

## ABSTRACT

SCHWARTZ, MEGHAN HELENE. Effects of Pro-nutrient Feed Additives on Live Performance and Gastrointestinal Health of Broilers and Turkeys. (Under the direction of Dr. Peter R. Ferket).

Nutrient digestibility directly influences intestinal health and growth performance of broilers and turkeys. Coccidiosis is among the most economically important enteric diseases in turkeys as it has adverse effects on performance and resistance to other enteric pathogens. Increasing public perception against the use of anticoccidials requires the industry to consider additional alternatives. Moreover, the potential economic value of supplemental enzymes that improve energy availability increases as dietary energy costs increase, especially when used in low energy diets that may contain grain by-products such as distiller's dried grains with solubles (DDGS). Increasing dietary inclusion of DDGS may contribute to variable performance of poultry because of its high nutritional variability and low energy value, but this may be remediated by dietary enzyme supplementation. Similarly, dietary phytase inclusion increases phosphorus availability, decreasing feed cost of inorganic phosphorus, and reducing fecal phosphorus emission. This dissertation evaluated two alternatives to antibiotics under a coccidia-challenge model in poult. Additionally, energy-releasing enzyme products were evaluated with and without DDGS inclusion in two experiments: one experiment evaluated pelleted diets fed to toms, and the other was mash fed to mixed-sex broilers. Finally, the efficacy a novel phytase was evaluated in a titration study with poult.

The first experiment evaluated Regano<sup>®</sup> on poult performance, relative intestinal weight (rIW) and histomorphology during a coccidia-challenge until 15 d of age. *Eimeria* challenge significantly reduced 14 d BW and increased 15 d rIW. Regano<sup>®</sup> supplementation significantly reduced 7 d BW but had no significant effect on 14 d BW. Growth performance

coincided with scanning electron microscopy observations and histomorphometrical analysis. Regano<sup>®</sup> supplementation did not improve poult response to *Eimeria* challenge perhaps due to excessive dose level.

Experiment 2 was similar to Experiment 1, but an all-natural proprietary composition product Natustat<sup>™</sup> (Alltech, Inc., Nicholasville, KY) was assessed. Natustat<sup>™</sup> supplementation did not improve poult performance during this cocci-challenge, but it may improve compensatory absorption of nutrients in the jejunum after challenge.

In Experiment 3, a phytase product with and without an enzyme product containing xylanase, amylase, and protease (XAP) activities was supplemented in corn-soy diets fed to toms with and without DDGS inclusion until 20 weeks. Increasing DDGS to 15 % had no negative effect on BW gain but negatively affected FCR and pellet quality. The addition of phytase +/- XAP enzymes resulted in an apparent uplift of over 150 kcal ME/kg, reducing the need for dietary fat and inorganic phosphate inclusion, regardless of the dietary DDGS level.

Similarly, Experiment 4 contained an enzyme product supplemented in corn-soy diets with and without DDGS inclusion. However, the enzymes in Experiment 4 were a xylanase and an enzyme with xylanase and  $\beta$ -glucanase activities fed in mash form to mixed-sex broilers until 49 d. Dietary supplementation of xylanase improved performance of broilers fed mash corn-soy diets with 10 % DDGS and in low-energy diets containing DDGS restored broiler performance to the level of the high-energy control at 49 d.

Experiment 5 was a dose titration study of a novel phytase in poults until 28 d. Phytase supplementation significantly improved turkey growth response, which was associated with improved feed intake and nutrient bioavailability. The optimum dose of this dietary phytase was identified to be approximately 1000 FTU/kg of feed.

In conclusion, the alternatives to antibiotics examined did not prevent performance reduction associated with the cocci-challenge. Furthermore, energy releasing enzymes improve nutrient utilization and growth performance in corn-soy diets but did not provide additional benefit with dietary DDGS inclusion.

Effects of Pro-nutrient Feed Additives on Live Performance and Gastrointestinal Health of  
Broilers and Turkeys

by  
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## **DEDICATION**

To my family

## **BIOGRAPHY**

Meghan Schwartz was raised in Barron, Wisconsin. She received her Bachelor of Science in Natural Sciences majoring in Biochemistry from the University of Wisconsin-Madison. One year later, she received her Master of Science in Animal Science from UW-Madison under the direction of Dr. Mark E. Cook. While at UW, her research focused on mechanisms by which egg yolk antibodies improve chick performance. Meghan moved to Raleigh, NC to attend North Carolina State University as a doctoral student in the Department of Poultry Science concentrating in Nutrition and Biotechnology under the direction of Dr. Peter R. Ferket. Her research at NC State focused on effects of pro-nutrient feed additives on live performance and gastrointestinal health of broilers and turkeys. Upon completion of her Ph.D., Meghan will continue to work as a poultry nutritionist at Schwartz Consulting Services, Inc. with her father, Dr. Robert W. Schwartz.

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## TABLE OF CONTENTS

LIST OF TABLES .....	ix
LIST OF FIGURES .....	xv
CHAPTER 1 .....	1
1.1 INTRODUCTION .....	2
1.2 NORMAL DIGESTION.....	3
Carbohydrates .....	3
Proteins and amino acids .....	3
Fats .....	4
1.3 ANALYSES.....	5
Sample type (fecal vs ileal).....	6
Common bioassays .....	7
1.4 MODIFICATION OF DIGESTION.....	10
Early access, delayed feeding, and feed restriction .....	10
Enteric challenges and stress .....	11
Anti-nutritional factors (ANFs) .....	14
Ingredient processing .....	18
1.5 FACILITATION OF DIGESTION .....	21
Feed processing and feed form .....	21
Antibiotic enteric conditioners.....	23
Alternatives to antibiotic enteric conditioners .....	24
Exogenous enzymes.....	27
1.6 CURRENT STUDY .....	30
1.7 REFERENCES .....	33
CHAPTER 2 .....	46
2.1 ABSTRACT.....	47
Treatments.....	49

Bird husbandry .....	49
Data collection .....	50
Histology analysis .....	51
Electron microscopy analysis .....	52
Statistical analysis .....	52
Animal ethics .....	53
2.4 RESULTS .....	53
2.5 DISCUSSION .....	55
2.6 TABLES AND FIGURES .....	60
2.7 REFERENCES .....	69
CHAPTER 3 .....	72
3.1 ABSTRACT .....	73
3.2 INTRODUCTION .....	74
3.3 MATERIALS AND METHODS .....	75
Bird husbandry .....	75
Data collection .....	76
Statistical analysis .....	76
Animal ethics .....	77
3.4 RESULTS .....	77
3.5 DISCUSSION .....	78
3.6 TABLES .....	82
3.7 REFERENCES .....	87
CHAPTER 4 .....	89
4.1 ABSTRACT .....	90
4.2 INTRODUCTION .....	91
4.3 MATERIALS AND METHODS .....	92
Treatments .....	92
Feed manufacturing .....	93

Bird husbandry .....	95
Growth performance, walking scores, and tibia ash .....	95
Statistical analysis .....	96
Animal ethics .....	96
4.4 RESULTS AND DISCUSSION .....	96
Feed manufacturing .....	96
Growth performance, walking scores, and tibia ash .....	98
4.5 TABLES .....	104
4.6 REFERENCES .....	135
CHAPTER 5 .....	138
5.1 ABSTRACT .....	139
5.2 INTRODUCTION .....	140
5.3 MATERIALS AND METHODS .....	141
Bird husbandry .....	141
Growth performance .....	142
Statistical analysis .....	143
Animal ethics .....	143
5.4 RESULTS .....	144
5.5 DISCUSSION .....	148
5.6 TABLES .....	152
5.7 REFERENCES .....	180
CHAPTER 6 .....	181
6.1 ABSTRACT .....	182
6.2 INTRODUCTION .....	183
6.3 MATERIALS AND METHODS .....	184
Bird husbandry .....	184
Experimental diets .....	185
Sample collection and laboratory analysis .....	186

Statistical analysis .....	189
Animal ethics .....	189
6.4 RESULTS .....	189
Bird performance .....	189
Tibia and toe ash .....	190
Fecal and ileal DM digestibility and nitrogen-corrected apparent metabolizable energy (AMEn) .....	191
Fecal and ileal sample analyses .....	191
Fecal and ileal mineral digestibility .....	192
6.5 DISCUSSION .....	194
6.6 TABLES .....	197
6.7 REFERENCES .....	214
CHAPTER 7 .....	216

## LIST OF TABLES

### CHAPTER 2

Table 1: Composition and nutrient content of the basal diet fed to turkey poults from 1 to 15 d of age.....	60
Table 2: Effect of cocci-challenge and Regano <sup>®</sup> supplementation on growth performance of tom poults until 14 d of age. ....	61
Table 3: Effect of cocci-challenge and Regano <sup>®</sup> supplementation on relative intestinal weight (rIW) of tom poults at 8 and 15 d of age.....	62
Table 4: Effect of cocci-challenge and Regano <sup>®</sup> supplementation on histological measurements of ileum of tom poults at 8 d of age. ....	63
Table 5: Effect of cocci-challenge and Regano <sup>®</sup> supplementation on histological measurements of ileum of tom poults at 15 d of age. ....	65

### CHAPTER 3

Table 1: Composition and nutrient content of the basal diet fed to turkey poults from 1 to 15 d of age.....	82
Table 2: Effect of dietary Natustat <sup>™</sup> supplementation on body weights of cocci-challenged and unchallenged poults at 7 and 14 d of age. ....	83
Table 3: Effect of dietary Natustat <sup>™</sup> supplementation on feed intake (FI) and feed conversion ratio (FCR) of cocci-challenged and unchallenged poults from 1 to 14 d of age.....	84
Table 3: Effect of dietary Natustat <sup>™</sup> supplementation on feed intake (FI) and feed conversion ratio (FCR) of cocci-challenged and unchallenged poults from 1 to 14 d of age.....	84
Table 4: Effect of dietary Natustat <sup>™</sup> supplementation on relative intestinal weight (rIW) of cocci-challenged and unchallenged poults at 8 d of age.....	85

Table 5: Effect of dietary Natustat™ supplementation on relative intestinal weight (rIW) of cocci-challenged and unchallenged poult at 15 d of age. ....	86
--	----

**CHAPTER 4**

Table 1: Composition and nutrient content of the experimental diets fed to turkey toms from 1 to 21 days of age with and without distillers dried grains with solubles (DDGS) .....	104
Table 2: Composition and nutrient content of the experimental diets fed to turkey toms from 22 to 42 days of age with and without distillers dried grains with solubles (DDGS). ....	106
Table 3: Composition and nutrient content of the experimental diets fed to turkey toms from 43 to 56 days of age with and without distillers dried grains with solubles (DDGS). ....	107
Table 4: Composition and nutrient content of the experimental diets fed to turkey toms from 57 to 70 days of age with and without distillers dried grains with solubles (DDGS). ....	108
Table 5: Composition and nutrient content of the experimental diets fed to turkey toms from 71 to 84 days of age with and without distillers dried grains with solubles (DDGS). ....	109
Table 6: Composition and nutrient content of the experimental diets fed to turkey toms from 85 to 98 days of age with and without distillers dried grains with solubles (DDGS). ....	110
Table 7: Composition and nutrient content of the experimental diets fed to turkey toms from 99 to 119 days of age with and without distillers dried grains with solubles (DDGS). ....	111
Table 8: Composition and nutrient content of the experimental diets fed to turkey toms from 120 to 137 days of age with and without distillers dried grains with solubles (DDGS). ....	112
Table 9: Feed manufacturing parameters of starter (phase 2) diets with and without distillers dried grains with solubles (DDGS) and enzyme supplementation. ....	113

Table 10: Feed manufacturing parameters of grower 1 (phase 3) diets with and without distillers dried grains with solubles (DDGS) and enzyme supplementation. ....	114
Table 11: Feed manufacturing parameters of finisher 1 (phase 6) diets with and without distillers dried grains with solubles (DDGS) and enzyme supplementation. ....	115
Table 12: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on BW of toms. ....	116
Table 14: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative feed intake of toms. ....	120
Table 15: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period FCR of toms. ....	122
Table 16: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative FCR of toms. ....	124
Table 17: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period mortality of toms. ....	126
Table 18: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative mortality of toms. ....	128
Table 19: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on incidence and severity of leg abnormalities of toms at 14 weeks of age. ....	130
Table 20: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on incidence and severity of leg abnormalities of toms at 17 weeks of age. ....	132
Table 21: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on tibia ash (%) at 21 d of age in toms. ....	134

## **CHAPTER 5**

Table 1: Enzyme premix formulations. ....	152
Table 2: Composition and nutrient content of the experimental starter diets fed to broilers from 1 to 14 d of age without distillers dried grains with solubles (DDGS). ....	153

Table 3: Composition and nutrient content of the experimental starter diets fed to broilers from 1 to 14 d of age with distillers dried grains with solubles (DDGS). .....	155
Table 4: Composition and nutrient content of the experimental grower diets fed to broilers from 14 to 35 d of age without distillers dried grains with solubles (DDGS). ...	157
Table 5: Composition and nutrient content of the experimental grower diets fed to broilers from 14 to 35 d of age with distillers dried grains with solubles (DDGS). .....	159
Table 6: Composition and nutrient content of the experimental finisher diets fed to broilers from 35 to 49 d of age without distillers dried grains with solubles (DDGS). ...	161
Table 7: Composition and nutrient content of the experimental finisher diets fed to broilers from 35 to 49 d of age with distillers dried grains with solubles (DDGS). .....	163
Table 8: Brooder temperatures.....	165
Table 9: Lighting program schedule. ....	165
Table 10: Effect of dietary energy level, inclusion of distiller’s dried grains with solubles (DDGS) and enzyme supplementation on BW of female broilers. ....	166
Table 11: Effect of dietary energy level, inclusion of distiller’s dried grains with solubles (DDGS) and enzyme supplementation on BW of male broilers.....	167
Table 12: Effect of dietary energy level, inclusion of distiller’s dried grains with solubles (DDGS) and enzyme supplementation on coefficient of variation of 42 d BW of male and female broilers. ....	168
Table 13: Effect of dietary energy level, inclusion of distiller’s dried grains with solubles (DDGS) and enzyme supplementation on period feed intake of mixed-sex broilers. ....	169
Table 14: Effect of dietary energy level, inclusion of distiller’s dried grains with solubles (DDGS) and enzyme supplementation on cumulative feed intake of mixed-sex broilers. ....	170
Table 15: Effect of dietary energy level, inclusion of distiller’s dried grains with solubles (DDGS) and enzyme supplementation on period feed conversion ratio (FCR) of mixed-sex broilers.....	171

Table 16: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative feed conversion ratio (FCR) of mixed-sex broilers. ....	172
Table 17: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period mortality of female broilers..	173
Table 18: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period mortality of male broilers. ....	174
Table 19: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative mortality of female broilers. ....	176
Table 20: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative mortality of male broilers. ....	178

## **CHAPTER 6**

Table 1: Room temperature schedule. ....	197
Table 2: Dietary treatment, enzyme identification and incorporation rate. ....	197
Table 3: Composition and nutrient content of diets fed to turkeys.....	198
Table 4: Post-pellet enzyme application formulation for experimental diets.....	200
Table 5: Effect of phytase supplementation on body weight of tom poults. ....	201
Table 6: Effect of phytase supplementation on average daily gain (ADG) of tom poults...	202
Table 7: Effect of phytase supplementation on feed intake (g/bird per d) of tom poults. ...	203
Table 8: Effect of phytase supplementation on periodic feed conversion ratio (FCR) of tom poults.....	204
Table 9: Effect of phytase supplementation on cumulative feed conversion ratio (FCR) of tom poults.....	205
Table 10: Effect of phytase supplementation on periodic mortality (%) of tom poults. ....	206

Table 11: Effect of phytase supplementation on cumulative mortality (%) of tom poult..	207
Table 12: Effect of phytase supplementation on tibia and toe ash (as is, %) of 28 d old tom poult.....	208
Table 13: Effect of phytase supplementation on apparent metabolizable energy (AME <sub>n</sub> ), apparent nitrogen retention (ANR), and digestible ash (Dash) as determined from fecal samples including an indigestible acid insoluble ash marker. ....	209
Table 14: Effect of phytase supplementation on fecal content of Ca, P, S, Mg, Na, K, Cu, Fe, Mn, and Zn (dry matter basis) of tom poult. ....	210
Table 15: Effect of phytase supplementation on ileal content of Ca, P, S, Mg, Na, K, Cu, Fe, Mn, Zn and Al (dry matter basis) of tom poult. ....	211
Table 16: Effect of phytase supplementation on fecal digestibility (%) of dry matter of Ca, P, S, Mg, Na, K, Cu, Fe, Mn and Zn of tom poult. ....	212
Table 17: Effect of phytase supplementation on ileal digestibility (%) of dry matter of Ca, P, S, Mg, K, Cu, Fe, Zn and Mn of tom poult. ....	213

## LIST OF FIGURES

### CHAPTER 2

Figure 1: Scanning electron micrographs (150 X and 850 X) of ileum sections of poult s at 8 d of age.....	67
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## **CHAPTER 1**

### **LITERATURE REVIEW**

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<sup>1</sup>The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service or the North Carolina Cooperative Extension Service of the products mentioned nor criticism of similar products not mentioned.

## 1.1 INTRODUCTION

In the process of digestion, the complex molecules that comprise the diet must be cleaved to facilitate absorption. Quantifying nutrient digestibility of feed ingredients is crucial in diet formulation. Chemical analysis can easily give a gross nutrient value but this does not account for nutrient availability. *In vivo* analyses have been developed over the years as a means to assess nutrient availability. Conclusions drawn from these analyses assumes that the value of the ingredient being tested is independent of other diet constituents and that the value remains constant regardless of age, type, and physiological status of the bird (Sibbald *et al.*, 1960).

Gastrointestinal tract development, including digestive enzyme production, may be a limiting factor in posthatch growth (Corless and Sell, 1999). The ability of these enzymes to be produced and function correctly in the bird directly influences bird health and performance. Furthermore, feed ingredient processing, feed formulation, and bird health and management can also impact feed digestibility and consequently affect bird performance and profitability. This review will highlight *in vitro* and *in vivo* analyses for digestibility values, normal digestion, digestion under stressed situations, modifications of digestive processes, and ways to help facilitate digestion in regard to gut health and performance.

## 1.2 NORMAL DIGESTION

### ***Carbohydrates***

Starch is the major form of storage in plant materials. Poultry lack amylase in their saliva, but amylase found in the crop is believed to be a result of digesta flow in reverse (Hoerr, 1998). However, questions arise about the quantity of starch digestion in the crop that is a result of true amylase activity or *Lactobacilli* action (Leeson and Summers, 2001). The jejunum is considered the major site of carbohydrate digestion. The pancreas secretes alpha-amylase into the duodenum. Interestingly, pancreatic enzyme activities have been related to body weight (BW) and intestinal size (Sklan and Noy, 2000). Amylase activity in the pancreas was highest at hatch and decreased until d 8, and amylase activity in the intestinal contents also increased with age (Nir *et al.*, 1993). The brush border enzymes of the jejunum are also secreted to facilitate digestion of complex sugars. Sell *et al.* (1989) reported that disaccharidases specific activities including maltase, sucrase, isomaltase, and trehalase were highest in jejunum segments from poults. Carbohydrates that are not absorbed in the small intestine become fuel for the microflora of the ceca and large intestine.

### ***Proteins and amino acids***

Protein is first digested in the proventriculus by gastric juice containing pepsinogen and HCl. Pepsinogen is converted to its active form pepsin when the pH of the proventriculus decreases. The activity of pepsin on the protein results in polypeptides that can be further cleaved by enzymes found in the intestine such as trypsin, chymotrypsin, and

elastase, which release terminal peptide bonds. Zymogens released from the pancreas are critical in protein digestion. Enterokinase from the duodenum cleaves trypsinogen into its active form, trypsin. Trypsin initiates activation of other zymogens. Carboxypeptidases A and B are exopeptidases secreted from the pancreas. They attack the carboxyl-terminal ends to sequentially remove amino acids. Both carboxypeptidase A and B are inhibited by proline. Carboxypeptidase A is also inhibited by charged side-chains. Conversely, carboxypeptidase B needs cationic side-chains for optimal activity. Collagenase is released from the pancreas, whereas the jejunal wall releases peptidase and polynucleotidase. Peptidases are also found in the intestinal mucosa and lumen. They hydrolyze small peptides. Other enzymes, such as disaccharidases, phosphatases, and aminopeptidases, are found in the brush border membrane of the intestine. Peptides are better absorbed in the jejunum and amino acids are better absorbed in the ileum (Leeson and Summers, 2001).

Trypsin specific activity in chickens was shown to increase from hatch until 11 d in the pancreas and increase with age in the intestinal contents (Nir *et al.*, 1993). The same authors reported that chymotrypsin specific activity in the pancreas decreased for the first 8 d but increased thereafter and increased with age in the intestinal contents.

### ***Fats***

Most fat digestion occurs in the small intestine. Emulsification with bile salts that are secreted into the small intestine from the gallbladder is required for fat digestion because lipase acts at the interface of the oil and water. Lipase hydrolyses the fat into fatty acids, monoglycerides, and glycerol. Next, cholesterol esterase from the pancreas cleaves

cholesterol-fatty esters. Short-chain fatty acids and glycerol can be directly absorbed into the mucosa, but other fatty acids, cholesterol, and monoglycerides must be emulsified with bile salts to form micelles for proper absorption. It is important for the amount of bile salts secreted to be adjusted for the quantity of dietary saturated and unsaturated fatty acids.

Ketels and De Groote (1989) demonstrated that the best fit between unsaturated:saturated and fat digestibility was exponential. The maximal value for fat AME<sub>n</sub> was found to be 8,227 kcal/kg and 92 % for the digestibility coefficient. Lipase specific activity increased with age in both pancreas and intestinal contents of chickens (Nir *et al.*, 1993), which is in agreement with Nitsan *et al.* (1991) where lipase levels in jejunum contents also increased with age.

### 1.3 ANALYSES

One of the first *in vivo* digestibility methods was total collection. This method required that all of the excreta, free of contaminants, be quantitatively collected, which poses numerous challenges including animal welfare concerns (Bakker and Jongbloed, 1994). Alternative methods involve markers. When using a marker, it is assumed that the marker is not absorbed or changed during its transit through the gut. Commonly used markers are chromic oxide, titanium dioxide, and acid-insoluble ash. The chromic oxide marker method was preferred over the total collection method after Sibbald *et al.* (1960) reported more precise metabolizability values with chromic oxide. One potential problem when comparing

these methods is the recovery of the chromic oxide in the feces. Mueller (1956) showed that 5 % of the chromic oxide could not be recovered, resulting in lower digestibility values for the marker method compared to the total collection method.

The acid-insoluble ash method most commonly used in poultry is Vogtmann *et al.* (1975). These authors reported that this method gave similar values as the total collection method. However, several authors have reported higher digestibility in poultry. Suggested explanations for higher digestibility values using acid-insoluble ash are: 0.25 % dietary inclusion of sand (Tillman and Waldroup, 1988) and dietary silica inclusion greater than 0.2 % (Cheng and Coon, 1990). Currently, the acid-insoluble ash method is more prevalent than the chromic oxide method because it is more economical and less hazardous. Conversely, titanium dioxide is a more acceptable marker when only a small sample size is available (Short *et al.*, 1996).

### ***Sample type (fecal vs ileal)***

Continued debate remains about the type of sample to obtain, be it of fecal or ileal origin. Much of this debate is concerning amino acid digestibility and the effect of hindgut bacteria that catabolize the excess dietary amino acids. When meat-type chickens were fed a protein-free diet, the site of digestibility measurement, ileal vs fecal, generally did not influence the endogenous amino acid production (Kadim *et al.*, 2002). Furthermore, fecal digestibility values tended to be higher than ileal digestibility values, which strongly indicated microbial catabolism of amino acids in the ceca and large intestine (Ravindran *et al.*, 1999; Kadim *et al.*, 2002; Saki *et al.*, 2009). Ravindran *et al.* (1999) showed that poorly

digestible feed ingredients, such as wheat, sunflower meal, and cotton meal, will give higher excreta digestibility than ileal digestibility. This is a result of the ingredients' carbohydrates being poorly utilized by the bird and then fermented in the hindgut for bacterial product synthesis. Similarly, excreta collection has been shown to overestimate apparent metabolizable energy (AME) and AME adjusted for nitrogen (AME<sub>n</sub>) as compared to ileal collection analyses (Saki *et al.*, 2009). Ten Doeschate *et al.* (1993) compared ingredient digestibility values in birds selected for either weight gain, or feed conversion or commercial strain, males vs females, and different ages (2, 4, and 6 weeks of age). Overall, females demonstrated higher digestibility values than males, and birds selected for feed conversion had higher digestibility coefficients than the birds selected for weight gain and the commercial strain. The effect of age on digestibility values was inconsistent. In other words, it is extremely critical that the age, genotype, and sex of the bird and the entire experimental diet be reported when comparing digestibility values.

### ***Common bioassays***

Traditionally, the chick growth bioassay is the preferred method (Crissey and Thomas, 1987). In this method, graded levels of the tested substance are added to a basal diet that is deficient in the nutrient being evaluated. The goal is to establish a relationship between the growth response and the level of the substance in the diet (Ravindran and Bryden, 1999). The disadvantages of this assay are that it is expensive, time consuming, and measures only one nutrient at a time. Assumptions made for this assay are that the growth response measured is due solely to the nutrient under analysis. One of the other commonly

used assays is the precision-fed cecectomized rooster assay. In this assay, the test diet is intubated into the crop of a fasted rooster, while another rooster is fasted over the same 24 hr period. Excreta are collected during this time and analyzed for true metabolizable energy (TME) (Sibald, 1976) and true amino acid availability (TAAA) (Sibald, 1979). A correction factor is made for the metabolic and endogenous excretions of the negative control birds. Advantages of this assay are that it's relatively quick and inexpensive, while disadvantages include questions about contributions of microbes in excreta collections and whether a fasted bird is the proper control (Engster *et al.*, 1985). Kessler *et al.* (1981) reported that amino acid availability values were higher in cecectomized roosters than intact roosters. Similarly, Engster *et al.* (1985) evaluated six different laboratories conducting the precision fed rooster assay. Even though different techniques were employed, TAAA values agreed and variation among labs was not any greater than variation within a lab. A third method used is the standardized ileal assay. For this method, apparent ileal digestibility values are corrected for basal endogenous secretions using enzymatically hydrolyzed casein (Garcia *et al.*, 2007). The treated casein is fed with the assumption that all of the free amino acids and peptides will be absorbed, and the excreta from these birds is assumed to match the endogenous losses. Ileal contents are collected at 21 d of age. A variation of this diet involves feeding a N-free diet to correct for endogenous losses (Adedokun *et al.*, 2007). Both methods of accounting for endogenous losses have been shown to give comparable digestibility values at 21 d of age in both chicks and poults fed various meat and bone meal sources (Adedokun *et al.*, 2007). Finally, a fourth method has been recently proposed, which is a precision-fed

chick assay. At 22 d of age, chicks are precision-fed 10 g of a sample pre-mixed with a chromic oxide marker. Ileal contents are collected 4 hours after feeding (Kim *et al.*, 2009).

Debate still remains about the determination of the best bioassay method. Crissey and Thomas (1987) fed soybean meal (SBM) and autoclaved SBM to chicks and roosters. The roosters were intact, cecectomized or cannulated. Excreta from intact birds was collected either on mats or in bags. For statistical significance, only a 4 % difference in chick weight gain was necessary, but in the intact roosters a 30 % or 41 % difference in lysine quantity was required for statistical significance when excreta was collected on mats and in bags, respectively. Cecectomized roosters and cannulated roosters required a 42 % and 67 % difference in lysine quantity to demonstrate significance. When looking for very small differences a chick assay would be preferable. Another concern about the type of assay chosen is age of the bird. Several authors (Huang *et al.*, 2005; Batal and Parsons, 2002) have reported increased digestibility and metabolizable energy adjusted for nitrogen (ME<sub>n</sub>) with rising age in chickens. Following-up on some of this research, Garcia *et al.* (2007) reported that amino acid digestibility coefficients from ileal digesta of chicks at 7 d of age were significantly less than digestibility coefficients from the precision fed rooster assay. Kim *et al.* (2009) reported that the standardized digestibility values for a few amino acids in corn diets were higher in the precision fed rooster assay than in the precision fed chick assay and the standardized ileal assay. Standardized digestibility values for SBM and meat and bone meal (MBM) did not significantly differ across the various assays. These authors concluded that all three assays should be suitable for amino acid digestibility studies. Looking at the

larger picture, it is imperative to consider the time, labor, cost, feed ingredient characteristics, bird type, standardization method, number of replicate treatment groups, control groups, and bird age when evaluating data and determining the appropriate bioassay.

#### 1.4 MODIFICATION OF DIGESTION

##### ***Early access, delayed feeding, and feed restriction***

Early feeding has been shown to improve intestinal development in poultry through increased villi height and crypt depth (Geyra *et al.*, 2001). Early feeding has also increased relative intestinal weight (Noy *et al.*, 2001). Taking this one step earlier, *in ovo* feeding (IOF) can increase the digestive ability of poults. Foye *et al.* (2007) reported that poults IOF arginine and  $\beta$ -hydroxy-  $\beta$ -methyl-butyrate (HMB) significantly increased jejunal sucrase, maltase and leucine aminopeptidase activities at 25 E and 14 d of age. This IOF technique may enhance the bird's ability to digest nutrients even before hatch.

Because early feeding has been shown to improve digestion, feed restriction should delay digestive functions. Delaying poults access to feed and water for 54 hours reduced relative small intestinal, proventriculus, and gizzard weight until 4 d of age (Corless and Sell, 1999). In addition, this 54 hour delay in feed and water access reduced the activities of pancreatic amylase, lipase, and trypsin activities at 2 d of age. These results are in general disagreement with those of Pinchasov and Noy (1993), where a 24- or 48-hour delay in placement did not reduce relative pancreas and gizzard weights. The relative pancreas

weights are of particular attention. Corless and Sell (1999) suggested that the changes in relative pancreatic weight may be reflective of changes in digestive ability. Applying this feed restriction to older birds, Siddons (1972) reported that 16 d old chicks exhibited a decrease in activities of small intestinal disaccharidases (maltase, sucrase, isomaltase, and palatinase) and in protein quantity of small intestinal content homogenates after 3 d of fasting. He concluded that dietary carbohydrates are necessary for the disaccharidases to develop but the carbohydrate form is irrelevant. Taking feed restriction one step further, Razdon and Pettersson (1994) restricted broiler feeding to once daily. Compared to *ad libitum* fed birds, the restricted fed birds had significantly improved ileal starch digestibility, whereas crude protein, crude fat, organic matter and nonstarch polysaccharide (NSP) digestibilities of ileal contents were numerically higher. Another interesting result of this experiment was an increase in cecal short chain fatty acids (SCFA) in the restricted fed birds. The authors concluded that the increased SCFA were a result of improved cecal fermentation capacity. One explanation of this improved digestibility is that the birds were in starvation mode, and they convert better when they are fed under their requirement. This is evident in the data that the restricted fed broilers had lower BW and improved feed conversion ratio.

### ***Enteric challenges and stress***

Several diseased and stressed states commonly reduce bird performance, which can be related to decreased digestion. One of the most costly diseases in poultry production is coccidiosis, which results in impaired weight gain and feed conversion (Williams, 2003). Only approximately 70 % of the impaired weight gain can be accredited to anorexia (Preston-

Mafham and Sykes, 1967). Coccidiosis has also been shown to decrease feed and water intake (Reid and Pitois, 1965; Ward *et al.*, 1995). Several groups have reported reductions in digestive enzymes during coccidiosis infections. For example, Turk (1972) suggested that protein digestion is impaired during a coccidiosis infection; however, the direct mechanism is undetermined. In another study, pancreatic weight and overall amylolytic activity in the same organ were reduced in broilers infected with coccidiosis. The amylolytic activity generally decreased as the severity of the coccidiosis infection increased, and the decrease in enzyme activity was determined to be independent of reduced feed intake during infection (Major and Ruff, 1978a). In another paper by the same authors, the disaccharidase (sucrase and maltase) activity of intestinal epithelial cell fractions was determined during a coccidia challenge in broilers. The uninoculated control birds had the highest disaccharidase activity in the jejunum followed by duodenum and then ileum sections. The disaccharidase activities only decreased in the intestinal area that was most severely infected (Major and Ruff, 1978b). These results are in agreement with those of Enigk and Dey-Hazra (1976), who found activities of maltase and saccharase to be highest in the mid-jejunum, and the extent of the decrease in disaccharidase activity corresponded with the degree of coccidia infection. One additional connection between coccidiosis and digestion involved the gizzard crushing the oocyst wall. The action of chymotrypsin and bile salts releases sporocysts from the sprozoites (McDougald, 2003). Therefore, if the digestive enzymes are reduced release of the sporocysts is also decreased.

Another costly disease that is increasingly more prevalent in commercial poultry production is necrotic enteritis (NE). NE is an enteric disease due to toxins produced by the Gram-positive bacterium *Clostridium perfringens* usually resulting in characteristic mucosal necrosis. Many predisposing factors of the diet have been suggested for NE, including type of cereal grain, animal by-products, feed particle size and feed form (Williams, 2003). Annett *et al.* (2002) reported an increase in *in vitro* *C. perfringens* type A proliferation in digested barley and wheat but not in digested corn or in non-digested barley, wheat and corn. These findings support observations of increased NE incidence in birds fed grains other than corn (Riddell and Kong, 1992). Moreover, use of all wheat diets *versus* all corn diets increased mortality due to NE 6-10X (Branton *et al.*, 1987). In the same experiment, birds fed roller-milled wheat had higher mortality than birds fed hammer-milled wheat, while there was no significant effect on bird weight gain and feed conversion ratio. This does not agree with Riddell and Kong (1992) who observed no significant link determined between particle size and mortality due to NE. Furthermore, birds fed pelleted diets had lower numbers of *C. perfringens* in the ceca and large intestine than birds fed mash diets (Engberg *et al.*, 2002). Similarly, feeding whole wheat instead of ground wheat reduced ileal and cecal prevalence of *C. perfringens* (Engberg *et al.*, 2004). The protein source and level also affects NE incidence. Feeding fish meal rather than soy protein concentrate increased *C. perfringens* numbers in the ileum and ceca of broilers, and *C. perfringens* increased when the level of protein increased without a source by inclusion level interaction effect (Drew *et al.*, 2004). One continuing debate surrounding NE is whether an increase in digesta viscosity or the

associated increase in nutrients available to feed the clostridia is the larger player in causing NE to proliferate (Williams, 2003).

