}	33.9±2.36^{ab}	40.7 ±2.85^{a}	33.2 ±2.1^{ab}	30.9±2.95^{b}
Ether extract (%)	82.0±5.57^{b}	89.4± 4.02^{a}	93.7± 5.46^{a}	84.8±7.78^{b}	82.7± 6.67^{b}

Values within the same row which have different superscripts are significantly different (p<0.05)

Table 4 – Effect of dietary inclusion of sodium bicarbonate on absorption (%) of the minerals in layers.

Variables	Treatment				
	A Control	B 0.5%NaHCO$_3$	C 1%NaHCO$_3$	D 1.5%NaHCO$_3$	E 2%NaHCO$_3$
Calcium (%)	56.1± 3.29^c	58.3±3.10^b	60.3±3.20^a	58.1± 3.28^b	54.8 ±3.19^c
Phosphorus (%)	50.8±2.32^c	53.5±3.09^b	57.6±3.27^a	53±3.33^b	49.6±2.40^c
Iron (%)	50.5 ± 4.36^d	53.1±3.38^c	60.8 ±5.34^a	55.6 ±5.2^b	50±4.27^d
Sodium (%)	51.6± 2.1^c	54.1± 1.36^b	59.2± 1.1^a	53.5±1.11^b	51.6.± 1.913^b
Potassium (%)	51.8±2.10^c	53.5±2.47^b	56.3±3.64^a	53.5±3.64^b	52±3.47^c

Values within the same row which have different superscripts are significantly different (p<0.05)

to the inclusion of NaHCO$_3$ in the diets of the layer when compared to those of the control group. Birds using diet containing 1% NaHCO$_3$ exhibited maximum absorption followed by those of group B, D and E, whereas, the lowest value was recorded in the controls.

The results revealed a significant effect on absorption of P due to the inclusion of NaHCO$_3$ in the diets of layer when compared to those of the control group. The birds using diet containing 1% NaHCO$_3$ exhibited maximum absorption followed by those of group B, D and E, whereas, the lowest P absorption was recorded in the control group. The results revealed a significant effect on absorption of Iron due to the inclusion of NaHCO$_3$ in the diets of layer when compared to those of the control group. Birds using diet containing 1% NaHCO$_3$ exhibited maximum absorption followed by those of group D, B and E, whereas the lowest iron absorption was recorded in the control group. The results revealed a significant effect on absorption of Na due to the inclusion of NaHCO$_3$ in the diets of layer when compared to those of control group. Birds using diet containing 1% NaHCO$_3$ exhibited maximum absorption followed by those of group B, D and E, whereas, the lowest Na absorption was recorded in the birds of the control group. The results revealed a significant effect on absorption of K due to the inclusion of NaHCO$_3$ in the diets of layer when compared to those of the control group. Birds using diet containing 1% NaHCO$_3$ exhibited maximum absorption followed by those of group B, D and E, whereas, the lowest digestibility of K was recorded in the control group.

DISCUSSION

Dietary inclusion of different levels of NaHCO$_3$ depicted a significant increase in antibody titer against Newcastle disease in layers when compared to those fed diet without its addition. Environmental stressors have been known to affect immunity and innate resistance of the host directly or indirectly (Rakib *et al.,* 2016). Therefore, increase in antibody titer against Newcastle disease virus in birds fed diets containing different levels of NaHCO$_3$ may probably be due either to less heat stress upon these birds because of reduction in their body temperature or lower cortisol concentration as compared to those of control group, or both. Results of the present study are compatible with the findings of Khatak *et al.* (2012) who reported higher hemagglutination inhibition titer against Newcastle disease virus in birds consuming diets containing NaHCO$_3$.

