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REGULAR ARTICLES

Potassium phosphate and potassium carbonate administration by feed or drinking water improved broiler performance, bone strength, digestive phosphatase activity and phosphorus digestibility under induced heat stress conditions

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Abstract

Potassium phosphate (K_2HPO_4) and potassium carbonate (K_2CO_3) administration by feed or water were evaluated on broiler performance, bone strength, alkaline phosphatase activity (ALP), and phosphorus digestibility under heat stress and high chloride condition. Experimental groups include control; 15 cc/kg K_2HPO_4; 30 cc/kg K_2HPO_4; 15 cc/l K_2HPO_4; and 3.7 g/kg K_2CO_3. Body weight (BW), feed and water consumption, plasma potassium, phosphorus, and calcium concentration along with plasma and digestive ALP and intestinal digesta pH were measured during the trial. Tibia ash, calcium and phosphorus content, and breaking strength were measured on days 21 and 42 and phosphorus digestibility on day 36 of age. As a result of this, study feed and water consumption was increased by supplementation of the feed or water with K_2HPO_4 ($P \leq 0.001$). K_2HPO_4 increased body weight at 42 days of age ($P \leq 0.001$). Tibia ash and phosphorus content was increased by K_2HPO_4 supplementation ($P \leq 0.004$; $P \leq 0.003$). K_2CO_3 did increased tibia ash but not changed tibia phosphorus content significantly. Tibia shear force, shear energy, extension, and length were improved by K_2HPO_4 administration at 42 days of age ($P \leq 0.001$). Administration of either feed or water with K_2HPO_4 increased plasma potassium, phosphorus, and calcium concentration at 21 days of age, whereas K_2CO_3 reduced plasma potassium at 21 days of age ($P \leq 0.05$). Plasma ALP reduced by addition of 15 cc K_2HPO_4 and K_2CO_3 to diets at 42 days of age, whereas digestive ALP was increased by inclusion of K_2HPO_4 and not by K_2CO_3. Supplementation of either feed or water with K_2HPO_4 increased phosphorus digestibility, whereas K_2CO_3 reduced phosphorus digestibility ($P \leq 0.003$). Jejunum and ileum pH was reduced by K_2HPO_4 or by K_2CO_3 at 21 and 42 days of age ($P \leq 0.006$; ($P \leq$ 0.05). Over all, results of current study revealed that K_2HPO_4 can be a suitable potassium salt choice instead of KCL in hot weather conditions especially when the water or diet contains high levels of chloride.

Keywords Potassium phosphate · Potassium carbonate · Heat stress · Chloride

Introduction

Global warming and surface and groundwater withdrawals are increasing across the world, particularly in tropical areas, where there is high population and prolonged drought (Kanga and Jackson 2016). One of the abundant salts found in the surface and underground water bodies is sodium chloride which its concentration is increasing by drought and water withdrawals. By increasing the salinity and total dissolved solids (TDS) in underground water, sodium (400 ≥ ppm) and chloride (600 ≥ ppm) levels have been raised, which is resulting water unsuitable for poultry and domestics. Leeson et al. (1995) mentioned that leg problems occurs more frequently when birds receiving an excess of sodium relative to potassium. High levels of chloride in such conditions emerge leg disorders rapidly. However, Zarrin-Kavyani et al. (2018) reported that high potassium level (0.94%) did not affect tibia and femur width, ash, calcium, and P

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content as well as tibia breaking strength. Therefore, potassium salts such as potassium phosphate or potassium carbonate in feed or water should be paid attention instead of potassium chloride to reduce chloride daily allowance of chloride by omitting the sodium chloride from diets without any disturbance of diet acid-base balance.

Luo et al. (2018) postulated that heat stress is the main environmental factor affecting the performance and economic benefit of livestock and poultry. Heat stress is a key factor that reduces feed and water intake and increases leg disorders by disturbing the acid-base balance and by inhibiting the activity of alkaline phosphatase (Ahmad and Sarwar 2006; Luo et al. 2018). Chronic heat stress causes respiratory alkalosis by CO_2 losses (Mongin 1981; Ahmad et al. 2008) and recompense of bicarbonate ions by excreting the sodium and potassium ions through the urine, ultimately leads to losses of sodium and potassium and thereby reducing the plasma concentration of these ions (Ahmad et al. 2008). Therefore, addition of potassium salts such as potassium chloride or potassium carbonate to feed or drinking water could be beneficial during hot weather condition (Ahmad et al. 2008). However, supplementation with potassium carbonate failed to alleviate harmful effects of heat stress because of water intake depression effects of this compound (Smith and Teeter 1987b, 1987c). On the other hand, potassium chloride also is not suitable for the saline water due to high chloride intake in underground water. Alternatively, chloride levels can be reduced by substitution of potassium chloride with potassium phosphate in broiler diets in hot climates.

