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## ***The Effects of Sodium Chloride versus Sodium Bicarbonate at the Same Dietary Sodium Concentration on Efficacy of Dietary Phytase and a Carbohydrase-Protease Cocktail in Broilers***

### **ABSTRACT**

A trial was conducted to determine the effect of phytase (PHY) or a carbohydrase/protease cocktail (CPX) on broilers fed diets with two different levels of chloride (0.28% or 0.43%) created by altering dietary salt (NaCl) and sodium bicarbonate (NaHCO<sub>3</sub>). There were 6 combination dietary treatments (3 enzyme x 2 NaCl treatments) applied to 4 replicate pens. The treatments were as follows: Control diet (CON), CON+PHY and CON+CPX, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate. The 0.5% salt versions had no sodium bicarbonate. Replicate pen BW, and feed consumption (FC) were measured at 1, 14, and 35 d, and mortality was weighed daily for feed conversion ratio (FCR) calculations. Feed consumption at 14 d tended to be lower ( $p < 0.10$ ) for CON+CPX diets compared to CON and CON+PHY diets. The birds fed CON+CPX diet consumed less feed but exhibited improved FCR in the presence of 0.5% NaCl at 14 d. The birds fed the CON, and CON+PHY diets exhibited higher BW at 14 d ( $p < 0.05$ ) and 35 d ( $p < 0.01$ ) of age than did CON+CPX birds. From 15 d to 35 d, birds fed the CON+CPX diet exhibited poorer BW gain (BWG) in the presence of 0.25% NaCl ( $p < 0.05$ ). In conclusion, Cl, as NaCl versus NaH<sub>2</sub>CO<sub>3</sub>, could affect CPX but not PHY feed enzyme function in broilers. Further, it may be suggested that certain feed enzymes may be best utilized at later broiler ages rather than in initial feeds.

### **INTRODUCTION**

Exogenous enzymes such as phytase, xylanase, amylase, and protease have been extensively scrutinized during the last two decades in studies designed to demonstrate their efficacy in broiler diets. The inclusion of phytase (PHY) has made a significant reduction of inorganic phosphorus in non-ruminant diets possible without compromising live performance, livability, or carcass traits (Adeola *et al.*, 2004; Beudeker *et al.*, 2005; Pieniazek *et al.*, 2017; Scholey *et al.*, 2018; Leyva-Jimenez *et al.*, 2019). Beyond the capacity of PHY to increase the availability of phosphorus via degradation of the anti-nutritive phytate molecule, this enzyme has demonstrated to increase the availability of other minerals as well as protein and starch. Previous researches showed that the addition of PHY to adequate or marginally deficient diets could increase the digestibility coefficients of phosphorus, calcium, amino acids, and metabolizable energy (Selle & Ravindran, 2007).

The purpose of exogenous dietary carbohydrases has been to digest cell walls to reduce viscosity of the digesta and expose macronutrients to further digestion by endogenous enzymes. This mechanism has been most easily observed in wheat-based diets as the structural and chemical composition of these diets was well suited for maximum efficacy of the enzymes (Yaghobfar *et al.*, 2017). It has also been suggested that a substantial amount of the starch present in feedstuffs such as corn was



embedded in protein matrixes (García-Lara *et al.*, 2019). This could limit access to critical digestive enzymes such as amylase. To solve these problems carbohydrase-protease cocktails (CPX) have been developed to ensure optimal digestion of all macronutrients in multiple types of diets. In addition, researchers have postulated that exogenous enzyme supplementation could reduce the synthesis of endogenous enzymes, decreasing the energy expenditure of the animal (Mahagna *et al.*, 1995; Yuang *et al.*, 2017). Still, most of the trials performed to assess enzyme effectiveness did not collect data beyond 21 d of age and, thus, before energy requirements have been generally recognized to increase substantially.