Borges *et al.* (2003) have observed that an increase in dietary electrolyte balance may cause a decrease in heterophil to lymphocyte ratio in blood, leading to an increase in antibody titer. Similarly, Santin *et al.* (2003) have reported a significant linear increase in Newcastle disease virus antibody titers with increasing DEB (40, 140, 240, 340mEq/kg), using NaCl, NaHCO$_3$ and NH$_4$Cl as supplements, Therefore, it may safely be concluded that the dietary addition of NaHCO$_3$ may improve antibody titter against Newcastle disease virus in layers.

Diets containing NaHCO$_3$ exhibited better digestibility of DM in layers. Increase in digestibility of dry matter (DM) of the treated groups may be due to more sodium ions concentration in the rations containing NaHCO$_3$. A similar effect of increased sodium ions concentration in broilers has been observed by Fethiere *et al.* (1994). Dietary inclusion of NaHCO$_3$ might have improved the electrolyte balance in the diet by creating favorable conditions for improvement in digestibility of nutrients (Borges *et al.,* 2003; Mahmud *et al.,* 2010).

Another probable explanation of better digestibility of DM in the birds fed diets containing NaHCO$_3$ may be that pancreatic juices which are involved in digestion of most of the nutrients essentially contain NaHCO$_3$. The presence of NaHCO$_3$ in pancreatic juice, neutralizes the high acidity of chyme and raises it to be alkaline to prepare the chyme for the process of nutrient absorption, which takes place in the small intestine (Leeson & Summer, 2001). Therefore, increased digestibility of DM in treated groups may have been due to higher bicarbonate and sodium levels.

Heat stress may exert a negative influence on digestion and absorption of dietary nutrients as well as their metabolism (Puvadolpirod & Thaxton, 2000; Deraz, 2018), as has been observed in the birds of the control group. Therefore, decreased digestibility of DM in the control group may have been due to lower bicarbonate and sodium levels. On the other hand, the presence of $NaHCO_3$ in the diets of treated birds might have improved their digestibility and prevented losses caused by heat stress (Mirsalimi *et al.,* 1993). However, beneficial effects of $NaHCO_3$ can be achieved only when its recommended optimum levels are incorporated in the diets. An excessive level of this chemical compound in the diet has been reported to be toxic in White Leghorn layers (Davison & Wideman, 1992). Therefore, this may be the probable reason for decreased digestibility of DM in group E, which was fed a diet containing 2% $NaHCO_3$. Another reason for decrease in digestibility of dry matter in these birds might have been increased passage rate of digesta (Ravindran *et al.,* 2008). However, Ahmad (1997) observed that DM digestibility in broilers was not influenced due to dietary inclusion of $NaHCO_3$.

The birds fed diet without the inclusion of $NaHCO_3$ (control) exhibited the lowest digestibility of protein. At an ambient temperature above 30°C, the thermoregulatory system is activated and causes an increase in blood flow to upper respiratory tract and other organs associated in heat excretion *i.e.,* combs and wattles, which causes a decrease in blood flow to the digestive tract (Wolfenson, 1986). Consequently, activities of proteolytic enzymes in the upper part of the digestive system are decreased, ultimately leading to a decrease in protein digestibility. Considering the fact that heat stressed birds use glucogenic amino acids for glucose production (Nelson & Cox, 2000) during the process of gluconeogenesis in the birds, which is a metabolically expensive process (Nelson & Cox, 2000), provision of $NaHCO_3$ in their diets can decrease glucose production from amino acids, which may lead to improved digestibility of protein during stress.

The addition of $NaHCO_3$ in the diet of layers exhibited more digestibility of protein in these birds as compared to those of the control group. Protein consumed by the birds is broken down by the action of certain enzymes in the gastrointestinal tract to its constituent amino acids prior to absorption, and most of these amino acids require sodium (Leeson & Summer, 2001) for this process. Therefore, the increase in digestibility of protein of the treated groups may probably be due to the presence of more sodium ions concentration in the rations containing sodium bicarbonate. Sodium containing compounds such as sodium bentonite has been successfully used in sorghum containing diets to prevent deleterious effects of tannins present in it, on digestibility of protein (Pasha *et al.,* 2008).