Similar to diseased states, stress situations usually affects digestion and performance in poultry. When broiler chicks were stressed with adrenocorticotropin (ACTH) for 7 d, they exhibited increased water intake and excreta production. Furthermore, fat digestibility did not change but digestibility of dry matter, proteins, gross energy and carbohydrates decreased (Puvadolpirod and Thaxton, 2000). These authors also reported that digestion was affected more than absorption during the stress period, but during the recovery period of one week, absorption parameters were more affected than digestion. This observation does not completely agree with other reports in laying hens and broilers, where amino acid digestibility was not affected by induced stress. Laying hens under heat stress did not show any adverse effects on amino acid digestibility. Only histidine and lysine digestibility increased during the heat stress period (Koelkebeck *et al.*, 1998).

#### ***Anti-nutritional factors (ANFs)***

Anti-nutritional factors are components of the diet that are not sufficiently digested in the bird's gastrointestinal tract (Bedford and Schulze, 1998) or block the ability of other nutrients to be digested (Hoerr, 1998). One of the most commonly encountered anti-nutritional factors in the industry is protease inhibitor, commonly referred to as trypsin inhibitor, found in SBM. Protease inhibitors are divided into two categories (Liener, 1994). Kunitz inhibitor has an approximate average molecular weight of 20,000 with two disulfide bridges and specificity mostly against trypsin. Bowman-Birk inhibitor is smaller with an

approximate average molecular weight of 8,000 with seven disulfide bridges with two active sites; one active site with specificity against chymotrypsin and the other active site with specificity against trypsin. Trypsin inhibitors are heat labile proteins, but caution is necessary to prevent over-processing soybeans which would degrade protein quality of the meal (Clarke and Wiseman, 2007). When full fat soybeans were extruded at different temperatures and fed at different concentrations to broilers from d 19 to 26, bird weight gain was directly correlated with increased extrusion temperatures of full fat soybeans. In addition, the pancreas enlarged after 6 d of feeding soybeans that were only extruded at low temperatures, 90 °C (Clarke and Wiseman, 2007). Lectin is another anti-nutritional factor found in soybeans, but it can be degraded at a lower temperature than trypsin inhibitor (Armour *et al.*, 1998). Dietary lectins, also called agglutinins or hemagglutinins, are glycoproteins that bind carbohydrates but are also capable of binding glycosyl receptors on enterocytes (Leeson and Summers, 2001). Fasina *et al.* (2006) reported that poults fed semi-purified diets containing 0.048 % soybean lectin had reduced total intestinal length but had superior feed efficiency, which was attributed to an improved absorptive efficiency per unit of intestinal weight. The industry mainly focuses on reducing the trypsin inhibitor content of soybean meal while maintaining protein quality and commonly uses an indirect urease test to estimate protease inhibitor content in SBM (Leeson and Summers, 2001).

Other anti-nutritional factors that are receiving considerable attention are non-starch polysaccharides (NSPs: cellulose,  $\beta$ -glucans, arabinoxylanase, and  $\beta$ -mannans) (Odetallah, 2000). NSPs comprise approximately 70-90 % of the plant cell wall (Knudsen, 2001) and

are classified based on their water solubility (Sasaki *et al.*, 2000). This is a heterogeneous group of polysaccharides with varying physiochemical properties, resulting in diverse nutritional effects in poultry. Soluble  $\beta$ -glucans and arabinoxylans have been identified to cause a viscous environment in the intestine, thereby hindering digestion (Antoniou and Marquardt, 1982; White *et al.*, 1981, 1983; Teitge *et al.*, 1991). As the inclusion level of grains (rye, wheat, barley, and oats) rich in NSPs increases, growth performance significantly decreases and litter moisture content increases (Classen *et al.*, 1985; Bedford and Classen, 1992). High viscosity is associated with the animal's inability to digest NSPs (Bedford, 1995). In order to digest feed and absorb the products of digestion, a complex between digestive enzyme and substrate must be formed, product of digestion must be released and travel through the intestinal lumen to an enterocyte for absorption. The diffusion rate of the products of digestion from the intestinal lumen to an enterocyte decreases with increasing intestinal viscosity (Fengler and Marquardt, 1988) and is exacerbated by larger molecular sizes (McCleary and Glennie-Holmes, 1985). Another problem commonly encountered with increased intestinal viscosity is reduced rate of feed passage. Birds fed rye and barley-based diets exhibit significantly reduced feed passage rates (Salih *et al.*, 1991). As a result of this reduced feed passage rate and the presence of large quantities of undigested feed due to high NSP content, a disturbance in intestinal microflora can occur (Bedford, 1995; Santos, Jr., 2006). With the reduced feed passage rate, microbes can migrate from the ceca into the small intestine and effectively compete for nutrients with the host. In addition, some intestinal microflora can excrete bile acid degrading enzymes that reduce the host's ability to

digest lipids (Feighner and Dashkevicz, 1988). Birds fed rye-based diets are prone to fat soluble vitamin deficiencies, especially rickets (Bedford, 1995). Administration of antibiotics or bile acids in rye-based diets can alleviate many of these problems (Campbell *et al.*, 1983). Many common feed ingredients contain NSPs that can negatively affect bird performance, but many of these effects can be overcome with dietary enzyme supplementation. There are many of these blended enzyme products available on the market. The choice of the appropriate enzyme product depends mainly on the diet composition, feed processing conditions, and type of bird being fed.

Like NSPs in recent years, dietary phytate content has been receiving increased attention with the increasing costs of dietary phosphorus. Phytic acid, *myo*-inositol hexaphosphate, is present in grains and oilseeds and is most known for chelating divalent cations (Deshpande and Cheryan, 1984). In relation to the digestive process, phytic acid has been shown to inhibit *in vitro* activities of  $\alpha$ -amylase (Knuckles and Betschart, 1987) and lipase (Knuckles, 1988). Suggested *in vivo* mechanisms where phytate inhibits digestive enzyme function include: binding products of digestion; chelation with necessary enzyme cofactors; and complex formation between proteins and phytate (Liu *et al.*, 2008). *In vivo* phytate has been shown to decrease amylase concentration in the duodenum and serum, decrease sucrase and maltase activity in the duodenum, and decrease amylase and sucrase activity in the jejunum (Liu *et al.*, 2008). Liu *et al.* (2008) also reported that broilers fed high levels of phytate (4.4 g/kg) had reduced growth performance. In a separate experiment, high dietary phytate concentrations reduced AME and apparent ileal amino acid digestibilities

(Ravindran *et al.*, 2006). Phytate X phytase interactions were significant on amylase and sucrase concentrations in the duodenum (Liu *et al.*, 2008) and on ileal protein and amino acid digestibilities (Ravindran *et al.*, 2006). In general, phytase supplementation has a greater effect on digestibilities at lower dietary phytate concentrations and the endogenous enzymes have higher concentrations at lower dietary phytate inclusion. However, many of these deleterious effects of dietary phytate can be overcome with dietary phytase supplementation, which will be discussed later in this review.

### ***Ingredient processing***

Many feed ingredients included in commercial poultry diets are co-products of foods processed for human consumption or re-processed to prevent viable nutrients from being wasted. Among these co-products, SBM is one of the most important protein sources for poultry production throughout the world. When SBM is combined with corn in poultry diets, usually methionine is the only limiting amino acid. Concerns regarding the use of SBM include variation in protein quality due to insufficient or excessive heating (underprocessing or overprocessing, respectively). During processing, raw soybeans are dehulled, cracked, heat conditioned at approximately 70 °C, and flaked before oil extraction, usually with hexane (Leeson and Summers, 2001). The hexane is removed from the SBM because it is extremely volatile and carcinogenic. Adequate heat processing removes the ANFs, trypsin inhibitors and lectin, as discussed earlier in this review. Excessive heat processing of SBM destroys lysine and reduces ME values. The urease index is the most commonly used assay to assess SBM processing (American Oil Chemists Society, 1980a). This assay is limited in

its ability to detect overprocessing of SBM (Araba and Dale, 1990; Batal *et al.*, 2000), but is useful for detecting underprocessing. Furthermore, controversy about the maximum level of urease in the urease assay is evident in the literature (McNaughton and Reese, 1980; Waldroup *et al.*, 1985), especially because its inconsistency and non-linear nature.

Therefore, another *in vitro* assay for determining protein quality was developed. Unlike the urease assay, which does not give a good indication of overprocessing, protein solubility in 0.2 % KOH is a good indicator of overprocessed SBM (Araba and Dale, 1990; Parsons *et al.*, 1991), but it is not a good indicator of underprocessed SBM (Anderson-Haferman *et al.*, 1992). A newer assay has been developed that is more consistent and sensitive than the urease and KOH assays (Batal *et al.*, 2000). The protein dispersibility index (American Oil Chemists Society, 1980b) measures protein solubility in water. Batal *et al.* (2000) reported that the protein dispersibility index had consistent decreases when soyflakes were heated, notably when soyflakes were only heated for a short amount of time. Conversely, the KOH and urease methods were unable to consistently determine the differences in protein quality when soyflakes were heated for only a short time (Batal *et al.*, 2000). It is essential that SBM be processed adequately to prevent inactivate trypsin inhibitors and other ANFs but not overprocessed to ensure maximal lysine and ME availability.

Another co-product from products made for human use is distillers dried grains with solubles (DDGS), which is a co-product of ethanol production from corn. During ethanol production, starch is fermented and the non-fermentable components of the corn are recovered in a more concentrated form, approximately 3 fold, as DDGS (Salim *et al.*, 2010).

Dietary formulation with DDGS presents numerous challenges. The nutrient availability and quality of DDGS can be quite variable (Cromwell *et al.*, 1993), and it can have poor handling characteristics. However, Noll *et al.* (2007) reported that the nutrient composition of DDGS from the same source is relatively consistent. One source of the variability is the amount of solubles added to the DDG before drying (Martinez-Amezcuca *et al.*, 2007). The solubles portion contains more than three times the amount of oil and phosphorus as the wet grains (Noll *et al.*, 2007), and the oil content of DDGS can vary from 2.5 to 16 % (Batal and Dale, 2006). Based on previously reported TME<sub>n</sub> values in the literature, Waldroup *et al.* (2007) suggested a TME<sub>n</sub> value of 2,851 kcal/kg for DDGS. Similarly, AME<sub>n</sub> for DDGS in laying hens was determined to be 2,770 kcal/kg (Roberson *et al.*, 2005). Because production of DDGS involves heat at multiple points in the production process, lysine content and availability require considerable interest. Lysine digestibility for DDGS has been reported to be the lowest, 69.6 %, when compared to other amino acids (Batal and Dale, 2006), mostly a result of the Maillard reaction. Like other nutrients in DDGS, phosphorus content of DDGS varies from 0.59 to 0.95 % (Spiehs *et al.*, 2002; Stein *et al.*, 2006). The bioavailability of P from DDGS is also variable. Dale and Batal (2005) hypothesized that the increased P availability may be a result of some phytase production during the fermentation process for ethanol production, where phytase would convert phytin P to more available forms. In addition, increasing DDGS drying temperature increased P bioavailability from 69 to 91 % but significantly reduced digestibility coefficients for all amino acids (Martinez-Amezcuca *et al.*, 2007). When fed to laying hens, DDGS inclusion up to 20 % showed no detrimental effect

on performance (Choen *et al.*, 2008; Rew *et al.*, 2009). In broilers, Lumpkins *et al.* (2004) found no adverse effect of 15 % DDGS inclusion on growth performance when fed from 0 to 18 d of age. However, in a subsequent experiment, the authors reported reduced body weight gain and feed utilization with 18 % DDGS inclusion but not with 12 % DDGS inclusion for broilers throughout the 42 d experiment. Likewise, Roberson (2003) fed increasing DDGS inclusion levels up to 27 % to turkey hens from 56 to 105 d of age, and reported that body weight decreased linearly with increasing DDGS inclusion. The effect of feeding DDGS in a full production length experiment has not been published. The published literature is also limited on the effect of enzyme supplementation to diets containing DDGS. Higher DDGS inclusion levels can be included in grower and finisher diets but inclusion levels should be lower in starter diets to prevent reduce growth performance. One additional issue to take into consideration when formulating with DDGS is handling characteristics and pellet quality. Wang *et al.* (2007) reported that increasing DDGS inclusion level visually reduced pellet quality. Nutritionists are successfully including DDGS in their dietary formulations but much is still to be learned about formulating with DDGS.

## 1.5 FACILITATION OF DIGESTION

### ***Feed processing and feed form***

Particle size reduction is performed to improve contact between endogenous digestive enzymes and nutrients. After pelleting, particle size reduction or grinding is the greatest

energy user in feed manufacturing (Reece *et al.*, 1985), and the finer the grind, the higher the energy costs. Coarse particle size has been shown to increase gizzard and reduce relative small intestinal weight (Nir *et al.*, 1994). Similarly, Engberg *et al.* (2004) observed that feeding whole wheat increased relative pancreas and gizzard weights, also increased ileal viscosity and lowered pancreatic amylase activity. Particle size and type of grain did not affect duodenal histomorphometric parameters of broilers (Amerah *et al.*, 2008).

Inconsistencies in the literature surround the effect of particle size on AME<sub>n</sub> in wheat diets. Peron *et al.* (2005) reported improved AME<sub>n</sub> with a fine grind, but Amerah *et al.* (2008) reported an improved AME<sub>n</sub> with a coarse grind. However, starch digestibility values increased with a finer grind (Peron *et al.*, 2008).

Pellets were first made to reduce feed wastage and improve feed handling (Calet, 1965). Pelleting mainly increases feed intake and reduces feed wastage, thereby increasing body weight gain and improving feed conversion (Calet, 1965; Choi *et al.*, 1986; Engberg *et al.*, 2002). Conversely, leghorn hens performed better on mash diets based on egg production rate and number and monetary returns (Hamilton and Proudfoot, 1995). In addition, pelleting has been shown to reduce the relative weights of the gizzard, and small intestine (Choi *et al.*, 1986; Nir *et al.*, 1994; Engberg *et al.*, 2002), and the weight of the small intestine and pancreas (Engberg *et al.*, 2002). As relative weight of the gastrointestinal tract decreases, maintenance cost decreases (Clissold *et al.*, 2010). Pelleting also lowers the activities of digestive enzymes. Birds fed pelleted diets have reduced activities of pancreatic amylase, lipase, chymotrypsin and trypsin activities when compared to mashed

fed birds (Nir *et al.*, 1994; Engberg *et al.*, 2002). All of this improves feed digestion and efficiency of utilization.

One concern about pelleting is that certain feed ingredients are not stable when exposed to the high heat, up to 120 °C, found in the conditioning and pelleting processes. For example, many exogenous enzymes will not survive the pelleting process. Silversides and Bedford (1999) reported that maximum broiler body weight and minimum feed conversion ratio for 55 second processing time was found for processing temperature between 80 and 85 °C. In order to overcome these thermostability issues, many enzymes either have a thermostable coating or are sprayed on post-pelleting. Another suggested concern about pelleting is the rapid growth rate of birds fed pellets and a proposed link to metabolic disorders (Havenstein *et al.*, 1994; Nir *et al.*, 1995; Engberg *et al.*, 2002). However, feeding whole triticale in pelleted diets has been shown to reduce acites related deaths (Jones and Taylor, 2001).

#### ***Antibiotic enteric conditioners***

Antibiotics have been commonly used in animal production to improve feed efficiency at the gut level for over forty years. Lev and Forbes (1959) reported that antibiotics had no benefit in gnotobiotic chicks but had a benefit in conventionally raised chicks. Antibiotics affect microbe metabolism, and most antibiotics are not even absorbed by the animal. In addition to improving feed conversion and body weight gain, antibiotics decrease intestinal length and weight (Miles *et al.*, 2006). Similarly, gnotobiotic birds have lower intestinal weights than conventionally raised birds (Coates *et al.*, 1955). These smaller

intestines reduce the bird's maintenance costs and allow more of its resources to be partitioned towards production.

Government and consumer concerns about antibiotic resistance transferring from food animals to humans have resulted in several bans against subtherapeutic use of antibiotics in food animals. In Denmark, which was the first country to ban subtherapeutic use of antibiotics in food animal diets, the ban has resulted in a 5 % increase in therapeutic use of antibiotics (Dibner and Richards, 2005). Furthermore, the use of ionophore salinomycin in Denmark increased after the ban (Dibner and Richards, 2005). The use of this ionophore, which has activity against CP, may explain why mortality due to NE did not rise after the ban. The search to find a suitable replacement for antibiotics continues.

#### ***Alternatives to antibiotic enteric conditioners***

Given the ban of antibiotics and consumer demands, the search for suitable alternatives has been given much attention. These alternatives must be economical and have consistent improvements in bird performance under conventional housing situations. Most feed additives including antibiotics have better effectiveness in unsanitary environmental conditions. Good sanitation is much more cost effective than treating for an outbreak. Suggested alternatives are numerous and diverse. They include: probiotics, prebiotics, organic acids, acidifiers, essential oils, antibodies, herbs, spices, and enzymes.

Antibodies, or immunoglobulins, are produced in response to an antigen in an animal. Humans and rabbits transfer maternal antibodies via the placenta, but other mammals transfer maternal antibodies (IgA) via colostrum. In avian species, however, maternal antibodies

(IgY) are transferred through the egg. The egg is a nice package that contains IgY that can be easily harvested on the commercial level (Cook, 2004) compared to obtaining immunoglobulins from mammals, which would require bleeding the animal. Furthermore, antibodies have naturally evolved to resist the difficult environment of the gastrointestinal tract, including reduced pH and proteolytic enzymes (Cook and Trott, 2010). In addition, egg antibodies are very safe. Orally fed antibodies have not been shown to be effectively absorbed (Carlander *et al.*, 2000). Two strategies are used in producing egg antibodies: pathogen specific and host-targeted. In pathogen specific, the pathogen (antigen) is injected into the hen to produce hyperimmunized eggs, and the eggs are later collected and processed. For host-targeted immunotherapy, the choice of antigen is chosen to prevent the adverse effects of inflammation on growth performance. For example, antibody to phospholipase A<sub>2</sub>, aPLA<sub>2</sub>, has been shown to improve body weight gain 5.4 % and improve feed conversion ratio by 6 points in broiler chick bioassays to 3 weeks of age (Cook, 2004). However, the mechanism by which aPLA<sub>2</sub> works is still unknown, but it's improvement in growth performance appears to be additive to antibiotics (Cook and Trott, 2010). Besides having to survive the harsh environment of the gastrointestinal tract, antibodies for feed-additives must also survive the high temperature and pressure of the pelleting process, which is difficult without a thermostable coating.

Essential oils, herbs, and spices are used throughout the world to make foods more appetizing. The ability to easily distinguish these additives is difficult because they are composed of a mixture of volatile compounds (Oyen and Dung, 1999). The term “essential”

is a remnant of medieval pharmacy, where Paracelsus believed that this was the effective constituent in the medicine (Oyen and Dung, 1999). Because essential oils are derived from natural sources, their composition varies and biological effects may also differ (Janssen *et al.*, 1987). Oregano essential oil has been recognized for having antibacterial activity (Si *et al.*, 2008) and has been suggested to have anticoccidial activity. Paster *et al.* (1990) reported that oregano essential oil was effective against *Campylobacter jejuni* and *Clostridium sporogenes* and somewhat inhibited *Staphylococcus aureus* and *Salmonella typhimurium*. Oregano essential oil has also been shown to increase apoptotic cell death (Dusan *et al.*, 2006). Furthermore, carvacrol, the main active component of oregano essential oil, has repressed proliferation of the porcine enterocyte cell line IPEC-1 *in vitro* (Bimczok *et al.*, 2008). Giannenas *et al.* (2003) observed broilers infected with *E. tenella* and given oregano essential oil in their diet exhibited better BW gain and FCR than the infected control group and similar growth performance to non-infected controls; but they exhibited worse BW gain and FCR than the cocci-infected birds fed lasalocid, an ionophore coccidiostat. These authors suggested that the phenolic constituents in oregano essential oil could have toxic effects on broiler health, and the oregano essential oil may affect membrane fluidity by being toxic to the mature enterocytes. Carvacrol, one of the active ingredients in oregano essential oil, is thought to increase turnover of the gastrointestinal tract lining, which would increase maintenance costs and hinder bird performance under a non-challenged environment (Ferket, 2004). Lee *et al.* (2004) concluded that essential oils can be toxic to poultry at high levels. If

essential oils are to replace an antibiotic enteric conditioner, the proper dose must be carefully considered because these essential oils are not regulated like antibiotics.

### ***Exogenous enzymes***

As mentioned earlier in this paper, exogenous or feed-additive enzymes have increasingly gained popularity in poultry diets with rising feed ingredient costs and stricter government regulations on excreta management. These enzymes improve nutrient digestibility. The classical suggested mode of action for enzymes is that they improve feed digestibility and therefore improve production performance. The second suggested mode of action is that enzymes reduce the amount of undigested nutrients in the proximal small intestine and subsequently reduce the quantity of substrate available for microbes in the gastrointestinal tract (Bedford, 2000b). Furthermore, exogenous enzymes affect digestive physiology, including gastrointestinal weight, and absorptive processes (Cowieson, 2005; Cowieson *et al.*, 2009). For exogenous enzymes to be effective, they must be stable for transportation and storage before feed production. One of the most difficult hurdles to overcome is surviving the high temperature and pressure of the pelleting process. Many enzymes are not tolerant of pelleting. The enzyme must also be stable in the feed. Furthermore, sufficient substrate must be present in the feed for the exogenous enzyme, and the enzyme's end product must be usable or benefit the animal (Dale, 2002). One caution about adding enzymes is that the efficacy of enzymes is lower in highly digestible diets, which follows the law of diminishing returns (Cowieson and Bedford, 2009). In general, for

every 10 % improvement in feed digestibility, enzyme efficacy drops 50 % and is essentially 0 for feed digestibility coefficients above 0.95 (Cowieson, 2010).

Dietary phytase (*myo*-inositol hexakisphosphate phosphohydrolase) was one of the first enzymes to become commercially available and to be utilized in most poultry diets. The ability of poultry to effectively hydrolyze phytate P is very variable (Ravindran *et al.*, 1995). This high variability in P bioavailability is associated with poultry's lack of endogenous enzymes to hydrolyze phytic acid (Bedford, 2000a). If inclusion of inorganic P in the diet can be reduced, the dietary costs can also be reduced. As the dietary level of phytase increases, the nutritional availability of P from phytate also increases (Cowieson *et al.*, 2006). Several phytases from either fungal or bacterial origin are available commercially. For example, Dilger *et al.* (2004) reported a linear increase in BW gain due to phytase supplementation in broilers from 8 to 22 d of age in one trial and from 22 to 43 d in a second trial. However, a growth performance effect is not always evident with phytase supplementation. Applegate *et al.* (2003) reported that phytase concentration did not affect poult performance. Pirgozliev *et al.* (2007) observed a 7.6 % FCR improvement in turkeys fed diets containing phytase. Similarly, other researchers have reported improved FCR in broilers with dietary phytase supplementation (Cowieson and Adeola, 2005; Cowieson *et al.*, 2006; Dilger *et al.*, 2004). Besides improved growth performance, phytase also improves mineral retention and reduces P excretion. Cowieson *et al.* (2006) observed dietary phytase supplementation improved the retention of P, K, Na, Fe, Mg, S, and Cu in broilers. Yan *et al.* (2001) reported that P excretion was reduced when commercial male chicks were fed low

levels of P with increasing supplementation of phytase. Phytase has the potential to have a positive effect on growth performance and environmental pollution (Rao *et al.*, 2009).

Because phytase is usually the first enzyme added to a diet, the choice in subsequent enzyme additions must be made with caution and must be evaluated for efficacy above that of only phytase in the diet. The recommended order of enzyme usage in order of decreasing relevance is: phytase, xylanase, amylase and glucanase (Coweison, 2010). Previously, it was believed that xylanase was of negligible benefit in corn-soy diets (Santos, 2006).

However, this has changed with the inclusion of alternative feed ingredients and the increasing costs of energy in the diet. Numerous studies are available on NSP degrading enzymes in wheat, barley, or rye based diets, but this review will focus on the more limited research of energy releasing enzymes in corn-soy diets. For example, Rutherford *et al.* (2007) reported an 80 kcal ME uplift when broilers were fed corn-soy diets supplemented with an enzyme product containing xylanase,  $\alpha$ -amylase and  $\beta$ -glucanase. Similarly, Meng and Slominski (2005) reported that feeding broilers a corn-soy diet supplemented with an enzyme product containing xylanase, glucanase, pectinase, cellulase, mannanase, and galactanase activities increased dietary AME<sub>n</sub> 2.3 %. Likewise, a commercially available product containing xylanase, amylase and protease supplemented to a corn-soy broiler diet improved BW gain 1.9 % and FCR 2.2 % (Zanella *et al.*, 1999). When phytase and a product containing xylanase, amylase and protease activities were administered to corn-soy diets, Cowieson and Adeola (2005) reported an additive beneficial effect on broiler performance. Again, not all enzyme combinations are additive or synergistic and careful consideration

must be made before choosing enzymes to use in combination. An example of this situation is when a phytase is used to improve phosphorus availability and when a blended enzyme product is used for improved energy uplift. The energy uplift of the phytase must not be accounted for in the nutritional matrix if the entire energy uplift of the energy releasing enzyme is expected. Overall, enzymes can successfully improve nutrient digestibility and dietary costs while reducing the environmental impact of commercial poultry operations.

## 1.6 CURRENT STUDY

This review examined the limitations to evaluating digestibility of feed ingredients and diets *in vitro*, and the several bioassays employed to determine *in vivo* nutrient digestibility. Furthermore, the method of nutrient digestibility determination must be considered before extrapolating data used for diet formulation. Many factors influence digestion, including ingredient choices, feed processing, bird genetics, environment and stress. The ways in which nutritionists attempt to account for these influences and strive to improve digestibility and consequently improve bird performance under increasing consumer scrutiny and government regulations will continue to be a challenge.

The objective of the first experiment, Chapter 2, was to determine the effects of feeding oregano essential oil during coccidia-induced gastrointestinal inflammation on early gastrointestinal development of male poult through 15 d of age. Gastrointestinal status was

evaluated through intestinal size, histomorphology and scanning electron microscopy. Growth performance results were also reported.

Because the feed-additive in Chapter 2 was a product only with activity from oregano essential oil, perhaps a blended product with activities from several organisms would promote better gastrointestinal health and therefore improve growth performance. In Chapter 3, the effects of feeding Natustat™, an “all-natural” product containing organic minerals, plant extracts, and at least one yeast-derived mannanoligosaccharide, was evaluated in coccidia-challenged poulters until 15 d of age.

In Chapter 4, turkey toms were grown to 20 weeks of age in a 2 X 4 factorial of 2 dietary DDGS inclusion levels (0 and 15 %) and 4 diets: 1) positive control, 2) negative control, 3) negative control plus phytase (1000 FTU/kg feed) and 4) negative control with phytase and a combination of xylanase, amylase, and protease (XAP) enzymes. The positive and negative control diets differed by 150 kcal ME/kg, 0.15 % available P, and 0.14 % Ca. The goal of this experiment was to determine if an energy releasing enzyme product had a better effect in diets containing DDGS than traditional corn-soy diets. Feed production parameters during pelleting were also measured to help evaluate the effect of low energy diets containing DDGS on feed production performance.

Chapter 5 also evaluated dietary enzyme supplementation and DDGS inclusion in corn-soy diets, but included a xylanase or a product containing xylanase and two  $\beta$ -glucanase activities. The experimental design was a 2 X 2 X 3 factorial design with 2 levels of DDGS inclusion (0 and 10 %), 2 energy levels (differing by 200 kcal ME/kg of diet) and 3 diets

(control, xylanase, and the product with multiple activities). Mash diets were fed to mixed-sex pens of broilers until 49 d of age. Diets were not pelleted to remove the pelleting effect, which allowed a true test of the enzymes. The efficacy of a novel phytase was tested over a range of doses on growth performance, digestibility and bone mineralization of tom poult in Chapter 6. The results of the experiments in this dissertation are discussed and summarized in Chapter 7.

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## **CHAPTER 2**

### **EFFECTS OF WATER AND FEED SUPPLEMENTATION OF OREGANO ESSENTIAL OIL (REGANO<sup>®</sup>) OF COCCIDIA-CHALLENGED TURKEY POULTS ON GROWTH PERFORMANCE AND GUT MORPHOLOGY<sup>1</sup>**

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<sup>1</sup>The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service or the North Carolina Cooperative Extension Service of the products mentioned nor criticism of similar products not mentioned.

## 2.1 ABSTRACT

Coccidiosis is among the most economically important enteric diseases in turkeys as it has adverse effects on growth performance and resistance to other enteric pathogens. Moreover, the increasing public perception against the use of chemical anticoccidials requires the industry to consider non-pharmaceutical “natural” alternatives. Dietary supplementation of oregano oil may be one “natural” alternative to increase the resistance of turkeys to coccidiosis by influencing mucosal barrier function. Two-hundred day-old Nicholas toms were assigned to 20 cages with two levels of coccidia (challenged *vs* unchallenged) and two dietary levels of oregano essential oils (0 or 300 mg Regano<sup>®</sup>/kg). The four treatments were as follows: control (CC), *Eimeria* challenged (EE), oregano essential oils (OEO) and *Eimeria* challenged plus oregano essential oils (E-OEO). Regano<sup>®</sup> (Ralco Nutrition, Inc., Marshall, MN) contains oregano oil in a soluble hemicellulose emulsifier. A stock solution of Regano<sup>®</sup> at 4.7 mL/L was given at 1:128 to drinking water for the first week. Each poult was gavaged at 3 d with either a 1mL solution containing *E. adenoides* and *E. meleagritidis* (total of 2500 oocysts/bird) or 1 mL water. On 8 d, proximal ileum samples were taken for scanning electron microscopy (SEM) and for histomorphometrical analysis on 8 and 15 d. Intestinal weight (rIW) between the gizzard and the cloaca relative to body weight (BW) was determined on one bird/cage at 8 and 15 d. There were no significant EE X OEO interaction effects on BW or rIW. *Eimeria* challenge significantly reduced 14d BW (366.4 *vs* 410.6 g,  $P < 0.0001$ ) and increased 15 d rIW (0.0836

vs 0.0941,  $P < 0.05$ ). Regano<sup>®</sup> supplementation significantly reduced 7 d BW (152.5 vs 161.7 g,  $P < 0.001$ ) but had no significant effect on 14 d BW (384.5 vs 392.5 g,  $P > 0.05$ ). Relative to SEM images from CC, E-OEO exacerbated villi tip erosion. Growth performance coincided with SEM observations and histomorphometrical analysis. Regano<sup>®</sup> supplementation did not improve poult response to *Eimeria* challenge perhaps due to excessive dose level.

(Key words: *Eimeria*, oregano essential oils, growth performance, intestinal morphology, poult)

## 2.2 INTRODUCTION

Gastrointestinal stress can inflame and compromise mucosal development in neonatal poultry, which adversely affects their growth, feed conversion efficiency, disease resistance, and welfare (Yun *et al.*, 2000). Coccidiosis is a common enteric stressor in turkey poult, and it is often associated with secondary infections that further challenge mucosal development and performance (Ruff *et al.*, 1981). Although a limited number of pharmaceutical coccidostats are available to control the adverse effects of coccidia challenge in turkeys, there is growing public demand to reduce their use in favor of more “natural” alternatives. Oregano essential oil has been suggested as one such natural alternative to control coccidiosis and other enteric pathogens (Giannenas *et al.*, 2003). Oregano oil does have an antibacterial effect (Si *et al.*, 2008), but how it affects the mucosal characteristics of cocci-challenged neonatal animals is not fully understood. The purpose of this study was to

evaluate the effect of coccidia challenge and dietary oregano essential oil on gastrointestinal histomorphometry, health and body weight in neonatal turkeys.

## 2.3 MATERIALS AND METHODS

### ***Treatments***

The dietary treatments were formulated to contain 0 or 300 mg of Regano<sup>®</sup>/kg of diet and were provided to the birds for *ad libitum* consumption throughout the trial. Regano<sup>®</sup> was added on top of the basal (control) diet in a double ribbon mixer. In addition to the corresponding Regano<sup>®</sup> feed treatment, a stock solution of Regano<sup>®</sup> at 4.7 mL/L was given at 1:128 to drinking water during the first 7 d. At 3 d of age, each bird in the challenged treatments was gavaged with a 1 mL solution containing *E. adenoeides* and *E. meleagrimitis* (total of 2,500 oocysts/bird) while each bird in the unchallenged treatment was gavaged with 1 mL of water to simulate a cocci-challenge. A plywood board approximately 1 cm thick X 10 cm wide X 30 cm long was placed on the floor in front of the feeder in each cage to allow the bird to naturally cycle some of the cocci.

### ***Bird husbandry***

Two hundred Nicholas poults were acquired from a commercial hatchery (Sleepy Creek Hatchery, Goldsboro, NC). Birds were placed in battery cages (0.363 m<sup>2</sup>/cage), each equipped with one trough feeder and one water trough. The experimental room had 24 hours of incandescent light per day and temperature was set at 37.8 °C at placement and reduced by 2.8 °C/week. Each cage was stocked with 10 poults at 1 d of age. The dietary treatments

were randomly assigned to 5 replicate pens within 2 isolation rooms: one cocci-challenged and one unchallenged. These identical isolation rooms were located in the Dearstyne Avian Health Center at North Carolina State University (Raleigh, NC), which was specifically designed and built for poultry disease studies. Birds were given access to mash feed (Table 1) and water *ad libitum*.

### ***Data collection***

Body weights (BW) were determined at 7 and 14 d of age, and relative intestinal weights (rIW, g intestine/g BW) and histomorphometry were determined at 8 and 15 d of age. In each pen, the bird closest to the average bird weight was selected for sampling. The selected bird was euthanized by cervical dislocation. The body weight of the sampled bird was determined. Next, the entire small intestine was collected, including the duodenum (from the gizzard to pancreatic and bile duct), the jejunum (from the bile duct to Meckel's diverticulum), and the ileum (from Meckel's diverticulum to the ileo-cecal-colonic junction) and weighed. A 1 cm segment of the proximal ileum, directly distal from Meckel's diverticulum, was collected for electron microscopy, only at 8 d of age. This 1 cm section was immediately processed into four tissue blocks, each approximately 3 x 3 mm, while submerged in 0.1 M sodium phosphate buffer (SPB), pH 7.2, placed on dental wax. The SPB was used to prevent the mucosa from drying. The small tissue blocks were fixed with cold solution of 3 % glutaraldehyde in SPB at approximately 4 °C for a minimum of 72 hours. About a 3 cm section of the remaining proximal ileum was collected for histological analysis.

The ileal section was immediately placed in 10 % buffered formalin solution for 24 hours, which was later used for histological analysis.