Di-potassium phosphate (K_2HPO_4) is a highly water-soluble potassium salt which is often used as a fertilizer, food and feed additive, and buffering agent. It is a valuable source of phosphorus and potassium combination which is used to make the urine more acid to prevent high blood pressure in humans. Potassium phosphate is an effective salt for preventing and treating most causes of hypophosphatemia in human beings.

Review of prior poultry heat stress-induced trials reveals that potassium salt especially potassium chloride and potassium carbonate has alleviating and beneficial effects in hot weather conditions; however, no experiments have been conducted to determine the efficacy of potassium phosphate as a new potassium salt choice instead of KCl in hot weather conditions especially when water or diet contain high levels of chloride. We hypothesized that the addition of this potassium salt would favor the performance, water consumption, and bone characteristics. The objective of our experiment was to evaluate the effects of potassium phosphate (K_2HPO_4) and potassium carbonate (K_2CO_3) administration via feed or water on broiler performance, bone strength, digestive phosphatase activity, and phosphorus digestibility under heat stress and high chloride content of drinking water.

Materials and methods

Experiments were carried out based on procedures and guidelines approved by the Animal Care Committee of the Iranian Council of Animal Care 1995.

Potassium phosphate and potassium carbonate

Potassium phosphate used in current study was synthesized using commercial phosphoric acid (98 g/mol, density = 1.57 g/l) containing 51% P_2O_5 and potassium hydroxide (56.1 g/mol). Briefly, to prepare 1 l of K_2HPO_4, 122.4 cc H_3PO_4 was diluted to 1000 ml in water that was followed by the addition of 112 g of KOH to the solution gently. Potassium carbonate which is used here contained 560 g/kg K^+ with 99% purity. Total phosphorus content of synthesized potassium phosphate and also for formulated diets was determined by the ammonium molybdate method as described by Davies et al. 1973. The calcium content of diets was also is digested by perchloric acid and calcium was quantified by triethanolamine (50%) measuring absorbance at 600 nm (method, 935.13, AOAC 2000). Potassium content of potassium phosphate and potassium carbonate, as well as potassium, sodium, and chloride content of experimental diets, was measured according the methods described by AOAC (AOAC International 2000). Analyzed and calculated compositions of the experimental diets are shown in Table 1.

Experimental design and housing management

Ross-308 broilers (500 1-day-old male chicks) with an initial body weight (BW) of 40.9 ± 1.03 g were allocated into five experimental groups: (1) No additive in water or feed (control); (2) 15 cc/kg feed DM of K_2HPO_4; (3) 30 cc/kg feed DM of K_2HPO_4; (4) 15 cc/lit water of K_2HPO_4; and (5) 3.7 g/kg feed DM of K_2CO_3. All treatment groups comprised 5 replicates with 20 chicks in each. Dicalcium phosphate and limestone were adjusted at the expense of K_2HPO_4 to keep dietary phosphorus and calcium content constant across treatments. Birds were reared in floor pens (2.5 × 1.5 m^2) with wood shaving as litter material over a concrete floor. The rearing house was conditioned (35 ± 1 °C and 60% relative humidity) by using the two heaters and one fogger machine. Temperature and relative humidity were recorded three times a day throughout the experiment using five digital thermo-hygrometers (CTH-168, Taiwan) at chick's level. Maximum, minimum, and average room temperature during the experimental period was shown in Fig. 1. Feed consumption for each pen of chicken was recorded throughout the experiment. Automatic bell drinkers with separate polyethylene tanks were used for measuring water consumption of each pen weekly. Drinking water was supplied from underground water source which contained 657 mg/l sodium and

Table 1 Composition and calculated analysis of the basal and experimental diets (g/kg)