Dietary inclusion of different levels of $NaHCO_3$ has depicted a significant increase in the digestibility of crude fibers in layers when compared to that fed diet without its addition. The increase in the digestibility of crude fibers of the treated groups may probably be due to the availability of more sodium and bicarbonate ions concentration in rations containing sodium bicarbonate. Dietary inclusion of $NaHCO_3$ has also shown to improve electrolyte balance in poultry diets by creating physiological conditions favorable for improvement in digestibility of nutrients. Pasha *et al.* (2008) used different levels of sodium bentonite in broiler rations and found an improvement in nutrient digestibility as compared to the control group (without sodium bentonite). Similarly, Salari *et al.* (2006) have also observed improvement in nutrient digestibility because of the addition of sodium bentonite in broiler diets.

Digestibility of ether extract was found to be significantly better in the birds using diets containing $NaHCO_3$ as compared to those of untreated group. Hyperthermia seems to be the most possible contributing factor for decreased digestibility and absorption of ether extract in the birds fed diet without sodium bicarbonate as have been observed by Koh & Macleod, (1999). Leeson & Summer, (2001), while discussing the factors affecting digestibility of fats, have also stated that fat digestibility is negatively affected in the birds exposed to heat stress. These findings are compatible with those observed in birds maintained under heat stress conditions.

Dietary inclusion of $NaHCO_3$ significantly influenced the absorption of all the minerals (Ca, P, Fe, Na and K), which were studied in this trial. Birds using diets containing $NaHCO_3$ exhibited better absorption of these minerals as compared to those of the untreated group. Minerals and trace elements are essential for optimum performance (Leeson & summers, 2001) of poultry birds. Therefore, increased mineral absorption in $NaHCO_3$ fed birds may probably be due either to more availability of minerals as a result of increased feed intake or due to improved electrolyte balance or both (Borges *et al.,* 2003).

CONCLUSIONS

The use of $NaHCO_3$ proved to be a better choice to be included in the diets of commercial layers to reduce or at least ameliorate the harmful effects of heat stress on immune response against ND virus and nutrient digestibility during summer conditions.

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REFERENCES

Abbas G, Mahmood S, Nawaz H. Effect of dietary inclusion of sodium bicarbonate on blood profile of caged layers during summer. Pakistan Journal of Agricultural Sciences 2017;54(2):443-450.

Ahmad T, Sarwar M, Nisa M, Haq A, Hasan Z. Influence of varying sources of dietary electrolytes on the performance of broilers reared in a high temperature environment. Animal Feed Science and Technology 2005;120:277–298.

Ahmad R. Growth performance and electrolyte balance of broiler as affected by two sources of sodium. *M. Sc. (Hons.)* [thesis]. Faisalabad (Punjab): Department of Poultry Science, University of Agriculture; 1997.

Al-Hassani DH, Al-Daraji HJ, Abdul-Hassan IA. Effect of dietary sodium bicarbonate on some physiological parameters in Hisex Brown layers reared under high environmental temperature. Recent Advances in Animal Nutrition in Australia 2001;13:32A.

AOAC- Official Methods of Analysis. Association of official analytical chemists. 19th ed. Washington; 2010.

Balnave D, Brake J. Different responses of broilers at low, high or cyclic moderate – high temperature to dietary sodium bicarbonate supplementation due to differences in dietary formulation. Australian Journal of Agriculture Research 2001;52:609-613.

Bayati S, Salari S, Tatar A, Sari M, Mirzadeh K. Effect of different levels of *Salvia mirzayanii* essential oil on performance, some blood and immunity parameters of broiler chickens under heat stress conditions. Animal Production Research 2017;6(4):69-80.

Belhadj Slimen I, Najar T, Ghram A, Abdrrabba M. Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review. Journal of Animal Physiology and Animal Nutrition 2016;100(3):401-412.