The addition of common table salt (NaCl) to the diet has typically been used to supply the sodium (Na) and chloride (Cl) requirements of birds, which have been estimated by some authors to be 0.28% Na and 0.25% Cl (Oviedo-Rondón *et al.* 2001) for birds from 1 to 21 d of age. Murakami *et al.* (2001) recommended levels of 0.15% Na and 0.23% Cl from 22 to 42 d of age. However, Aviagen (2019) recommends levels of Na between 0.16 – 0.23% and 0.16% - 0.20%, from 1 – 24 d and 25 d – market age, respectively. Recommended Cl levels range between 0.16 – 0.23% for the whole growing period. It has been established that minerals such as Na, Cl, and potassium (K) are crucial for nutrient absorption in the intestine (Boron & Boulpaep, 2016). As a consequence, any imbalance could affect the digestive process due to resulting changes in the digesta pH.

Antinutritive components, such as phytate, have the capacity to reduce ileal availability of Na by attracting the mineral to the lumen (Ravindran *et al.*, 2006). They suggested that the decrease in pH due to the presence of high concentrations of phytate could stimulate the secretion of  $\text{NaHCO}_3$ , compromising the mineral availability. Due to the influence of phytate and PHY on Na availability, researchers have suggested that the dietary electrolyte balance (DEB;  $\text{mEq Na}^+ + \text{mEq K}^+ - \text{mEq Cl}^-$ ) could interact with the efficacy of the PHY. In a study conducted by Ravindran *et al.* (2008), it was observed that the addition of PHY to marginally energy-protein deficient diets at low to moderate DEB levels improved broiler performance and availability of nutrients. No benefit of the enzyme was observed at a high DEB level. However, the design of the diets did not permit Ravindran *et al.* (2008) to determine if the interaction between PHY and DEB was due to the DEB *per se*, or to the different Na levels in the diets.

It has been reported that carbohydrases and CPX “cocktails” could improve the digestibility of amino

acids and minerals (Kim *et al.*, 2005; Olukosi *et al.*, 2007; Selle *et al.*, 2009; Liu & Kim, 2017). Absorption of these nutrients could be closely related, not only to the optimal requirement of Na but also to an optimum ratio between Na, K, and Cl (DEB). It is well known that most absorption process of amino acids, sugars, and some minerals, have shown to indirectly depend upon the electrochemical difference between the digesta and the intracellular space of absorptive cells in the intestine (Boron & Boulpaep, 2016).

The study aimed to determine if the response to PHY or CPX supplementation on productive performance of broiler was affected by different dietary levels of Cl, produced by different inclusion levels of NaCl versus  $\text{NaHCO}_3$  at the same dietary Na.

## MATERIALS AND METHODS

### Broiler Progeny Management and Data Collection

Care for birds in this study was in compliance with the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Eggs from a resident 60-wk-old Ross 344 x 708SF breeder flock were collected, stored, and incubated under standard conditions. The chicks (average BW at d 1 of 45.51g + 0.65g) were hatched, identified by breeder pen (a total of 16) and maintained separately at all times. The chicks were sexed and 32 (2 per breeder pen) male chicks were permanently identified with neck tags and placed in one of 32 floor pens. Each 4.83 m<sup>2</sup> pen had 3 feeder trays from 1 – 7 d. Then 1 tray was removed at 10 d and 14 d, allowing an appropriate transition from trays to tube feeders. All pen floors were covered with wood shavings, and each pen had one tube feeder and one bell-type drinker, plus a plastic font drinker during the first 7 d. The lighting program during the first 7 d consisted of 23 h of light, then 21 h to 21 d. After 21 d of age only natural light was employed. Starter feed per bird was equalized to 1.14 kg per live chick at 7 d, to ensure equal consumption of nutrients. Grower feed was then added beginning at 14 d of age. Feeders were shaken twice daily to maintain feed flow from the tubes into the pans. Feed consumption and pen BW were measured at 14 and 35 d, and BWG was calculated by time interval. Mortality was weighed daily and recorded to be included in FCR calculations.

### Broiler Dietary Treatments

The 6 broiler dietary treatments tested were comprised of 2 levels of sodium chloride (NaCl; 0.5



and 0.25%) that provided two levels of chloride (Cl; 0.43 and 0.28%), and three enzyme amendments that included a control (CON), a CON plus phytase enzyme (CON+PHY), and CON plus a carbohydrase/protease cocktail (CON+CPX) (Tables 1 and 2). The PHY was assumed to contribute 0.12% and 0.10% of available phosphorus (AvP) and calcium (Ca), respectively. To diets with 0.25% NaCl, 0.35% NaHCO<sub>3</sub> was added to equalize the Na content. Intended differences

in mineral content of the diets were confirmed by analyses. Birds were provided feed for *ad libitum* consumption with starter feed offered as crumbles, while grower feed was in pellet form. All the treatment diets were made from common batches of ingredients and were corn-soybean meal based with 10 – 10.9% corn distiller's dried grains with solubles (DDGS), 4 – 4.5% poultry by-product meal (PBM), and formulated to be isonitrogenous with similar amino acid levels.