Ingredients	K_2HPO4 Control	K_2HPO_4(15)	K_2HPO_4(30)	K_2CO_3
Corn	517.3	517.3	517.3	517.3
Soybean meal, 440 g/kg CP	380	380	380	380
Soybean oil	30	30	30	30
Limestone	10	10.8	12	10
Dicalcium phosphate	20	18.4	16.8	20
Sodium chloride	3	3	3	3
Vitamin and mineral premix[1]	5	5	5	5
L-Lys-HCL, 780 g/kg	2	2	2	2
DL-Met, 990 g/kg	2.7	2.7	2.7	2.7
Rice bran	30	15.8	1.2	26.3
K_2PO_4	–	15	30	
Potassium carbonate (560 g/kg K)	–			3.7
Calculated analysis (g/kg)				
Calculated analysis				
ME (MJ/kg)	12.359	12.350	12.347	12.342
CP	212	211	210	211
Dig Lys	14.4	14.2	14.1	14.3
Available P	4.93	4.95	4.97	4.93
Sodium	1.6	1.6	1.6	1.6
Potassium	8.63	9.61	10.6	10.6
Calcium	9.5	9.42	9.47	9.52
Chloride	1.7	1.7	1.7	1.7
(Na + K)-CL mEq/kg	242.89	268	293.49	293.49
Analyzed composition (g/kg)				
CP	217	209	218	217
Total P	6.5	7.3	7.2	7.1
Calcium	11.0	10.5	11.7	9.8
Sodium	1.59	1.81	1.53	1.73
Potassium	9.01	9.74	11.3	10.8
Chloride	1.83	1.61	1.92	1.67

[1] Vitamin and mineral mix supplied the following per kilogram of diet: transretinol, 11 mg; cholecalciferol, 0.5 mg; a tocopherol acetate, 80 mg; menadione, 3 mg; thiamine, 3 mg, riboflavin, 8 mg; pyridoxine, 5 mg; cyanocobalamin, 0.024 mg; nicotinic acid, 60 mg; folic acid, 2 mg; Ca pantothenate, 15 mg; choline, 1000 mg; Mn, 120 mg; Zn, 100 mg; Cu, 15 mg; Se, 0.3 mg; I, 1 mg; and Fe, 30 mg

634 mg/l chloride. The body weight (BW) was measured weekly and cumulative feed intake was measured at 7, 28, and 42 days of age for each pen, which allows for calculating the feed conversion ratio (FCR).

Bone characteristics

On day 21 and 42, two chicks per pen (ten birds/treatment) were chosen randomly, weighed, and euthanized in accordance with the procedure and guidelines approved by the animal care committee of the university. The whole bones were collected into plastic bags and stored at − 20 °C for later analysis. Tibia breaking strength was determined by an All-Digital Electronic Universal Testing Machine (Santam Instrument Co. Model-MRT-5, serial no. 628415) according to the recommendations from the American Society of Agricultural Engineers (2001) shear and 3-point bending test of animal bone (standard S459). For determination of tibial ash, calcium, and phosphorus content, fat contamination was first removed from the tibia using ethyl alcoholin a 36-h Soxhlet extraction procedure followed by a 36-h extraction with diethyl ether and subsequent drying of the bones at 100 °C for 24 h. Tibial ash content was determined by combusting the bones in a muffle furnace for 4 h at 600 °C. Phosphorus and calcium content of tibia was determined according to procedures described by Davies et al. 1973 and AOAC (2000) respectively, as mentioned earlier.

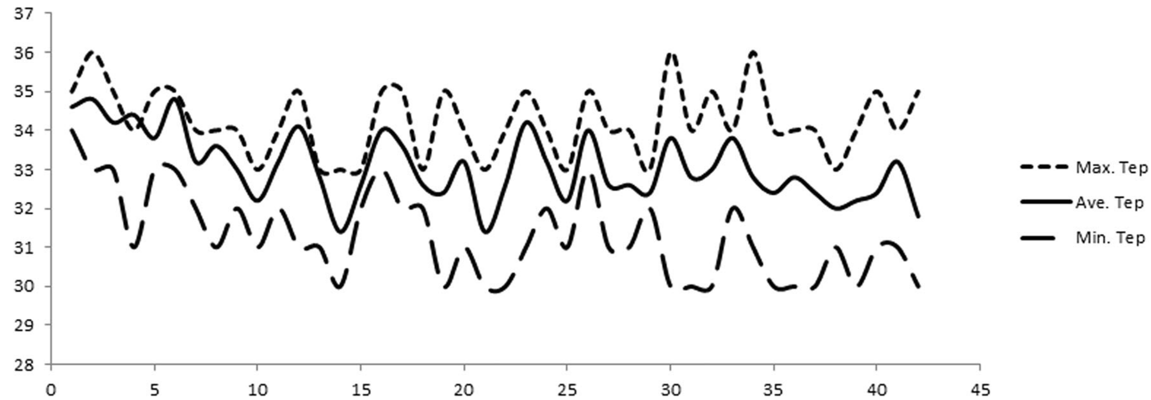

Fig. 1 Maximum, minimum, and average room temperature during the experimental period