Bonnet S, Geraert PA, Lessire M, Carre B, Guillaumin S. Effect of high ambient temperature on feed digestibility in broilers. Poultry Science 1997;76(6):857-863.

Borges SA, Fisher da Silva AV, Ariki J, Hooge DM, Cummings KR. Dietary electrolyte balance for broiler chickens under moderately high ambient temperatures and relative humidities. Poultry Science 2003;82:301–308.

Davison S, Wideman RF. Excess sodium bicarbonate in diet and its effect on Leghorn chicken. British Poultry Science 1992;33:859–870.

Ekanayake S, Silva SS, Priyankarage N, Herath UT, Jayasekara MU, Horadagoda NU, *et al.* Effect of excess sodium in feed on haematological parameters and plasma sodium level in broiler chickens. British Poultry Science 2004;1:53-54.

Fethiere R, Miles RD, Harms RH. The utilization of sodium in sodium zeolite A by broilers. Poultry Science 1994;73:118-121.

Fouad AM, Chen W, Ruan D, Wang S, Xia WG, Zheng CT. Impact of heat stress on meat, egg quality, immunity and fertility in poultry and nutritional factors that overcome these effects: a review. International Journal of Poultry Science 2016;15(3):81-95.

Goff JP. Invited review: mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of Dairy Science 2018;101(4):2763-2813.

Gorman I, Balnave D. Effects of dietary mineral supplementation on the performance and mineral excretions of broilers at high ambient temperatures. British Poultry Science 1994;35:563–572.

Hai L, Rong D, Zhang ZY. The effect of thermal environment on the digestion of broilers. Journal of Animal Physiology and Animal Nutrition 2000;83(2):57-64.

Jiang MJ, Zhao JP, Jiao HC, Wang XJ, Zhang Q, Lin H. Dietary supplementation with sodium bicarbonate improves calcium absorption and eggshell quality of laying hens during peak production. British Poultry Science 2015;56(6):740-747.

Kaya I, Karademir B, Ukar O. The effects of diet supplemented with sodium bicarbonate upon blood pH, blood gases and eggshell quality in laying geese. Veterinary Medicine Czech 2004;49:201–206.

Khattak FM, Acamovic T, Sparks N, Pasha TN, Hussain MH, Joiya M, *et al.* Comparative efficacy of different supplements used to reduce heat stress in broilers. Pakistan Journal of Zoology 2012;44:31-41.

Koh K, Macleod MG. Effects of ambient temperature on heat increment of feeding and energy retention in growing broilers maintained at different food intakes. British Poultry Science,1999;40:511-516.

Kurtoglu V, Kurtoglu F, Balevi T. Effects of sodium bicarbonate, potassium chloride and sodium chloride supplementation on some blood biochemical parameters in laying hens. Proceedings of the European Symposium on Poultry Nutrition; 2007 Aug 26-30; Strasbourg. France. p.189-192.

Leeson S, Summers JD. Scott's nutrition of the chicken. 4th ed. Guelph: University Books; 2001.

Li M, Wu J, Chen Z. Effects of heat stress on the daily behavior of wenchang chickens. Revista Brasileira de Ciência Avícola 2015;17(4):559-566.

Lin H, Jiao HC, Buyse J, Decuypere E. Strategies for preventing heat stress in poultry. World's Poultry Science Journal 2006;62(1):71-86.

Deraz SF. Synergetic effects of multispecies probiotic supplementation on certain blood parameters and serum biochemical profile of broiler chickens. Journal of Animal Health and Production 2018;6(1):27-34.

Maff A . Manual of veterinary investigation. 3rded. London: Her Majesty Stationary Officer; 1984.

Mahmud A, Hayat Z, Khan MZ, Khalique A, Younus M. Comparison of source and levels of sodium in broilers under low temperature conditions. Pakistan Journal of Zoology 2010;42:383-388.