**Table 1** – Ingredient composition and calculated analysis for broiler dietary treatments for the starter period.

Ingredients	Basal Dietary Treatment <sup>1</sup>		
	CON	CON+PHY	CON+CPX
	(%)		
Corn	51.04	51.03	50.88
Soybean meal (48% CP)	25.87	25.86	25.79
Corn DDGS (26% CP)	10.60	10.80	10.60
Poultry by-product meal	4.48	4.40	4.55
Poultry Fat	3.32	3.32	3.39
Sodium chloride <sup>1</sup>	-	-	-
Sodium bicarbonate <sup>1</sup>	-	-	-
Limestone	0.88	1.05	0.88
Dicalcium phosphate	2.22	1.47	2.22
Premixes <sup>2</sup>	0.60	0.60	0.60
Filler (sand)	0.05	0.50	0.05
Phytase premix <sup>3</sup>	0.00	0.03	0.00
Avizyme 1502 <sup>4</sup>	0.00	0.00	0.10
L-Lysine	0.11	0.11	0.11
DL-Methionine	0.15	0.15	0.15
L-Threonine	0.09	0.09	0.09
	100.00	100.00	100.00
Calculated nutrients <sup>5</sup>			
ME (kcal/g)	2.80	2.80	2.80
Crude protein	23.00	23.00	23.00
Lysine	1.26	1.26	1.26
Methionine + Cysteine	0.91	0.91	0.91
Threonine	0.84	0.84	0.84
Calcium	0.90	0.90 <sup>4</sup>	0.90
Available phosphorus	0.45	0.45 <sup>4</sup>	0.45
Sodium	0.21	0.21	0.21

<sup>1</sup>The were six dietary treatments consisted of: CON = Control, CON+PHY = CON plus 500 FTU/kg feed (Phyzyme XP), and CON+CPX = CON plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate. The 0.5% salt versions had no sodium bicarbonate.

<sup>2</sup>Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D<sub>3</sub>, 2,000 IU; vitamin E, 33 IU; vitamin B<sub>12</sub>, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K<sub>3</sub>), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na<sub>2</sub>SeO<sub>3</sub>), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

<sup>3</sup>Phyzyme XP TPT (Dupont Animal Nutrition, Marlborough, UK), an *Escherichia coli*-derived phytase, was added at 500 FTU /kg to replace 0.12% available phosphorus (AvP) and 0.1% calcium (Ca).

<sup>4</sup>Avizyme 1502 (Dupont Animal Nutrition, Marlborough, UK), is composed of xylanase (300 U/kg), *Trichoderma longibrachiatum*, protease (4000 U/kg), *Bacillus subtilis* and amylase (400 U/kg), and *Bacillus amyloliquefaciens*.

<sup>5</sup>The nutrient compositions were calculated from proximate analyses of all ingredients, and final diet composition was confirmed by proximate analyses.

**Table 2** – Ingredient composition and calculated analysis for broiler dietary treatments for the grower period.

Ingredients	Basal Dietary Treatment <sup>1</sup>		
	CON	CON+PHY	CON+CPX
	(%)		
Corn	55.17	55.17	55.17
Soybean meal (48% CP)	18.96	18.96	18.96
Corn DDGS (26% CP)	10.00	10.00	10.00
Poultry by-product meal	4.40	4.40	4.40
Poultry Fat	5.00	5.00	5.00
Sodium chloride <sup>1</sup>	-	-	-
Sodium bicarbonate <sup>1</sup>	-	-	-
Limestone	0.92	1.09	0.92
Dicalcium phosphate	2.01	1.27	2.01
Premixes <sup>2</sup>	0.60	0.60	0.60
Filler (sand)	1.67	2.21	1.57
Phytase premix <sup>3</sup>	0.00	0.03	0.00
Avizyme 1502 <sup>4</sup>	0.00	0.00	0.10
L-Lysine	0.30	0.30	0.30
DL-Methionine	0.20	0.20	0.20
L-Threonine	0.16	0.16	0.16
	100.00	100.00	100.00
Calculated nutrients <sup>5</sup>			
ME (kcal/g)	2.90	2.90	2.90
Crude protein	20.00	20.00	20.00
Lysine	1.20	1.20	1.20
Methionine + Cysteine	0.86	0.86	0.86
Threonine	0.80	0.80	0.80
Calcium	0.85	0.85 <sup>4</sup>	0.85
Available phosphorus	0.40	0.40 <sup>4</sup>	0.40
Sodium	0.21	0.21	0.21