Blood characteristics

On day 21 and 42, two chicks were chosen and randomly picked from each pen and blood samples were taken 3 ml into heparin containing tubes by puncturing the brachial vein. The samples were centrifuged for 10 min at 3000×*g* at 20 °C and the plasma were harvested and stored at − 20 °C for later analysis. Plasma calcium, phosphorus, and potassium concentration were assayed colorimetrically using Sysmex KX-21N automated hematology analyzer (KX-21N, Sysmex Corp., Kobe, Japan) according to the method described by Robertson and Marshall 1979). A plasma specimen, free of clot, was collected into a bottle as quickly as possible to avoid hemolysis and elevation of serum phosphorus from hydrolysis or leakage of phosphate present in erythrocytes and then transferred into a bottle containing 10 mL of 10% v/v hydrochloric acid to avoid phosphate precipitation, adjusted to pH 2, diluted with distilled water, and phosphorous was determined by direct method using Audit Diagnostic kit (catalog no. I.874, Ireland (by reaction with ammonium molybdate as a phosphomolybdate and measuring the color intensity at wave length 340 nm. Alkaline phosphatase activity was determined according to the procedure describe by Morgenstern et al. (1965) with an automatic biochemical analyzer (Hitachi 917, Boehringer Mannheim, Ingelheim am Rhein, Germany) using a Bionik Diagnostic kit (catalog no. A.110537(and measuring color intensity at 405 nm.

Digestive phosphatase activity

On 42 days of age, two chicks per pen (ten birds/treatment) were chosen randomly, weighed, and euthanized in accordance with the procedure and guidelines approved by the animal care committee of the university. The birds were deprived of feed 6 h prior to slaughter to ensure intestinal emptying. The duodenum and a 10-cm segment of the jejunum adjacent to the distal pancreas, free of residual feed, were excised and snap frozen in liquid nitrogen and stored in liquid nitrogen until preparation for the assay. The samples were prepared by partially thawing the frozen intestinal segments in the refrigerator at 4 °C for 2 h. Subsequently, the duodenum and jejunum samples were homogenized separately (dilution 1:5, w/v) in cold buffer (50 mMTris–HCl, pH 8.0 containing 10 mM $CaCl_2$) on ice at 11000 rpm for 2 min according to the procedure devised by Beyranvand et al. (2019). Thereafter, the homogenates were centrifuged at 14000*g* for 45 min at 4 °C. The resultant supernatants were collected and aliquots were stored at − 80 °C until digestive enzyme analysis. Phosphatase activity was measured at 37 °C by spectrophotometer (company name) following the procedure described by Walter and Schutt (1974). Briefly, homogenates were incubated with p-nitrophenyl phosphate as substrate and the increase in absorbance was measured continuously over 30 min at 405 nm.

Phosphorus digestibility and gastrointestinal pH

On day 35 to 36 of the experiment, two chicks per pen (ten birds/treatment) were chosen randomly, weighed, and euthanized as described previously. Afterwards, the abdominal cavity of birds was dissected and ileum was cut from the Meckel's diverticulum up to 1 cm proximal to the ileocecal junction and the ileum was dried using chick paper. Finally, the digesta content from half of the illeum was gently flushed into a plastic cup using distilled water. The samples from two chicks of each pen were pooled into one sample which was frozen and stored until being lyophilized. Thereafter, diet and lyophilized digesta samples were ground through 1 mm screen grinder and dry matter content was measured by oven drying at 105 °C for 24 h. The concentration of acid insoluble ash in the feed and excreta was measured as proposed by Vogtmann et al. (1975). Phosphorous contents of the feed and digesta samples were determined as described previously. Finally, having the AIA and phosphorous content of diet and digesta, digestibility of phosphorus was calculated using the indirect-ratio method.

pH of the intestinal content was measured on day 21 and 42. Two chicks from each pen were killed by cutting the neck, and the gizzard, duodenum, jejunum, and ileum were cut longitudinally, and pH of their contents was measured in triplicates by using a digital pH meter (Testo 205, Testo-Strabe 1, 79853 Lenzkirch, Germany).

Statistical design and analysis

Data were analyzed in a completely randomized design with 5 treatments of 5 replicates and 20 chicks in each pen and the effects were estimated using the GLM procedure of SAS (SAS Institute 2003). Normal distribution of the residual was tested by the UNIVARIATE procedure of SAS (SAS Institute 2003). When the effect was significant, differences among treatment means were tested using the Duncan multiple comparison test. Results are reported as means in tables, and differences among treatments were considered significant at a threshold of $P < 0.05$.