### ***Histology analysis***

After submerged in 10 % buffered formalin solution for at least 24 hours, the 3 cm ileal section was processed. A total of four sections about 2 to 3 mm in length were taken from the 3 cm fixed ileal section of each bird. These smaller sections were placed in tissue cassettes and submerged in 10 % buffered formalin solution until processed at the Histopathology Laboratory (North Carolina State University, College of Veterinary Medicine, Raleigh, NC). Next, the fixed ileal sections were embedded in paraffin wax, and 5  $\mu\text{m}$  thick transverse sections were cut with a microtome. After these sections were placed on slides, they were stained with Lilee Meyer haematoxylin and counter-stained with eosin yellow.

A Leica-DMR light-microscope (LEICA-DMR light-microscope, Leica Camera AG, Solms, Germany) was used to visualize the transverse sections placed on slides. The images were captured using a Spot-RTCR digital camera (Spot-LTCR digital camera, Diagnostic Instruments, Inc., Sterling Heights, MI) and analyzed using Image Tool (UTHSCSA Image Tool Software, Version 3.0, The University of Texas, San Antonio, TX) software. Villus height, villus apical width at the tip of the villus, villus basal width at the crypt-villus junction, crypt depth, and muscularis depth were measured on 15 villi per sampled poult. The

following mathematical formula was used to determine apparent villus surface area  $(((\text{villus tip} + \text{villus base}) / 2) * \text{villus height})$ .

### ***Electron microscopy analysis***

Fixed tissues were processed for evaluation at the North Carolina State University Center for Electron Microscopy in the Microbiology Department. Tissue blocks that were earlier fixed in 3 % glutaraldehyde solution with SPB were washed in SPB 3 times at 4 °C for 20 minutes. Next, tissue blocks were post-fixed using 1 % osmium tetroxide (OsO<sub>4</sub>) at 4 °C for 1 hour. After post-fixing, SPB was used to rinse the tissue blocks. Then, the tissue blocks were submerged for 30 minutes each in a graded series of alcohol solutions (30, 50, 70, 95, and 100 %) to dehydrate the tissue. Subsequently, the samples were critical point dried with liquid carbon dioxide in a Samdri PVT-2. Aluminum stubs with silver paint were used to mount the samples. Samples were then coated with a total of 50-60 kÅ of gold/palladium in a Hummer 6.2 Sputtering Device. A JEOL 5900LV at 20 kV accelerating voltage was utilized during SEM evaluation.

### ***Statistical analysis***

The experiment was arranged as a completely randomized design and data were analyzed using the GLM procedure for ANOVA of SAS (2005). Pen means served as the experimental units for growth performance, and individual bird means served as the experimental unit for villi measurements. The experimental rooms, challenged and unchallenged, were identical and were considered a single unit for statistical analysis.

Replicate rooms were cost prohibited. Means were separated using the LSMEANS procedure of SAS at  $P < 0.05$ .

### *Animal ethics*

This experiment was conducted according to the protocol approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC).

## 2.4 RESULTS

Cocci-challenge significantly reduced poult BW at 7 d BW by 8.8 % (164.4 vs 149.7 g,  $P < 0.005$ ) and significantly reduced 14 d poult BW by 10.8 % (410.5 vs 364.4 g,  $P < 0.001$ ) (Table 2). Regano<sup>®</sup> supplementation significantly reduced 7 d poult BW by 5.7 % as compared to control fed poult (161.6 vs 152.4 g,  $P < 0.05$ ), but there was no significant Regano<sup>®</sup> supplementation effect on 14 d BW. There were no significant main effects observed with any of the feed parameters (feed intake and FCR, Table 2).

Cocci-challenge also significantly increased rIW by 20 % (0.1320 vs 0.1054 g/g,  $P < 0.001$ ) at 8 d and by 11.2 % (0.0836 vs 0.0941 g/g,  $P < 0.05$ ) at 15 d (Table 3). Regano<sup>®</sup> supplementation significantly increased rIW 10 % at 8 d (0.1125 vs 0.1250 g/g,  $P = 0.05$ ), but not at 15 d. At 8 d of age, cocci-challenge significantly increased villus height 9.9 % (332 vs 365  $\mu\text{m}$ ,  $P < 0.01$ ) but significantly reduced villus basal width 9.5 % (66.3 vs 60.0  $\mu\text{m}$ ,  $P < 0.05$ ) (Table 4). There were no significant effects of cocci-challenge on villus tip width, crypt depth, muscularis depth, and villus surface area at 8 d. Furthermore, there were no

significant interactions of diet X cocci-challenge on any of the histology measurements at 8 d. At 15 d, cocci-challenge significantly increased villus tip width 6.4 % (53.9 vs 57.6  $\mu\text{m}$ ,  $P<0.05$ ) and villus surface area 12 % (26,081 vs 29,641  $\mu\text{m}^2$ ,  $P<0.05$ ). Significant main effects of diet on any of the histological measurements of the ileum were not observed at 8 and 15 d (Tables 4 and 5).

The SEM images of ileum villi at 150 X and 850 X magnifications from the four treatment groups are illustrated in Figure 1. The ileum villi from the control treatment group appeared normal: they were finger-like, uniform in shape and size, and the villi tip epithelial surface (850X) appeared smooth with a dimpled extrusion zone at the apex. In contrast to controls, the ileum villi from the Regano<sup>®</sup> treatment group appeared less organized in orientation, more variable in size and shape (150 X), and the villi epithelial surface appeared more dimpled without a defined extrusion zone at the tip (850X). Moreover, unidentified spherical-shaped bacteria covered much of the villi epithelial surface in the Regano<sup>®</sup> treatment group. The ileum villi of the cocci-challenged group appeared irregular in shape and size, and more spread apart than villi of the control group (150X), and the epithelial surface appeared even more dimpled than the Regano<sup>®</sup> treatment group (850X). The extrusion zone was also easily viewed in the cocci-challenged bird. Mucus secretions from goblet cells appeared as irregular drops on the villi and were much more readily visible among the cocci-challenged treatment groups, especially when combined with the Regano<sup>®</sup> treatment. The SEM images of villi from the cocci-challenged poult fed oregano, appeared to be retracted from the intestinal lumen, resulting in a more cone-shaped form than the

finger-shaped villi typically observed. Moreover, the extrusion zone on the apical tip of the villi in this treatment group encompassed a larger percentage of the villus than in the other treatment groups because the villi appeared shorter.

## 2.5 DISCUSSION

Coccidiosis does not cause the distinctive lesions of the intestine in turkeys as observed in chickens, and infections, which result in reduced weight gain, are not recognized until control measures are correctly established (McDougald, 2003). In agreement with other research reports with turkeys (Augustine and Thomas, 1980; Girdhar *et al.*, 2006), the cocci-challenge induced in this study reduced weight gain of poults at 8 and 15 d. Although BW gain during the two-week trial was significantly compromised after the cocci-challenge, significant adverse effects on feed intake and FCR could not be detected. Coccidial infection has also been previously shown to reduce feed intake and FCR in turkeys (Augustine and Thomas, 1980; Ward *et al.*, 1995; Girdhar *et al.*, 2006).

The observed reduction in poult performance can be related to changes in the bird's gastrointestinal system. Anderson *et al.* (1977) demonstrated that turkeys infected with *E. meleagrimitis* exhibit reduced pH in the duodenum and jejunum, but *E. adenoides* increased cecal pH. They found muscle and plasma 3-methylhistidine were elevated in a dose-response manner 6 d after infection with a field isolate containing a mixture of *Eimeria* species, which suggests muscle degradation during acute infection (Fetterer and Augustine, 2001). Poults

infected with *E. adenoeides* had malabsorption of methionine and glucose in the jejunum, although parasites were not found in this part of the small intestine (Ruff *et al.*, 1981). These authors also concluded that the malabsorption was not correlated to the extent of mucosal damage or the number of parasites. In the current study, we expected the cocci-challenge to increase rIW, which agrees with Allen (1984) who observed broilers infected with *E. acervulina* had increased fresh weight of the jejunum and ileum at 7 d post infection. However, our observation that the cocci-challenge increased villus height and decreased villus basal width in poult samples at 8 d and increased villus tip width and villus surface area at 15 d disagrees with previous reports (Fernando and McCraw, 1973; Ruff *et al.*, 1981). Ruff *et al.* (1981) reported that coccidia in turkeys decreased villus height and increased crypt depth. Similarly, chickens infected with *E. acervulina* had decreased villus height and increased crypt depth (Fernando and McCraw, 1973). The increased villus surface area we observed at 15 d may be associated with the increase in the villus tip width. Perhaps this increase in villus tip width, villus surface area and rIW, may be a compensatory response of nutrient absorption compromised during the cocci-challenge, as concluded by Allen (1984).

Although a mild cocci challenge may exhibit little effect on intestinal lesions and villi histologically in turkeys, subtle morphological aberrations that affect nutrient absorption may be observed by SEM. Previous reports (Madden and Ruff, 1979; Ruff *et al.*, 1981) show that many coccidiosis infections can go undetected with SEM. Madden and Ruff (1979) reported that *E. meleagridis* infection disrupted villi tips in the small intestine, but most villi appeared normal despite large numbers of parasites. This observation agrees with the SEM

images obtained in our experiment (Figure 1). In comparison to the non-challenged controls, the SEM image of a villus from the cocci-challenged poult appear irregular in shape and size, and the extrusion zone at the tip of the villi is more easily visible. Apparently, the cocci-challenge caused some degree of distress.

Although dietary supplementation of oregano essential oil may have antibacterial effects that benefits gut health, excessive dietary levels may compromise growth performance in turkeys. Regano<sup>®</sup> supplementation significantly reduced BW at 7 d but this effect did not persist through to 14 d. The adverse effect of Regano<sup>®</sup> consumption during the first week post-hatch may be due to excessive dosage. The Regano<sup>®</sup> treatment included the dry product in the feed and the liquid product in the water according to the manufacturer's recommendations, but the liquid product was not given after 7 d of age. A dose response study should be done to determine optimum dosage and when compromised growth performance begins. Botsoglou *et al.* (2002) also reported that dietary oregano essential oil did not promote growth in broilers when provided at 50 or 100 mg/kg of diet. Primarily due to its adverse effects on BW during the first week post-hatch, dietary supplementation of Regano<sup>®</sup> significantly increased rIW at 8 d, but this effect dissipated by 15 d. Again, the Regano<sup>®</sup> dosage in the feed and water was likely too high during the first 7 days post-hatch. The increased rIW with Regano<sup>®</sup> supplementation does not agree with a report by Hernández *et al.* (2004) where feeding 200 ppm of an essential oil extract from oregano, cinnamon, and pepper to broilers did not affect small or large intestine weights at 21 and 42 d. The

conflicting intestinal weight results may again be a dose effect. Similarly, Lee *et al.* (2004) concluded that essential oils can be toxic in poultry when given at high levels.

Although the Regano treatment had no significant effect on the histological measurements of the ileum brush boarder at 8 d (Table 4) or 15 d (Table 5), its adverse effect on growth performance was still likely associated with an enteric distress during the first week of the experiment. SEM images obtained from ileal villi at 8 d of age revealed that the Regano treatment was associated with increased mucin secretion and villi retraction that was exacerbated by the cocci-challenge (Figure 1), which may be a protective mechanism against the brush boarder irritation or distress. Dusan *et al.* (2006) demonstrated that oregano essential oil increased apoptotic cell death. Likewise, Bimczok *et al.* (2008) reported that carvacrol, a compound in oregano essential oil, provoked apoptosis and repressed proliferation of the porcine enterocyte cell line IPEC-1 *in vitro*.

Oregano essential oil has been suggested to have anticoccidial activities. Giannenas *et al.* (2003) observed broilers infected with *E. tenella* and given oregano essential oil in their diet exhibited better BW gain and FCR than the infected control group and similar growth performance to non-infected controls; but they exhibited worse BW gain and FCR than the cocci-infected birds fed lasalocid, an ionophore coccidiostat. These authors suggested that the phenolic constituents in oregano essential oil could have toxic effects on broiler health, and the oregano essential oil may affect membrane fluidity by being toxic to the mature enterocytes. In the current study, no significant diet X challenge interaction was present on poult growth performance, rIW or histology. However, the SEM images provided a clearer

assessment of the diet X challenge interaction occurring at the mucosal level. The SEM image of the ileal mucosa of the cocci-challenged bird given Regano<sup>®</sup> (Figure 1) clearly illustrates a treatment interaction effect on mucosal distress even if it could not be measured by histological measurements.

Stressed intestinal mucosa can have an increased weight. Allen (1987) reported increased dry mucosal weights at sites distant from *E. acervulina* and *E. mitis* 5 and 7 d post infection. Furthermore, significant inverse correlations have been reported between ileal weight, feed/gain, and the number of *C. perfringens* in the ileum and weight gain (Stutz and Lawton, 1984). In other words, broilers with increased ileal weight had poorer growth performance. Likewise, it is important for intestinal mass to be relatively small because the small intestinal mucosa is the quickest regenerating tissue in the body (LeBlond and Walker, 1956). A larger intestinal mucosa would require greater partitioning of nutrients towards maintenance rather than growth. Antibiotics fed for growth promotion have been shown to decrease intestinal weight (Coates *et al.*, 1955) mostly from thinning of mucosa (Gordon and Bruckner-Kardoss, 1958-1959).

In conclusion, cocci-challenge compromised the growth of poults likely because of increased maintenance costs to support greater relative intestinal mass and mucosa. The oregano essential oil (Regano<sup>®</sup>) treatment had minimal benefit as a natural coccidiostat for turkey poults. Although histomorphometric analysis may be a valuable measurement of mucosal development, SEM provided additional assessment of gut health.

## 2.6 TABLES AND FIGURES

Table 1: Composition and nutrient content of the basal<sup>1</sup> diet fed to turkey poult from 1 to 15 d of age.

Ingredients	Basal Diet (%)
Corn	43.66
Soybean meal (48 % CP)	41.94
Poultry meal (60 % CP)	5.00
Dicalcium phosphate (18.5 % P)	3.34
Poultry fat	3.26
Limestone	0.94
D,L-methionine	0.40
L-lysine HCl	0.34
L-threonine	0.28
Salt (NaCl)	0.22
Choline chloride (60 %)	0.20
Mineral premix <sup>2</sup>	0.15
Vitamin premix <sup>3</sup>	0.15
Selenium premix <sup>4</sup>	0.11
<b><i>Calculated analysis</i></b>	
Metabolizable energy (ME), kcal/kg	3000
Crude protein, %	27.5
Calcium, %	1.45
Non-phytate phosphorus, %	0.85
Sodium, %	0.18
Lysine, %	1.80
Methionine + cysteine, %	1.25
Fat, %	5.83
Fiber, %	2.33

<sup>1</sup>Basal diet = control diet, but for OEO diet 300 mg of Regano<sup>®</sup> / kg diet was added in a double-ribbon mixer.

<sup>2</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>3</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>4</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

Table 2: Effect of cocci-challenge and Regano<sup>®</sup> supplementation on growth performance of tom poult until 14 d of age.

Treatment	Diet	Challenge	Body Weight		FI	FCR
			7 d	14 d	1 – 14 d	
			(g)	(g)	(g/bird)	(g:g)
CC	-	-	169.4	419.8	376.3	1.13
EE <sup>1</sup>	-	+	153.8	361.9	377.5	1.20
OEO <sup>2</sup>	+	-	159.3	401.2	383.3	1.19
E-OEO	+	+	145.5	366.9	374.4	1.19
SEM(16) <sup>3</sup>			4.1	11.2	12.0	0.032
<i>Main effects of treatment</i>						
Control diet			161.6 <sup>a</sup>	390.9	376.9	1.17
Regano <sup>®</sup> diet			152.4 <sup>b</sup>	384.0	378.9	1.19
Unchallenged			164.4 <sup>a</sup>	410.5 <sup>a</sup>	379.8	1.16
Cocci-challenged			149.7 <sup>b</sup>	364.4 <sup>b</sup>	376.0	1.20
<i>Source of variation among treatments</i>			<i>P-value</i>			
Diet			<b>0.0416</b>	0.5487	0.8749	0.4842
Challenge			<b>0.0027</b>	<b>0.0008</b>	0.7524	0.3195
Diet X challenge			0.8181	0.3057	0.6799	0.2662

<sup>a,b</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Cocci challenge (EE) = day old poult gavaged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird). Unchallenged birds were gavaged with 1 mL water.

<sup>2</sup>Regano<sup>®</sup> (OEO) = 300 mg of Regano<sup>®</sup>/kg of diet throughout experiment and stock solution of Regano<sup>®</sup> at 4.7 mL/L was given at 1:128 to drinking water during the first 7 d.

<sup>3</sup>SEM(16) = Standard error of the mean with 16 degrees of freedom.

Table 3: Effect of cocci-challenge and Regano<sup>®</sup> supplementation on relative intestinal weight (rIW)<sup>1</sup> of tom poult at 8 and 15 d of age.

Treatment	Diet	Challenge	Days of Age	
			8	15
			(g/g)	
CC	-	-	0.1000	0.0822
EE <sup>2</sup>	-	+	0.1249	0.0930
OEO <sup>3</sup>	+	-	0.1109	0.0850
E-OEO	+	+	0.1391	0.0952
SEM(16) <sup>4</sup>			0.0059	0.0041
<i>Main effects of treatment</i>				
Control diet			0.1125 <sup>b</sup>	0.0876
Regano <sup>®</sup> diet			0.1250 <sup>a</sup>	0.0901
Unchallenged			0.1054 <sup>b</sup>	0.0836 <sup>b</sup>
Cocci-challenged			0.1320 <sup>a</sup>	0.0941 <sup>a</sup>
<i>Source of variation among treatments</i>			<b><i>P-value</i></b>	
Diet			<b>0.0515</b>	0.5563
Challenge			<b>0.0004</b>	<b>0.0211</b>
Diet X challenge			0.7785	0.9501

<sup>a,b</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Relative intestinal weight = Bird intestinal segment weight/Bird body weight.

<sup>2</sup>Cocci challenge (EE) = day old poult gavaged with 1 mL solution containing *E. adenoeides* and *E. meleagrimitis* (total of 2500 oocysts/bird). Unchallenged birds were gavaged with 1 mL water.

<sup>3</sup>Regano<sup>®</sup> (OEO) = 300 mg of Regano<sup>®</sup>/kg of diet throughout experiment and stock solution of Regano<sup>®</sup> at 4.7 mL/L was given at 1:128 to drinking water during the first 7 d.

<sup>4</sup>SEM(16) = Standard error of the mean with 16 degrees of freedom.

Table 4: Effect of cocci-challenge and Regano<sup>®</sup> supplementation on histological measurements<sup>1</sup> of ileum of tom poult at 8 d of age.

Treatment	Diet	Challenge	Villus Tip	Villus	Villus Basal	Crypt	Muscularis	Villus
			Width	Height	Width	Depth	Depth	Surface Area
			(µm)			(µm <sup>2</sup> )		
CC	-	-	50.3	333	68.0	49.7	79.2	19,692
EE <sup>2</sup>	-	+	49.8	365	61.6	45.0	80.0	20,506
OEO <sup>3</sup>	+	-	52.8	331	64.7	49.7	89.9	19,413
E-OEO	+	+	45.8	364	58.4	47.5	78.6	18,577
SEM (32) <sup>4</sup>			1.9	11	2.8	2.0	3.8	1,089
<i>Main effects of treatment</i>								
Control diet			50.0	349	64.8	47.3	79.6	20,099
Regano <sup>®</sup> diet			49.3	348	61.6	48.6	84.2	18,995
Unchallenged			51.5	332 <sup>b</sup>	66.3 <sup>a</sup>	49.7	84.5	19,552
Cocci-challenged			47.8	365 <sup>a</sup>	60.0 <sup>b</sup>	46.3	79.3	19,542
<i>Source of variation among treatments</i>						<b>P-value</b>		
Diet			0.7302	0.8974	0.2916	0.5526	0.2650	0.3553
Challenge			0.0723	<b>0.0106</b>	<b>0.0432</b>	0.1126	0.2106	0.9929
Diet X challenge			0.1180	0.9493	0.9902	0.5404	0.1517	0.4883

Table 4 (continued)

<sup>a,b</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Histological measurements:

Villus tip width = Measurement of the width of the villus apical tip;

Villus height = Measured from the villus tip to the villus base, not including the intestinal crypt;

Villus basal width = Measurement of the width of the villus base;

Crypt depth = Measured from the villus base to the muscularis, not including intestinal musculares;

Villus surface area = [(villus apical width + villus basal width) / 2] \* villus height;

Muscularis depth = Measured from the crypt to the serosa layer, not including intestinal serosa.

<sup>2</sup>Cocci challenge (EE) = day old poults gavaged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird). Unchallenged birds were gavaged with 1 mL water.

<sup>3</sup>Regano<sup>®</sup> (OEO) = 300 mg of Regano<sup>®</sup>/kg of diet throughout experiment and stock solution of Regano<sup>®</sup> at 4.7 mL/L was given at 1:128 to drinking water during the first 7 d.

<sup>4</sup>SEM (32) = Standard error of the mean with 32 degrees of freedom.

Table 5: Effect of cocci-challenge and Regano<sup>®</sup> supplementation on histological measurements<sup>1</sup> of ileum of tom poult at 15 d of age.

Treatment	Diet	Challenge	Villus Tip	Villus	Villus Basal	Crypt	Muscularis	Villus
			Width	Height	Width	Depth	Depth	Surface Area
			(μm)			(μm <sup>2</sup> )		
CC	-	-	51.9	469	59.8	53.3	119	26,165
EE <sup>2</sup>	-	+	56.7	470	64.0	55.3	105	28,455
OEO <sup>3</sup>	+	-	55.9	442	61.7	57.3	127	25,998
E-OEO	+	+	58.6	493	66.4	60.4	119	20,827
SEM (32) <sup>4</sup>			1.5	13	2.4	2.3	9	1,243
<i>Main effects of treatment</i>								
Control diet			54.2	469	61.9	54.3	112	27,310
Regano <sup>®</sup> diet			57.2	467	64.1	58.9	123	28,412
Unchallenged			53.9 <sup>b</sup>	455	60.8	55.3	123	26,081 <sup>b</sup>
Cocci-challenged			57.6 <sup>a</sup>	481	65.2	57.9	112	29,641 <sup>a</sup>
<i>Source of variation among treatments</i>						<b>P-value</b>		
Diet			0.0799	0.8932	0.4029	0.0681	0.3112	0.4094
Challenge			<b>0.0284</b>	0.0604	0.0930	0.2869	0.2823	<b>0.0110</b>
Diet X challenge			0.5164	0.0767	0.9234	0.8072	0.7564	0.3430

Table 5 (continued)

<sup>a,b</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Histological measurements:

Villus tip width = Measurement of the width of the villus apical tip;

Villus height = Measured from the villus tip to the villus base, not including the intestinal crypt;

Villus basal width = Measurement of the width of the villus base;

Crypt depth = Measured from the villus base to the muscularis, not including intestinal musculares;

Villus surface area = [(villus apical width + villus basal width) / 2] \* villus height;

Muscularis depth = Measured from the crypt to the serosa layer, not including intestinal serosa.

<sup>2</sup>Cocci challenge (EE) = day old poults gavaged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird). Unchallenged birds were gavaged with 1 mL water.

<sup>3</sup>Regano<sup>®</sup> (OEO) = 300 mg of Regano<sup>®</sup>/kg of diet throughout experiment and stock solution of Regano<sup>®</sup> at 4.7 mL/L was given at 1:128 to drinking water during the first 7 d.

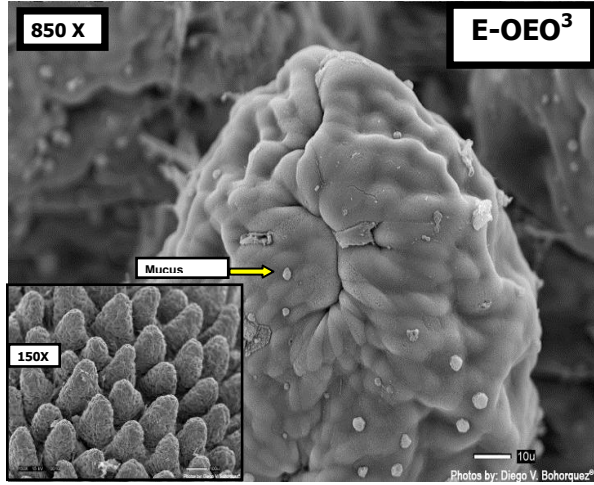
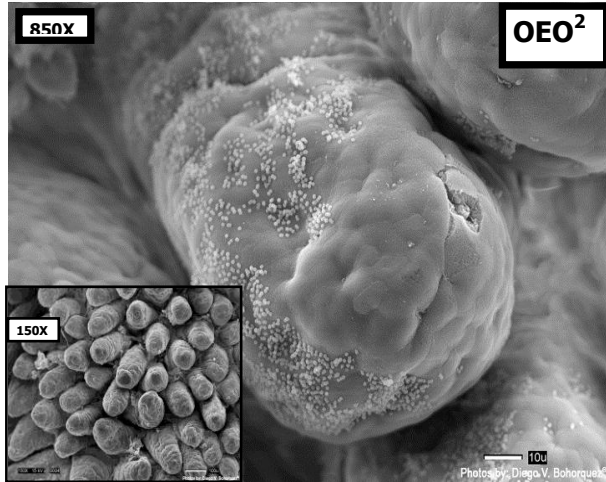
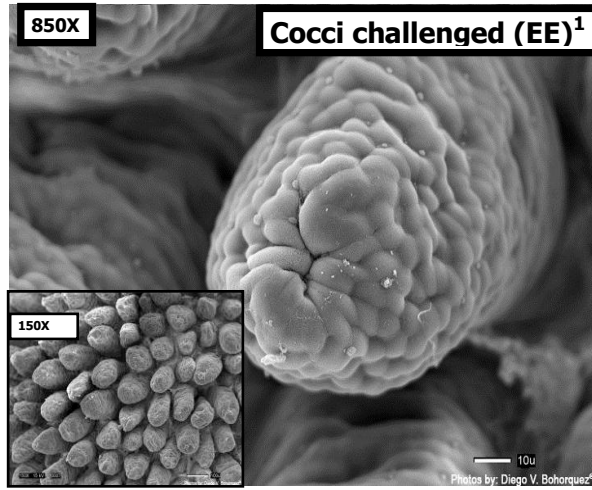
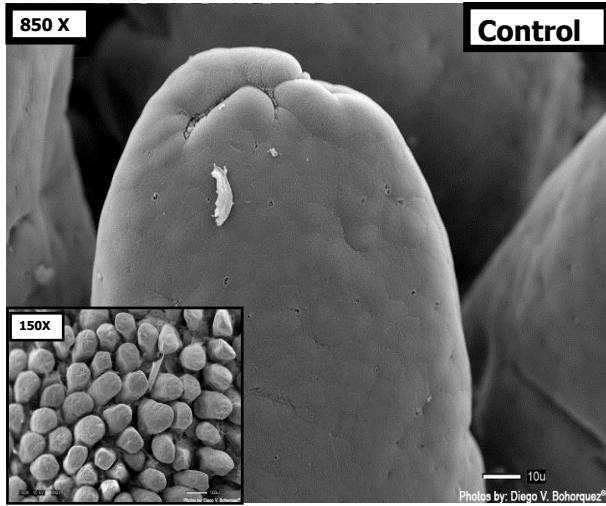
<sup>4</sup>SEM (32) = Standard error of the mean with 32 degrees of freedom.

Figure 1: Scanning electron micrographs (150 X and 850 X) of ileum sections of poult at 8 d of age.

<sup>1</sup>Cocci challenge (EE) = day old poult gaviged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird). Unchallenged birds were gaviged with 1 mL water.

<sup>2</sup>Regano<sup>®</sup> (OEO) = 300 mg of Regano<sup>®</sup>/kg of diet throughout experiment and stock solution of Regano<sup>®</sup> at 4.7 mL/L was given at 1:128 to drinking water during the first 7 d.

<sup>3</sup>(E-OEO) = Regano<sup>®</sup> + cocci challenge.



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## **CHAPTER 3**

### **EFFECTS OF NATUSTAT™ AND COCCIDIA-INDUCED GASTROINTESTINAL INFLAMMATION ON EARLY (1-15 D) GUT DEVELOPMENT OF POULTS<sup>1</sup>**

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<sup>1</sup>The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service or the North Carolina Cooperative Extension Service of the products mentioned nor criticism of similar products not mentioned.

### 3.1 ABSTRACT

Coccidiosis is one of the most costly enteric diseases in commercial turkey production because of its effects on growth performance and resistance to other gut challenges. Increasing public perception against the use of pharmaceutical anticoccidials requires the industry to consider “natural” alternatives. Dietary inclusion of Natustat™, an “all-natural” product containing organic minerals, plant extracts, and at least one yeast-derived mannoligosaccharide, may increase the resistance of turkeys to coccidiosis. Four hundred day-old Nicholas toms were assigned to 40 cages with two levels of cocci-challenge (challenged vs unchallenged) and two dietary levels of Natustat™ (0 or 1000 mg of Natustat™/kg) in a 2 X 2 factorial arrangement of treatments. At placement, each poult was gavaged with either a 1 mL solution containing *E. adenoeides* and *E. meleagrimitis* (total of 2500 oocysts/bird) or 1 mL water. Small intestinal weight (rIW) relative to body weight (BW) was determined on one bird/cage at 8 and 15 d. The cocci-challenge reduced BW at 7 and 14 d by 6.8 % ( $P < 0.001$ ) and 4.4 % ( $p < 0.10$ ), respectively. Cocci-challenge also increased 1-14 d FCR by 5.9 % ( $P < 0.05$ ). Cocci-challenge reduced 8 d rIW of duodenum, ileum and thus small intestine (SI) was reduced by 10, 8.4, and 8.1 %, respectively ( $P < 0.05$ ). In contrast, the cocci-challenge significantly increased 15 d rIW of jejunum by 12.7 % ( $P < 0.05$ ). There were no significant diet effects on BW, feed intake (FI), feed conversion ratio (FCR) and rIW. The only significant diet X challenge interaction observed was on the jejunum rIW at 15 d: dietary supplementation of Natustat™ increased rIW among cocci-

challenged poultry but not among the unchallenged birds. Although dietary Natustat™ supplementation did not improve poult performance during this cocci-challenge, it may improve compensatory absorption of nutrients by promoting jejunum development after challenge. Additional studies on Natustat™ are necessary to confirm its effect on the intestinal development and growth response after a challenge.

(*Key words:* poult, growth performance, *Eimeria*, relative intestinal weight)

### 3.2 INTRODUCTION

Coccidiosis is a gastrointestinal disease caused by protozoa that are ubiquitous to commercial poultry and it adversely affects growth performance and resistance to other pathogens. This disease costs the world poultry industry US\$3 billion annually (Bal, 2009). Coccidiosis in commercial poultry is typically controlled by dietary supplementation of prophylactic antibiotics and anticoccidials; but recently, governments and consumers have become increasingly critical of using these pharmaceutical feed additives. Consequently, alternative methods to control coccidiosis must be evaluated.

Natustat™ (Alltech, Inc., Nicholasville, KY), a proprietary blend of non-pharmaceutical components, may be a natural anticoccidial that increases resistance to coccidiosis in poultry. Duffy *et al.* (2005a,b) demonstrated that this product helps tolerance against *Histomonas* in turkeys and coccidiosis in broilers. In turkeys exposed to *Histomonas*, Natustat™ supplementation improved 42 d FCR and BW compared to infected control birds. Furthermore, when broilers exposed to litter infected with *Eimeria* were fed diets

supplemented with Natustat™, they performed similarly to broilers also exposed to infected litter but fed Salinomycin-supplemented diets (Duffy *et al.*, 2005b). The hypothesis of this experiment is that dietary supplementation of corn-soybean meal diets with Natustat™ will improve growth performance and gastrointestinal characteristics in both coccidiosis challenged and unchallenged poult. The objective of the current experiment was to study the effect of dietary Natustat™ supplementation on growth performance and gastrointestinal characteristics of turkeys challenged and unchallenged by coccidiosis.

### 3.3 MATERIALS AND METHODS

#### ***Bird husbandry***

Four-hundred Nicholas 700 X 300 toms were obtained from a commercial hatchery (Sleepy Creek Hatchery, Goldsboro, NC) and randomly assigned to one of 40 Petersime battery cages (0.363 m<sup>2</sup>/cage) with 10 poult per cage. Each cage contained one trough feeder and one water trough. Mash feed (Table 1) and water were available *ad libitum*. The dietary treatments (0 or 1000 mg of Natustat™/kg of diet) were assigned to 5 replicate cages within 2 isolation rooms: one cocci-challenged and one unchallenged. These identical isolation rooms were located in the Dearstyne Avian Health Center at North Carolina State University (Raleigh, NC), which was specifically designed and built for poultry disease studies and have been demonstrated to have no statistical block effect on poultry performance. At 1 d of age, each bird was gavaged with a 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) or 1mL water to mitigate a

cocci challenge. A plywood board, approximately 1 cm thick X 10 cm wide X 30 cm long, was placed on the floor in front of the feeder in each cage to allow the birds to naturally cycle some of the cocci. Each isolation room had 24 hours of incandescent light per day, and the temperature was set at 37.8 °C at placement.

### ***Data collection***

Body weight (BW) and feed intake (FI) were determined at 7 and 14 d of age. Feed conversion ratio (g:g, FCR) was calculated for the periods of 1-7 and 1-14 d after adjusting for the weights of mortality or culls. Relative intestinal weights (rIW, g intestine/g BW) were determined at 8 and 15 d of age. From each pen, the bird with a BW closest to the pen average was selected and euthanized by cervical dislocation for tissue sampling. The intestinal segments evaluated were from the duodenum (from the gizzard to pancreatic and bile duct), the jejunum (from the bile duct to Meckel's diverticulum), and the ileum (from Meckel's diverticulum to the ileo-cecal-colonic junction).

### ***Statistical analysis***

This experiment was arranged as a completely randomized design. Data were analyzed using the GLM procedure for ANOVA of SAS (2005). Cage mean values served as the experimental unit for the statistical analysis of BW, FI, FCR and rIW. The experimental rooms that housed the cocci-challenged and unchallenged separately were identical and not considered as separate blocks for statistical analysis. Treatment means were separated using the LSMEANS procedure of SAS at  $P < 0.05$ .

### *Animal ethics*

This experiment was conducted according to the protocol approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC).

## 3.4 RESULTS

The experimental treatment effects on growth performance are summarized in Tables 2 and 3. There were no significant effects of dietary Natustat™ supplementation on BW (Table 2), FI and FCR (Table 3) throughout the duration of the experiment. The cocci-challenge reduced BW at 7 d and 14 d by 6.8 % (154.9 vs. 144.4 g,  $P<0.001$ ) and 4.4 % (374.8 vs. 358.4,  $P<0.10$ ), respectively. The cocci-challenge significantly increased the FCR 5.9 % (1.28 vs. 1.36), but there was no significant cocci-challenge effect on feed intake.