<sup>1</sup>The were six dietary treatments consisted of: CON = Control, CON+PHY = CON plus 500 FTU/kg feed (Phyzyme XP), and CON+CPX = CON plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate. The 0.5% salt versions had no sodium bicarbonate.

<sup>2</sup>Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D<sub>3</sub>, 2,000 IU; vitamin E, 33 IU; vitamin B<sub>12</sub>, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K<sub>3</sub>), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na<sub>2</sub>SeO<sub>3</sub>), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

<sup>3</sup>Phyzyme XP TPT (Dupont Animal Nutrition, Marlborough, UK), an *Escherichia coli*-derived phytase, was added at 500 FTU /kg to replace 0.12% available phosphorus (AvP) and 0.1% calcium (Ca).

<sup>4</sup>Avizyme 1502 (Dupont Animal Nutrition, Marlborough, UK), is composed of xylanase (300 U/kg), *Trichoderma longibrachiatum*, protease (4000 U/kg), *Bacillus subtilis* and amylase (400 U/kg), and *Bacillus amyloliquefaciens*.

<sup>5</sup>The nutrient compositions were calculated from proximate analyses of all ingredients, and final diet composition was confirmed by proximate analyses.



The phytase enzyme product (PHY) (Phyzyme XP TPT, Dupont Animal Nutrition, Marlborough, UK), an *Escherichia coli*-derived phytase, was added at 500 FTU/kg. The CPX enzyme cocktail (Avizyme 1502, Dupont Animal Nutrition, Marlborough, UK), provided 300 U of xylanase/kg from *Trichoderma longibrachiatum*, 4000 U of protease from *Bacillus subtilis*, and 400 U of amylase from *Bacillus amyloliquofaciens*. Enzyme activity assays of finisher feed were performed by the enzyme manufacturer, and the expected activity for each enzyme was confirmed.

### Statistical Methods

A randomized complete block design with a 2 x 3 factorial arrangement of treatments was used (dietary enzyme x NaCl level) with the general linear model of SAS (SAS Institute, 2008) used to analyze live performance. Variable means were partitioned by LSMEANS and were considered statistically different when  $p < 0.05$ , while  $p < 0.10$  was considered to be numerical trends.

## RESULTS

The effects of dietary treatments on broiler BW, BWG, FC, FCR, and mortality at 14 and 35 d of age are shown in Table 3. Even when no main effects were found for FC and FCR, interactions were detected ( $p < 0.05$ ) for FC (Figure 1) and FCR (Figure 2) at 14 d. Feeding CON+CPX in the presence of 0.5% NaCl decreased FC, but improved FCR at 14 d. No differences in FC were observed after 14 d. At 14 d ( $p < 0.05$ ) and 35 d ( $p < 0.05$ ) broilers fed CON and CON+PHY diets exhibited greater BW and BWG than the birds fed the CON+CPX diet. However, a significant interaction was detected where broilers fed CON+CPX diets with 0.25% NaCl exhibited reduced BW at 35 d ( $p < 0.05$ ; Figure 3) as compared to CON and CON+PHY. At 35 d an interaction was observed that demonstrated that BWG ( $p < 0.05$ ; Figure 4) of broilers fed CON+CPX was greater when the diet contained 0.5% NaCl. None of the dietary factors studied affected mortality (Table 3).

**Table 3** – Effect of dietary treatment on broiler body weight (BW), body weight gain (BWG), feed consumption (FC), feed conversion ratio (FCR), and mortality.