Results

Performance

Effect of K_2HPO_4 and K_2CO_3 on broiler performance and water consumption are shown in Table 2. Feed intake was reduced in chicks received 30 cc/kg feed DM of K_2HPO_4; however, there were no differences in feed intake among other treatments on day 7 of age ($P \leq 0.01$). The increase in feed intake was more pronounced with supplementation of 15 cc K_2HPO_4 per liter in drinking water. Potassium carbonate supplementation increased FCR and the lowest FCR was observed on day 7 of age in chicks receiving 30 cc K_2HPO_4 per kg of feed ($P \leq 0.01$). Water consumption was reduced by inclusion of 15 and 30 cc K_2HPO_4 in feed, versus K_2HPO_4 inclusion in drinking water increased water intake at 7 days of age ($P \leq 0.05$). Body weight showed no difference among treatments at 7 and 28 days of age. Feed conversion ratio was reduced by addition of K_2HPO_4 to feed and water at 28 days of age ($P \leq 0.02$). Both feed intake and water consumption were increased by supplementing of the feed and drinking water with K_2HPO_4 compared to the control diet and diet supplemented with K_2CO_3 at 42 days of age ($P \leq 0.009$; $P \leq 0.001$). The highest feed intake and water consumption at 42 days of age were observed when K_2HPO_4 was added to the drinking water. No differences were detected in feed intake and water consumption between control and diet supplemented with K_2CO_3 at 42 days of age. Inclusion of feed or drinking water with K_2HPO_4 significantly increased body weight at 42 days of age ($P \leq 0.001$).

Bone characteristics

When measured on day 21 of age, tibial shear force, shear energy, extension, and length showed no differences among treatments (Table 3). However, birds supplemented with K_2HPO_4 either via water or feed showed significantly higher tibial shear force and shear energy compare to other treatments on 42 days of age ($P \leq 0.05$ and $P \leq 0.01$, respectively: Table 3). K_2HPO_4 supplementation increased tibial length compared to other treatments on day 42 of age only when supplemented at 15 cc per kg feed DM or per liter of water. Tibia extension was positively influenced by supplementation of both K_2HPO_4 and K_2CO_3 ($P < 0.05$). Tibial ash content was significantly higher in birds supplemented with either K_2HPO_4 or K_2CO_3 compared to the control on day 21 of age ($P \leq 0.01$). The highest tibial ash was observed when K_2HPO_4 was added to drinking water. However, these differences in ash content of the tibia disappeared on day 42 of age and there was no difference among treatment groups at this time point. Administration of K_2HPO_4 increased phosphorus content of the tibia compared to other treatments on day 21 and 42 of age ($P \leq 0.01$and $P \leq 0.05$, respectively). The highest tibia phosphorus was observed when 15 cc K_2HPO_4 is included in feed. Tibial calcium percentage showed no prominent differences among treatments ($P > 0.05$).

Blood characteristics

The effects of K_2HPO_4 and K_2CO_3 on blood characteristics are shown in Table 4. On day 21 and 42 of age, birds supplemented with K_2HPO_4 at any dose in feed had significantly higher plasma calcium and phosphorus and potassium concentrations than those in other treatments ($P \leq 0.05$). However, when added to water, K_2HPO_4 did increase plasma calcium and phosphorous content only on day 42 of age ($P > 0.05$). K_2HPO_4 also increased plasma potassium concentration on 21 days of age when delivered either via feed or water ($P < 0.05$). In contrast, the administration of K_2CO_3 reduced plasma calcium, phosphorus, and potassium levels compared to the control on day 21 of age ($P > 0.05$) but elevated plasma content of phosphorous and potassium on day 42 of age. The alkaline phosphatase activity showed no differences among treatments on 21 days of age but it was reduced when K_2HPO_4 and K_2CO_3 were added to the diet at 15 cc per kg DM on day 42 ($P < 0.01$).

Digestive phosphatase activity

Digestive alkaline phosphatase activity was increased by inclusion of K_2HPO_4 in feed or water; however, no differences were observed on phosphatase activity of control and K_2CO_3 supplemented diets at 42 days of age (Table 5; $P \leq 0.001$). The

Table 2 Effect of potassium phosphate and potassium carbonate on broiler performance