Treatment effects observed on rIW at 8 d and 15d are summarized in Tables 4 and 5, respectively. There were no significant main effects of dietary Natustat™ supplementation on rIW of any gut segment sampled at 8 d or 15 d of age. However, significant cocci-challenge affects were observed on rIW of duodenum and ileum at 8 d of age (Table 4) and jejunum at 15 d (Table 5). At 8 d, the cocci-challenge reduced rIW of duodenum by 10.0 % (2.39 vs. 2.15 %) and rIW of ileum by 8.4 % (2.18 vs. 2.38 %) in comparison to the unchallenged controls. Although the cocci-challenge had no significant effect on rIW of the jejunum, the rIW of the entire small intestine decreased by 8.1 % (7.07 vs. 7.69 %,  $P<0.05$ ). Interestingly, the challenge significantly increased relative jejunum weight at 15 d by 12.7 %

(2.62 vs. 3.00 %, Table 5). The only significant diet X cocci-challenge interaction effect ( $P<0.05$ ) observed in our experiment was on the relative jejunum weight at 15 d: Natustat™ significantly increased the relative jejunum weight among the cocci-challenged poult and not among those that were not challenged.

### 3.5 DISCUSSION

Our observations on the effects of dietary Natustat™ supplementation on the growth performance of turkeys challenged by an enteric disease agent agree with the results observed by other researchers. As reported by Duffy *et al.* (2005a), we did not observe a significant effect of dietary Natustat™ supplementation on BW of turkey poult during the first 2 weeks post-hatch. Dietary supplementation of Natustat™ has been shown to improve FCR in broilers during a cocci-challenge and in turkeys during a *Histomonas* challenge (Duffy *et al.*, 2005a, b). In our study, the cocci-challenge reduced BW by 6.8 % ( $P<0.001$ ) at 7 d and 4.4 % ( $P=0.07$ ) at 14 d. Previous research has also shown that coccidiosis reduces BW and hinders FCR in turkeys (Augustine and Thomas, 1980; Girdhar *et al.*, 2006). The adverse effect of the cocci-challenge on FCR was expected, as growth performance is greatly influenced by gut health. An immune response in the gastrointestinal tract diverts resources away from growth and feed efficiency (Yegani and Korver, 2008). Birds can be especially vulnerable during a coccidiosis infection because damage to the intestinal mucosa due to coccidiosis is considered a significant predisposing factor to necrotic enteritis (McDevitt *et al.*, 2006). The economic impact of an enteric challenge includes reduced growth

performance, additional time spent to diagnose and treat the birds, and medication expenses. Chapman (2009) estimated that the U.S. poultry industry spends \$127 million on anticoccidial medications annually. Clearly, an enteric challenge can have many significant implications on growth performance and profitability.

The changes in poult growth performance due to the cocci-challenge can be related to changes in gastrointestinal health, enteric function and digestive capacity. The segments of the small intestine that showed a decrease in relative weight are the locations where the coccidia species given in the challenge infect. Allen (1984) observed that the fresh weights of the middle and lower small intestines of chickens dosed with *E. acervulina* were significantly greater than those that were uninfected. She concluded that the parts of the intestine not severely damaged by a cocci-challenge increased in weight as to compensate for the parts of the intestine that were damaged earlier. In the current study, the cocci-challenge comprised of two species: *E. meleagrimitis*, which infects the duodenum, and *E. adenoides*, which infects the ileum and ceca of turkey poults. Ruff and Wilkins (1980) reported compensatory absorption of methionine in the regions of the intestine that were not infected with *E. acervulina*. Furthermore, they reported that that it was more difficult to distinguish malabsorption when weight of the intestinal segment was used as the unit of measure.

In the current study, ileal samples were collect for histological analysis. Unfortunately, samples of the jejunum were not collected. Histology measurements should be taken to determine which tissue type (muscularis or mucosa) is contributing to the increase in jejunum weight after a cocci-challenge. If hypertrophy of the mucosal tissues is enhanced by

Natustat™ after a cocci-challenge, it may indicate greater digestive and nutrient absorptive capacity due to increased mucosal surface area. Jamroz *et al.* (2006) reported that feeding broilers maize-based diets supplemented with a mixture of carvacrol, cinnamaldehyde, and cesaicin increased the release of mucus, which created a thick wall of mucus in the jejunum that covered up to two-thirds of the villi. Feeding this extract from plant origins also significantly increased villi height and reduced crypt depth in the jejunum at 21 d of age.

Feed restriction, amino acid or protein or energy deficiency, or enteric challenge has been shown to precede compensatory growth (Ferket and Sell, 1990; Shapiro *et al.*, 1999; Odetallah *et al.*, 2001). At times, the compensatory absorption of nutrients after an enteric challenge is not apparent in the growth performance characteristics of birds until a few weeks after they recover. Odetallah *et al.* (2001) showed that largest turkeys that survived poult enteritis and mortality syndrome (PEMS) grew at a faster rate than uninfected control turkeys. These PEMS-affected birds had BW gains relative to the starting BW (40 to 56 d gain/BW at 40 d) of 113 %, whereas the uninfected control was only 95 %. Given enough time, the largest turkeys that survived PEMS would have attained the same BW as the uninfected control. Conversely, the smallest turkeys that survived PEMS were severely stunted and did not exhibit compensatory growth. Similarly, Zhan *et al.* (2007) reported that broilers feed restricted until 21 d of age had reduced average daily body weight gain and feed intake. These birds were then given feed *ad libitum* after 21 d of age. At 63 d of age, the broilers in the earlier feed restricted growth were numerically, but not significantly, lower in average daily body weight gain and feed intake than the broilers that were fed *ad libitum*

throughout the experiment. There were no reported differences in FCR among the two feed treatments. Because the birds in the current study were only kept until 15 d, the effect of Natustat™ supplementation on compensatory growth could not be confirmed.

Based on the performance data in this experiment, dietary Natustat™ supplementation did not help improve poult performance during the cocci-challenge, but it may help the bird recover from the challenge. Dietary Natustat™ supplementation may improve compensatory absorption of nutrients in the jejunum after challenge. Natustat™ supplementation may increase villi height and reduce crypt depth in the jejunum, allowing the bird to exhibit compensatory growth after an enteric challenge. Additional studies on Natustat™ are necessary to confirm its effect on the intestine after a challenge.

### 3.6 TABLES

Table 1: Composition and nutrient content of the basal<sup>1</sup> diet fed to turkey poult from 1 to 15 d of age.

Ingredients	Basal Diet (%)
Corn	43.66
Soybean meal (48 % CP)	41.94
Poultry meal (60 % CP)	5.00
Dicalcium phosphate (18.5 % P)	3.34
Poultry fat	3.26
Limestone	0.94
D,L-methionine	0.40
L-lysine HCl	0.34
L-threonine	0.28
Salt (NaCl)	0.22
Choline chloride (60 %)	0.20
Mineral premix <sup>2</sup>	0.15
Vitamin premix <sup>3</sup>	0.15
Selenium premix <sup>4</sup>	0.11
<b><i>Calculated analysis</i></b>	
Metabolizable energy (ME), kcal/kg	3000
Crude protein, %	27.5
Calcium, %	1.45
Non-phytate phosphorus, %	0.85
Sodium, %	0.18
Lysine, %	1.80
Methionine + cysteine, %	1.25
Fat, %	5.83
Fiber, %	2.33

<sup>1</sup>Basal diet = control diet, but for Natustat<sup>TM</sup> diet 1000 mg of Natustat<sup>TM</sup>/kg diet was added in a double-ribbon mixer.

<sup>2</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>3</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>4</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

Table 2: Effect of dietary Natustat™ supplementation on body weights of cocci-challenged and unchallenged poult at 7 and 14 d of age.

Treatment	Diet	Challenge	Days of Age	
			7	14
			(g)	
Control <sup>1</sup>	-	-	155.5	376.7
Cocci-challenge <sup>2</sup>	-	+	142.1	359.8
Natustat™ <sup>3</sup>	+	-	154.4	372.9
Natustat™ + cocci <sup>4</sup>	+	+	146.7	357.1
SEM(36) <sup>5</sup>			2.8	9.0
<i>Main effects of treatment</i>				
Control diet			148.8	368.3
Natustat™ diet			150.5	365.0
Unchallenged			154.9 <sup>a</sup>	374.8
Cocci-challenged			144.4 <sup>b</sup>	358.4
<i>Source of variation among treatments</i>			<b>P-value</b>	
Diet			0.5320	0.7210
Challenge			<b>0.0005</b>	0.0776
Diet X challenge			0.3069	0.9562

<sup>a,b</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Unsupplemented corn/SBM-basal diet.

<sup>2</sup>Control diet and challenged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) at 1 d. Unchallenged birds were gavaged with 1 mL water.

<sup>3</sup>1000 mg Natustat™/kg of basal diet.

<sup>4</sup>1000 mg Natustat™/kg of basal diet and challenged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) at 1 d.

<sup>5</sup>SEM(36) = standard error of the mean with 36 degrees of freedom.

Table 3: Effect of dietary Natustat™ supplementation on feed intake (FI) and feed conversion ratio (FCR) of cocci-challenged and unchallenged poults from 1 to 14 d of age.

Treatment	Diet	Challenge	FI (g)	FCR (g:g)
Control <sup>1</sup>	-	-	3425	1.26
Cocci-challenge <sup>2</sup>	-	+	3672	1.35
Natustat™ <sup>3</sup>	+	-	3672	1.31
Natustat™ + cocci <sup>4</sup>	+	+	3591	1.38
SEM(36) <sup>5</sup>			108	0.02
<i>Main effects of treatment</i>				
Control diet			3549	1.31
Natustat™ diet			3631	1.34
Unchallenged			3549	1.28 <sup>b</sup>
Cocci-challenged			3632	1.36 <sup>a</sup>
<i>Source of variation among treatments</i>			<i>P-value</i>	
Diet			0.4501	0.0709
Challenge			0.4469	<b>0.0002</b>
Diet X Challenge			0.1378	0.7087

<sup>a,b</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Unsupplemented corn/SBM-basal diet.

<sup>2</sup>Control diet and challenged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) at 1 d. Unchallenged birds were gavaged with 1 mL water.

<sup>3</sup>1000 mg Natustat™/kg of basal diet.

<sup>4</sup>1000 mg Natustat™/kg of basal diet and challenged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) at 1 d.

<sup>5</sup>SEM(36) = standard error of the mean with 36 degrees of freedom.

Table 4: Effect of dietary Natustat™ supplementation on relative intestinal weight (rIW)<sup>7</sup> of cocci-challenged and unchallenged poult at 8 d of age.

Treatment	Diet	Challenge	Relative Intestinal Weight (%)			
			Duodenum	Jejunum	Ileum	SI <sup>1</sup>
Control <sup>2</sup>	-	-	2.36	2.99	2.33	7.68
Cocci-challenge <sup>3</sup>	-	+	2.15	2.75	2.27	7.18
Natustat™ <sup>4</sup>	+	-	2.42	2.84	2.43	7.70
Natustat™ + cocci <sup>5</sup>	+	+	2.16	2.71	2.09	6.96
SEM(36) <sup>6</sup>			0.09	0.15	0.10	0.27
<i>Main effects of treatment</i>						
Control diet			2.26	2.87	2.30	7.43
Natustat™ diet			2.29	2.78	2.26	7.33
Unchallenged			2.39 <sup>a</sup>	2.92	2.38 <sup>a</sup>	7.69 <sup>a</sup>
Cocci-challenged			2.15 <sup>b</sup>	2.73	2.18 <sup>b</sup>	7.07 <sup>b</sup>
<i>Source of variation among treatments</i>			<b>P-value</b>			
Diet			0.7195	0.5398	0.6753	0.7014
Challenge			<b>0.0162</b>	0.2348	<b>0.0497</b>	<b>0.0255</b>
Diet X challenge			0.7433	0.7203	0.1591	0.6552

<sup>1</sup>SI = Small intestine.

<sup>a,b</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Unsupplemented corn/SBM-basal diet.

<sup>2</sup>Control diet and challenged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) at 1 d. Unchallenged birds were gavaged with 1 mL water.

<sup>3</sup>1000 mg Natustat™/kg of basal diet.

<sup>4</sup>1000 mg Natustat™/kg of basal diet and challenged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) at 1 d.

<sup>5</sup>SEM(36) = standard error of the mean with 36 degrees of freedom.

<sup>7</sup>Relative intestinal weight = bird intestinal segment weight/bird body weight.

Table 5: Effect of dietary Natustat™ supplementation on relative intestinal weight (rIW)<sup>7</sup> of cocci-challenged and unchallenged poult at 15 d of age.

Treatment	Diet	Challenge	Duodenum Jejunum Ileum SI <sup>1</sup>			
			————— (%) —————			
Control <sup>2</sup>	-	-	1.88	2.80 <sup>b</sup>	1.81	6.48
Cocci-challenge <sup>3</sup>	-	+	1.98	2.88 <sup>b</sup>	1.71	6.57
Natustat™ <sup>4</sup>	+	-	2.02	2.44 <sup>c</sup>	1.83	6.29
Natustat™ + cocci <sup>5</sup>	+	+	2.06	3.12 <sup>a</sup>	1.80	6.98
SEM(36) <sup>6</sup>			0.11	0.14	0.10	0.23
<i>Main effects of treatment</i>						
Control diet			1.93	2.84	1.76	6.53
Natustat™ diet			2.04	2.78	1.81	6.63
Unchallenged			1.95	2.62 <sup>b</sup>	1.82	6.39
Cocci-challenged			2.02	3.00 <sup>a</sup>	1.75	6.77
<i>Source of variation among treatments</i>			<b>P-value</b>			
Diet			0.3098	0.6729	0.5928	0.6400
Challenge			0.4975	<b>0.0120</b>	0.5282	0.0930
Diet X challenge			0.7766	<b>0.0454</b>	0.7353	0.1944

<sup>1</sup>SI = Small intestine.

<sup>a,b</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Unsupplemented corn/SBM-basal diet.

<sup>2</sup>Control diet and challenged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) at 1 d. Unchallenged birds were gavaged with 1 mL water.

<sup>3</sup>1000 mg Natustat™/kg of basal diet.

<sup>4</sup>1000 mg Natustat™/kg of basal diet and challenged with 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) at 1 d.

<sup>5</sup>SEM(36) = standard error of the mean with 36 degrees of freedom.

<sup>7</sup>Relative intestinal weight = bird intestinal segment weight/bird body weight.

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## CHAPTER 4

### **EFFECT OF PHYTASE AND CARBOHYDRASE SUPPLEMENTATION OF CORN-SOY DIETS WITH OR WITHOUT THE INCLUSION OF DISTILLERS DRIED GRAINS WITH SOLUBLES IN TURKEYS<sup>1</sup>**

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<sup>1</sup>The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service or the North Carolina Cooperative Extension Service of the products mentioned nor criticism of similar products not mentioned.

#### 4.1 ABSTRACT

Increasing the dietary inclusion of DDGS may contribute to variable growth performance of turkeys because of its high nutritional variability and low energy value, but this may be remediated by dietary enzyme supplementation. Turkey toms were assigned to eight dietary treatments arranged as a factorial of two dietary inclusion levels of DDGS (0 and 15 %) and four diets: 1) positive control (PC), 2) negative control (NC), 3) NC with phytase (1000 FTU/kg of feed), and 4) NC with phytase and a combination of xylanase, amylase, and protease (XAP) enzymes (Avizyme 1502, Danisco Animal Nutrition). The PC and NC diets differed by 150 kcal metabolizable energy (ME)/kg of diet, 0.15 % available phosphorus and 0.14 % calcium. Turkeys were fed *ad libitum* eight phases of pelleted feed that approximated requirements of digestible nutrients by age. Body weight (BW) and feed conversion ratio (FCR) were determined at 23, 43, 57, 85, 98, 119, and 137 d of age. There were neither DDGS X diet interactions on growth performance, nor DDGS effects on BW throughout the experiment. DDGS reduced 1 to 57 d FCR (1.79 vs. 1.76,  $P<0.05$ ), but this effect reversed after 85 d, such that 1 to 137 d FCR was significantly increased by DDGS (2.47 vs. 2.55,  $P<0.05$ ). The effect of DDGS on FCR was associated with its effect on crumble and pellet quality of the respective feed phases. Supplementation of the NC diets with phytase and/or XAP significantly ( $P<0.05$ ) increased BW by 3 % over the PC and NC diets through to 98 d without effect on FCR. DDGS significantly reduced the efficiency of

manufacturing low-energy starter feeds (fed from 1 to 57 d), and reduced feed pellet durability of high-energy finisher feeds. Increasing DDGS to 15 % had no negative effect on BW gain but negatively affected FCR and pellet quality. The addition of phytase and/or XAP enzymes resulted in an apparent uplift of over 150 kcal ME/kg of diet, reducing the need for dietary fat and inorganic phosphate inclusion, regardless of the dietary DDGS level.

*(Key words: turkey growth performance, DDGS, phytase, carbohydrase)*

## 4.2 INTRODUCTION

In 2008, the U.S. government passed the Renewable Fuels Act that increased public attention to corn-based ethanol. DDGS is a co-product from corn-based ethanol production. Currently, the use of DDGS in poultry feed is limited because of its variability in nutritional value (Cromwell *et al.*, 1993) and its poor handling characteristics during transport and feed manufacturing.

Dietary supplementation of phytase and carbohydrase has become a common strategy to reduce feed formula costs by enhancing the nutritional bioavailability of phosphorus and energy, thereby displacing the dietary inclusion of phosphorus and fat. Because turkey feeds typically contain higher concentrations of phosphorus and fat than feeds for other animal species, the potential feed costs saving from supplemental enzymes are significant. However, as the dietary inclusion level of inorganic phosphate (dicalcium phosphate or deflorinated phosphate) and fat are displaced as a result of phytase and carbohydrase

supplementation, the feed pellet manufacturing efficiency decreases, especially in diets containing DDGS. Inclusion of deflourinated phosphate and fat in feed formulations has positive effects on feed manufacturing efficiency and pellet quality. Pellet mill production rate and pellet quality is compromised when dietary DDGS inclusion exceeds 5 to 7 % (Batal, 2007), which may be due to DDGS's high concentration of zein, an insoluble protein found in corn (Behnke, 2007), and heat-modified proteins. The amount of denatured protein in the feed may be negatively correlated with pellet mill production rate and pellet quality (Wood, 1987).

The general objective of this study is to optimize the dietary inclusion of DDGS with solubles for turkeys using supplemental enzymes. Dietary enzyme supplementation may significantly increase the energy value of DDGS as well as decrease phosphorus emissions. The primary objective is to determine the effect of dietary inclusion of DDGS with and without phytase and carbohydrase enzymes on growth performance of turkeys. A secondary objective is to determine the effect DDGS and feed formulation on feed manufacturing efficiency and feed quality.

#### 4.3 MATERIALS AND METHODS

##### ***Treatments***

Eight-hundred and sixty-four male Nicholas 85 X 700 turkeys were obtained from a commercial hatchery (Sleepy Creek Hatchery, Goldsboro, NC) and randomly assigned among 48 9 m<sup>2</sup> floor pens with 15 birds per pen. Eight experimental treatment groups were

randomly assigned among two blocks of 24 pens in a curtain-sided house. The eight experimental treatments consisted of a 2 X 4 factorial arrangement of two dietary inclusion levels of DDGS (0 or 15 % of complete feed) and four diet formulations [positive control (PC), negative control (NC), NC with phytase, and NC with phytase and carbohydrase]. The caloric density difference between the PC and NC diets was 150 kcal ME/kg of diet. Phytase was supplemented as Phyzyme XP (PXP) at a rate of 1000 FTU/kg of diet and the carbohydrase was supplemented as Avizyme 1502 (XAP) at a rate of 500 mg/kg of diet (Danisco Animal Nutrition, Inc., Marlborough, UK). The turkeys were fed eight feed phases of each dietary treatment group *ad libitum*. Phase 1 feed, fed from 1-21 d, was manufactured as pelletized fine crumbs. Phase 2 feed, fed from approximately 21-42 d, was manufactured as pelletized course crumbs. Phases 3 to 8, fed from 43-137 d, were manufactured as whole 4 mm pellets.

### ***Feed manufacturing***

All of the feed was manufactured at the North Carolina State University Feed Mill Educational Unit in accordance with current Good Manufacturing Processes (cGMP's). Ingredients were ground with a hammer mill (Model 06036EP3E364TS, 8 ton/hr capacity, California Pellet Mill Co., Crawfordsville, IN) with one 2.38 mm (6/64 inch) and one 3.18 mm (8/64 inch) screen. Diets were produced in a double ribbon mixer (Model TRDB 126-0604, 2 ton capacity, Hayes & Stolz Ind. Mfg. Co., Fort Worth, TX). All diets were pellet processed at 185-195 °F (85-91 °C) with a 30 s retention time in the conditioner (Master Model HD, Size 46 cm X 122 cm (18 " X 48 " ), 1 ton/hr capacity, California Pellet Mill Co.,

Crawfordsville, IN). Pellets were manufactured with a pellet mill (Model PM 1112-2, 1 ton/hr capacity, California Pellet Mill Co., Crawfordsville, IN) with a 4.4 mm X 35 mm die (11/64 " X 1 3/8 "). Ambient air was used to cool pellets in a counter-flow cooler (Model VK09x09KL, 1 ton/hour capacity, Geelen counter-flow USA, Inc., Orlando, Florida). Enzyme was applied onto the cooled whole pellets as a liquid in a double ribbon mixer. Enzyme activities were confirmed by analysis of all manufactured feeds to be within 10 % of target. During feed manufacturing of phases 2, 3, and 6, feed manufacturing characteristics were measured. This included pellet production rate (kg/hour), conditioning temperature (°C), and hot pellet temperature (°C). Electrical consumption (hp) on the pellet mill main motor was also recorded. Pellet durability index (PDI), which is the percentage of pellets remaining after 10 minutes of tumbling, was performed according to ASAE S269.4.

All the experimental diets (Tables 1-8) within each feed phase were formulated to meet or exceed digestible amino acid requirements and other nutrients for commercial Nicholas 700 turkey males (Aviagen Turkeys, Inc., Lewisburg, WV). The NC and PC diets were formulated to be similar in nutrient content except for metabolizable energy: the NC diets were formulated to be about 150 kcal ME/kg of diet less than the PC diets. Among the NC diets supplemented with phytase and/or carbohydrase, calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively so as to maintain similar digestible calcium and phosphorus in all NC diets.

### ***Bird husbandry***

Each replicate pen was equipped with one tube feeder, one Plasson drinker (Plasson, Ltd., Menashe, Israel), and soft pine shavings as litter. All the birds were raised according to typical management practices. The birds received 23 h incandescent lights from 1-10 d and natural lighting subsequently until the experiment was terminated at 137 d of age.

### ***Growth performance, walking scores, and tibia ash***

Individual BW and pen group feed consumption were measured at 23, 43, 57, 85, 98, 119, and 137 d of age. Incidences of leg abnormalities (valgus, varus, and shaky legs) and gait scores of birds exhibiting valgus and varus deformities were evaluated at 89 and 119 d of age as described by Ferket et al. (2009). Gait score (3 = down and crippled; 2 = limited mobility with significant limp; 1 = mobile with mild limp; 0 = no leg-associated mobility problems) was evaluated by a single skilled observer unaware of treatment identity. The gait of each turkey was observed and those identified with a valgus or varus leg deformation were scored for severity as described by Abourachid, 1993; Resch-Magras *et al.*, 1993; and Corr *et al.*, 2003. The incidence of birds that exhibited leg tremors while walking or standing were classified as “shaky leg” as described by Julian (2005). At 21 d of age, one bird per pen was randomly selected and euthanized for tibia ash. The right legs were collected and stored in labeled sample bags at -20 °C for subsequent ash content determination. The legs were allowed to thaw for approximately four hours and then the tibia was dissected from the muscle. Each tibia was broken in half. Tibias were placed in individual crucibles and ashed for approximately 16 hours at 600 °C in a muffle furnace (Thermolyn, Model # F-A1730,

Serial # 6483, Sybron Corp., Dubuque, IA). Mortality rate and cause of mortality were recorded daily and analyzed by period and cumulatively. Weights of all mortalities were recorded as they occurred to correct for feed conversion ratio (FCR).

### ***Statistical analysis***

All data were analyzed as a completely randomized block design using 2 X 4 factorial arrangement of dietary DDGS inclusion level and diet (PC, NC, NC + phytase, and NC + phytase + carbohydrase) as main effects. Pen means were used as the experimental unit for all variables evaluated. Percentage mortality and leg abnormality incidence data were transformed to the arcsine square root before analysis, and final data are presented as natural numbers. All measures of statistical significance were based on a probability of  $P \leq 0.05$  unless otherwise stated. Data were subjected to ANOVA using the GLM procedure of SAS system (SAS Institute, 2003). Means separation was accomplished using LSMEANS procedure when a significant F statistic was indicated by ANOVA.

### ***Animal ethics***

This experiment was conducted according to the protocol approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC).

## 4.4 RESULTS AND DISCUSSION

### ***Feed manufacturing***

Statistics are not available for the feed manufacturing characteristics. Only a single run of each diet was made and subsequently there are not any replications of the diets.

However, the feed manufacturing parameters did give us insight into the manufacturing costs of each diet. Pettersson *et al.*, (1991) observed that feed form was a confounding factor when evaluating feed-additives. They showed that pellet processing increased ileal digestibility of organic matter, whereas enzyme supplementation also increased ileal digestibility of unpelleted diets. These researchers observed significant interactions between type of diet and enzyme supplementation on ileal digestibility of organic matter, starch digestibility, and incidence of sticky droppings. When pelleting the starter (phase 2) diet containing phytase and DDGS, pellet mill production rate (kg/hr) decreased by 59 % (380.4 vs 925.8 kg/hr). Similarly, the diet containing XAP and PXP with DDGS reduced pellet mill production rate 46.6 % (Table 9). Removing dietary energy, mostly as fat, from the PC to produce the NC diets improved PDI in grower 1 (phase 3) and finisher 1 (phase 6) diets without DDGS inclusion 34.6 % and 62.4 %, respectively (Tables 10 and 11). Adding PXP to these diets further improved PDI 11.8 % in the grower 1 and 6.0 % in the finisher 1 diet. The reduced pellet mill production rate observed with diets containing PXP and DDGS was directly related to the improved PDI observed in the diets containing PXP. When the inorganic phosphorus (dicalcium phosphorus) was removed from the diets, the pellet mill die throughput was reduced. Dietary inclusion of inorganic phosphorus, an abrasive feed ingredient, polishes the die, thus reducing contact friction between the die surface and the feed and increasing pelleting rate (Behnke, 2007). Reduced pellet durability had also been reported when dietary fat inclusion exceeded 2 % in corn-soy broiler finisher diets (Richardson and Day, 1976) and above 7.5 % fat in most other diets (Briggs et al., 1999).

### ***Growth performance, walking scores, and tibia ash***

The overall growth performance of the turkeys in this study was similar to those observed under industry conditions (Nicholas 700 Commercial Performance Objectives, Aviagen Turkeys, Inc., Lewisburg, WV). Average daily gain over the experiment was about 125.5 g/d with a final live weight at 137 d market age of 17.20 kg and adjusted FCR of 2.51. There were no significant diet X DDGS interaction effects observed on BW throughout the experiment (Table 12): the enzyme supplementation effects were independent of dietary DDGS inclusion. Moreover, DDGS had no significant ( $P<0.05$ ) effect on BW at any age. However, dietary inclusion of DDGS marginally reduced 71 d BW (6.304 vs 6.190 kg,  $P=0.0563$ ). Subsequently, turkeys fed diets containing 15 % DDGS continued to weigh marginally ( $P<0.20$ ) less than those fed diets that did not contain DDGS to 137 d, although this could not be statistically validated. This observation agrees with Noll and Brannon (2006) who observed dietary DDGS inclusion at 20 % did not affect turkey performance when fed typical levels of poultry by-product. Similarly, Roberson (2003) reported that turkey hens could be fed 10 % DDGS in the grower and finisher stages without any detrimental effects on performance.

Significant diet main effects were observed on BW on each day of measurement until 119 d. Although the PC diets contained 150 kcal ME/kg of diet more energy than the NC diets, there were no significant differences in BW between these two treatments. Apparently, the NC toms were able to compensate for the lower caloric density in their feed by improving FCR (Tables 12 and 15). The apparent ability of the toms in this experiment to compensate

for the reduced energy in the NC diets agrees with those reported by Cowieson *et al.* (2006), where broilers fed a NC diet reduced in ME, Ca, P, and amino acids did not demonstrated poorer growth performance than broilers fed a balanced PC diet.

Dietary supplementation of the NC diet with 1000 FTU phytase/kg of diet or the combination of phytase and carbohydrase significantly ( $P<0.05$ ) increased BW until 98 d. Evidently, there was no further benefit on growth response by dietary supplementation of carbohydrase in addition to the dietary phytase supplementation. The beneficial effect of enzyme supplementation was no longer significant at 119 and 137 d, likely because of the toms' reduced feed intake in response to the apparent energy uplift from the enzymes, as indicated by the significant reduction in feed intake from 99-137 d among the enzyme treated toms (Table 13). Conversely, Cowieson and Adeola (2005) reported an additive beneficial effect of PXP and XAP on broiler performance. Clearly, these toms are consuming feed to satisfy total caloric intake.

As observed with BW, there were no diet X DDGS interactions throughout the experiment. However, significant DDGS and diet main effects were observed. From 1-23 d and 24-43 d periods, toms consumed less of the crumbled feed containing 15 % DDGS than those fed diets without DDGS. Because DDGS had no effect on BW, this reduced feed intake corresponded with a significant reduction in 1-23 d and 24-43 d FCR (Table 15). This effect of DDGS on reducing cumulative feed intake persisted until 85 d (Table 16). In contrast, toms fed the pelleted diets containing 15 % DDGS from 99-137 d consumed significantly ( $P<0.05$ ) more feed than those that consumed feed without DDGS (Table 13).

Because toms consume about 50 % of their feed during the finisher period (99-137 d), the DDGS effect on increasing cumulative feed intake (1-137 d) was significant, thus increasing FCR by over 3 % (8 points in FCR). The effect of DDGS on FCR was determined to be related to crumble and pellet quality. Dietary inclusion of DDGS improved crumble/pellet quality as pellet mill production rate decreased for the feeds fed from 1 to 57 d; whereas DDGS reduced pellet quality in feeds fed from 85 to 137 d. This decrease in pellet quality as dietary inclusion of DDGS increases agrees with the observations of Wang *et al.* (2007). DDGS worsened cumulative FCR and pellet quality.

There were no significant diet X DDGS interactions on tibia ash (Table 21) at 21 d. However, DDGS inclusion significantly ( $P<0.05$ ) reduced tibia ash (37.05 vs 35.57 %). This reduced tibia ash may have been a result of excessive P levels relative to calcium levels in the diets containing DDGS. Reduced calcium to available P ratio has been shown to reduce bone ash content in chicks (Letourneou-Montminy *et al.*, 2008). Likewise, higher toe ash values have been observed for lower calcium to total P ratios, especially with higher levels of dietary phytase (Qian *et al.*, 1997). Apparently, the matrix value that was assigned for the available P in DDGS was not high enough. Suggested sources of available P that were not considered when determining the matrix value for available P in DDGS include phytate bond breakage during fermentation and highly available P in any residual yeast in DDGS (Waldroup *et al.*, 2007). No diet effect was observed on tibia ash. Therefore, the 0.10 % Ca reduction and 0.12 % available P reduction in the NC diet compared to the PC diet did not demonstrate a negative effect on tibia ash.

Gait abnormalities may be due to many causes, such as leg deformation, pain in articulations, and changes in body conformation (Abourachid, 1993, Resch-Magras *et al.*, 1993, Corr *et al.*, 2003). Leg problems are of concern for economic and welfare reasons. The bird needs to be able to obtain access to both feed and water easily in order to have good growth performance. No diet X DDGS interactions were observed in leg abnormalities at 14 and 17 weeks. At 14 weeks (Table 19), a significant ( $P<0.05$ ) diet effect was observed on the incidence of toms exhibiting with mild varus leg abnormalities (severity score 1) and total varus leg abnormalities. Toms fed the NC+PXP diet had a higher incidence of varus leg abnormalities than those fed the PC diet, regardless of DDGS inclusion (1.40 vs 6.79 %). The increased incidence of varus abnormalities at 14 weeks of age may be a result of tibia dyschondroplasia (TD) also being higher at 14 weeks. The excess available P with the addition of phytase to the diet may have exacerbated the TD. Hulan *et al.* (1985) reported that total leg abnormalities increased as the ratio of calcium to available P narrowed from decreased calcium or increased available P. At 17 weeks (Table 20), the only significant effect was a decrease in “shaky legs” with DDGS inclusion (2.80 vs 0.28 %,  $P<0.05$ ). “Shakey leg” behavior is defined as leg tremors and resistance to walk. Ferket *et al.* (2009) reported that supplemental organic trace minerals also significantly decreased the incidence of shaky legs at 17 weeks, and they related it to increased flexibility or reduced calcification of tendons. Perhaps the increased available P in the DDGS diets may have resulted in reduced leg tendon calcification in the current study. Moreover, the significant diet effect

observed on the incidence of varus leg abnormalities observed at 14 weeks were not observed at 17 weeks, most likely because these affected birds were culled by 17 weeks.

Treatment effects observed on mortality was in part due to the culling of birds exhibiting leg abnormalities. No diet X DDGS interactions were observed in periodic mortality (Table 17), but a diet X DDGS interaction was found in 1-137 d mortality (Table 18). A DDGS effect was also observed for 1-137 d mortality, where the diets containing DDGS had significantly less mortality (10.94 vs 4.43 %). Dietary inclusion of DDGS significantly reduced mortality rate during the periods of 72-85 d (1.83 vs 0.00 %) and 120-137 d (5.81 vs 1.53 %). The only significant diet effect on mortality was observed during 120-137 d, where the NC had the lowest mortality, NC+PXP+XAP was intermediate, and the PC and NC+PXP had higher mortality.

In conclusion, dietary inclusion at a level of 15 % DDGS reduced pellet mill performance by approximately 25 %, but it had no adverse effects on growth performance when digestibility was properly taken into consideration. Lower dietary fat in the NC diet improved pellet characteristics and resulted in a similar performance to the positive control diet with additional 150 kcal ME/kg. No additional benefit of enzyme inclusion in diets with 15 % DDGS was observed. However, phosphorus availability must also be considered when including DDGS in dietary formulations. The reduction in dietary fat inclusion levels in the NC compared to the PC diets can have very significant economic benefits. In addition to reduced feed costs, this reduction in dietary fat inclusion improved pellet quality, which should result in better bird performance. Also, the toms were able to maintain BW and adapt

to the caloric reduction in the NC diets by increasing feed intake. Dietary inclusion of 15 % DDGS reduced pellet mill performance and resulted in marginally lower market weights of toms. Although the adverse effects of this level dietary DDGS inclusion on pellet mill performance was not statistically validated, it may have significant economic consequences on a commercial scale of production.