Variable	Age (d)	Treatment <sup>1</sup>							Source of variability		
		Diet			SEM <sup>2</sup>	NaCl level		SEM <sup>3</sup>	Diet	NaCl Level	Diet x NaCl
		CON	CON+PHY	CON+CPX		0.5%	0.25%				
(g)											
BW	1	46	46	45	0.2	45	46	0.2	NS	NS	NS
	14	521 <sup>a</sup>	520 <sup>a</sup>	506 <sup>b</sup>	4.7	518	513	3	<0.05	NS	NS
	35	2531 <sup>A</sup>	2533 <sup>A</sup>	2481 <sup>B</sup>	12.0	2520	2509	9	<0.01	NS	<0.05
(p-value)											
BWG	1-14	476 <sup>a</sup>	475 <sup>a</sup>	460 <sup>b</sup>	4.7	472	468	3	<0.05	NS	NS
	15-35	2009 <sup>a</sup>	2012 <sup>a</sup>	1976 <sup>b</sup>	10.0	2003	1995	8	<0.05	NS	<0.05
	1-35	2485 <sup>a</sup>	2487 <sup>a</sup>	2436 <sup>b</sup>	13.43	2475	2464	11	<0.05	NS	NS
FC	1-14	710 <sup>x</sup>	711 <sup>x</sup>	675 <sup>y</sup>	12.0	697	700	6	<0.10	NS	<0.05
	15-35	2672	2676	2774	69.0	2662	2753	50	NS	NS	NS
	1-35	3388	3377	3446	72.66	3361	3447	59	NS	NS	NS
(g:g)											
FCR <sup>4</sup>	1-14	1.49	1.49	1.46	0.02	1.46	1.49	0.02	NS	NS	<0.05
	15-35	1.69	1.69	1.75	0.04	1.69	1.73	0.03	NS	NS	NS
	1-35	1.65	1.65	1.71	0.04	1.65	1.69	0.03	NS	NS	NS
(%)											
Mortality	1-14	1.56	1.56	1.71	0.65	1.82	1.04	0.59	NS	NS	NS
	1-35	2.34	2.73	3.13	1.31	3.65	1.83	1.07	NS	NS	NS

<sup>A, B</sup> Means across columns lacking a common superscript are significantly different at the  $p < 0.01$ .

<sup>a, b</sup> Means across columns lacking a common superscript are significantly different at the  $p < 0.05$ .

<sup>x, y</sup> Means across columns lacking a common superscript are significantly different at the  $p < 0.10$ .

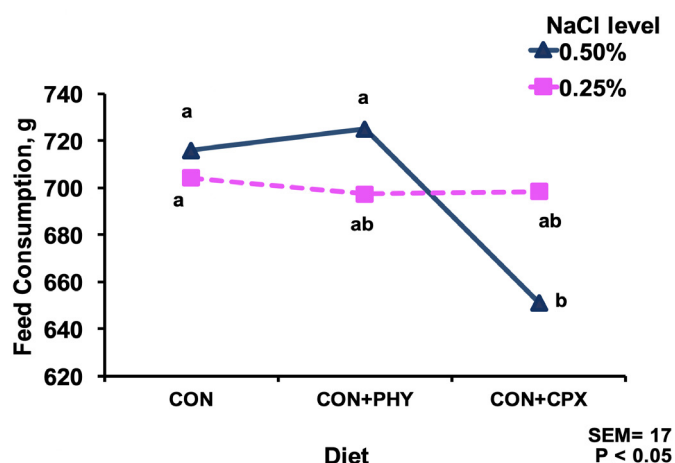
<sup>1</sup>The six dietary treatments consisted of CON = Control, CON+PHY = CON plus 500 FTU/kg feed (Phyzyme XP), and CON+CPX = CON plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate. The 0.5% salt versions had no sodium bicarbonate.

<sup>2</sup>SEM for n=8 pens.