Mirsalimi SM, Brlen PJO, Julian RJ. Changes in erythrocyte deformabillty in NaCI-induced right sided cardiac failure in broiler chickens. American Journal of Veterinary Research 1992;53:2359-2363.

Mohammed AA, Jacobs JA, Murugesan GR, Cheng HW. Effect of dietary synbiotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. Poultry science 2018;97(4):1101-1108.

Nelson DL, Cox MM. Lehninger principles of biochemistry. New York: Worth Publishers; 2000.

NRC. Nutrient requirements of poultry. 9th ed. rev. Washington: National Academy Press; 1994.

Orhan C, Tuzcu M, Deeh PB, Sahin N, Komorowski JR, Sahin K. Organic chromium form alleviates the detrimental effects of heat stress on nutrient digestibility and nutrient transporters in laying hens. Biological Trace Element Research 2018;21:1-9.

Pasha TN, Mahmood A, Khattak FM, Jabbar MA, Khan AD. The effect of feed supplemented with different sodium bentonite treatments on broiler performance. Turkish Journal of Veterinary and Animal Science 2008;32:245-248.

Peng Y, Wang Y, Ning D, Guo Y. Estimation of dietary sodium bicarbonate dose limit in broiler under high ambient temperatures. The Journal of Poultry Science 2013;50(4):346-353.

Puvadolpirod S, Thaxton JP. Model of physiological stress in chickens: digestion and metabolism. Poultry Science 2000;79:383–390.

Rakib TM, Hassan MM, Faruq AA, Erfan R, Barua SR, *et al*. Effect of transport on physical and haematological status of cattle in Bangladesh. Journal of Animal Health and Production 2016;4(3):78-86.

Ravindran VJ, Cowieson A, Selle PH. Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization, and excreta quality of broiler chickens. Poultry Science 2008;87:677-688

Salari S, Kermanshahi H, Nasiri MH. Effect of sodium bentonite and comparison of pellet vs mash on performance of broiler chickens. International Journal of Poultry Science 2006;5:31–34.

Saeed M, Babazadeh D, Naveed M, Arain MA, Hassan FU, Chao S. Reconsidering betaine as a natural anti-heat stress agent in poultry industry: a review. Tropical Animal Health and Production 2017;1-10.

Sales J, Janssens JPJ. Methods to determine metabolizable energy and digestibility of feed ingredients in the domestic pigeon (Columba liviadomestica). Poultry Science 2003;82:1457–1461.

Saeed M, Xu YT, Zhang TT, Ren Q, Sun C. 16S ribosomal RNA sequencing reveals a modulation of intestinal microbiome and immune response by dietary L-theanine supplementation in broiler chickens. Poultry Science 2018b;98(2):842-854.

Santin E, Borges SA, Fischer da Silva AV, Hooge DM, Cummings KR. Effect of dietary electrolyte balance on the immune response (Newcastle disease virus antibody titers) of broiler chickens at various ages following vaccination and during heat stress. Abstractss of the International Poultry Science.; 2003; Georgia.

Saeed M, Xu YT, Hassan FU , Arain M, Elhack MA, Ahamed N, *et al*. Influence of graded levels of l-theanine dietary supplementation on growth performance, carcass traits, meat quality, organs histomorphometry, blood chemistry and immune response of broiler chickens. International Journal of Molecular Sciences 2018a;19(2):462.

Steel RGD, Torrie JH, Dickey DA. Principles and procedures of Statistics, a biometrical approach. 3rd ed. New York: McGraw Hill Book; 1997.

Whiting TS, Andrews LS, Adams MH, Stamps L. Effects of sodium bicarbonate and potassium chloride drinking water supplementation. Meat and carcass characteristics of broilers grown under thermoneutral and cyclic heat-stress conditions. Poultry Science 1991;70:60-66.

Wolfenson D. The effect of acclimatization on blood flow and its distribution in normothermic and hyperthermic domestic fowl. Comparative Biochemistry and Physiology 1986;85:739-742.