## 4.5 TABLES

Table 1: Composition and nutrient content of the experimental diets fed to turkey toms from 1 to 21 days of age with and without distillers dried grains with solubles (DDGS).

Ingredients	PC <sup>1</sup>	NC <sup>2</sup>	NC + PXP <sup>3</sup>	NC + PXP + XAP <sup>4</sup>	PC	NC	NC + PXP	NC + PXP + XAP
	(%)							
Corn	40.76	44.94	46.56	46.56	33.39	37.44	38.91	38.91
Soybean meal (48 % CP)	48.83	43.01	42.69	42.69	36.31	35.60	35.41	35.41
DDGS	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00
Poultry meal (60 % CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dical phosphate (18.5 % P)	3.16	3.15	2.28	2.28	2.84	2.83	1.95	1.95
Limestone	1.07	1.07	1.21	1.21	1.27	1.29	1.43	1.43
L-lysine HCl	0.24	0.26	0.26	0.26	0.41	0.42	0.42	0.42
Poultry fat	4.49	1.08	0.48	0.48	4.33	0.94	0.37	0.37
D,L-methionine	0.32	0.32	0.31	0.31	0.31	0.31	0.30	0.30
Salt (NaCl)	0.33	0.33	0.33	0.33	0.28	0.28	0.28	0.28
L-threonine	0.16	0.16	0.17	0.17	0.21	0.21	0.21	0.21
Mineral premix <sup>5</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (60 %)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1502	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Phyzyme XP	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02
Filler (Sand)	0.00	0.05	0.05	0.00	0.00	0.05	0.05	0.00
<b>Calculated analysis</b>								
ME, kcal/kg	2950	2800	2800	2800	2950	2800	2800	2800
Crude protein, %	28.9	28.9	28.9	28.9	28.9	28.9	29.0	29.0
Calcium, %	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Non-phytate P, %	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Sodium, %	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Lysine, %	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Methionine + cysteine, %	1.21	1.21	1.21	1.21	1.22	1.22	1.22	1.22
<b>Chemical analysis (DM<sup>8</sup> basis)</b>								
Dry matter, %	88.15	88.55	88.17	88.28	88.72	89.03	88.94	88.91
Crude protein, %	29.86	30.84	31.66	32.53	31.16	31.02	32.55	30.76
Fat (ether extraction), %	7.36	4.28	3.48	3.49	8.16	5.52	4.98	5.36
Ash, %	8.35	9.23	7.99	7.71	7.95	8.52	8.13	8.21
Total fiber, %	13.26	20.61	15.75	18.46	23.42	20.47	22.77	20.86

Table 1 (continued)

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg Mn as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>6</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>7</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>8</sup>DM = Dry matter.

Table 2: Composition and nutrient content of the experimental diets fed to turkey toms from 22 to 42 days of age with and without distillers dried grains with solubles (DDGS).

Ingredients	PC <sup>1</sup>	NC <sup>2</sup>	NC + PXP <sup>3</sup>	NC + PXP + XAP <sup>4</sup>	PC	NC	NC + PXP	NC + PXP + XAP
	(%)							
Corn	43.78	49.61	49.58	49.58	36.40	41.72	41.70	41.70
Soybean meal (48 % CP)	41.48	40.33	40.34	40.34	33.96	33.26	33.27	33.27
DDGS	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00
Poultry meal (60 % CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dical phosphate (18.5 % P)	2.32	1.45	1.44	1.44	2.00	1.13	1.11	1.11
Limestone	1.17	1.30	1.31	1.31	1.39	1.52	1.53	1.53
L-lysine HCl	0.24	0.26	0.26	0.26	0.40	0.41	0.41	0.41
Poultry fat	4.56	0.55	0.56	0.56	4.41	0.47	0.48	0.48
D,L-methionine	0.29	0.28	0.28	0.28	0.28	0.27	0.27	0.27
Salt (NaCl)	0.38	0.38	0.38	0.38	0.34	0.34	0.34	0.34
L-threonine	0.13	0.14	0.14	0.14	0.18	0.18	0.18	0.18
Mineral premix <sup>5</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (60 %)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1502	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Phyzyme XP	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02
Filler (Sand)	0.00	0.05	0.05	0.00	0.00	0.05	0.05	0.00
<b>Calculated analysis</b>								
ME, kcal/kg	3000	2850	2850	2850	3000	2850	2850	2850
Crude protein, %	28.0	28.0	28.0	28.0	28.0	28.2	28.2	28.2
Calcium, %	1.30	1.16	1.30	1.30	1.30	1.16	1.30	1.30
Non-phytate P, %	0.65	0.50	0.65	0.65	0.65	0.50	0.65	0.65
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Lysine, %	1.74	1.74	1.74	1.74	1.74	1.74	1.74	1.74
Methionine + cysteine, %	1.15	1.16	1.16	1.16	1.16	1.17	1.17	1.17
<b>Chemical analysis (DM<sup>8</sup> basis)</b>								
Dry matter, %	87.76	87.64	87.88	89.92	88.60	88.43	88.08	88.10
Crude protein, %	31.06	32.52	30.15	32.20	32.22	30.98	31.86	30.66
Fat (ether extraction), %	7.65	3.88	3.67	3.47	8.77	5.18	5.09	5.11
Ash, %	7.51	7.30	7.24	7.17	7.65	7.70	7.44	7.38
Total fiber, %	17.32	14.40	13.67	15.55	13.70	24.44	20.43	20.27

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>6</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>7</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>8</sup>DM = Dry matter.

Table 3: Composition and nutrient content of the experimental diets fed to turkey toms from 43 to 56 days of age with and without distillers dried grains with solubles (DDGS).

Ingredients	PC <sup>1</sup>	NC <sup>2</sup>	NC + PXP <sup>3</sup>	NC + PXP + XAP <sup>4</sup>	PC	NC	NC + PXP	NC + PXP + XAP
	(%)							
Corn	46.31	52.14	52.11	52.11	38.94	44.28	44.25	44.25
Soybean meal (48 % CP)	39.08	37.94	37.94	37.94	31.56	30.85	30.86	30.86
DDGS	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00
Poultry meal (60 % CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dical phosphate (18.5 % P)	2.05	1.18	1.17	1.17	1.73	0.86	0.85	0.85
Limestone	1.07	1.21	1.21	1.21	1.29	1.42	1.43	1.43
L-lysine HCl	0.24	0.26	0.26	0.26	0.40	0.41	0.41	0.41
Poultry fat	4.90	0.88	0.89	0.89	4.74	0.80	0.81	0.81
D,L-methionine	0.27	0.26	0.26	0.26	0.26	0.24	0.24	0.24
Salt (NaCl)	0.38	0.38	0.38	0.38	0.34	0.34	0.34	0.34
L-threonine	0.05	0.05	0.05	0.05	0.10	0.09	0.09	0.09
Mineral premix <sup>5</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (60 %)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1502	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Phyzyme XP	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02
Filler (Sand)	0.00	0.05	0.05	0.00	0.00	0.05	0.05	0.00
<b>Calculated analysis</b>								
ME, kcal/kg	3050	2900	2900	2900	3050	2900	2900	2900
Crude protein, %	27.0	27.0	27.0	27.0	27.0	27.2	27.2	27.2
Calcium, %	1.20	1.06	1.20	1.20	1.20	1.06	1.20	1.20
Non-phytate P, %	0.60	0.45	0.60	0.60	0.60	0.45	0.60	0.60
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Lysine, %	1.67	1.67	1.67	1.67	1.69	1.69	1.69	1.69
Methionine + cysteine, %	1.11	1.11	1.11	1.11	1.12	1.12	1.12	1.12
<b>Chemical analysis (DM<sup>8</sup> basis)</b>								
Dry matter, %	90.34	90.50	89.12	90.18	90.09	90.01	89.62	89.75
Crude protein, %	30.01	27.54	29.81	27.97	29.83	30.18	29.99	30.92
Fat (ether extraction), %	7.51	4.63	3.92	3.90	8.49	5.85	5.00	4.88
Ash, %	7.44	7.15	6.73	6.51	7.24	7.09	6.86	6.72
Total fiber, %	16.76	11.78	12.13	12.70	14.47	16.77	16.95	15.32

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>6</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>7</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>8</sup>DM = Dry matter.

Table 4: Composition and nutrient content of the experimental diets fed to turkey toms from 57 to 70 days of age with and without distillers dried grains with solubles (DDGS).

Ingredients	PC <sup>1</sup>	NC <sup>2</sup>	NC + PXP <sup>3</sup>	NC + PXP + XAP <sup>4</sup>	PC	NC	NC + PXP	NC + PXP + XAP
	(%)							
Corn	49.63	55.46	55.43	55.43	42.25	47.78	47.76	47.76
Soybean meal (48 % CP)	35.83	34.68	34.68	34.68	28.31	27.42	27.43	27.43
DDGS	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00
Poultry meal (60 % CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dical phosphate (18.5 % P)	1.79	0.92	0.91	0.91	1.47	0.60	0.59	0.59
Limestone	1.10	1.24	1.25	1.25	1.32	1.46	1.47	1.47
L-lysine HCl	0.22	0.24	0.24	0.24	0.38	0.39	0.39	0.39
Poultry fat	5.10	1.08	1.09	1.09	4.95	0.98	0.99	0.99
D,L-methionine	0.25	0.24	0.24	0.24	0.24	0.23	0.23	0.23
Salt (NaCl)	0.38	0.39	0.39	0.39	0.34	0.34	0.34	0.34
L-threonine	0.05	0.05	0.05	0.05	0.10	0.10	0.10	0.10
Mineral premix <sup>5</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (60 %)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1502	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Phyzyme XP	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02
Filler (Sand)	0.00	0.05	0.05	0.00	0.00	0.05	0.05	0.00
<b>Calculated analysis</b>								
ME, kcal/kg	3100	2950	2950	2950	3100	2950	2950	2950
Crude protein, %	25.7	25.7	25.7	25.7	25.7	25.8	25.8	25.8
Calcium, %	1.15	1.01	1.15	1.15	1.15	1.01	1.15	1.15
Non-phytate P, %	0.55	0.40	0.55	0.55	0.55	0.40	0.55	0.55
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Lysine, %	1.56	1.56	1.56	1.56	1.58	1.58	1.58	1.58
Methionine + cysteine, %	1.06	1.06	1.06	1.06	1.07	1.07	1.07	1.07
<b>Chemical analysis (DM<sup>8</sup> basis)</b>								
Dry matter, %	89.11	90.14	90.19	89.12	89.41	89.99	88.99	90.42
Crude protein, %	29.72	29.31	30.23	29.20	30.00	29.22	29.02	29.93
Fat (ether extraction), %	7.44	5.26	3.81	4.92	8.71	6.62	5.24	5.88
Ash, %	7.05	7.12	7.01	6.83	7.11	7.29	7.03	6.91
Total fiber, %	13.50	13.68	12.11	13.34	17.22	16.92	18.76	16.67

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>6</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>7</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>8</sup>DM = Dry matter.

Table 5: Composition and nutrient content of the experimental diets fed to turkey toms from 71 to 84 days of age with and without distillers dried grains with solubles (DDGS).

Ingredients	PC <sup>1</sup>	NC <sup>2</sup>	NC + PXP <sup>3</sup>	NC + PXP + XAP <sup>4</sup>	PC	NC	NC + PXP	NC + PXP + XAP
	(%)							
Corn	53.34	59.17	59.14	59.14	45.96	51.54	51.52	51.52
Soybean meal (48 % CP)	31.81	30.67	30.67	30.67	24.29	23.37	23.37	23.37
DDGS	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00
Poultry meal (60 % CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dical phosphate (18.5 % P)	1.54	0.67	0.66	0.66	1.22	0.35	0.34	0.34
Limestone	0.87	1.01	1.02	1.02	1.09	1.23	1.24	1.24
L-lysine HCl	0.17	0.19	0.19	0.19	0.33	0.35	0.35	0.35
Poultry fat	6.02	2.00	2.02	2.02	5.87	1.89	1.90	1.90
D,L-methionine	0.19	0.19	0.19	0.19	0.18	0.17	0.17	0.17
Salt (NaCl)	0.39	0.39	0.39	0.39	0.34	0.34	0.34	0.34
L-threonine	0.02	0.02	0.02	0.02	0.06	0.06	0.06	0.06
Mineral premix <sup>5</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (60 %)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1502	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Phyzyme XP	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02
Filler (Sand)	0.00	0.05	0.05	0.00	0.00	0.05	0.05	0.00
<b>Calculated analysis</b>								
ME, kcal/kg	3200	3050	3050	3050	3200	3050	3050	3050
Crude protein, %	24.0	24.0	24.0	24.0	24.0	24.1	24.1	24.1
Calcium, %	1.00	0.86	1.00	1.00	1.00	0.86	1.00	1.00
Non-phytate P, %	0.50	0.35	0.50	0.50	0.50	0.35	0.50	0.50
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Lysine, %	1.41	1.41	1.41	1.41	1.43	1.43	1.43	1.43
Methionine + cysteine, %	0.96	0.96	0.96	0.96	0.97	0.97	0.97	0.97
<b>Chemical analysis (DM<sup>8</sup> basis)</b>								
Dry matter, %	88.18	88.55	89.92	89.37	67.63	89.63	90.15	90.37
Crude protein, %	27.42	26.75	26.97	26.77	25.80	27.39	26.73	26.56
Fat (ether extraction), %	7.02	5.06	3.93	5.70	8.52	6.31	7.17	6.06
Ash, %	6.86	7.23	6.32	6.08	6.68	6.86	6.24	6.35
Total fiber, %	11.31	13.69	11.48	17.85	20.03	17.64	17.35	16.72

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg Mn as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>6</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>7</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>8</sup>DM = Dry matter.

Table 6: Composition and nutrient content of the experimental diets fed to turkey toms from 85 to 98 days of age with and without distillers dried grains with solubles (DDGS).

Ingredients	PC <sup>1</sup>	NC <sup>2</sup>	NC + PXP <sup>3</sup>	NC + PXP + XAP <sup>4</sup>	PC	NC	NC + PXP	NC + PXP + XAP
	(%)							
Corn	61.15	66.97	66.94	66.94	53.77	59.21	59.18	59.18
Soybean meal (48 % CP)	24.00	22.86	22.86	22.86	16.48	15.68	15.69	15.69
DDGS	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00
Poultry meal (60 % CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dical phosphate (18.5 % P)	1.31	0.45	0.43	0.43	0.99	0.12	0.11	0.11
Limestone	0.78	0.91	0.92	0.92	1.00	1.13	1.14	1.14
L-lysine HCl	0.19	0.21	0.21	0.21	0.35	0.36	0.36	0.36
Poultry fat	6.28	2.26	2.27	2.27	6.12	2.17	2.18	2.18
D,L-methionine	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15
Salt (NaCl)	0.39	0.39	0.39	0.39	0.34	0.34	0.34	0.34
L-threonine	0.09	0.09	0.09	0.09	0.13	0.13	0.13	0.13
Mineral premix <sup>5</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (60 %)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1502	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Phyzyme XP	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02
Filler (Sand)	0.00	0.05	0.05	0.00	0.00	0.05	0.05	0.00
<b>Calculated analysis</b>								
ME, kcal/kg	3300	3150	3150	3150	3300	3150	3150	3150
Crude protein, %	21.0	21.0	21.0	21.0	21.0	21.1	21.1	21.1
Calcium, %	0.90	0.76	0.90	0.90	0.90	0.76	0.90	0.90
Non-phytate P, %	0.50	0.35	0.50	0.50	0.50	0.35	0.50	0.50
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Lysine, %	1.21	1.21	1.21	1.21	1.23	1.23	1.23	1.23
Methionine + cysteine, %	0.86	0.86	0.86	0.86	0.87	0.87	0.87	0.87
<b>Chemical analysis (DM<sup>8</sup> basis)</b>								
Dry matter, %	89.42	90.41	89.51	89.56	89.86	90.53	89.49	89.62
Crude protein, %	19.21	23.23	21.40	21.64	21.59	23.31	22.64	23.12
Fat (ether extraction), %	9.60	6.82	6.77	6.88	11.23	8.22	7.94	7.87
Ash, %	5.55	6.61	5.51	5.66	6.02	5.99	5.68	5.55
Total fiber, %	13.92	11.74	18.24	17.47	20.59	18.51	21.32	25.82

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>6</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>7</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>8</sup>DM = Dry matter.

Table 7: Composition and nutrient content of the experimental diets fed to turkey toms from 99 to 119 days of age with and without distillers dried grains with solubles (DDGS).

Ingredients	PC <sup>1</sup>	NC <sup>2</sup>	NC + PXP <sup>3</sup>	NC + PXP + XAP <sup>4</sup>	PC	NC	NC + PXP	NC + PXP + XAP
	(%)							
Corn	64.38	68.56	70.18	70.18	57.01	60.94	62.41	62.41
Soybean meal (48 % CP)	19.32	18.49	18.17	18.17	11.79	11.19	11.01	11.01
DDGS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Poultry meal (60 % CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dical phosphate (18.5 % P)	1.35	1.34	1.05	1.05	1.03	1.02	0.15	0.15
Limestone	0.91	0.91	1.05	1.05	1.13	1.13	1.27	1.27
L-lysine HCl	0.17	0.18	0.19	0.19	0.33	0.34	0.34	0.34
Poultry fat	7.60	4.18	3.59	3.59	7.43	4.07	3.50	3.50
D,L-methionine	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15
Salt (NaCl)	0.39	0.39	0.39	0.39	0.34	0.34	0.35	0.35
L-threonine	0.07	0.07	0.07	0.07	0.11	0.11	0.11	0.11
Mineral premix <sup>5</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (60 %)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1502	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Phyzyme XP	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02
Filler (Sand)	0.00	0.05	0.05	0.00	0.00	0.05	0.05	0.00
<b>Calculated analysis</b>								
ME, kcal/kg	3400	3250	3250	3250	3400	3250	3250	3250
Crude protein, %	19.0	19.0	19.0	19.0	19.0	19.1	19.1	19.1
Calcium, %	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Non-phytate P, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Sodium, %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Lysine, %	1.06	1.06	1.06	1.06	1.08	1.08	1.08	1.08
Methionine + cysteine, %	0.81	0.81	0.81	0.81	0.82	0.82	0.82	0.82
<b>Chemical analysis (DM<sup>8</sup> basis)</b>								
Dry matter, %	89.84	90.52	89.55	89.46	90.25	89.13	89.48	90.31
Crude protein, %	21.62	20.26	19.57	18.62	19.08	20.30	20.69	19.67
Fat (ether extraction), %	10.10	7.58	7.35	7.47	12.20	8.98	8.71	8.91
Ash, %	5.55	5.63	5.34	5.32	5.50	5.65	5.29	5.13
Total fiber, %	10.37	11.85	15.40	16.26	19.85	17.66	18.98	14.80

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>6</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>7</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>8</sup>DM = Dry matter.

Table 8: Composition and nutrient content of the experimental diets fed to turkey toms from 120 to 137 days of age with and without distillers dried grains with solubles (DDGS).

Ingredients	PC <sup>1</sup>	NC <sup>2</sup>	NC + PXP <sup>3</sup>	NC + PXP + XAP <sup>4</sup>	PC	NC	NC + PXP	NC + PXP + XAP
	(%)							
Corn	67.61	73.39	73.34	73.34	60.24	65.40	65.36	65.36
Soybean meal (48 % CP)	16.21	15.07	15.08	15.08	8.69	7.89	7.89	7.89
DDGS	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00
Poultry meal (60 % CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dical phosphate (18.5 % P)	0.81	0.00	0.00	0.00	0.49	0.00	0.00	0.00
Limestone	0.58	0.68	0.68	0.68	0.80	0.71	0.71	0.71
L-lysine HCl	0.15	0.17	0.17	0.17	0.31	0.33	0.33	0.33
Poultry fat	8.46	4.46	4.48	4.48	8.30	4.47	4.48	4.48
D,L-methionine	0.10	0.09	0.09	0.09	0.09	0.08	0.08	0.08
Salt (NaCl)	0.42	0.42	0.42	0.42	0.37	0.37	0.37	0.37
L-threonine	0.02	0.02	0.02	0.02	0.06	0.06	0.06	0.06
Mineral premix <sup>5</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (60 %)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1502	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.05
Phyzyme XP	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02
Filler (Sand)	0.00	0.05	0.05	0.00	0.00	0.05	0.05	0.00
<b>Calculated analysis</b>								
ME, kcal/kg	3500	3350	3350	3350	3500	3350	3350	3350
Crude protein, %	17.7	17.7	17.7	17.7	17.7	17.8	17.8	17.8
Calcium, %	0.70	0.56	0.70	0.70	0.70	0.56	0.70	0.70
Non-phytate P, %	0.35	0.21	0.36	0.36	0.35	0.27	0.42	0.42
Sodium, %	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Lysine, %	0.96	0.96	0.96	0.96	0.98	0.98	0.98	0.98
Methionine + cysteine, %	0.71	0.71	0.71	0.71	0.72	0.72	0.72	0.72
<b>Chemical analysis (DM<sup>8</sup> basis)</b>								
Dry matter, %	89.89	90.07	89.69	89.07	90.31	89.47	89.70	90.12
Crude protein, %	19.45	18.40	19.04	19.18	19.92	19.01	18.84	18.27
Fat (ether extraction), %	11.18	8.30	8.45	8.00	12.52	9.14	9.51	9.65
Ash, %	4.62	4.15	4.19	4.25	4.80	4.31	4.36	4.23
Total fiber, %	12.08	12.46	11.63	11.86	15.09	16.11	15.13	16.22

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg Mn as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>6</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>7</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>8</sup>DM = Dry matter.

Table 9: Feed manufacturing parameters of starter (phase 2) diets with and without distillers dried grains with solubles (DDGS) and enzyme supplementation.

Diet	DDGS	Conditioning Temperature	Hot Pellet Temperature	Pellet Mill Production Rate	Average Pellet Mill Load	Max. Pellet Mill Load	Min. Pellet Mill Load
	(%)	(°C)	(°C)	(kg/hr)	(hp)	(hp)	(hp)
PC <sup>1</sup>	0	81.4	84.8	901.2	8.0	9.0	7.0
NC <sup>2</sup>	0	81.6	88.5	922.8	11.0	12.5	9.0
NC + PXP <sup>3</sup>	0	82.2	88.3	910.8	12.0	14.5	7.0
NC + PXP + XAP <sup>4</sup>	0	82.6	89.8	931.8	12.0	23.0	10.0
PC	15	81.5	85.1	966.0	8.5	8.8	8.0
NC	15	80.7	86.7	925.8	14.0	30.0	4.0
NC + PXP	15	79.4	87.8	<b>380.4</b>	11.0	30.0	4.0
NC + PXP + XAP	15	80.2	88.8	<b>494.4</b>	10.0	26.0	8.0

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

Table 10: Feed manufacturing parameters of grower 1 (phase 3) diets with and without distillers dried grains with solubles (DDGS) and enzyme supplementation.

Diet	DDGS	Conditioning Temperature	Hot Pellet Temperature	Pellet Mill Production Rate	Average Pellet Mill Load	Max. Pellet Mill Load	Min. Pellet Mill Load	Standard PDI <sup>1</sup>
	(%)	(°C)	(°C)	(kg/hr)	(hp)	(hp)	(hp)	(%)
PC <sup>2</sup>	0	79.1	83.3	919.8	8.0	8.5	7.5	41.6
NC <sup>3</sup>	0	78.9	84.8	921.6	10.0	10.5	9.0	63.6
NC + PXP <sup>4</sup>	0	79.4	86.1	967.2	10.5	11.0	8.0	70.1
NC + PXP + XAP <sup>5</sup>	0	80.0	86.1	935.4	11.0	13.0	8.0	70.6
PC	15	78.5	82.9	945.6	7.5	8.0	6.5	32.3
NC	15	79.8	86.3	924.0	9.0	9.5	8.5	64.7
NC + PXP	15	79.1	86.4	913.8	10.0	11.0	8.5	72.6
NC + PXP + XAP	15	79.6	85.2	945.0	10.0	11.0	8.5	70.5

<sup>1</sup>PDI = Pellet durability index.

<sup>2</sup>PC = Positive control.

<sup>3</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>4</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>5</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

Table 11: Feed manufacturing parameters of finisher 1 (phase 6) diets with and without distillers dried grains with solubles (DDGS) and enzyme supplementation.

Diet	DDGS	Conditioning Temperature	Hot Pellet Temperature	Pellet Mill Production Rate	Average Pellet Mill Load	Max. Pellet Mill Load	Min. Pellet Mill Load	Standard PDI <sup>1</sup>
	(%)	(°C)	(°C)	(kg/hr)	(hp)	(hp)	(hp)	(%)
PC <sup>2</sup>	0	79.1	81.1	949.8	7.5	9.0	7.0	21.2
NC <sup>3</sup>	0	79.4	85.2	921.0	10.0	11.0	9.0	56.4
NC + PXP <sup>4</sup>	0	79.8	86.3	953.4	10.5	11.5	8.5	60.0
NC + PXP + XAP <sup>5</sup>	0	79.4	85.7	931.8	11.0	14.0	8.0	59.7
PC	15	78.7	81.7	960.0	7.0	7.5	6.5	17.0
NC	15	80.2	85.2	960.6	9.0	11.0	8.0	53.6
NC + PXP	15	79.4	85.2	966.6	9.0	11.0	8.0	56.1
NC + PXP + XAP	15	79.8	84.4	951.0	10.0	11.0	8.0	58.6

<sup>1</sup>PDI = Pellet durability index.

<sup>2</sup>PC = Positive control.

<sup>3</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>4</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>5</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

Table12: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on BW of toms.

Diet	DDGS	Days of Age							
		23	43	57	71	85	98	119	137
	(%)	(kg)							
PC <sup>2</sup>	0	0.587	2.18	3.83	6.12	8.52	10.66	14.38	17.42
NC <sup>3</sup>	0	0.600	2.20	3.98	6.23	8.53	10.62	14.07	16.93
NC + PXP <sup>4</sup>	0	0.626	2.33	4.17	6.51	8.97	11.12	14.69	17.82
NC + PXP + XAP <sup>5</sup>	0	0.630	2.30	4.17	6.37	8.81	10.96	14.33	17.15
PC	15	0.589	2.14	3.82	6.04	8.42	10.51	14.29	16.97
NC	15	0.598	2.19	3.95	6.16	8.62	10.70	14.35	17.05
NC + PXP	15	0.605	2.21	4.04	6.19	8.63	10.72	14.21	17.02
NC + PXP + XAP	15	0.638	2.30	4.14	6.36	8.83	10.89	14.26	17.27
<i>Main effects of treatment</i>									
PC		0.588 <sup>c</sup>	2.16 <sup>c</sup>	3.83 <sup>c</sup>	6.08 <sup>b</sup>	8.47 <sup>b</sup>	10.58 <sup>b</sup>	14.33	17.19
NC		0.599 <sup>bc</sup>	2.19 <sup>bc</sup>	3.97 <sup>bc</sup>	6.19 <sup>b</sup>	8.57 <sup>b</sup>	10.66 <sup>ab</sup>	14.21	17.00
NC + PXP		0.615 <sup>b</sup>	2.27 <sup>ab</sup>	4.11 <sup>ab</sup>	6.35 <sup>a</sup>	8.80 <sup>a</sup>	10.92 <sup>a</sup>	14.45	17.42
NC + PXP + XAP		0.634 <sup>a</sup>	2.29 <sup>a</sup>	4.15 <sup>a</sup>	6.37 <sup>a</sup>	8.82 <sup>a</sup>	10.93 <sup>a</sup>	14.29	17.21
0 % DDGS		0.610	2.24	4.04	6.30	8.62	10.84	14.37	17.33
15 % DDGS		0.607	2.21	3.99	6.19	8.71	10.70	14.28	17.08
<i>Source of variation among treatments</i>		<i>P-value</i>							
Diet		<b>0.0385</b>	<b>0.0054</b>	<b>0.0003</b>	<b>0.0032</b>	<b>0.0086</b>	<b>0.0328</b>	0.7489	0.4209
DDGS		0.7794	0.2060	0.3562	0.0563	0.3232	0.1666	0.5729	0.1722
Diet X DDGS		0.8190	0.3357	0.8300	0.2584	0.2733	0.3935	0.4227	0.2095
SEM(39) <sup>5</sup>		0.017	0.04	0.07	0.08	0.12	0.14	0.22	0.26

Table 12 (continued)

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>SEM(39) = pooled standard error of the mean with 39 degrees of freedom.

Table 13: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period feed intake of toms.

Diet	DDGS	Days of Age							
		1-23	24-43	44-57	58-71	72-85	86-98	99-119	120-137
	(%)	(kg)							
PC <sup>1</sup>	0	1.009	2.66	3.09	4.30	5.33	5.44	11.88	10.38
NC <sup>2</sup>	0	1.052	2.66	3.29	4.34	5.29	4.86	9.51	9.41
NC + PXP <sup>3</sup>	0	1.051	2.82	3.49	4.59	5.63	5.18	10.08	9.75
NC + PXP + XAP <sup>4</sup>	0	1.077	2.83	3.40	4.50	5.57	4.95	10.73	8.95
PC	15	0.965	2.50	3.08	4.05	5.45	5.50	11.85	10.16
NC	15	0.993	2.60	3.26	4.47	5.73	5.19	11.09	10.28
NC + PXP	15	0.999	2.65	3.37	4.44	5.67	5.06	10.31	10.41
NC + PXP + XAP	15	1.049	2.73	3.37	4.52	5.62	5.14	11.16	9.79
<i>Main effects of treatment</i>									
PC		0.987 <sup>b</sup>	2.58 <sup>b</sup>	3.08 <sup>c</sup>	4.35	5.39 <sup>b</sup>	5.47 <sup>a</sup>	10.86 <sup>a</sup>	10.27 <sup>a</sup>
NC		1.022 <sup>ab</sup>	2.63 <sup>b</sup>	3.27 <sup>b</sup>	4.41	5.51 <sup>ab</sup>	5.03 <sup>b</sup>	10.30 <sup>b</sup>	9.84 <sup>ab</sup>
NC + PXP		1.025 <sup>ab</sup>	2.74 <sup>a</sup>	3.43 <sup>a</sup>	4.52	5.65 <sup>a</sup>	5.12 <sup>b</sup>	10.19 <sup>c</sup>	10.08 <sup>a</sup>
NC + PXP + XAP		1.063 <sup>a</sup>	2.79 <sup>a</sup>	3.39 <sup>ab</sup>	4.51	5.60 <sup>a</sup>	5.04 <sup>b</sup>	10.94 <sup>a</sup>	9.37 <sup>b</sup>
0 % DDGS		1.047 <sup>a</sup>	2.74 <sup>a</sup>	3.32	4.43	5.45 <sup>b</sup>	5.11	10.55 <sup>b</sup>	9.62 <sup>b</sup>
15 % DDGS		1.002 <sup>b</sup>	2.62 <sup>b</sup>	3.27	4.46	5.62 <sup>a</sup>	5.22	11.10 <sup>a</sup>	10.16 <sup>a</sup>
<i>Source of variation among treatments</i>		<i>P-value</i>							
Diet		<b>0.0264</b>	<b>0.0001</b>	<b>0.0001</b>	0.1714	0.0917	<b>0.0382</b>	<b>&lt;0.0001</b>	<b>0.0166</b>
DDGS		<b>0.0095</b>	<b>0.0003</b>	0.3815	0.6397	<b>0.0330</b>	0.3309	<b>0.0149</b>	<b>0.0099</b>
Diet X DDGS		0.9230	0.5571	0.8875	0.6397	0.2239	0.5806	0.0623	0.1867
SEM(39) <sup>5</sup>		0.021	0.04	0.07	0.09	0.11	0.17	0.31	0.28

Table 13 (continued)

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>SEM(39) = pooled standard error of the mean with 39 degrees of freedom.

Table 14: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative feed intake of toms.

Diet	DDGS	Days of Age							
		1-23	1-43	1-57	1-71	1-85	1-98	1-119	1-137
	(%)	(kg)							
PC <sup>1</sup>	0	1.009	3.67	6.75	11.05	16.38	21.82	33.69	44.07
NC <sup>2</sup>	0	1.052	3.71	7.00	11.34	16.62	21.48	30.99	40.40
NC + PXP <sup>3</sup>	0	1.051	3.87	7.36	11.95	17.58	22.76	32.84	42.59
NC + PXP + XAP <sup>4</sup>	0	1.077	3.91	7.31	11.82	17.38	22.33	33.06	42.02
PC	15	0.965	3.47	6.54	10.95	16.40	21.91	33.76	43.92
NC	15	0.993	3.59	6.85	11.32	17.05	22.24	33.33	43.61
NC + PXP	15	0.999	3.65	7.02	11.46	17.14	22.76	32.50	42.92
NC + PXP + XAP	15	1.049	3.78	7.15	11.68	17.30	22.44	33.60	43.39
SEM(39) <sup>5</sup>		0.021	0.06	0.12	0.15	0.24	0.34	0.54	0.69
<i>Main effects of treatment</i>									
PC		0.987 <sup>b</sup>	3.57 <sup>b</sup>	6.65 <sup>c</sup>	11.00 <sup>c</sup>	16.39 <sup>b</sup>	21.86	32.73 <sup>b</sup>	44.00 <sup>a</sup>
NC		1.022 <sup>ab</sup>	3.65 <sup>b</sup>	6.92 <sup>b</sup>	11.33 <sup>b</sup>	16.83 <sup>b</sup>	21.86	32.16 <sup>c</sup>	42.01 <sup>b</sup>
NC + PXP		1.025 <sup>ab</sup>	3.76 <sup>ab</sup>	7.19 <sup>a</sup>	11.71 <sup>a</sup>	17.36 <sup>a</sup>	22.48	32.67 <sup>b</sup>	42.75 <sup>ab</sup>
NC + PXP + XAP		1.063 <sup>a</sup>	3.86 <sup>a</sup>	7.24 <sup>a</sup>	11.75 <sup>a</sup>	17.34 <sup>a</sup>	22.39	33.33 <sup>a</sup>	42.70 <sup>ab</sup>
0 % DDGS		1.047 <sup>a</sup>	3.79 <sup>a</sup>	7.11 <sup>a</sup>	11.54	16.99	22.10	32.65	42.27 <sup>b</sup>
15 % DDGS		1.002 <sup>b</sup>	3.62 <sup>b</sup>	6.89 <sup>b</sup>	11.35	16.97	22.20	33.30	43.46 <sup>a</sup>
<i>Source of variation among treatments</i>		<i>P-value</i>							
Diet		<b>0.0264</b>	<b>0.0003</b>	<b>0.0001</b>	<b>&lt;0.0001</b>	<b>0.0004</b>	0.1518	<b>0.0314</b>	<b>0.0464</b>
DDGS		<b>0.0095</b>	<b>0.0005</b>	<b>0.0143</b>	0.0951	0.9095	0.6899	0.0970	<b>0.0188</b>
Diet X DDGS		0.9230	0.7901	0.8416	0.4400	0.3515	0.3064	0.0865	0.0863

Table 14 (continued)

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>SEM(39) = pooled standard error of the mean with 39 degrees of freedom.