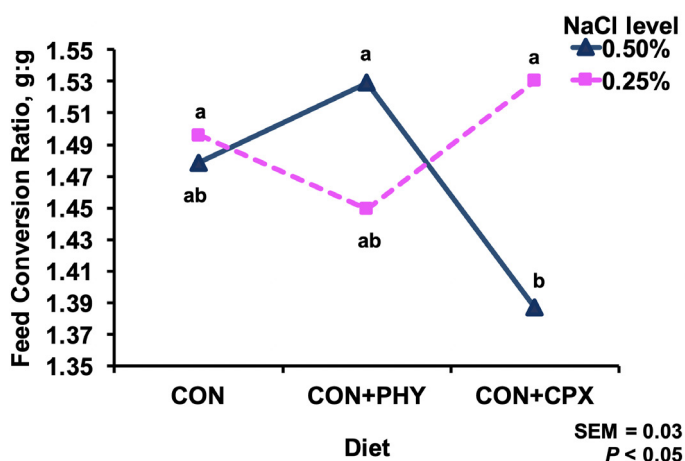
<sup>3</sup>SEM for n=12 pens.

<sup>4</sup>FCR included BW of mortality





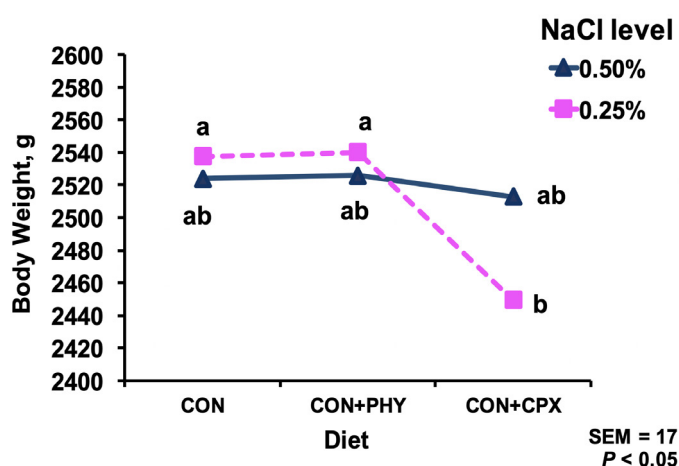
**Figure 1** – Effect of dietary treatment on broiler feed consumption from 1 to 14 d of age. The six dietary treatments consisted of CON = Control, CON+PHY = CON plus 500 FTU/kg feed (Phyzyme XP), and CON+CPX = CON plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl (square) diets contained 0.35% sodium bicarbonate. The 0.5% salt (diamond) diets had no sodium bicarbonate. Pooled standard error of the mean (SEM) for n=24 pens. Means lacking a common superscript are significantly different at  $p < 0.05$ .



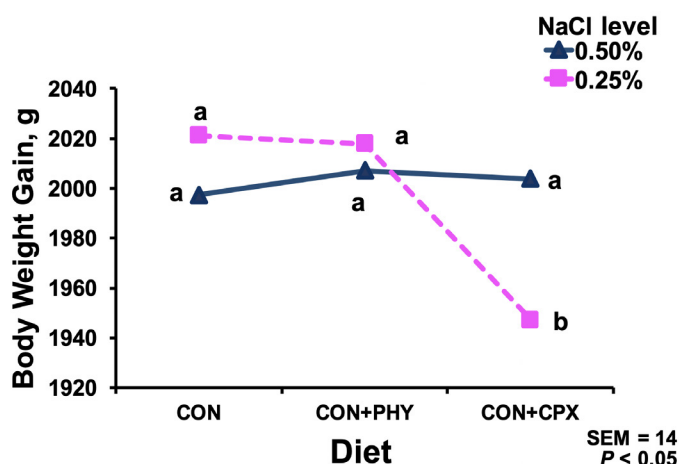
**Figure 2** – Effect of dietary treatment on broiler adjusted feed conversion ratio from 1 to 14 d of age. The six dietary treatments consisted of CON = Control, CON+PHY = CON plus 500 FTU/kg feed (Phyzyme XP), and CON+CPX = CON plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl (square) diets contained 0.35% sodium bicarbonate. The 0.5% salt (diamond) diets had no sodium bicarbonate. Pooled standard error of the mean (SEM) for n=24 pens. Means lacking a common superscript are significantly different at  $p < 0.05$ .