Treatments	7 days				28 days				42 days			
	FCR,[2] g of feed/g of BW	FI,[1] (g)	BW (g)	Water consumption (cc)	FCR,[2] g of feed/g of BW	FI,[1] (g)	BW (g)	Water consumption (cc)	FCR,[2] (g of feed/g of BW)	FI,[1] (g)	BW (g)	Water consumption (cc)
Control	1.08^{b}	135.83^{a}	125.03	244.5^{a}	1.82^{a}	1641	749.29	2964	2.02	3619.8^{c}	1789.49^{c}	6515^{c}
15 cc/kg/feed K_2PO_4	1.1^{b}	128.74^{a}	116.56	231.7^{ab}	1.81^{a}	1508	730.63	2714	2.04	3948.2^{bc}	1834.84^{bc}	7106^{bc}
30 cc/kg/feed K_2PO_4	0.98^{c}	112.08^{b}	114.21	201.7^{b}	1.63^{b}	1416	770.71	2550	1.98	3982.2^{b}	1956.90^{b}	7168^{b}
15 cc/lit/water K_2PO_4	1.1^{b}	142.56^{a}	128.53	256.7^{a}	1.74^{ab}	1585	798.81	2853	2.06	4335.8^{a}	2101.51^{a}	7804^{a}
Potassium carbonate	1.24^{a}	139.58^{a}	112.53	251.2^{a}	1.84^{a}	1567	750.57	2820	2	3811.9^{bc}	1900.67^{bc}	6861^{bc}
SEM	0.03	4.42	0.18	11.9	0.04	79.94	28.06	134	0.07	105.96	43.69	190
P value	0.002	0.004	5.05	0.05	0.02	0.33	0.52	0.33	0.95	0.009	0.001	0.001

$^{a–d}$Means within a column without a common superscript significantly differ ($P < 0.05$). Pens means were used to calculate dietary averages. [1] FI, feed intake. [2] FCR, feed conversion ratio

highest digestive phosphatase activity was observed when K_2HPO_4 supplemented in drinking water.

Phosphorus digestibility and gastrointestinal pH

K_2HPO_4 caused an increase in phosphorus digestibility regardless of the route of administration, whereas K_2CO_3 reduced phosphorus digestibility in comparison to the control diet ($P \leq 0.01$). The pH of jejunum and ileum was reduced significantly in all supplemented chicks compared to the control on day 21 and 42 of age ($P \leq 0.01$ and $P \leq 0.05$, respectively) (Table 5).

Discussion

Current trial showed that potassium phosphate interestingly increased feed and water consumption especially when included in drinking water and such an increase in feed and water consumption caused meaningful increases in final body weight. Reduction in weight gains of broilers raised at high temperatures are due to decrease in feed intake and fluctuation of acid-base balance (Borges et al. 2003a, 2003b; Mushtaq et al. 2007; Ahmad and Sarwar 2006; Luo et al. 2018). Additionally, Porto et al. (2015) and Song et al. (2017) indicated that high environmental temperature reduces ileal villi proliferation and absorptive area of the intestinal mucosa and limit the secretion of digestive enzymes. Also, the high chloride level reported has detrimental effect on broiler growth (Leeson et al. 1995). During such conditions, also, either acidosis or alkalosis has adverse effect on growth; therefore, the total quantity of water consumption is an important consideration. It is demonstrated that supplementation of diet or drinking water with electrolytes resulting higher water consumption in birds reared under heat stress conditions (Borges et al. 2003a, 2003b, 2007; Ahmad et al. 2005; Mushtaq et al. 2007). Therefore, addition of potassium salts such as potassium chloride or potassium carbonate to feed or drinking water was associated with benefits in warm weather. (Smith and Teeter 1987a; Ahmad et al. 2008). However, supplementation with potassium carbonate because of water depression effects of this compound has got not to succeed in this concept (Smith and Teeter 1987b). Potassium carbonate did not reduce water intake in current trial; however, body weight losses and an increase in FCR were observed at early phase of rearing but not at whole experimental period. Ahmad et al. (2008) reported a better broiler body weight gain by addition of KCl to diets under conditions of severe heat stress. However, the results for KCl effects on feed intake were not consistent during heat stress condition (Ait-Boulahsen et al. 1995; Ahmad et al. 2008). On the other hand; potassium chloride is not suitable for the saline water due to high chloride levels in underground water. Overall, results of current study demonstrated that

Table 3 Effect of potassium phosphate and potassium carbonate on tibial strength, ash, calcium, and phosphorus content at 21 and 42 days of age