Table 15: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period FCR of toms.

Diet	DDGS	Days of Age							
		1-23	24-43	44-57	58-71	72-85	86-98	99-119	120-137
	(%)	(kg)							
PC <sup>1</sup>	0	1.92	1.65	1.88	1.92	2.26	2.56	3.16	3.57
NC <sup>2</sup>	0	1.95	1.68	1.85	1.97	2.32	2.42	2.91	3.43
NC + PXP <sup>3</sup>	0	1.87	1.67	1.89	1.97	2.29	2.42	3.26	3.41
NC + PXP + XAP <sup>4</sup>	0	1.89	1.72	1.81	2.04	2.29	2.48	3.26	3.48
PC	15	1.83	1.62	1.83	1.99	2.30	2.65	3.18	3.76
NC	15	1.85	1.63	1.86	2.03	2.30	2.61	3.04	3.83
NC + PXP	15	1.85	1.65	1.84	2.07	2.33	2.43	3.05	3.71
NC + PXP + XAP	15	1.81	1.65	1.85	2.02	2.28	2.48	3.06	3.68
SEM(39) <sup>5</sup>		0.02	0.02	0.04	0.04	0.03	0.09	0.08	0.10
<i>Main effects of treatment</i>									
PC		1.88	1.63	1.85	1.96	2.27	2.60	3.17 <sup>a</sup>	3.67
NC		1.90	1.66	1.85	2.00	2.31	2.52	2.97 <sup>b</sup>	3.63
NC + PXP		1.85	1.66	1.87	2.02	2.31	2.42	2.99 <sup>b</sup>	3.56
NC + PXP + XAP		1.85	1.68	1.83	2.03	2.29	2.48	3.16 <sup>a</sup>	3.58
0 % DDGS		1.91 <sup>a</sup>	1.68 <sup>a</sup>	1.86	1.98	2.29	2.47	3.06	3.47 <sup>b</sup>
15 % DDGS		1.84 <sup>b</sup>	1.64 <sup>b</sup>	1.84	2.03	2.30	2.54	3.16	3.74 <sup>a</sup>
<i>Source of variation among treatments</i>		<i>P-value</i>							
Diet		0.2398	0.0801	0.8301	0.2752	0.5823	0.2366	<b>0.0356</b>	0.1878
DDGS		<b>0.0003</b>	<b>0.0025</b>	0.6794	0.1005	0.5061	0.2484	0.1268	<b>0.0048</b>
Diet X DDGS		0.3901	0.5825	0.5816	0.5318	0.6377	0.6743	0.9017	0.0723

Table 15 (continued)

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>SEM(39) = pooled standard error of the mean with 39 degrees of freedom.

Table 16: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative FCR of toms.

Diet	DDGS	Days of Age							
		1-23	1-43	1-57	1-71	1-85	1-98	1-119	1-137
	(%)	(kg)							
PC <sup>1</sup>	0	1.92	1.72	1.79	1.83	1.95	2.07	2.34	2.53
NC <sup>2</sup>	0	1.95	1.73	1.79	1.86	1.98	2.05	2.24	2.42
NC + PXP <sup>3</sup>	0	1.87	1.72	1.80	1.86	1.98	2.06	2.26	2.44
NC + PXP + XAP <sup>4</sup>	0	1.89	1.77	1.78	1.87	1.99	2.07	2.33	2.49
PC	15	1.83	1.68	1.74	1.83	1.96	2.10	2.38	2.59
NC	15	1.85	1.69	1.76	1.86	1.98	2.09	2.32	2.55
NC + PXP	15	1.85	1.71	1.76	1.87	2.00	2.08	2.30	2.52
NC + PXP + XAP	15	1.81	1.69	1.76	1.85	1.97	2.06	2.28	2.51
SEM(39) <sup>5</sup>		0.02	0.02	0.02	0.01	0.01	0.02	0.03	0.03
<i>Main effects of treatment</i>									
PC		1.88	1.70	1.76	1.83 <sup>b</sup>	1.96 <sup>b</sup>	2.08	2.36 <sup>a</sup>	2.56 <sup>a</sup>
NC		1.90	1.72	1.78	1.86 <sup>a</sup>	1.98 <sup>a</sup>	2.07	2.28 <sup>b</sup>	2.49 <sup>b</sup>
NC + PXP		1.85	1.71	1.78	1.86 <sup>a</sup>	1.99 <sup>a</sup>	2.07	2.28 <sup>b</sup>	2.48 <sup>b</sup>
NC + PXP + XAP		1.85	1.73	1.77	1.86 <sup>a</sup>	1.98 <sup>a</sup>	2.07	2.30 <sup>a</sup>	2.50 <sup>b</sup>
0 % DDGS		1.91 <sup>a</sup>	1.74 <sup>a</sup>	1.79 <sup>a</sup>	1.86	1.97	2.06	2.29 <sup>b</sup>	2.47 <sup>b</sup>
15 % DDGS		1.84 <sup>b</sup>	1.69 <sup>b</sup>	1.76 <sup>b</sup>	1.85	1.98	2.08	2.32 <sup>a</sup>	2.55 <sup>a</sup>
<i>Source of variation among treatments</i>		<b>P-value</b>							
Diet		0.2398	0.3094	0.7542	<b>0.0249</b>	<b>0.0277</b>	0.8664	<b>0.0076</b>	<b>0.0294</b>
DDGS		<b>0.0003</b>	<b>0.0005</b>	<b>0.0143</b>	0.5920	0.5612	0.0973	<b>0.0278</b>	<b>0.0008</b>
Diet X DDGS		0.3901	0.4886	0.9492	0.3825	0.2583	0.5628	0.6282	0.2986

Table 16 (continued)

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>SEM(39) = pooled standard error of the mean with 39 degrees of freedom.

Table 17: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period mortality of toms.

Diet	DDGS	Days of Age							
		1-23	24-43	44-57	58-71	72-85	86-98	99-119	120-137
	(%)	(%)							
PC <sup>1</sup>	0	0.00	0.00	2.56	1.28	3.76	1.19	2.77	11.0
NC <sup>2</sup>	0	0.00	1.19	0.00	1.19	2.56	1.19	2.67	1.51
NC + PXP <sup>3</sup>	0	1.11	1.19	0.00	0.00	1.11	1.19	2.57	7.97
NC + PXP + XAP <sup>4</sup>	0	0.00	0.00	0.00	1.19	0.00	2.78	1.19	2.70
PC	15	0.00	1.19	0.00	0.00	0.00	0.00	1.28	0.00
NC	15	0.00	1.19	0.00	1.19	0.00	0.00	0.00	0.00
NC + PXP	15	1.11	1.19	0.00	0.00	0.00	0.00	1.28	3.75
NC + PXP + XAP	15	0.00	2.22	0.00	0.00	0.00	0.00	3.86	2.38
SEM(39) <sup>5</sup>		0.02	0.04	0.02	0.03	0.04	0.04	0.05	0.06
<i>Main effects of treatment</i>									
PC		0.00	0.59	1.28	0.64	1.84	0.59	2.03	5.52 <sup>a</sup>
NC		0.00	1.19	0.00	1.19	1.28	0.59	1.33	0.75 <sup>b</sup>
NC + PXP		1.11	1.19	0.00	0.00	0.55	0.59	1.93	5.86 <sup>a</sup>
NC + PXP + XAP		0.00	1.11	0.00	0.59	0.00	1.39	2.53	2.54 <sup>ab</sup>
0 % DDGS		0.28	0.59	0.64	0.91	1.83 <sup>a</sup>	1.58	2.30	5.81 <sup>a</sup>
15 % DDGS		0.28	1.45	0.00	0.30	0.00 <sup>b</sup>	0.00	1.61	1.53 <sup>b</sup>
<i>Source of variation among treatments</i>		<i>P-value</i>							
Diet		0.1375	0.9325	0.4031	0.5850	0.5488	0.9826	0.8609	<b>0.0320</b>
DDGS		1.0000	0.2619	0.3235	0.3189	<b>0.0497</b>	0.0604	0.5110	<b>0.0087</b>
Diet X DDGS		1.0000	0.6727	0.4031	0.7964	0.5488	0.9826	0.2986	0.1267

Table 17 (continued)

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>SEM(39) = pooled standard error of the arcsin(sqrt(%)) mean with 39 degrees of freedom.

Table 18: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative mortality of toms.

Diet	DDGS	Days of Age							
		1-23	1-43	1-57	1-71	1-85	1-98	1-119	1-137
	(%)	(%)							
PC <sup>1</sup>	0	0.00	0.00	2.08	3.12	6.25	7.29	9.37	16.7 <sup>a</sup>
NC <sup>2</sup>	0	0.00	1.04	1.04	2.08	3.12	5.21	7.23	8.33 <sup>c</sup>
NC + PXP <sup>3</sup>	0	1.11	2.08	2.08	2.08	4.35	4.95	6.25	12.5 <sup>b</sup>
NC + PXP + XAP <sup>4</sup>	0	0.00	0.00	0.00	1.04	1.04	3.12	4.17	6.25 <sup>e</sup>
PC	15	0.00	1.04	1.04	1.04	1.04	1.04	2.08	2.08 <sup>f</sup>
NC	15	0.00	1.04	1.04	2.08	2.08	2.08	2.08	2.08 <sup>f</sup>
NC + PXP	15	1.11	2.08	2.08	2.08	2.08	2.08	3.12	6.25 <sup>e</sup>
NC + PXP + XAP	15	0.00	2.08	2.08	2.08	2.08	2.08	5.21	7.29 <sup>d</sup>
SEM(39) <sup>5</sup>		0.02	0.04	0.05	0.06	0.06	0.06	0.07	0.06
<i>Main effects of treatment</i>									
PC		0.00	0.52	1.56	2.08	3.65	4.17	5.73	9.37
NC		0.00	1.04	1.04	2.08	3.12	3.64	4.69	5.21
NC + PXP		1.11	2.08	2.08	2.08	2.60	3.12	4.69	9.37
NC + PXP + XAP		0.00	1.04	1.04	1.56	1.56	2.60	4.68	6.77
0 % DDGS		0.28	0.78	1.30	2.08	3.65	4.95	6.77	10.9 <sup>a</sup>
15 % DDGS		0.28	1.56	1.56	1.82	1.82	1.82	3.12	4.43 <sup>b</sup>
<i>Source of variation among treatments</i>		<b>P-value</b>							
Diet		0.1375	0.5045	0.7837	0.9441	0.8054	0.9266	0.9973	0.3894
DDGS		1.0000	0.2946	0.6240	0.9507	0.2434	0.0665	0.0877	<b>0.0034</b>
Diet X DDGS		1.0000	0.7119	0.7039	0.9242	0.5129	0.7348	0.4489	<b>0.0470</b>

Table 18 (continued)

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>SEM(39) = pooled standard error of the arcsin(sqrt(%)) mean with 39 degrees of freedom.

Table 19: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on incidence and severity of leg abnormalities of toms at 14 weeks of age.

Diet	DDGS	Valgus <sup>1</sup>				Varus <sup>1</sup>				Shaky Leg	Total LP <sup>2</sup>
		1	2	3	Total	1	2	3	Total		
	(%)										
PC <sup>3</sup>	0	2.22	0.00	0.00	2.22	1.28	0.00	0.00	1.28	4.52	8.02
NC <sup>4</sup>	0	2.58	1.28	3.97	7.83	0.00	2.78	1.12	3.97	4.88	16.68
NC + PXP <sup>5</sup>	0	3.50	0.00	0.00	3.50	5.73	1.11	0.00	6.84	4.44	14.79
NC + PXP + XAP <sup>6</sup>	0	1.39	1.11	1.11	3.61	3.49	3.41	0.00	6.90	3.69	14.21
PC	15	2.22	0.00	0.00	2.22	1.11	0.00	0.00	1.11	2.22	5.55
NC	15	2.30	0.00	2.70	5.00	2.30	2.30	0.00	4.60	1.20	10.80
NC + PXP	15	1.11	0.00	1.11	2.22	4.53	4.53	1.11	6.75	3.41	12.38
NC + PXP + XAP	15	2.30	0.00	0.00	2.30	0.00	0.00	0.00	1.11	6.07	9.48
SEM(39) <sup>7</sup>		0.06	0.02	0.04	0.06	0.05	0.05	0.02	0.06	0.06	0.07
<i>Main effects of treatment</i>											
PC		2.22	0.00	0.00	2.22	1.20 <sup>c</sup>	0.00	0.00	1.20 <sup>d</sup>	3.37	6.79
NC		2.44	0.64	3.33	6.42	1.15 <sup>d</sup>	2.53	0.59	4.28 <sup>b</sup>	3.05	13.74
NC + PXP		2.31	0.00	0.55	2.86	5.12 <sup>a</sup>	1.11	0.55	6.79 <sup>a</sup>	3.92	13.58
NC + PXP + XAP		1.84	0.55	0.55	2.95	1.75 <sup>b</sup>	2.26	0.00	4.01 <sup>c</sup>	4.88	11.84
0 % DDGS		1.98	0.00	0.95	2.94	1.98	1.10	0.28	3.39	3.22	9.55
15 % DDGS		2.42	0.60	1.23	4.29	2.62	1.82	2.97	4.75	4.38	13.42
<i>Source of variation among treatments</i>											
						<b>P-value</b>					
Diet		0.9419	0.5666	0.0619	0.1698	<b>0.0140</b>	0.3437	0.5670	<b>0.0224</b>	0.8535	0.1149
DDGS		0.7292	0.1602	0.8690	0.5523	0.7556	0.7437	0.9799	0.5160	0.3896	0.2054
Diet X DDGS		0.7063	0.5666	0.7455	0.9840	0.2653	0.8431	0.2667	0.4689	0.7427	0.9684

Table 19 (continued)

<sup>1</sup>Valgus and varus leg abnormality severity reported as walking ability score: 1 = mobile with mild limp, 2 = resistant mobility with significant limp, and 3 = down and crippled.

<sup>2</sup>Total LP = total leg problems = percentage of all the birds with leg problems including valgus, varus and shaky legs.

<sup>3</sup>PC = Positive control.

<sup>4</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>5</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>6</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>7</sup>SEM(39) = pooled standard error of the mean with 39 degrees of freedom.

Table 20: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on incidence and severity of leg abnormalities of toms at 17 weeks of age.

Diet	DDGS	Valgus <sup>1</sup>				Varus <sup>1</sup>				Shaky Leg	Total LP <sup>2</sup>
		1	2	3	Total	1	2	3	Total		
	(%)										
PC <sup>3</sup>	0	1.12	1.19	0.00	2.38	2.70	0.00	0.00	2.70	0.00	5.09
NC <sup>4</sup>	0	2.47	2.47	1.28	6.23	0.00	1.39	1.39	2.78	6.15	15.16
NC + PXP <sup>5</sup>	0	5.30	0.00	0.00	5.30	1.28	1.19	1.28	3.75	0.00	9.05
NC + PXP + XAP <sup>6</sup>	0	2.67	0.00	0.00	2.67	1.39	1.19	2.38	4.96	5.05	12.68
PC	15	4.52	0.00	0.00	4.52	0.00	0.00	0.00	0.00	0.00	4.52
NC	15	2.30	0.00	1.19	3.49	4.05	0.00	0.00	4.05	1.11	8.65
NC + PXP	15	3.66	0.00	0.00	3.66	2.47	2.38	1.19	6.04	0.00	9.70
NC + PXP + XAP	15	2.63	0.00	0.00	2.63	1.11	2.30	0.00	3.41	0.00	6.04
SEM(39) <sup>7</sup>		0.06	0.03	0.02	0.07	0.05	0.04	0.04	0.07	0.05	0.08
<i>Main effects of treatment</i>											
PC		2.89	0.59	0.00	3.45	1.35	0.00	0.00	1.35	0.00	4.80
NC		2.39	1.23	1.23	4.86	2.02	0.69	0.69	3.41	3.63	11.90
NC + PXP		4.48	0.00	0.00	4.48	1.88	1.78	1.23	4.90	0.00	9.38
NC + PXP + XAP		2.64	0.00	0.00	2.64	1.25	1.75	1.20	4.18	2.53	9.36
0 % DDGS		2.91	0.00	0.32	3.56	1.97	1.17	1.26	3.55	2.80 <sup>a</sup>	10.49
15 % DDGS		3.28	0.91	0.30	4.14	1.34	0.94	0.30	3.38	0.28 <sup>b</sup>	7.23
<i>Source of variation among treatments</i>											
		<i>P-value</i>									
Diet		0.5542	0.2560	0.1224	0.7531	0.9628	0.2593	0.5307	0.4174	0.0847	0.3179
DDGS		0.7720	0.0722	0.9781	0.9659	0.6616	0.7262	0.1684	0.8383	<b>0.0538</b>	0.3514
Diet X DDGS		0.5836	0.2560	1.0000	0.8515	0.1944	0.6070	0.5359	0.6543	0.2395	0.7937

Table 20 (continued)

<sup>1</sup>Valgus and varus leg abnormality severity reported as walking ability score: 1 = mobile with mild limp, 2 = resistant mobility with significant limp, and 3 = down and crippled.

<sup>2</sup>Total LP = total leg problems = percentage of all the birds with leg problems including valgus, varus and shaky legs.

<sup>3</sup>PC = Positive control.

<sup>4</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>5</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>6</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>7</sup>SEM(39) = pooled standard error of the mean with 39 degrees of freedom.

Table 21: Effect of dietary inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on tibia ash (%) at 21 d of age in toms.

Diet	DDGS (%)	Tibia Ash (%)
PC <sup>1</sup>	0	37.21
NC <sup>2</sup>	0	36.77
NC + PXP <sup>3</sup>	0	36.88
NC + PXP + XAP <sup>4</sup>	0	37.36
PC	15	36.08
NC	15	36.12
NC + PXP	15	36.07
NC + PXP + XAP	15	34.03
SEM(39) <sup>5</sup>		0.83
<i>Main effects of treatment</i>		
PC		36.64
NC		36.44
NC + PXP		36.47
NC + PXP + XAP		35.64
0 % DDGS		37.05 <sup>a</sup>
15 % DDGS		35.57 <sup>b</sup>
<i>Source of variation among treatments</i>		<b>P-value</b>
Diet		0.7694
DDGS		<b>0.0378</b>
Diet X DDGS		0.4781

<sup>1</sup>PC = Positive control.

<sup>2</sup>NC = Negative control and 150 kcal ME/kg of diet less than the PC diets.

<sup>3</sup>NC + PXP = NC diet supplemented with Phyzyme XP and calcium and non-phytate phosphorus content was reduced by 0.10 % and 0.12 %, respectively.

<sup>4</sup>NC + PXP + XAP = NC diet supplemented with Phyzyme XP and Avizyme 1502.

<sup>5</sup>SEM(39) = pooled standard error of the mean with 39 degrees of freedom.

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## CHAPTER 5

### **ENZYME SUPPLEMENTATION OF CORN-BASED DIETS WITH OR WITHOUT DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS) FOR BROILERS<sup>1</sup>**

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<sup>1</sup>The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service or the North Carolina Cooperative Extension Service of the products mentioned nor criticism of similar products not mentioned.

## 5.1 ABSTRACT

The potential economic value of supplemental enzymes that improve energy availability increases as dietary energy costs increase, especially when they are used in low energy diets that may contain grain co-products such as distiller's dried grains with solubles (DDGS). Ross 708 broilers chicks were randomly distributed among 72 pens (16 males and 16 females/pen), each assigned to one of 12 dietary treatments arranged as a factorial of 2 dietary energy levels (low and high), 2 dietary DDGS inclusion levels (0 and 10 %), and 3 dietary enzyme treatments (control, Ronozyme<sup>®</sup> WX, Roxazyme<sup>®</sup> G2; DSM Nutritional Products, Inc.). Ronozyme<sup>®</sup> WX is a xylanase product, while Roxazyme<sup>®</sup> G2 contains a broad array of enzymes. The high and low dietary energy treatment levels differed by 200 kcal ME/kg. Broilers were fed 3 phases of mash feed that approximated requirements of digestible nutrients by age. BW and feed conversion ratio (FCR) were determined at 14, 35, 42, and 49 d of age. There were no treatment interactions on BW beyond 35 d. The high energy diets significantly increased 49 d BW of females (2.60 vs 2.69 kg,  $P<0.01$ ) and males (3.21 vs 3.35 kg,  $P<0.01$ ), and improved mixed sex 1-49 d FCR (2.06 vs 1.98,  $P<0.01$ ). Dietary enzyme supplementation improved 14 d BW of females (0.42 vs 0.44 vs 0.44 kg,  $P<0.05$ ) and males (0.45 vs 0.46 vs 0.47 kg,  $P<0.05$ ) and improved 1-14 d FCR (1.81 vs 1.66 vs 1.73,  $P<0.05$ ). Dietary DDGS inclusion had no significant effect on 14 d BW, but improved 1-14 d FCR (1.78 vs 1.68,  $P<0.05$ ). There were significant energy X enzyme interaction effects for 14 d male and female BW, but not for 1-14 d FCR. Dietary enzyme

supplementation improved both male and female BW by approximately 6 % in the low energy diets but by only 2 to 5 % in the high energy diets. FCR improved at 14 d by 11.4 % and 4.7 % with enzyme supplementation in the low and high energy diets, respectively. Enzyme and DDGS supplementation in starter diets were most effective on broiler growth performance.

*(Key words: growth performance, DDGS, xylanase, carbohydrase, broiler)*

## 5.2 INTRODUCTION

As dietary energy costs escalate, the potential economic value of supplemental enzymes that improve dietary energy availability increases. Passage of the 2008 Renewable Fuels Act in the USA encouraged immense interest in renewable fuels, including corn-based ethanol which produces distillers dried grains with solubles (DDGS) as a co-product. DDGS can be highly variable in nutrient content and bioavailability (Cromwell et al., 1993), but the use of supplemental enzymes may improve the nutrient bioavailability of DDGS. Martinez-Amezcuca et al. (2006) reported that phytase and citric acid supplementation to diets containing 30 % DDGS improved P bioavailability by 10 %, but it did not improve growth performance.

Pelleting reduces feed wastage and improves handling characteristics while also improving body weight (BW) gain and feed conversion ratio (FCR) (Calet, 1965).

Supplemental enzymes can be destroyed through the conditioning and pelleting processes of

feed manufacturing (Silversides and Bedford, 1999). Further complicating matters, DDGS inclusion can also hinder the pelleting process by decreasing pellet manufacturing efficiency and pellet quality (Wang et al., 2007). The objective of this experiment was to determine the dietary nutrient uplift value of a xylanase-based enzyme product (Ronozyme<sup>®</sup> WX) and a multiple-enzyme blend product (Roxazyme<sup>®</sup> G2) in moderate and low energy diets containing corn-soybean meal (SBM) and corn-SBM-DDGS. All diets were provided as mash to avoid enzyme degradation during pellet manufacturing and to avoid any interactions of the feed form and DDGS inclusion.

### 5.3 MATERIALS AND METHODS

#### ***Bird husbandry***

Ross 708 broiler chicks were randomly distributed among 72 pens with 16 males and 16 females per pen. Pens were grouped in two blocks of house sides, north and south. Each pen was assigned to one of twelve dietary treatments arranged as a factorial of two dietary energy levels (low and high), two dietary DDGS inclusion levels (0 and 10%), and three dietary enzyme treatments (control, Ronozyme<sup>®</sup> WX, Roxazyme<sup>®</sup> G2; DSM Nutritional Products, Inc.). Ronozyme<sup>®</sup> WX is an endo-1, 4- $\beta$ -xylanase product derived from *Thermomyces langinosus* and expressed in *Aspergillus oryzae*. Roxazyme<sup>®</sup> G2 also contains the an endo-1, 4- $\beta$ -xylanase in addition to an endo-1, 4- $\beta$ -glucanase and an endo-1, 2(3)- $\beta$ -glucanase derived from and expressed in *T. longibrachiatum*. Ronozyme<sup>®</sup> WX was added to

each dietary phase at 201 mg/kg, and Roxazyme<sup>®</sup> G2 was added to each dietary phase at 121 mg/kg. Enzyme premixes (Table 1) were prepared before mixing the experimental diets. Low energy diets were formulated to be 200 kcal of metabolizable energy (ME)/kg of feed less than the high energy diets in all three phases. High dietary energy level starter, grower, and finisher diets contained 3000, 3050, and 3100 kcal ME/kg diet, respectively. Water and each phase of roller-milled (800µm) mash diets were available *ad libitum*. Starter diets (Tables 2 and 3) were fed from 1 to 14 d of age. Grower diets (Tables 4 and 5) were fed from 14 to 35 d of age, and finisher diets (Tables 6 and 7) were fed from 35 to 49 d of age.

Each pen contained two tube feeders, and one Plasson drinker (Plasson, Ltd., Menashe, Israel). Supplemental chick drinkers were removed at 7 d of age. Three supplemental feeders (egg flats) were placed in each pen until 7 d of age, two flats until 10 d of age, and only one supplemental flat until 14 d of age. Before switching to a new feed phase, the previous phase was equalized between the two feeders in each pen. Brooding temperature at reception was 35 to 36.1 °C on the litter. This initial brooding temperature was maintained throughout the first night (Table 8). Birds were reared at ambient temperature by 22 days of age. Florescent light was not used after 21 d of age to control excessively rapid growth rate and improve livability (Table 9). All birds were reared according to typical management practices.

### ***Growth performance***

Pen feed consumption was determined at 14, 35, 42, and 49 d of age. Pen BW separated by sex was measured at the same time as feed intake, except at 42 d of age when

individual BW was determined to calculate pen variability. Mortality and cause of mortality was recorded daily, and their BW was recorded to adjust FCR. Mortality, feed intake, and FCR were analyzed periodically and cumulatively.

### ***Statistical analysis***

All data were analyzed as a completely randomized block design using a 2 x 2 x 3 factorial arrangement of 2 dietary energy levels (low and high), 2 dietary DDGS inclusion levels (0 and 10 %), and 3 dietary enzyme treatments (control, Ronozyme<sup>®</sup> WX, Roxazyme<sup>®</sup> G2). A group of birds within a pen was defined as the experimental unit for all statistically analyzed variables. Percentage mortality data were transformed to the arcsine square root before analysis, and final data are presented as natural numbers. Statistical significance was based on a probability of  $P < 0.05$  unless otherwise stated. Data were subjected to ANOVA using GLM procedure of SAS system (SAS Institute, 2003). Means separation was performed using LSMEANS procedure when a significant F statistic was indicated by ANOVA.

### ***Animal ethics***

The experiment herein reported was conducted in accordance to the guidelines of the Institutional Animal Care and Use Committee at North Carolina State University. All husbandry practices and euthanasia were done with full consideration of animal welfare.

## 5.4 RESULTS

Dietary treatment effects on female and male broilers are summarized in Tables 10 and 11, respectively. Treatment interactions on female and male BW did not occur after 35 d. Significant DDGS X energy X enzyme interactions ( $P<0.05$ ) were observed in female BW at 14 and 35 d. As expected, the high energy diets significantly ( $P<0.001$ ) increased both female and male BW throughout the entire experiment. A significant enzyme effect on BW of females ( $P<0.001$ ) and males ( $P<0.05$ ) only occurred at 14 d. In females, dietary supplementation of Ronozyme<sup>®</sup> WX and Roxazyme<sup>®</sup> G2 improved BW by 4.1 % and 4.6 % over controls ( $P<0.001$ ), respectively, but there were no significant differences among the two enzyme treatment groups. In the males, the same enzymes improved BW 2.6 % and 3.6 % over controls ( $P<0.01$ ), respectively, but the response to the two enzymes were not significantly different. The only significant DDGS effect observed on BW was on males at 49 d, where the DDGS inclusion improved male BW 2.1 % ( $P<0.01$ ). A significant ( $P<0.05$ ) enzyme X energy interaction effect on BW was observed on broilers at 14 d: dietary enzyme supplementation improved female and male BW by approximately 6 % among those fed the low energy diets but improved BW by only 2 to 5 % among those fed the high energy diets. At 14 d, male and female birds had similar BW responses to the energy X enzyme interaction. Among birds fed the low energy diets, dietary Ronozyme<sup>®</sup> WX supplementation resulted in higher BW than those fed the Roxazyme<sup>®</sup> G2 supplemented diets. Among birds the high-energy diets, those fed diets supplemented with Roxazyme<sup>®</sup> G2 had higher BW than

those fed diets supplemented with Ronozyme<sup>®</sup> WX. A significant DDGS X enzyme interaction was observed in BW of females at 14 d. Among females fed diets without DDGS inclusion, dietary Ronozyme<sup>®</sup> WX supplementation improved BW 6.3 %, but Roxazyme<sup>®</sup> G2 improved BW by only 3.7 % at 14 d. Conversely, among females fed diets with 10 % DDGS inclusion, dietary supplementation of Roxazyme<sup>®</sup> G2 improved 14 d BW 6.0 %, whereas Ronozyme<sup>®</sup> WX supplementation only improved BW 1.9 %. Finally, significant ( $P<0.05$ ) DDGS X enzyme interaction effects were observed on BW of females at 14 d and males at 35 d.

Coefficient of variation (CV) 42 d BW for male and females is summarized in Table 12. There were no significant main factor effects on flock variability throughout the experiment. However, a significant energy X enzyme interaction effect was observed on 42 d BW of males. Among the females that did not receive enzyme-supplemented diets, CV increased as the dietary energy level increased, but those birds that received enzyme supplemented diets, CV decreased as dietary energy increased.

Dietary treatment effects on mixed-sex feed intake are summarized on Table 13. Periodic feed intake had significant DDGS and enzyme effects at 1-14 d and 14-35 d. DDGS inclusion decreased the 1-14 d feed intake 4.9 % ( $P<0.001$ ) but increased 14-35 d feed intake 3.5 % ( $P<0.001$ ). Dietary Ronozyme<sup>®</sup> WX supplementation decreased 1 to 14 d feed intake 4.1% ( $P<0.01$ ) but increased 14-35 d feed intake 3.5 % ( $P<0.05$ ). Roxazyme<sup>®</sup> G2 supplementation had no significant response on feed intake over in comparison to controls. There was none but one significant energy X enzyme interaction effect observed on mixed-

sex feed intake at 35-42 d. Enzyme supplementation decreased periodic feed intake in birds fed the low energy diet but increased periodic feed intake in birds fed the high-energy diet.

Cumulative feed intake data is summarized in Table 14. DDGS inclusion significantly increased cumulative feed intake by approximately 1.7 % throughout the experiment. A significant energy X enzyme interaction effect was observed 1-42 and 1-49 d cumulative feed intake. Dietary Roxazyme<sup>®</sup> G2 supplementation decreased 42d and 49 d cumulative feed intake among birds fed the low energy diets but increased it among birds fed the high energy diets. Conversely, dietary Ronozyme<sup>®</sup> WX supplementation increased 42 and 49 d cumulative feed intake among birds fed both the high and low energy diets.

As expected, the high energy diets resulted in significantly better period FCR through to 42 d than the low energy diets (Table 15). DDGS inclusion improved 1-14 d FCR by 5.6 % ( $P<0.05$ ) but deteriorated 14-35 d FCR by 2.7 % ( $P<0.001$ ). Both supplemental enzymes had a positive effect on 1-14 d FCR but a negative effect on 14-35 d FCR. Ronozyme<sup>®</sup> WX and Roxazyme<sup>®</sup> G2 supplementation improved 1-14 d FCR by 8.3 % and 4.4 %, respectively, but they hindered 14 to 35 d FCR by 4.3 % and 1.1 %, respectively. A significant DDGS X enzyme interaction effect was observed for 14-35 d FCR. When DDGS were included in the diet, the period FCR was impaired, and enzyme supplementation even further impaired the 14-35 d FCR. A significant three way interaction of DDGS X energy X enzyme was found for 14-35 d FCR.

Cumulative FCR data is summarized in Table 16. As observed with the periodic FCR data, the high energy diet significantly improved cumulative FCR throughout the experiment

by about 8 points (2.06 vs 1.98,  $P < 0.001$ ). Dietary inclusion of DDGS increased cumulative FCR to 35 d by 5 points (1.83 vs 1.78,  $P < 0.05$ ). Dietary supplementation of Ronozyme<sup>®</sup> WX significantly increased cumulative FCR at 35 d in comparison to control and Roxazyme<sup>®</sup> G2 (1.81 vs 1.78 and 1.78,  $P < 0.05$ ). A significant DDGS X enzyme interaction effect was observed on 1-35 d FCR ( $P < 0.001$ ). When DDGS were not included in the ration, the cumulative FCR for each of the enzyme treatments were similar, but when DDGS were included in the ration, the 1-35 d FCR increased. A significant DDGS X energy X enzyme interaction effect on 1-49 d FCR was also observed.

Periodic and cumulative mortality data for females and males is presented in Tables 17-20. There were no significant treatment effects on mortality except for a DDGS X energy and energy X enzyme interaction effects on 14-35 d female mortality (Table 16). Among birds fed the low energy diets, there was no mortality from 14 to 35 d for birds that received the enzyme treatments, in comparison 1.76 % mortality among females that did not receive an enzyme. In contrast, females fed the high energy diets had approximately 1 % greater mortality when the diets included an enzyme than without enzyme supplementation. When fed 10 % DDGS, the high energy diets had greater mortality, but without DDGS the low energy diets had higher mortality.

## 5.5 DISCUSSION

As expected, significant differences in growth performance of male and female broilers fed the high and low energy diets, which differed by 200 kcal ME/kg. Dietary inclusion 10 % DDGS did not have any adverse effect on female or male BW. Surprisingly, DDGS inclusion significantly improved male BW at 49 d. Lumpkins *et al.* (2004) concluded that 15 % DDGS inclusion can be used in commercial broiler diets without detrimental effects on growth performance. Wang *et al.* (2007) also concluded that 15 % DDGS inclusion did not hinder broiler growth performance. The improved 1-14 d mixed-sex FCR is consistent with the observed decrease in 1-14 d mixed-sex feed intake. This observation disagrees with Lumpkins *et al.* (2004) who reported 15 % DDGS inclusion increased 1-7 d FCR. The disagreement may be related to the additional 5 % DDGS inclusion in the previous report as compared to the current research. Similarly, the increased 1-35 d mixed-sex feed intake is consistent with the increased 1-35 d FCR. The birds fed DDGS consumed more feed but did not result in increased BW. Overall, DDGS inclusion increased feed intake but had no detrimental effects on cumulative FCR and BW, which generally agrees with reports by Lumpkins *et al.* (2004) and Wang *et al.* (2007).