## DISCUSSION

This study determined the effects of the addition of PHY or CPX to a corn-soybean meal-DDGS-PBM based diet formulated with two different levels of Cl created by amending basal diets with either NaCl or NaHCO<sub>3</sub> on broiler live performance to 35 d of age. In this experiment, a marginally low level of dietary ME and commercially typical levels of AvP and Ca for CON and CON+CPX diets (Tables 1 and 2) were used. Only in the CON+PHY diets were AvP and Ca reduced by 0.12% and 0.10%, respectively, expecting the PHY to compensate for these reductions. Also, Na and K



**Figure 3** – Effect of dietary treatment on broiler BW at 35 d of age. The six dietary treatments consisted of CON = Control, CON+PHY = CON plus 500 FTU/kg feed (Phyzyme XP), and CON+CPX = CON plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl (square) diets contained 0.35% sodium bicarbonate. The 0.5% salt (diamond) diets had no sodium bicarbonate. Pooled standard error of the mean (SEM) for n=24 pens. Means lacking a common superscript are significantly different at  $p < 0.05$ .



**Figure 4** – Effect of dietary treatment on broiler BW gain from 15 to 35 d of age. The six dietary treatments consisted of CON = Control, CON+PHY = CON plus 500 FTU/kg feed (Phyzyme XP), and CON+CPX = CON plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl (square) diets contained 0.35% sodium bicarbonate. The 0.5% salt (diamond) diets had no sodium bicarbonate. Pooled standard error of the mean (SEM) for n=24 pens. Means lacking a common superscript are significantly different at  $p < 0.05$ .

were the same for all the diets (0.22% and 0.69%, respectively) as all feeds were made from a common basal, and only Cl differed, being 0.40% and 0.25% for the 0.50% and 0.25% NaCl diets, respectively.

Broilers fed the CON+PHY diet maintained live performance similar to the CON diet, which demonstrated that the PHY enzyme restored AvP and Ca to normal levels as reported by others (Donato *et al.*, 2013; dos Santos *et al.*, 2017; Walk & F. Poernama, 2018). However, no other nutritional benefits due to the PHY were observed. Calculated electrolyte balance (DEB) for the 0.50% NaCl level diets was 164 mEq/kg, contrasting with the 203 mEq/kg of the 0.25% NaCl



diet, with only Cl being different. By way of comparison, Ravindran *et al.* (2008) studied the effect of DEB and the addition of phytase to marginally deficient non-phytate phosphorus and Ca diets. Four DEB levels of 150, 225, 300, and 375 mEq/kg (or 0.15, 0.18, 0.35, and 0.52% Na, respectively) and two levels of phytase (0 and 500 FTU/kg) were studied. They reported that FCR was improved when phytase was added to lower DEB diets (150 – 300 mEq), but not to the 375 mEq/kg diet. However, they could not determine if this response was because of the DEB or due to the differences in the Na level of the different diets. Nonetheless, they attributed the response at 375 mEq to an excess in Na (0.52%) that negatively affected nutrient absorption and metabolism.

Broilers fed CON+CPX grew more slowly than CON and CON+PHY birds during both 1-14 d and 15-35 d periods ( $p < 0.05$ ; Table 3). The reduced growth rate could be partially explained by a trend observed in which FC during the 1-14 d age period for the CON+CPX diet tended to be lower compared to the other two treatments ( $p < 0.10$ ; Table 3). However, the interaction of diet x NaCl level (Figure 1) demonstrated that broilers fed the CON+CPX diet at the 0.5% NaCl level consumed numerically less feed, albeit at an improved FCR ( $p < 0.05$ ; Figure 2) probably due to the lower maintenance required for their reduced BW, than the broilers fed the same diet at the 0.25% NaCl level. Thus, the initially reduced FC observed with the CPX supplementation (Figure 1) was apparently ameliorated by the replacement of NaCl by  $\text{NaHCO}_3$  but reduced BWG and BW ( $p < 0.05$ ) persisted to 35 d (Table 3). Previous researches in various animal species demonstrated that the addition of  $\text{NaHCO}_3$  increased FC (Tripathi *et al.*, 2004; Yoruk *et al.*, 2004ab; Ahmad *et al.*, 2006). However, the consistency of this effect has been debated by others (Fuentes *et al.*, 1998; Balnave *et al.*, 1999; Zakaria *et al.*, 2008) as a multiple dietary factor and physiology of the animal could have modifying effects. Indeed, this effect was only observed during the 1-14 d period of the present study.