Treatments	21 days of age							42 days of age						
	Shear force (N)	Shear energy (J)	Extension (mm)	Length (mm)	Ash (g/100 g)	Calcium (g/100 g)	Phosphorous (g/100 g)	Shear force (N)	Shear energy (J)	Extension (mm)	Length (mm)	Ash (g/100 g)	Calcium (g/100 g)	Phosphorous (g/100 g)
Control	49.63	83.42	3.402	69.56	28.84^{b}	35.89	16.553^{b}	200.87^{b}	248.46^{b}	2.486^{b}	99.12^{b}	39.71	36.53	16.62^{b}
15 cc/kg/feed K_2PO_4	59.24	93.07	2.958	62.5	33.63^{a}	35.37	19.683^{a}	232.56^{b}	311.04^{b}	2.820^{b}	106.75^{a}	38.60	36.08	17.74^{a}
30 cc/kg/feed K_2PO_4	58.95	122.10	4.289	65.2	32.07^{a}	34.93	17.683^{ab}	334.73^{a}	472.90^{a}	2.934^{b}	95.38^{b}	41.79	36.13	17.09^{ab}
15 cc/lit/water K_2PO_4	80.72	125.24	3.068	68.3	34.32^{a}	35.90	17.673^{ab}	266.73^{ab}	586.26^{a}	4.422^{a}	105.92^{a}	39.03	35.90	16.85^{b}
Potassium carbonate	50.33	90.42	3.493	66.78	32.53^{a}	36.49	16.857^{b}	197.67^{b}	321.50^{b}	3.363^{ab}	99.05^{b}	38.94	36.11	17.25^{ab}
SEM	13.52	26.02	0.326	2.16	0.76	0.47	0.64	29.76	44.109	0.367	1.99	1.89	0.22	0.22
P value	0.51	0.7	0.1	0.24	0.004	0.26	0.003	0.04	0.001	0.03	0.009	0.76	0.4	0.04

$^{a–d}$Means within a column without a common superscript significantly differ ($P < 0.05$)

K_2HPO_4 supplementation via drinking water showed significant effects on water consumption. Ahmad et al. (2008) reported that increases in weight gain by inclusion of potassium salt supplementation may be attributed to low body temperature by more water consumption which is resulting in more energy diversion toward BW gains. Collectively, these findings indicate that K_2HPO_4 supplementing alleviates heat stress and high water chloride level and improves performance.

The effects of K_2HPO_4 on bone characteristics are noteworthy. Maximum shear force, shear energy, extension, and tibia length were obtained with K_2HPO_4 inclusion in drinking water. Potassium phosphate inclusion in feed also increased these parameters but potassium carbonate did not improved tibia feature in comparison to the control group. Leg problem is an indicator of high chloride levels in diets for broiler. Lesson et al. (1995) reported that incidence of leg problems such as development of cartilage plug which reduce breaking strength of long bones can be greatly enhanced by metabolic acidosis induced by high levels of chloride. Tibial dyschondroplasia seems most problematic when a high chloride level is used in diets (Leeson et al. 1995). There is no tibial dyschondroplasia with low diet chloride levels regardless of electrolyte balance and also addition of potassium or sodium to diets where a high level of chloride in feed or water causes an electrolyte imbalance could reduce leg problems (Lesson et al. 1995). Therefore, addition of potassium salt especially K_2HPO_4 in this situation increased plasma potassium level and ameliorate harmful effect of chloride on bone mineralization. Nonetheless, Zarrin-Kavyani et al. (2018) reported that high potassium level (0.94%) did not affect tibia and femur width, ash, calcium, and P content as well as tibia breaking strength. As it is noticeable, there was a great difference in tibia ash and phosphorus content as bone mineralization criteria among treatments in this study. It seems that increase in phosphorus digestibility and accessibility with highly water-soluble K_2HPO_4 may affected tibia ash and phosphorus content. On the other hand, increase in digestive alkaline phosphatase activity and thereafter increase in phosphorus digestibility can increase plasma and intestinal phosphorus access to bone mineralization. It is reported that dietary electrolyte balance can improve phosphorus availability and tibia breaking strength by increasing the phytase activity (Ravindran et al. 2008; Kornegay et al. 1999). Reduction in tibia length is associated with decrease in growth due to reduced feed intake by induced heat stress. So that, increase in tibia length may be the consequence of increase in feed intake by inclusion of K_2HPO_4 in feed or drinking water with a resultant increase in body weight gain. Bruno et al. (2000) reported decreases in femur, tibia, and humerus lengths in the feed-restricted broiler compared with the ad libitum group.