The supplemental enzymes evaluated in this study had different responses on broiler performance, as expected because the Ronozyme<sup>®</sup> WX only contained xylanase activity whereas the Roxazyme<sup>®</sup> G2 contained xylanase and two glucanase activities. Both enzymes increased male and female BW at 14 d but not thereafter. This positive effect of dietary enzyme supplementation on early growth may be due to the young bird's limited capacity of

endogenous enzymes. The activity of endogenous digestive enzymes in chickens has been observed to increase with age. Nitsan *et al.* (1991) reported maximal activities in the small intestine at 4 d for lipase, 11 d of age for trypsin and chymotrypsin, and 17 d of age for amylase. Dietary supplementation of Ronozyme<sup>®</sup> WX decreased 1-14 d FI, but it increased 14-35 and 1-35 d FI. In contrast, dietary supplementation of Roxazyme<sup>®</sup> G2 had no significant effect on FI. Both enzymes improved 1-14 d FCR mostly as a result of improved 14 d BW. No significant beneficial effect of enzyme supplementation on FCR was evident beyond 14 d.

Although the supplemental enzymes did not demonstrate any main effects on broiler growth performance beyond 35 d of age, it is important to note the significant energy X enzyme interaction effects observed among the diets containing DDGS. At 49 d, female BW in the low-energy diets with Ronozyme<sup>®</sup> WX supplementation was similar to the BW of the female broilers fed the high-energy control diets (2.64 vs 2.65 kg, respectively), even though there was a 200 kcal ME/kg of diet difference between the high and low energy diets in all feed phases. A similar response was observed for 35-49 d FCR. The FCR for broilers fed the low energy diet supplemented with Ronozyme<sup>®</sup> WX was identical to the FCR for broilers fed the high-energy diet. The apparent 200 kcal ME/kg uplift with dietary enzyme supplementation observed in this experiment exceeds the 80 kcal ME uplift observed by Rutherford *et al.* (2007) who fed broilers corn-soy diets supplemented with an enzyme product containing xylanase,  $\alpha$ -amylase and  $\beta$ -glucanase. Similarly, Meng and Slominski (2005) reported that feeding broilers a corn-soy diet supplemented with an enzyme product

containing xylanase, glucanase, pectinase, cellulase, mannanase, and galactanase activities increased dietary AME<sub>n</sub> 2.3 %.

In the current study, 49 d BW among males did not respond to dietary energy as among females. The 49 d BW of males fed the high-energy control diet was greater than the BW of males fed the low-energy diet supplemented with Ronozyme<sup>®</sup> WX (3.39 vs 3.29 kg, respectively). Male BW at 49 d was the same among those fed the high-energy diets, regardless of dietary enzyme supplementation (control, Ronozyme<sup>®</sup> WX, and Roxazyme<sup>®</sup> G2). The males likely consumed less feed, and the females likely consumed additional feed, but this could not be determined because feed parameters were mixed-sex. Furthermore, the males fed the high-energy diets with 10 % DDGS inclusion had identical BW at 49 d. This may have occurred because the males reached their maximum growth potential. If the male broilers reached their maximum growth potential when fed the high-energy diets, the benefit of the supplemental enzymes would not be detected in male BW.

In conclusion, the high-energy diets significantly improved male and female BW and FCR compared to the low-energy diets, which were reduced by 200 kcal ME/kg of diet. DDGS inclusion at 10 % significantly increased 49 d male BW 2.1 %, decreased 1-14 d feed intake 4.9 % but increased overall feed intake 1.7 %. DDGS inclusion also significantly increased cumulative FCR 5 points. Dietary supplementation with Ronozyme<sup>®</sup> WX, which only contained xylanase activity, improved growth performance of broilers fed 10 % DDGS, but dietary supplementation with Roxazyme<sup>®</sup> G2, which contained xylanase and  $\beta$ -glucanase activities, had variable responses. Ronozyme<sup>®</sup> WX supplemented in low-energy diets

containing 10 % DDGS restored broiler growth performance to the level of the high-energy control at 49 d. In other words, Ronozyme<sup>®</sup> WX, but not Roxazyme<sup>®</sup> G2, provided a 200 kcal ME uplift.

## 5.6 TABLES

Table 1: Enzyme premix formulations.

Component	Control Premix		WX Premix <sup>1</sup>		G2 Premix <sup>2</sup>	
	(%)	(g)	(%)	(g)	(%)	(g)
Ronozyme <sup>®</sup> WX	0	0	40	2000	0	0
Roxazyme <sup>®</sup> G2	0	0	0	0	24.2	1210
Carrier <sup>3</sup>	100	5000	60	3000	75.8	3790
Total	100	5000	100	5000	100	5000

<sup>1</sup>WX Premix provided 201 mg Ronozyme<sup>®</sup> WX/kg when added to the diet at 0.05 % of the total mix.

<sup>2</sup>G2 Premix provided 121 mg Roxazyme<sup>®</sup> G2/kg when added to the diet at 0.05 % of the total mix.

<sup>3</sup>Carrier is inert filler (vermiculite).

Table 2: Composition and nutrient content of the experimental starter diets fed to broilers from 1 to 14 d of age without distillers dried grains with solubles (DDGS).

Ingredients	HE <sup>1</sup>	HE + WX <sup>2</sup>	HE + G2 <sup>3</sup>	LE <sup>4</sup>	LE + WX <sup>5</sup>	LE + G2 <sup>6</sup>
	(%)					
Corn	58.28	58.28	58.28	53.05	53.05	53.05
Soybean meal (48 % CP)	35.67	35.67	35.67	36.54	36.54	36.54
DDGS	0.00	0.00	0.00	0.00	0.00	0.00
Poultry fat	1.56	1.56	1.56	1.00	1.00	1.00
Dical. (18.5 % P)	1.99	1.99	1.99	2.00	2.00	2.00
Limestone	1.19	1.19	1.19	1.18	1.18	1.18
L-lysine HCl	0.05	0.05	0.05	0.03	0.03	0.03
D,L-methionine	0.31	0.31	0.31	0.31	0.31	0.31
Salt (NaCl)	0.53	0.53	0.53	0.53	0.53	0.53
L-threonine	0.02	0.02	0.02	0.00	0.00	0.00
Mineral premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride (60 %)	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix <sup>8</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>9</sup>	0.05	0.05	0.05	0.05	0.05	0.05
Control premix <sup>10</sup>	0.05	0.00	0.00	0.05	0.00	0.00
Ronozyme <sup>®</sup> WX premix <sup>11</sup>	0.00	0.05	0.00	0.00	0.05	0.00
Roxazyme <sup>®</sup> G2 premix <sup>12</sup>	0.00	0.00	0.05	0.00	0.00	0.05
Filler (Vermiculite)	0.00	0.00	0.00	4.96	4.96	4.96
<b><i>Calculated analysis</i></b>						
ME, kcal/kg	3000	3000	3000	2800	2800	2800
Crude protein, %	21.5	21.5	21.5	21.5	21.5	21.5
Crude fat, %	3.90	3.90	3.90	3.19	3.19	3.19
Crude fiber, %	2.35	2.35	2.35	2.29	2.29	2.29
Sodium, %	0.25	0.25	0.25	0.25	0.25	0.25
Lysine, %	1.23	1.23	1.23	1.23	1.23	1.23
Methionine + cysteine, %	1.01	1.01	1.01	1.01	1.01	1.01
<b><i>Chemical analysis (dry matter basis)</i></b>						
Dry matter, %	89.69	90.33	89.85	90.11	90.30	90.22
Crude protein, %	24.87	25.73	24.56	24.61	23.90	23.99
Fat (ether extraction), %	4.14	4.01	4.25	2.84	2.77	3.02
Ash, %	6.64	6.98	6.92	11.67	12.03	12.22
Total fiber, %	13.49	11.59	11.71	16.82	14.51	16.99

Table 2 (continued)

<sup>1</sup>HE = High energy control.

<sup>2</sup>HE + WX = HE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>3</sup>HE + G2 = HE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>4</sup>LE = Low energy control, which was reduced by 200 kcal ME/kg of diet compared to HE control.

<sup>5</sup>LE + WX = LE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>6</sup>LE + G2 = LE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>7</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>8</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>9</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>10</sup>Control premix completely inert filler (vermiculite).

<sup>11</sup>Ronozyme<sup>®</sup> WX premix provided 201 mg Ronozyme<sup>®</sup> WX/kg of feed.

<sup>12</sup>Roxazyme<sup>®</sup> G2 premix provided 121 mg Roxazyme<sup>®</sup> G2/kg of feed.

Table 3: Composition and nutrient content of the experimental starter diets fed to broilers from 1 to 14 d of age with distillers dried grains with solubles (DDGS).

Ingredients	HE <sup>1</sup>	HE + WX <sup>2</sup>	HE + G2 <sup>3</sup>	LE <sup>4</sup>	LE + WX <sup>5</sup>	LE + G2 <sup>6</sup>
	(%)					
Corn	53.41	53.41	53.41	48.16	48.16	48.16
Soybean meal (48 % CP)	30.54	30.54	30.54	31.38	31.38	31.38
DDGS	10.00	10.00	10.00	10.00	10.00	10.00
Poultry fat	1.56	1.56	1.56	1.00	1.00	1.00
Dical. (18.5 % P)	1.78	1.78	1.78	1.79	1.79	1.79
Limestone	1.33	1.33	1.33	1.32	1.32	1.32
L-lysine HCl	0.15	0.15	0.15	0.14	0.14	0.14
D,L-methionine	0.29	0.29	0.29	0.30	0.30	0.30
Salt (NaCl)	0.51	0.51	0.51	0.51	0.51	0.51
L-threonine	0.03	0.03	0.03	0.03	0.03	0.03
Mineral premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride (60 %)	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix <sup>8</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>9</sup>	0.05	0.05	0.05	0.05	0.05	0.05
Control premix <sup>10</sup>	0.05	0.00	0.00	0.05	0.00	0.00
Ronozyme <sup>®</sup> WX premix <sup>11</sup>	0.00	0.05	0.00	0.00	0.05	0.00
Roxazyme <sup>®</sup> G2 premix <sup>12</sup>	0.00	0.00	0.05	0.00	0.00	0.05
Filler (Vermiculite)	0.00	0.00	0.00	4.97	4.97	4.97
<b><i>Calculated analysis</i></b>						
ME, kcal/kg	3000	3000	3000	2800	2800	2800
Crude protein, %	21.5	21.5	21.5	21.5	21.5	21.5
Crude fat, %	4.76	4.76	4.76	3.05	3.05	3.05
Crude fiber, %	2.74	2.74	2.74	2.67	2.67	2.67
Sodium, %	0.25	0.25	0.25	0.25	0.25	0.25
Lysine, %	1.24	1.24	1.24	1.24	1.24	1.24
Methionine + cysteine, %	1.01	1.01	1.01	1.01	1.01	1.01
<b><i>Chemical analysis (dry matter basis)</i></b>						
Dry matter, %	89.85	90.18	89.96	90.45	90.73	90.44
Crude protein, %	25.54	26.18	25.31	23.82	25.28	24.71
Fat (ether extraction), %	5.71	5.48	5.50	4.31	4.10	4.26
Ash, %	7.48	7.34	7.28	12.40	12.30	12.19
Total fiber, %	14.01	20.32	16.83	21.30	22.16	22.97

Table 3 (continued)

<sup>1</sup>HE = High energy control.

<sup>2</sup>HE + WX = HE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>3</sup>HE + G2 = HE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>4</sup>LE = Low energy control, which was reduced by 200 kcal ME/kg of diet compared to HE control.

<sup>5</sup>LE + WX = LE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>6</sup>LE + G2 = LE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>7</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>8</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>9</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>10</sup>Control premix completely inert filler (vermiculite).

<sup>11</sup>Ronozyme<sup>®</sup> WX premix provided 201 mg Ronozyme<sup>®</sup> WX/kg of feed.

<sup>12</sup>Roxazyme<sup>®</sup> G2 premix provided 121 mg Roxazyme<sup>®</sup> G2/kg of feed.

Table 4: Composition and nutrient content of the experimental grower diets fed to broilers from 14 to 35 d of age without distillers dried grains with solubles (DDGS).

Ingredients	HE <sup>1</sup>	HE + WX <sup>2</sup>	HE + G2 <sup>3</sup>	LE <sup>4</sup>	LE + WX <sup>5</sup>	LE + G2 <sup>6</sup>
	(%)					
Corn	62.09	62.09	62.09	57.09	57.09	57.09
Soybean meal (48 % CP)	32.04	32.04	32.04	33.45	33.45	33.45
DDGS	0.00	0.00	0.00	0.00	0.00	0.00
Poultry fat	1.81	1.81	1.81	1.00	1.00	1.00
Dical. (18.5 % P)	1.75	1.75	1.75	1.75	1.75	1.75
Limestone	1.07	1.07	1.07	1.07	1.07	1.07
L-lysine HCl	0.04	0.04	0.04	0.00	0.00	0.00
D,L-methionine	0.26	0.26	0.26	0.25	0.25	0.25
Salt (NaCl)	0.54	0.54	0.54	0.56	0.56	0.56
L-threonine	0.00	0.00	0.00	0.00	0.00	0.00
Mineral premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride (60 %)	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix <sup>8</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>9</sup>	0.05	0.05	0.05	0.05	0.05	0.05
Control premix <sup>10</sup>	0.05	0.00	0.00	0.05	0.00	0.00
Ronozyme <sup>®</sup> WX premix <sup>11</sup>	0.00	0.05	0.00	0.00	0.05	0.00
Roxazyme <sup>®</sup> G2 premix <sup>12</sup>	0.00	0.00	0.05	0.00	0.00	0.05
Filler (Vermiculite)	0.00	0.00	0.00	4.43	4.43	4.43
<b><i>Calculated analysis</i></b>						
ME, kcal/kg	3050	3050	3050	2850	2850	2850
Crude protein, %	20.00	20.00	20.00	20.26	20.26	20.26
Crude fat, %	4.22	4.22	4.22	3.28	3.28	3.28
Crude fiber, %	2.29	2.29	2.29	2.25	2.25	2.25
Sodium, %	0.25	0.25	0.25	0.26	0.26	0.26
Lysine, %	1.12	1.12	1.12	1.12	1.12	1.12
Methionine + cysteine, %	0.92	0.92	0.92	0.92	0.92	0.92
<b><i>Chemical analysis (dry matter basis)</i></b>						
Dry matter, %	89.96	90.14	90.00	90.96	90.39	90.49
Crude protein, %	21.95	23.78	22.66	22.38	22.95	23.17
Fat (ether extraction), %	4.39	5.09	4.19	2.97	3.80	3.62
Ash, %	6.23	6.91	6.47	9.98	10.17	10.33
Total fiber, %	12.93	14.84	11.34	14.50	21.37	15.43

Table 4 (continued)

<sup>1</sup>HE = High energy control.

<sup>2</sup>HE + WX = HE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>3</sup>HE + G2 = HE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>4</sup>LE = Low energy control, which was reduced by 200 kcal ME/kg of diet compared to HE control.

<sup>5</sup>LE + WX = LE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>6</sup>LE + G2 = LE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>7</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>8</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>9</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>10</sup>Control premix completely inert filler (vermiculite).

<sup>11</sup>Ronozyme<sup>®</sup> WX premix provided 201 mg Ronozyme<sup>®</sup> WX/kg of feed.

<sup>12</sup>Roxazyme<sup>®</sup> G2 premix provided 121 mg Roxazyme<sup>®</sup> G2/kg of feed.

Table 5: Composition and nutrient content of the experimental grower diets fed to broilers from 14 to 35 d of age with distillers dried grains with solubles (DDGS).

Ingredients	HE <sup>1</sup>	HE + WX <sup>2</sup>	HE + G2 <sup>3</sup>	LE <sup>4</sup>	LE + WX <sup>5</sup>	LE + G2 <sup>6</sup>
	(%)					
Corn	56.04	56.04	56.04	51.76	51.76	51.76
Soybean meal (48 % CP)	28.02	28.02	28.02	28.95	28.95	28.95
DDGS	10.00	10.00	10.00	10.00	10.00	10.00
Poultry fat	1.95	1.95	1.95	1.00	1.00	1.00
Dical. (18.5 % P)	1.54	1.54	1.54	1.54	1.54	1.54
Limestone	1.21	1.21	1.21	1.20	1.20	1.20
L-lysine HCl	0.10	0.10	0.10	0.08	0.08	0.08
D,L-methionine	0.23	0.23	0.23	0.23	0.23	0.23
Salt (NaCl)	0.51	0.51	0.51	0.54	0.54	0.54
L-threonine	0.00	0.00	0.00	0.00	0.00	0.00
Mineral premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride (60 %)	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix <sup>8</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>9</sup>	0.05	0.05	0.05	0.05	0.05	0.05
Control premix <sup>10</sup>	0.05	0.00	0.00	0.05	0.00	0.00
Ronozyme <sup>®</sup> WX premix <sup>11</sup>	0.00	0.05	0.00	0.00	0.05	0.00
Roxazyme <sup>®</sup> G2 premix <sup>12</sup>	0.00	0.00	0.05	0.00	0.00	0.05
Filler (Vermiculite)	0.00	0.00	0.00	4.30	4.30	4.30
<b>Calculated analysis</b>						
ME, kcal/kg	3050	3050	3050	2850	2850	2850
Crude protein, %	20.39	20.39	20.39	20.50	20.50	20.50
Crude fat, %	5.19	5.19	5.19	4.13	4.13	4.13
Crude fiber, %	2.69	2.69	2.69	2.65	2.65	2.65
Sodium, %	0.25	0.25	0.25	0.26	0.26	0.26
Lysine, %	1.13	1.13	1.13	1.14	1.14	1.14
Methionine + cysteine, %	0.92	0.92	0.92	0.92	0.92	0.92
<b>Chemical analysis (dry matter basis)</b>						
Dry matter, %	90.55	90.37	90.22	90.43	90.64	90.44
Crude protein, %	22.64	23.98	23.90	25.25	21.52	23.79
Fat (ether extraction), %	5.70	6.09	5.33	4.56	4.14	4.33
Ash, %	6.52	6.19	6.45	9.91	10.43	9.61
Total fiber, %	13.41	23.12	12.72	18.37	23.18	15.14

Table 5 (continued)

<sup>1</sup>HE = High energy control.

<sup>2</sup>HE + WX = HE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>3</sup>HE + G2 = HE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>4</sup>LE = Low energy control, which was reduced by 200 kcal ME/kg of diet compared to HE control.

<sup>5</sup>LE + WX = LE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>6</sup>LE + G2 = LE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>7</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>8</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>9</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>10</sup>Control premix completely inert filler (vermiculite).

<sup>11</sup>Ronozyme<sup>®</sup> WX premix provided 201 mg Ronozyme<sup>®</sup> WX/kg of feed.

<sup>12</sup>Roxazyme<sup>®</sup> G2 premix provided 121 mg Roxazyme<sup>®</sup> G2/kg of feed.

Table 6: Composition and nutrient content of the experimental finisher diets fed to broilers from 35 to 49 d of age without distillers dried grains with solubles (DDGS).

Ingredients	HE <sup>1</sup>	HE + WX <sup>2</sup>	HE + G2 <sup>3</sup>	LE <sup>4</sup>	LE + WX <sup>5</sup>	LE + G2 <sup>6</sup>
	(%)					
Corn	67.28	67.28	67.28	63.10	63.10	63.10
Soybean meal (48 % CP)	27.19	27.19	27.19	27.84	27.84	27.84
DDGS	0.00	0.00	0.00	0.00	0.00	0.00
Poultry fat	1.92	1.92	1.92	1.00	1.00	1.00
Dical. (18.5 % P)	1.53	1.53	1.53	1.54	1.54	1.54
Limestone	0.96	0.96	0.96	0.95	0.95	0.95
L-lysine HCl	0.00	0.00	0.00	0.00	0.00	0.00
D,L-methionine	0.18	0.18	0.18	0.18	0.18	0.18
Salt (NaCl)	0.54	0.54	0.54	0.54	0.54	0.54
L-threonine	0.00	0.00	0.00	0.00	0.00	0.00
Mineral premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride (60 %)	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix <sup>8</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>9</sup>	0.05	0.05	0.05	0.05	0.05	0.05
Control premix <sup>10</sup>	0.05	0.00	0.00	0.05	0.00	0.00
Ronozyme <sup>®</sup> WX premix <sup>11</sup>	0.00	0.05	0.00	0.00	0.05	0.00
Roxazyme <sup>®</sup> G2 premix <sup>12</sup>	0.00	0.00	0.05	0.00	0.00	0.05
Filler (Vermiculite)	0.00	0.00	0.00	4.45	4.45	4.45
<b><i>Calculated analysis</i></b>						
ME, kcal/kg	3100	3100	3100	2900	2900	2900
Crude protein, %	18.00	18.00	18.00	18.00	18.00	18.00
Crude fat, %	4.41	4.41	4.41	3.38	3.38	3.38
Crude fiber, %	2.20	2.20	2.20	2.15	2.15	2.15
Sodium, %	0.73	0.73	0.73	0.25	0.25	0.25
Lysine, %	0.96	0.96	0.96	0.97	0.97	0.97
Methionine + cysteine, %	0.79	0.79	0.79	0.79	0.79	0.79
<b><i>Chemical analysis (dry matter basis)</i></b>						
Dry matter, %	89.76	89.86	90.02	90.40	90.44	88.96
Crude protein, %	20.63	20.61	21.10	20.87	20.36	21.22
Fat (ether extraction), %	8.70	4.22	4.41	2.77	2.75	3.00
Ash, %	7.74	7.46	7.19	11.82	11.62	10.79
Total fiber, %	14.42	13.75	14.79	20.08	17.19	23.11

Table 6 (continued)

<sup>1</sup>HE = High energy control.

<sup>2</sup>HE + WX = HE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>3</sup>HE + G2 = HE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>4</sup>LE = Low energy control, which was reduced by 200 kcal ME/kg of diet compared to HE control.

<sup>5</sup>LE + WX = LE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>6</sup>LE + G2 = LE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>7</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>8</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>9</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>10</sup>Control premix completely inert filler (vermiculite).

<sup>11</sup>Ronozyme<sup>®</sup> WX premix provided 201 mg Ronozyme<sup>®</sup> WX/kg of feed.

<sup>12</sup>Roxazyme<sup>®</sup> G2 premix provided 121 mg Roxazyme<sup>®</sup> G2/kg of feed.

Table 7: Composition and nutrient content of the experimental finisher diets fed to broilers from 35 to 49 d of age with distillers dried grains with solubles (DDGS).

Ingredients	HE <sup>1</sup>	HE + WX <sup>2</sup>	HE + G2 <sup>3</sup>	LE <sup>4</sup>	LE + WX <sup>5</sup>	LE + G2 <sup>6</sup>
	(%)					
Corn	62.40	62.40	62.40	58.20	58.20	58.20
Soybean meal (48 % CP)	22.09	22.09	22.09	22.76	22.76	22.76
DDGS	10.00	10.00	10.00	10.00	10.00	10.00
Poultry fat	1.91	1.91	1.91	1.00	1.00	1.00
Dical. (18.5 % P)	1.32	1.32	1.32	1.33	1.33	1.33
Limestone	1.10	1.10	1.10	1.09	1.09	1.09
L-lysine HCl	0.10	0.10	0.10	0.09	0.09	0.09
D,L-methionine	0.16	0.16	0.16	0.17	0.17	0.17
Salt (NaCl)	0.52	0.52	0.52	0.52	0.52	0.52
L-threonine	0.00	0.00	0.00	0.00	0.00	0.00
Mineral premix <sup>7</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride (60 %)	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix <sup>8</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>9</sup>	0.05	0.05	0.05	0.05	0.05	0.05
Control premix <sup>10</sup>	0.05	0.00	0.00	0.05	0.00	0.00
Ronozyme <sup>®</sup> WX premix <sup>11</sup>	0.00	0.05	0.00	0.00	0.05	0.00
Roxazyme <sup>®</sup> G2 premix <sup>12</sup>	0.00	0.00	0.05	0.00	0.00	0.05
Filler (Vermiculite)	0.00	0.00	0.00	4.44	4.44	4.44
<b>Calculated analysis</b>						
ME, kcal/kg	3100	3100	3100	2900	2900	2900
Crude protein, %	18.00	18.00	18.00	18.00	18.00	18.00
Crude fat, %	5.26	5.26	5.26	4.24	4.24	4.24
Crude fiber, %	2.59	2.59	2.59	2.54	2.54	2.54
Sodium, %	0.25	0.25	0.25	0.25	0.25	0.25
Lysine, %	0.97	0.97	0.97	0.97	0.97	0.97
Methionine + cysteine, %	0.79	0.79	0.79	0.79	0.79	0.79
<b>Chemical analysis (dry matter basis)</b>						
Dry matter, %	90.28	90.27	90.36	90.53	90.63	90.40
Crude protein, %	20.42	19.96	20.53	20.96	20.70	20.01
Fat (ether extraction), %	4.29	5.98	5.59	4.04	4.64	4.53
Ash, %	7.38	6.03	7.82	11.38	10.86	11.25
Total fiber, %	17.28	16.11	21.50	21.37	21.42	25.13

Table 7 (continued)

<sup>1</sup>HE = High energy control.

<sup>2</sup>HE + WX = HE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>3</sup>HE + G2 = HE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>4</sup>LE = Low energy control, which was reduced by 200 kcal ME/kg of diet compared to HE control.

<sup>5</sup>LE + WX = LE diet supplemented with Ronozyme<sup>®</sup> WX.

<sup>6</sup>LE + G2 = LE diet supplemented with Roxazyme<sup>®</sup> G2.

<sup>7</sup>Supplied the following per kilogram of feed: 120 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 120 mg MN as MnSO<sub>4</sub>·H<sub>2</sub>O; 80 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 10 mg Cu as CuSO<sub>4</sub>; 2.5 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1.0 mg Co as CoSO<sub>4</sub>.

<sup>8</sup>Selenium premix provided 0.3 ppm Se from sodium selenate.

<sup>9</sup>Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B<sub>12</sub>, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL- $\alpha$ -tocopheryl acetate.

<sup>10</sup>Control premix completely inert filler (vermiculite).

<sup>11</sup>Ronozyme<sup>®</sup> WX premix provided 201 mg Ronozyme<sup>®</sup> WX/kg of feed.

<sup>12</sup>Roxazyme<sup>®</sup> G2 premix provided 121 mg Roxazyme<sup>®</sup> G2/kg of feed.

Table 8: Brooder temperatures<sup>1</sup>.

Day	Temperature (°F)	Temperature (°C)
1 - 7	90 – 93	32.2 – 33.5
8 - 14	85	29.4
15 - 21	80	26.7
22 - 49	Ambient	Ambient

<sup>1</sup>Brooding temperature at reception was 35 to 36.1° C (95 to 97 °F), and was maintained throughout the first night.

Table 9: Lighting program schedule<sup>1</sup>.

Day	Hours	Off / On
1 - 7	23 L : 1 D	0500 / 0600
8 - 21	21 L : 3 D	0300 / 0600
22 - 49	natural light only	

<sup>1</sup>Natural light was used after 21 d of age to control excessively rapid growth and improve livability.

Table 10: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on BW of female broilers.

Dietary treatment	14 d	35 d	42 d	49 d
	(kg)			
High energy <sup>1</sup>	0.424	1.743	2.281	2.720
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	0.459	1.817	2.288	2.713
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	0.452	1.734	2.225	2.655
Low energy <sup>4</sup>	0.411	1.686	2.183	2.609
Low energy + Ronozyme <sup>®</sup> WX	0.433	1.677	2.156	2.547
Low energy + Roxazyme <sup>®</sup> G2	0.416	1.705	2.135	2.568
High energy + 10 % DDGS	0.442	1.790	2.245	2.650
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	0.428	1.717	2.240	2.699
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.468	1.798	2.306	2.691
Low energy + 10 % DDGS	0.401	1.665	2.168	2.610
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	0.432	1.699	2.208	2.641
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.429	1.701	2.167	2.604
SEM(59) <sup>5</sup>	0.007	0.025	0.025	0.028
<i>Main effects of treatment</i>				
High energy	0.446 <sup>a</sup>	1.766 <sup>a</sup>	2.264 <sup>a</sup>	2.688 <sup>a</sup>
Low energy	0.420 <sup>b</sup>	1.689 <sup>b</sup>	2.169 <sup>b</sup>	2.596 <sup>b</sup>
0 % DDGS	0.433	1.727	2.211	2.635
10 % DDGS	0.433	1.728	2.222	2.649
Control	0.420 <sup>b</sup>	1.721	2.219	2.647
Ronozyme <sup>®</sup> WX	0.438 <sup>a</sup>	1.727	2.223	2.650
Roxazyme <sup>®</sup> G2	0.441 <sup>a</sup>	1.735	2.208	2.629
<i>Source of variation among treatments</i>				
	<b>P-value</b>			
Energy	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
DDGS	0.8649	0.9226	0.4640	0.4004
Enzyme	<b>0.0001</b>	0.7443	0.7038	0.5479
DDGS X energy	0.9446	0.8648	0.4198	0.0726
DDGS X enzyme	<b>0.0149</b>	0.1348	0.0787	0.1163
Energy X enzyme	<b>0.0414</b>	0.7276	0.6290	0.6478
DDGS X energy X enzyme	<b>0.0224</b>	<b>0.0118</b>	0.1318	0.3987

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom.

Table 11: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on BW of male broilers.

Dietary treatment	14 d	35 d	42 d	49 d
	(kg)			
High energy <sup>1</sup>	0.458	2.080	2.740	3.294
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	0.478	2.171	2.786	3.324
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	0.486	2.074	2.738	3.290
Low energy <sup>4</sup>	0.424	1.899	2.608	3.203
Low energy + Ronozyme <sup>®</sup> WX	0.454	2.003	2.636	3.218
Low energy + Roxazyme <sup>®</sup> G2	0.443	1.987	2.612	3.143
High energy + 10 % DDGS	0.489	2.194	2.809	3.387
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	0.461	2.073	2.774	3.386
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.486	2.134	2.800	3.390
Low energy + 10 % DDGS	0.425	2.007	2.640	3.249
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	0.450	1.975	2.673	3.293
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.449	1.984	2.623	3.182
SEM(59) <sup>5</sup>	0.008	0.041	0.035	0.045
<i>Main effects of treatment</i>				
High energy	0.476 <sup>a</sup>	2.121 <sup>a</sup>	2.775 <sup>a</sup>	3.345 <sup>a</sup>
Low energy	0.441 <sup>b</sup>	1.976 <sup>b</sup>	2.631 <sup>b</sup>	3.215 <sup>b</sup>
0 % DDGS	0.460	2.036	2.687	3.245 <sup>b</sup>
10 % DDGS	0.457	2.061	2.720	3.315 <sup>a</sup>
Control	0.449 <sup>b</sup>	2.045	2.699	3.283
Ronozyme <sup>®</sup> WX	0.461 <sup>a</sup>	2.055	2.717	3.305
Roxazyme <sup>®</sup> G2	0.466 <sup>a</sup>	2.045	2.693	3.251
<i>Source of variation among treatments</i>				
	<i>P-value</i>			
Energy	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
DDGS	0.5532	0.2919	0.1063	<b>0.0096</b>
Enzyme	<b>0.0130</b>	0.9179	0.6033	0.2375
DDGS X energy	0.7292	0.9989	0.7478	0.5389
DDGS X enzyme	0.0921	<b>0.0156</b>	0.7381	0.9997
Energy X enzyme	<b>0.0208</b>	0.5066	0.8453	0.4336
DDGS X energy X enzyme	0.1413	0.5284	0.5504	0.8296

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom.

Table 12: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on coefficient of variation of 42 d BW of male and female broilers.

Dietary treatment	Male	Female
	(%)	
High energy <sup>1</sup>	8.54	7.32
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	8.15	7.73
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	7.36	6.93
Low energy <sup>4</sup>	7.01	7.02
Low energy + Ronozyme <sup>®</sup> WX	9.33	7.91
Low energy + Roxazyme <sup>®</sup> G2	7.80	7.63
High energy + 10 % DDGS	10.18	8.49
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	5.76	8.20
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	7.77	7.74
Low energy + 10 % DDGS	9.45	8.02
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	9.87	7.83
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	9.88	8.56
SEM(59) <sup>5</sup>	1.05	0.70
<i>Main effects of treatment</i>		
High energy	7.96	7.73
Low energy	8.89	7.83
0 % DDGS	8.03	7.42
10 % DDGS	8.82	8.14
Control	8.79	7.72
Ronozyme <sup>®</sup> WX	8.28	7.92
Roxazyme <sup>®</sup> G2	8.20	7.71
<i>Source of variation among treatments</i>		
	<b>P-value</b>	
Energy	0.1287	0.8093
DDGS	0.2016	0.0810
Enzyme	0.6868	0.8941
DDGS X energy	0.1444	0.8110
DDGS X enzyme	0.1255	0.6474
Energy X enzyme	<b>0.0422</b>	0.4895
DDGS X energy X enzyme	0.7725	0.9463

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom.

Table 13: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period feed intake of mixed-sex broilers.

Dietary treatment	1-14 d	14-35 d	35-42 d	42-49 d
	(kg/bird)			
High energy <sup>1</sup>	0.701	2.56	1.29	1.29
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	0.680	2.65	1.20	1.19
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	0.730	2.53	1.36	1.34
Low energy <sup>4</sup>	0.755	2.49	1.33	1.27
Low energy + Ronozyme <sup>®</sup> WX	0.677	2.64	1.28	1.25
Low energy + Roxazyme <sup>®</sup> G2	0.709	2.58	1.23	1.20
High energy + 10% DDGS	0.668	2.68	1.23	1.19
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	0.645	2.72	1.35	1.27
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.698	2.65	1.31	1.25
Low energy + 10 % DDGS	0.672	2.63	1.40	1.26
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	0.673	2.68	1.34	1.29
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.679	2.65	1.23	1.31
SEM(59) <sup>5</sup>	0.017	0.04	0.05	0.05
<i>Main effects of treatment</i>				
High energy	0.687	2.63	1.29	1.25
Low energy	0.694	2.61	1.30	1.26
0 % DDGS	0.709 <sup>a</sup>	2.58 <sup>b</sup>	1.28	1.26
10 % DDGS	0.673 <sup>b</sup>	2.67 <sup>a</sup>	1.31	1.26
Control	0.699 <sup>a</sup>	2.59 <sup>b</sup>	1.31	1.25
Ronozyme <sup>®</sup> WX	0.667 <sup>b</sup>	2.67 <sup>a</sup>	1.29	1.25
Roxazyme <sup>®</sup> G2	0.704 <sup>a</sup>	2.60 <sup>b</sup>	1.28	1.27
<i>Source of variation among treatments</i>				
Energy	0.4736	0.2697	0.6693	0.7923
DDGS	<b>0.0004</b>	<b>&lt;0.0001</b>	0.2979	0.9104
Enzyme	<b>0.0078</b>	<b>0.0022</b>	0.7156	0.7015
DDGS X energy	0.7700	0.7273	0.5402	0.1341
DDGS X enzyme	0.2421	0.3642	0.1903	0.2156
Energy X enzyme	0.1236	0.2524	<b>0.0142</b>	0.4285
DDGS X energy X enzyme	0.2240	0.7552	0.2949	0.2088

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom.