Even though CON+CPX diets exhibited statistically similar responses in terms of FC relative to the differences in dietary NaCl versus  $\text{NaHCO}_3$  (Figure 1), it seemed that the addition of CPX tended to reduce FC prior to 14 d ( $p < 0.10$ ; Table 3) primarily in the presence of 0.5% NaCl when compared to the CON diet (Figure 1). Interestingly, although broilers fed the CON diet continued to exhibit greater BWG than CON+CPX fed birds from 15 to 35 d ( $p < 0.05$ ; Table 3), the diet x NaCl interaction for BWG ( $p < 0.05$ ; Figure 3) demonstrated that it was only the CON+CPX birds receiving 0.25%

NaCl that did not grow normally. Thus, it was evident that Cl was interacting with some component of the CPX enzyme cocktail as dietary Cl had no effect in the CON or CON+PHY diets. Buonocore *et al.* (1977) originally characterized the chicken pancreatic  $\alpha$ -amylase and described it as a  $\text{Ca}^{+2}$  glycoprotein. Calcium was necessary for the structural stability of the enzyme while Cl was an activator, which was also true for amylases in general because they have very well conserved three-dimensional structure among species including mammals, other birds, insects, and some bacteria (D'Amico *et al.*, 2000). On the other hand,  $\alpha$ -amylase from most bacteria has been reported to be Ca-dependent but Cl-independent (Machius *et al.*, 1998; Mehta and Satyanarayana, 2016) as the amylase that was present in CPX.

The inclusion of exogenous enzymes such as alpha-amylase at an early age has led to down-regulation and reduced excretion of potentially essential endogenous enzymes associated with a reduced pancreas weight (Mahagna *et al.*, 1995; Gracia *et al.*, 2003; Onderci *et al.*, 2006). Furthermore, Jiang *et al.* (2008) found that supplementation with alpha amylase to broiler diets negatively affected mRNA expression pancreatic amylase. On the other hand, Yuan *et al.* (2017) demonstrated that the inclusion of bacterial amylases not only affected the activity of disaccharidases in the small intestine but also reduced the mRNA expression of sodium/glucose cotransporter 1 (SGLT 1) in the duodenum and jejunum. Such an effect may have modulated digestive physiology. We could hypothesize that the downregulation or reduction of endogenous amylase and SGLT1 (in addition to the fact that there could be a lower Cl level available for the endogenous amylase activation) would result in reduced digestion of starch and absorption of glucose, affecting BW and BWG.

It appeared that the ME that was expected to come from the addition of CPX (mainly from carbohydrates) was not apparent, which could have been due to dietary Cl. Dietary NaCl has also been demonstrated to affect intestinal amino acid absorption (Chen *et al.*, 2005), which could have been related to some additional interaction with the protease in the CPX enzyme cocktail.

Cowieson & Ravindran (2008) fed male broilers in cages to 21 d either a nutritionally adequate diet or a marginally ME and amino acid-deficient diet with or without CPX in a 2 x 2 factorial design. They reported that BWG and FCR were improved by the addition of the CPX enzyme cocktail, something that



was not observed in the present experiment. However, Cowieson & Ravindran (2008) fed mash diets, which were 30% lower in total phosphorus and 20% lower in NaCl than the present 0.5% NaCl diet. It was important to point out that the ME in the present CON starter and grower diets were 181 and 121 kcal/kg, respectively, less than those of Cowieson & Ravindran (2008). In addition, the present diets contained approximately 9% less corn, limiting the availability of substrate upon which certain enzymes could work. This supported the concept that the effectiveness of feed enzymes could depend upon many factors in the diet and animal digestive tract (Bedford, 2018). It was concluded that dietary Cl, as NaCl versus  $\text{NaH}_2\text{CO}_3$ , could affect CPX but not PHY feed enzyme function in broilers. Further, it may be suggested that certain feed enzymes may be best utilized at later broiler ages rather than in initial feeds.

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## ***The Effects of Sodium Chloride versus Sodium Bicarbonate at the Same Dietary Sodium Concentration on Efficacy of Dietary Phytase and a Carbohydrase-Protease Cocktail in Broilers***

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