An important finding of this experiment was the increase in plasma potassium, phosphorus, and calcium levels by

Table 4 Effect of potassium phosphate and potassium carbonate on blood calcium, phosphorus, potassium, and alkaline phosphatase activity content at 21 and 42 days of age

Treatments	21 days of age				42 days of age			
	Calcium mg/dl	Phosphorus mg/dl	Potassium mg/dl	ALP[1] (U/L)	Calcium mg/dl	Phosphorus mg/dl	Potassium mg/dl	ALP (U/L)
Control	10.09^{a}	9.43^{b}	4.57^{b}	194	10.21^{b}	6.61^{c}	4.45^{b}	5189^{a}
15 cc/kg/feed K_2PO_4	10.19^{a}	10.89^{a}	5.88^{a}	194	11.04^{a}	8.00^{b}	5.45^{a}	3899^{b}
30 cc/kg/feed K_2PO_4	10.34^{a}	10.62^{a}	5.9^{a}	191	10.8^{ab}	7.93^{b}	4.89^{ab}	6247^{a}
15 cc/lit/water K_2PO_4	9.64^{ab}	9.93^{b}	5.23^{ab}	180	10.28^{b}	9.16^{a}	5.40^{a}	6116^{a}
Potassium carbonate	9.12^{b}	8.42^{b}	3.56^{b}	249	10.16^{b}	8.29^{ab}	5.43^{a}	3772^{b}
SEM	0.25	0.32	0.33	23	0.19	0.34	0.19	396.62
P value	0.04	0.02	0.001	0.35	0.02	0.005	0.01	0.002

[a–d] Means within a column without a common superscript significantly differ ($P < 0.05$). [1] ALP, alkaline phosphatase

K_2HPO_4 administration of feed or drinking water. Ahmad et al. (2008) mentioned that heat stress can reduce the plasma potassium concentration, so that, increase in plasma potassium level by potassium salt administration would be a practical method to stimulate water consumption and enhance the growth. In support of this hypothesis, Ahmad et al. (2005) reported that sodium, potassium, and chloride supplements resulted in higher blood sodium, potassium, and chloride levels, respectively. Also, Takahashi and Akiba (2002) reported that balancing the blood electrolyte through potassium administration can lead to an improvement in broiler performance. Potassium phosphate and not potassium carbonate inclusion increased digestive alkaline phosphatase activity in this trial. Increase in plasma phosphorus and calcium concentration may occur in response to increase in digestive alkaline phosphatase activity, and thereafter increased phosphorus digestibility. Chen et al. (2011) reported that heat stress can significantly reduce the activities of digestive enzymes especially alkaline phosphatase activity and can cause serious damage of absorption function in the intestine. Both K_2HPO_4 and K_2CO_3 administration had a surprising acidogenic effect on intestinal pH. A consistent reduction in jejunum and ileum pH was observed by inclusion of these compounds in feed or drinking water. McPherson (1990) postulated that alkaline phosphatase is so active to remove phosphate moieties from myo-inositol when the media contain slightly acidic properties. Therefore, reduction in intestinal pH by K_2HPO_4 and K_2CO_3 administration were concomitant with increased in digestive alkaline phosphatase activity and increased in phosphorus digestibility, and this did enhanced plasma phosphorus and improved the bone features in heat stress induced chicks.

Conclusions

Over all, results of current study showed that K_2HPO_4 administration via feed or drinking water alleviate heat stress in broilers and improved performance by increasing the feed and water consumption, and obviously improved bone characteristics by enhancing the plasma potassium, calcium, and phosphorus level through the changes in intestinal

Table 5 Effect of potassium phosphate and potassium carbonate on intestinal pH, digestive phosphatase activity, and phosphorus digestibility

Treatments	21 days of age			42 days of age				35 days of age
	pH			pH			ALP	Phosphorus digestibility
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	(UA/mg of protein)	%
Control	4.38	5.43^{a}	5.49^{a}	4.43	5.26^{a}	6.00^{ab}	3.26^{c}	81.00^{b}
15 cc/kg/feed K_2PO_4	4.34	5.27^{b}	5.38^{ab}	4.23	5.12^{b}	5.64^{bc}	4.38^{b}	85.00^{a}
30 cc/kg/feed K_2PO_4	4.36	5.19^{b}	5.35^{b}	4.32	5.17^{b}	5.68^{abc}	4.61^{b}	87.66^{a}
15 cc/lit/water K_2PO_4	4.25	5.21^{b}	5.33^{b}	4.27	5.15^{b}	5.46^{c}	5.51^{a}	88.00^{a}
Potassium carbonate	4.31	5.3^{b}	5.25^{b}	4.34	5.15^{b}	5.96^{ab}	3.39^{c}	78.33^{b}
SEM	0.09	0.02	0.03	0.07	0.02	0.11	0.09	1.08
P value	0.89	0.006	0.01	0.41	0.03	0.01	0.001	0.003

[a–d] Means within a column without a common superscript significantly differ ($P < 0.05$). [1] Ca, calcium; P, phosphorus phosphatase activity and nutrient digestibility. Therefore, K_2HPO_4 can be a suitable potassium salt choice instead of potassium carbonate and chloride in hot weather conditions especially when the water or diet contains high levels of chloride.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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