Table 14: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative feed intake of mixed-sex broilers.

Dietary treatment	1-35 d	1-42 d	1-49 d
	(kg/bird)		
High energy <sup>1</sup>	3.27	4.55	5.84
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	3.34	4.54	5.73
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	3.27	4.63	5.97
Low energy <sup>4</sup>	3.24	4.56	5.84
Low energy + Ronozyme <sup>®</sup> WX	3.32	4.60	5.84
Low energy + Roxazyme <sup>®</sup> G2	3.28	4.51	5.70
High energy + 10 % DDGS	3.34	4.67	5.76
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	3.37	4.71	5.98
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	3.35	4.66	5.91
Low energy + 10 % DDGS	3.30	4.70	5.95
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	3.35	4.69	5.98
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	3.33	4.56	5.86
SEM(59) <sup>5</sup>	0.02	0.05	0.07
<i>Main effects of treatment</i>			
High energy	3.32	4.61	5.86
Low energy	3.30	4.60	5.86
0 % DDGS	3.28 <sup>b</sup>	4.56 <sup>b</sup>	5.82 <sup>b</sup>
10 % DDGS	3.34 <sup>a</sup>	4.65 <sup>a</sup>	5.91 <sup>a</sup>
Control	3.29 <sup>b</sup>	4.59	5.85
Ronozyme <sup>®</sup> WX	3.34 <sup>a</sup>	4.63	5.88
Roxazyme <sup>®</sup> G2	3.31 <sup>b</sup>	4.59	5.86
<i>Source of variation among treatments</i>		<i>P-value</i>	
Energy	0.2125	0.8191	0.9753
DDGS	<b>0.0002</b>	<b>0.0056</b>	<b>0.0277</b>
Enzyme	<b>0.0046</b>	0.3712	0.7489
DDGS X energy	0.5375	0.7357	0.1885
DDGS X enzyme	0.4716	0.4067	0.1456
Energy X enzyme	0.6084	<b>0.0417</b>	<b>0.0242</b>
DDGS X energy X enzyme	0.8267	0.3727	0.1429

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom.

Table 15: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period feed conversion ratio (FCR) of mixed-sex broilers.

Dietary treatment	1-14 d	14-35 d	35-42 d	42-49 d
	(g:g)			
High energy <sup>1</sup>	1.77	1.72	2.24	2.59
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	1.61	1.75	2.23	2.48
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	1.73	1.77	2.46	2.67
Low energy <sup>4</sup>	2.02	1.78	2.31	2.58
Low energy + Ronozyme <sup>®</sup> WX	1.70	1.87	2.47	2.61
Low energy + Roxazyme <sup>®</sup> G2	1.83	1.79	2.49	2.48
High energy + 10 % DDGS	1.60	1.75	2.29	2.65
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	1.61	1.88	2.24	2.40
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	1.62	1.78	2.24	2.53
Low energy + 10 % DDGS	1.83	1.83	2.57	2.39
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	1.71	1.91	2.25	2.46
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	1.73	1.84	2.42	2.55
SEM(59) <sup>5</sup>	0.06	0.01	0.11	0.12
<i>Main effects of treatment</i>				
High energy	1.66 <sup>b</sup>	1.77 <sup>b</sup>	2.28 <sup>b</sup>	2.55
Low energy	1.80 <sup>a</sup>	1.84 <sup>a</sup>	2.42 <sup>a</sup>	2.51
0 % DDGS	1.78 <sup>a</sup>	1.78 <sup>b</sup>	2.36	2.57
10 % DDGS	1.68 <sup>b</sup>	1.83 <sup>a</sup>	2.33	2.50
Control	1.81 <sup>a</sup>	1.77 <sup>c</sup>	2.35	2.55
Ronozyme <sup>®</sup> WX	1.66 <sup>c</sup>	1.85 <sup>a</sup>	2.30	2.49
Roxazyme <sup>®</sup> G2	1.73 <sup>b</sup>	1.79 <sup>b</sup>	2.40	2.56
<i>Source of variation among treatments</i>				
	<b>P-value</b>			
Energy	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0454</b>	0.5289
DDGS	<b>0.0080</b>	<b>&lt;0.0001</b>	0.6262	0.2897
Enzyme	<b>0.0035</b>	<b>&lt;0.0001</b>	0.4654	0.6405
DDGS X energy	0.9734	0.4488	0.7219	0.7909
DDGS X enzyme	0.0863	<b>0.0066</b>	0.1590	0.8872
Energy X enzyme	0.1889	0.1871	0.9010	0.3632
DDGS X energy X enzyme	0.9875	<b>0.0061</b>	0.3516	0.3798

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom.

Table 16: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative feed conversion ratio (FCR) of mixed-sex broilers.

Dietary treatment	1-35 d	1-42 d	1-49 d
	(g:g)		
High energy <sup>1</sup>	1.73	1.85	1.97
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	1.71	1.83	1.93
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	1.76	1.91	2.04
Low energy <sup>4</sup>	1.83	1.95	2.05
Low energy + Ronozyme <sup>®</sup> WX	1.83	1.97	2.07
Low energy + Roxazyme <sup>®</sup> G2	1.80	1.94	2.04
High energy + 10 % DDGS	1.72	1.84	1.95
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	1.82	1.92	2.01
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	1.74	1.86	1.96
Low energy + 10 % DDGS	1.83	2.00	2.07
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	1.87	1.96	2.05
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	1.81	1.94	2.05
SEM(59) <sup>5</sup>	0.02	0.03	0.03
<i>Main effects of treatment</i>			
High energy	1.75 <sup>b</sup>	1.87 <sup>b</sup>	1.98 <sup>b</sup>
Low energy	1.83 <sup>a</sup>	1.96 <sup>a</sup>	2.06 <sup>a</sup>
0 % DDGS	1.78 <sup>b</sup>	1.91	2.02
10 % DDGS	1.83 <sup>a</sup>	1.92	2.02
Control	1.78 <sup>b</sup>	1.91	2.01
Ronozyme <sup>®</sup> WX	1.81 <sup>a</sup>	1.92	2.02
Roxazyme <sup>®</sup> G2	1.78 <sup>b</sup>	1.91	2.02
<i>Source of variation among treatments</i>		<i>P-value</i>	
Energy	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
DDGS	<b>0.0284</b>	0.5009	0.8228
Enzyme	<b>0.0129</b>	0.8419	0.9010
DDGS X energy	0.6402	0.8697	0.8507
DDGS X enzyme	<b>0.0014</b>	0.1957	0.3076
Energy X enzyme	0.1043	0.2443	0.2537
DDGS X energy X enzyme	0.1034	0.0808	<b>0.0318</b>

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom.

Table 17: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period mortality of female broilers.

Dietary treatment	1-14 d	14-35 d	35-42 d	42-49 d
	(%)			
High energy <sup>1</sup>	2.23	2.40	1.12	0.00
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	1.12	0.00	1.12	1.12
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	0.00	0.00	1.12	0.00
Low energy <sup>4</sup>	3.35	0.00	2.30	0.00
Low energy + Ronozyme <sup>®</sup> WX	2.23	0.00	0.00	0.00
Low energy + Roxazyme <sup>®</sup> G2	0.00	0.00	2.62	0.00
High energy + 10 % DDGS	2.23	1.12	0.00	0.00
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	1.12	0.00	0.00	0.00
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	1.04	0.00	1.12	0.00
Low energy + 10 % DDGS	1.12	0.00	2.30	0.00
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	2.23	2.30	2.30	1.12
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.00	2.88	1.05	0.95
SEM(59) <sup>5</sup>	0.04	0.03	0.04	0.02
<i>Main effects of treatment</i>				
High energy	0.96	0.59	0.74	0.19
Low energy	1.64	0.86	1.76	0.35
0 % DDGS	1.31	0.40	1.38	0.19
10 % DDGS	1.29	1.05	1.13	0.34
Control	2.27	0.88	1.43	0.00
Ronozyme <sup>®</sup> WX	1.40	0.58	0.85	0.56
Roxazyme <sup>®</sup> G2	0.23	0.72	1.48	0.24
<i>Source of variation among treatments</i>				
	<i>P-value</i> <sup>6</sup>			
Energy	0.2063	0.5531	0.1196	0.6255
DDGS	0.8762	0.1720	0.6853	0.6372
Enzyme	0.0614	0.8841	0.6750	0.3798
DDGS X energy	0.4661	<b>0.0302</b>	0.4478	0.1121
DDGS X enzyme	0.5855	0.1776	0.6414	0.8004
Energy X enzyme	0.8005	<b>0.0160</b>	0.7268	0.7878
DDGS X energy X enzyme	0.3510	0.7675	0.2849	0.3787

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the arcsin(sqrt(%)) mean with 59 degrees of freedom.

<sup>6</sup>P-values based on mortality transformed to arcsin.

Table 18: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on period mortality of male broilers.

Dietary treatment	1-14 d	14-35 d	35-42 d	42-49 d
	(%)			
High energy <sup>1</sup>	2.23	0.00	0.00	0.00
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	1.12	1.18	0.00	0.00
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	0.00	0.00	1.12	0.00
Low energy <sup>4</sup>	3.35	1.12	0.00	0.00
Low energy + Ronozyme <sup>®</sup> WX	2.23	1.12	1.12	0.00
Low energy + Roxazyme <sup>®</sup> G2	0.00	1.49	0.00	0.00
High energy + 10 % DDGS	2.23	0.00	0.00	0.00
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	1.12	1.12	1.18	0.00
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	1.04	0.00	2.23	0.00
Low energy + 10 % DDGS	1.12	0.00	1.12	0.00
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	2.23	0.00	1.12	1.12
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.00	2.95	0.00	0.00
SEM(59) <sup>5</sup>	0.04	0.04	0.03	0.01
<i>Main effects of treatment</i>				
High energy	0.93	0.38	0.76	0.00
Low energy	0.94	1.11	0.56	0.19
0 % DDGS	1.33	0.82	0.38	0.00
10 % DDGS	0.54	0.68	0.94	0.18
Control	1.12	0.28	0.28	0.00
Ronozyme <sup>®</sup> WX	1.12	0.85	0.85	0.28
Roxazyme <sup>®</sup> G2	0.57	1.11	0.84	0.00
<i>Source of variation among treatments</i>				
	<i>P-value</i> <sup>6</sup>			
Energy	0.9897	0.2370	0.6932	0.3202
DDGS	0.1573	0.6583	0.2578	0.3367
Enzyme	0.6464	0.5334	0.5431	0.3766
DDGS X energy	0.4532	0.6780	0.6654	0.3367
DDGS X enzyme	0.1842	0.6640	0.9982	0.3646
Energy X enzyme	0.2548	0.1637	0.1046	0.3766
DDGS X energy X enzyme	0.6632	0.6754	0.5318	0.3646

Table 18 (continued)

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom based on the mortality transformed to arcsin.

<sup>6</sup>*P*-values based on mortality transformed to arcsin.

Table 19: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative mortality of female broilers.

Dietary treatment	1-35 d	1-42 d	1-49 d
	(%)		
High energy <sup>1</sup>	4.97	6.08	6.08
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	0.00	1.12	2.23
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	0.00	1.12	1.12
Low energy <sup>4</sup>	3.35	5.73	5.73
Low energy + Ronozyme <sup>®</sup> WX	2.23	2.23	2.23
Low energy + Roxazyme <sup>®</sup> G2	0.00	2.51	2.52
High energy + 10 % DDGS	2.23	2.23	2.23
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	2.23	2.23	2.23
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.00	2.22	2.22
Low energy + 10 % DDGS	2.23	4.62	4.62
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	3.50	5.88	7.00
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	3.92	5.04	5.98
SEM(59) <sup>5</sup>	0.05	0.06	0.06
<i>Main effects of treatment</i>			
High energy	1.57	2.50	2.68
Low energy	2.52	4.34	4.68
0 % DDGS	1.73	3.13	3.32
10 % DDGS	2.35	3.70	4.05
Control	3.20	4.67	4.67
Ronozyme <sup>®</sup> WX	1.99	2.87	3.43
Roxazyme <sup>®</sup> G2	0.95	2.72	2.96
<i>Source of variation among treatments</i>		<i>P-value<sup>6</sup></i>	
Energy	0.1496	0.0828	0.0559
DDGS	0.3160	0.6743	0.5553
Enzyme	0.1586	0.3595	0.5483
DDGS X energy	0.6307	0.5394	0.2378
DDGS X enzyme	0.2279	0.3043	0.2558
Energy X enzyme	0.5923	0.9141	0.8114
DDGS X energy X enzyme	0.3009	0.9975	0.8788

Table 19 (continued)

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom based on the mortality transformed to arcsin.

<sup>6</sup>*P*-values based on mortality transformed to arcsin.

Table 20: Effect of dietary energy level, inclusion of distiller's dried grains with solubles (DDGS) and enzyme supplementation on cumulative mortality of male broilers.

Dietary treatment	1-35 d	1-42 d	1-49 d
	(%)		
High energy <sup>1</sup>	2.23	2.23	2.23
High energy + Ronozyme <sup>®</sup> WX <sup>2</sup>	2.38	2.38	2.38
High energy + Roxazyme <sup>®</sup> G2 <sup>3</sup>	0.00	1.12	1.12
Low energy <sup>4</sup>	3.34	3.35	3.35
Low energy + Ronozyme <sup>®</sup> WX	2.23	3.35	3.35
Low energy + Roxazyme <sup>®</sup> G2	2.88	2.93	2.93
High energy + 10 % DDGS	0.00	0.00	0.00
High energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	3.35	3.50	4.61
High energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	0.00	2.23	2.23
Low energy + 10 % DDGS	0.00	1.12	1.12
Low energy + Ronozyme <sup>®</sup> WX + 10 % DDGS	0.00	1.12	2.23
Low energy + Roxazyme <sup>®</sup> G2 + 10 % DDGS	2.99	3.91	3.91
SEM(59) <sup>5</sup>	0.05	0.06	0.06
<i>Main effects of treatment</i>			
High energy	1.33	1.91	2.10
Low energy	1.91	2.63	2.81
0 % DDGS	2.18	2.56	2.56
10 % DDGS	1.06	1.98	2.35
Control	1.40	1.68	1.68
Ronozyme <sup>®</sup> WX	1.99	2.59	3.15
Roxazyme <sup>®</sup> G2	1.47	2.55	2.55
<i>Source of variation among treatments</i>		<i>P-value<sup>6</sup></i>	
Energy	0.5217	0.3956	0.4028
DDGS	0.1773	0.5177	0.8425
Enzyme	0.7059	0.8359	0.5621
DDGS X energy	0.2726	0.5177	0.5240
DDGS X enzyme	0.1842	0.2558	0.2219
Energy X enzyme	0.1239	0.8377	0.8421
DDGS X energy X enzyme	0.4524	0.6286	0.6374

Table 20 (continued)

<sup>1</sup>High dietary energy starter, grower and finisher diets containing 3000, 3050 and 3100 kcal/kg, respectively.

<sup>2</sup>Ronozyme<sup>®</sup> WX inclusion 182 g/t.

<sup>3</sup>Roxazyme<sup>®</sup> G2 inclusion 110 g/t.

<sup>4</sup>Low dietary energy starter, grower and finisher diets containing 2800, 2850 and 2900 kcal/kg, respectively.

<sup>5</sup>SEM(59) = Standard error of the mean with 59 degrees of freedom based on the mortality transformed to arcsin.

<sup>6</sup>*P*-values based on mortality transformed to arcsin.

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## **CHAPTER 6**

### **EFFECT OF INCREASING DOSE RATE ON THE EFFICACY OF A PHYTASE ON THE NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF POULTS FED CORN SOY BASED DIETS<sup>1</sup>**

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<sup>1</sup>The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service or the North Carolina Cooperative Extension Service of the products mentioned nor criticism of similar products not mentioned.

## 6.1 ABSTRACT

As the cost of phosphate increases, the dietary supplementation of phytase becomes more economically attractive. However, as new feed additive phytases are developed for commercial poultry applications, optimum dosage must be determined. Nicholas toms were assigned to dietary treatments including a positive control (PC), negative control (NC), NC plus 250, 500, 1000 or 2000 phytase units (FTU) /kg of feed. BW and FCR were determined at 7, 14, 21, and 27 or 28 d of age. NC diet contained 0.2 % less non-phytate phosphorus (NPP) and 0.15 % less Ca than PC diet. Excreta were collected daily from 22 to 24 d of age. Ileal contents, tibias and toes were collect at either 27 or 28 d of age. BW at 14 and 28 d increased linearly in the negative control diets as the level of phytase supplementation increased. Average daily gain (ADG) was also primarily linear in response to phytase supplementation from 7 to 14 d. Average daily feed intake increased by a quadratic function as dietary phytase supplementation level increased. No significant treatment effects were observed on periodic and cumulative FCR. Tibia ash content increased by a significant ( $P<0.05$ ) quadratic function as phytase inclusion increased but not in toe ash. Tibia and toe ash contents and  $AME_n$  were greatest at 1000 FTU/kg. NC diet had significantly lower  $AME_n$  content than PC diet (2704 vs 2743 kcal  $AME_n$ /kg,  $P<0.05$ ). Phytase supplementation significantly improved turkey growth response, which was associated with improved feed

intake and nutrient bioavailability. The optimum dose of this dietary phytase was identified to be approximately 1000 FTU/kg of feed.

(*Key words:* poults, phytase, ileal digestibility, fecal digestibility, growth performance)

## 6.2 INTRODUCTION

Phosphate is a limited resource and the majority of phosphate is utilized as fertilizer (Abelson, 1999). Phosphorus (P) is an essential mineral in non-ruminant diets, and inorganic phosphate must be added to the diet to meet the animal's nutritional requirement. Most P in commercial poultry diets is present as phytate P (Eeckhout and De Paepe, 1994), but the ability of poultry to effectively hydrolyze phytate P is very variable (Ravindran *et al.*, 1995). This high variability in P bioavailability is associated with poultry's lack of endogenous enzymes to hydrolyze phytic acid (Bedford, 2000). Further complicating matters, phytic acid is a polyanionic molecule that can chelate di- and trivalent cations (Wise, 1983), and interact with dietary carbohydrates, proteins (Angel *et al.*, 2002), and endogenous compounds (Cowieson *et al.*, 2004).

Because inorganic phosphate is a common dietary component in turkey feeds, feed costs can be reduced substantially if the dietary inclusion of P can be reduced by a measure made available from phytate P in other feedstuffs. As the dietary level of phytase increases, the nutritional availability of P from phytate also increases (Cowieson *et al.*, 2006). The objective of this research was to titrate the amount of a novel supplemental phytase using

growth performance (body weight, feed intake, and FCR), digestibility (ileal and fecal minerals and AME<sub>n</sub>) and bone mineralization of the young turkey in order to determine the optimal dietary dosage of this phytase.

### 6.3 MATERIALS AND METHODS

#### ***Bird husbandry***

Four-hundred-eighty Nicholas 300 X 700 tom poults were obtained from a commercial hatchery (Prestage Farms Hatchery, Clinton, NC) and randomly assigned to one of 48 cages with 10 poults per cage (Alternative Design cages, Alternative Design Manufacturing and Supply, Inc., Siloam Springs, AR). Each bird was identified with a numbered neck-tag in sequence for each replicate cage group. Six experimental diets were randomly assigned among 4 blocks of 12 cages, with each block having similar environmental variation. Feed and water from nipple drinkers was available *ad libitum* throughout the experiment. Birds were kept in a temperature-controlled room (Table 1) until 27 or 28 d of age, and supplemental heat was provided at the back of each cage to maintain thermal comfort for the poults. From 1 to 7 d, the birds were provided 23 hours light:1 hour dark, and 14L:10D after 7 d. Feed consumption, group weight and number of birds per cage were recorded on 0, 7, 14, and 21 d. Individual bird weights and cage feed consumptions were determined on 27 or 28 d. Morality rate and cause of mortality were recorded daily and

analyzed by weekly period and cumulatively after arcsine square root (%) transformation. Weight of each dead bird was recorded to correct the FCR.

### ***Experimental diets***

The dietary treatments consisted of corn-soybean meal-based diets formulated as a positive control (PC), negative control (NC), negative control + 250 FTU phytase/kg (a bacterial phytase supplied by Danisco Animal Nutrition), negative control + 500 FTU phytase/kg, negative control + 1000 FTU phytase/kg, and negative control + 2000 FTU phytase/kg (Table 2). NC diet contained 0.2 % less non-phytate phosphours (NPP) and 0.15 % less Ca than PC diet. One FTU is defined as the amount of enzyme required to hydrolyze one  $\mu\text{mol}$  of inorganic P per minute at pH 5.5 from an excess of 15  $\mu\text{M}$  Na phytate at 37 °C (International Union of Biochemistry, 1979). All experimental diets were formulated to meet or exceed NRC (1994) requirements for turkeys except for calcium and non-phytate phosphorus (NPP) content. The NPP content in the NC diet was 0.20 % less than in the PC diet (0.4 vs 0.6 % NPP), and Ca in the NC diet was reduced 0.15 % compared to the PC (1.05 vs 1.20 % Ca) (Table 3). The entire PC and NC diets were each mixed as a single batch (Table 4), and then each of the NC diets was split into 100 kg lots for the liquid phytase solutions as described below. The experimental phytase was added to the NC diet post-pelleting with a hand-held sprayer that delivered 2-5  $\mu\text{m}$  droplets in a double ribbon mixer. The phytase product containing 5000 FTU/g was blended with a water diluent to achieve the desired enzyme concentration. The sprayer was weighed before and after enzyme application to ensure that the correct amount of liquid (200 g) was applied to the pellets. The

enzyme application was confirmed by laboratory analysis (DAN lab Feed Enzyme Services, Brabrand, Denmark) within 10 % of target values. All diets were supplemented with 1.5 % Celite™ (Celite Corp. Lompoc, CA) to serve as an acid insoluble ash indigestible reference marker for determination of digestibility coefficients. The calculated and analyzed compositions of the experimental diets including the supplemental enzymes are reported in Table 3. All diets were manufactured at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC).

### ***Sample collection and laboratory analysis***

**Excreta collection and content analysis.** Excreta was collected over 24 hour intervals and pooled for 3 consecutive intervals to evaluate apparent nutrient digestibility of the diets. Collections occurred from 22 to 24 d of age. Samples of approximately 500 grams from each day were stored at -20 °C until later analysis. The frozen excreta were later defrosted for approximately 16 hours at room temperature (~20 °C) before further processing. For each pen, approximately 300 g, 100 g subsample from each the 3 collection days, of representative clean excreta was blended (Waring® Commercial Blender Model 31BL92, Waring Commercial, New Hartford, CT 06057) with 100 mL of water until a slurry was obtained and it was acidified to pH < 5.4 with the addition of HCl (0.1 N, Fisher Scientific, Fairlawn, NJ 07410) to prevent volatilization of nitrogen during overnight drying at 70 °C in a forced air convection oven (Blue-M, Model # DC-326F, Serial # DC-509, Blue M, Atlanta, GA). Once dried, excreta was blended into a fine powder and placed in storage bags at -20 °C until further analysis. Diet and excreta gross energy content were determined

with an adiabatic oxygen bomb calorimeter (IKA Calorimeter System C5000 Control, IKA<sup>®</sup> Werke Labortechnik, Staufen, Germany). Recovery of acid insoluble ash in excreta, ileal contents, and feeds were performed according to the method described by Vogtmann *et al.* (1975).

**Ileal digesta collection and content analysis.** On the evening before ileal sample collection, the birds were not given access to feed for at least 6 hours. Then, starting at 4 and 6 AM of 27 d, block 1 and block 2 was respectively given *ad libitum* access to feed. Similarly at starting at 4 and 6 am of 28 d, blocks 3 and 4 were respectively given *ad libitum* access to feed. Thus, each bird from each block had at 4-6 hours of *ad libitum* feed consumption before they were euthanized for ileal digesta sample collection. Ileal sections from Meckel's diverticulum to the cecal junction were dissected and contents collected in 50 mL centrifuge tubes. The ileal contents of the birds were pooled by pen and stored at -20 °C until later analysis.

Ileal samples were defrosted for approximately 16 hours at room temperature (~20 °C). The entire sample was acidified to pH < 5.4 with the addition of HCl (0.1 N, Fisher Scientific, Fairlawn, NJ 07410) to prevent volatilization of nitrogen during overnight drying in a forced air oven (Blue-M, Model # DC-326F, Serial # DC-509, Blue M, Atlanta, GA). After the dried ileal content samples were thoroughly mixed, they were analyzed by ICP mineral analysis (P, K, Ca, Mg, Na, S, Al, Cu, Fe, Mn, and Zn). One-half gram of dried ileal digesta sample was weighed and placed in a 125 mL beaker. The samples were then digested in the following sequence at approximately 95 °C: 1:1 solution of HNO<sub>3</sub>:deionized water,

two additions of concentrated HNO<sub>3</sub>, 3 mL deionized water and 30 % hydrogen peroxide which was added in 1 mL increments up to a total of 6 mL added, 5 mL concentrated HCl and 5 mL deionized water. Next, they were filtered on a 0.2 µm filter, brought to 0.100 L volume, and analyzed on a Perkin Elmer 2000 DV Optical Emission Spectrometer.

**Tibia and toe collection and ash determination.** Immediately after ileal digesta samples were collected from each pen of birds at 27 or 28 d, the entire right leg from 3 euthanized birds per pen was collected and stored in labeled sample bags at -20 °C for subsequent ash content determination of the tibia and toe. The right legs were allowed to thaw at room temperature for approximately 4 hours and then the tibia was dissected from the muscle and the middle toe was removed from the foot. Each tibia was broken in half. Tibias and toes were placed in individual crucibles and ashed for approximately 16 hours at 600 °C in a muffle furnace (Thermolyn, Model # F-A1730, Serial # 6483, Sybron Corp., Dubuque, IA). Mean ash content of tibia and toe from 3 birds per cage was used as the experimental unit for statistical analysis.

**Chemical analyses.** Representative samples of each diet and dried excreta were submitted to the North Carolina Department of Agriculture and Consumer Services Food and Drug Protection Division Forage Laboratory (Raleigh, NC) for AOAC approved analysis of dry matter (DM), crude protein (combustion nitrogen analyzer, AOAC 990.03), acid detergent fiber and neutral detergent fiber (Ankom Fiber System, Ankom Technology, Macedon, NY), crude fat (Soxtec ethyl ether extraction method), ash (muffle oven method),

Ca, P, S, Mg, Na, K, Cu, Fe, Mn, and Zn (CEM microwave digestion and Inductively Coupled Plasma (ICP) 5300 System).

### ***Statistical analysis***

This experiment was analyzed as a completely randomized block design. Pen means were used as the experimental unit. Percentage mortality was transformed to the arcsine  $\sqrt{\text{percentage}}$  before analysis, and final data are presented as natural numbers. Data were analyzed using the GLM procedure for ANOVA of SAS (2005). Means were separated using the LSMeans procedure of SAS at  $P < 0.05$ . Regression analysis was performed on the negative control diets containing the different levels of phytase.

### ***Animal ethics***

This experiment was conducted according to the guidelines of the Institutional Animal Care and Use Committee at North Carolina State University. All husbandry practices and euthanasia were performed with full consideration of animal welfare.

## 6.4 RESULTS

### ***Bird performance***

The overall BW gain of the birds in this experiment exceeded breed standards by about 10 %. In contrast to expectations, however, the poult fed the PC diet had lower BW at 14 d and 28 d than the birds fed the NC diets (Table 5). BW at 14 and 28 d increased linearly as the level of phytase supplementation increased in the NC basal diet. This linear phytase

level effect was primarily observed on ADG (after adjustment for mortality) from 7 to 14 d, and not during the other observational periods (Table 6).

Significant treatment effects on average daily feed intake per bird after accounting for mortality were observed only from 7 to 14 d (Table 7). Average daily feed intake among poult s consuming the PC diet was significantly higher than among those consuming the NC diet (24.58 vs 24.03,  $P<0.05$ ). Among the poult s fed the NC diets, average daily feed intake increased by a quadratic function as dietary phytase supplementation level increased, with maximum feed intake achieved at  $>500$  FTU/kg. There were no significant effects on periodic and cumulative FCR after adjustment for pen mortality (Tables 8 and 9). Cumulative mortality rate from 1 to 28 d over all treatment groups averaged 7.5 %. No significant treatment effects were observed on mortality rate during each period (Table 10) or cumulatively (Table 11). There was no unusual pathology associated with bird mortality.

### ***Tibia and toe ash***

Tibia and toe ash content data, summarized in Table 12 on an “as is basis”. Significant treatment effects on tibia bone and toe ash sampled from poult s at 28 d were observed although the treatment response in these two tissues differed. As expected, ash content of both tibia and toe were significantly greater among poult s that consumed the PC diet than the NC diet. Among the poult s that received the NC basal diet, tibia ash content increased by a significant quadratic function as the phytase supplementation level increased ( $P<0.05$ ), with the maximum ash content achieved at 1000 FTU/kg. Among the NC diets, maximum toe ash was also observed at 1000 FTU/kg, although the response to dietary

phytase supplementation level did not follow a significant quadratic function because the 250 FTU/kg treatment group was significantly lower than expected.

***Fecal and ileal DM digestibility and nitrogen-corrected apparent metabolizable energy (AMEn)***

The treatment effects on apparent metabolizable energy (AMEn), apparent nitrogen retention (ANR), and digestible ash (Dash) as determined from fecal samples including an indigestible acid insoluble ash marker are summarized in Table 13. Although the PC and NC diets were formulated to contain similar AMEn content, the NC diet was determined to contain significantly lower AMEn content (2704 vs 2743 kcal AMEn/kg,  $P<0.05$ ). However, as the level of phytase supplementation increased in the NC basal diet, AMEn significantly increased by a quadratic function, such that the maximum AMEn was observed among poult consuming the NC diet supplemented with 1000 FTU phytase/kg. Consequently, dietary supplementation of 500 FTU/kg provided an AMEn uplift to the NC basal diet of about 40 kcal/kg, similar to the PC, and 1000 FTU/kg provided an AMEn uplift of 80 kcal/kg. Interestingly, phytase supplementation to 2000 FTU/kg resulted in a significant depression of AMEn in comparison to the 1000 FTU/kg level (2783 vs 2634 kcal AMEn/kg,  $P<.001$ ).

***Fecal and ileal sample analyses***

The dietary treatment effects on mineral composition of fecal and ileal digesta samples are summarized on Tables 14 and 15, respectively. Similar significant treatment effects were observed on fecal and ileal content of Ca and P. Ca content of fecal and ileal samples decreased linearly ( $P<0.001$ ) as the dietary level of phytase increased in the NC

diets, indicating greater body retention of Ca. Similarly, P content decreased quadratically in fecal samples and linearly in ileal samples as the level of phytase supplementation increased. Evidently, fecal P emission decreased and P retention increased as dietary phytase supplementation level increased.

There were no significant treatment effects on fecal content of S, Mg, or Cu. Fecal content of Fe, Mn, and Zn was significantly higher among poult fed the PC than NC basal diets (with or without phytase). Among birds fed the NC diets, fecal K and S content increased linearly ( $P < 0.0001$ ) and Na increased by a quadratic function as the phytase supplementation level increased. A quadratic decrease in fecal DM content as dietary phytase level increased ( $P = 0.06$ ) (Table 14) was also observed. There were no dietary phytase level effects on the fecal content of Mg, Cu, Fe, Mn, or Zn.

There were no significant treatment effects observed on the content of S, K, and Cu in ileal samples. Although there was a significant treatment effect on ileal Na content, it was not associated with dietary phytase supplementation level. Mg, Fe, Mn, Zn, and Al content of ileal samples was significantly greater among the poult fed the PC diet than the NC basal diets, and the ileal content of these minerals decreased linearly as the phytase supplementation level increased.

#### ***Fecal and ileal mineral digestibility***

Using acid insoluble ash as the indigestible marker, the dietary treatment effects on mineral digestibility was determined from both fecal and ileal samples and summarized in Tables 16 and 17, respectively. Average digestibility values for Ca, P, Fe, and Mn are

similar for fecal and ileal samples. In contrast, average ileal digestibility values for S, Mg, K, Cu and Zn were much higher than the corresponding fecal digestibility values. As expected, average ileal DM digestibility was lower than the average fecal DM digestibility (75.6 vs 82.6 %,  $P<0.05$ ).

Table 16 summarizes the dietary treatment effects on fecal DM and mineral digestibility. There were small but significant differences in fecal digestibility values between the PC and NC diets. Dietary phytase supplementation level effects were observed to be most significant, but unlike the ileal digestibility values, the fecal digestibility values for DM and minerals increased in a quadratic manner as phytase supplementation level increased, with the maximum values between 500 and 1000 FTU/kg. The fecal S digestibility coefficient was the only fecal mineral that did not have a significant response for regression analysis.

As observed with fecal digestibility, significant treatment responses were observed on ileal digestibility values for DM and all measured minerals (Table 17). There were no or minimal differences in ileal DM or mineral digestibility among the PC or NC diets. In contrast, dietary phytase supplementation of the NC diet resulted in significant increases in ileal digestibility of DM and all minerals. Ileal digestibility of DM, Ca, S, K, Fe, Zn, and Mn increased linearly as the level of phytase supplementation increased ( $P<0.05$ ). Ileal digestibility of P, Mg, and Cu increased by a quadratic function as the level of phytase supplementation increased ( $P<0.05$ ), with a plateau in digestibility approaching at about 1000 FTU/kg.

## 6.5 DISCUSSION

BW differences of the birds fed PC diets and those fed all the other diets may be due to slightly higher analyzed crude protein content of the NC diet or insignificantly higher mortality rate of weaker birds among the birds fed the NC diets. Dilger *et al.* (2004) also reported a linear increase in BW gain due to phytase supplementation in broilers from 8 to 22 d of age in one trial and from 22 to 43 d in a second trial. In contrast, Applegate *et al.* (2003) reported that phytase concentration did not affect poult performance. In agreement with the current study, other authors have reported a quadratic response of feed intake to additive dietary phytase in broilers (Dilger *et al.*, 2004; Nyannor and Adeola, 2008). Evidently, dietary phytase supplementation improved feed intake, which corresponded with improved BW gain from 7 to 14 d and subsequent BW through 28 d. The lack of dietary phytase supplementation response on FCR does not agree with Pirgozliev *et al.* (2007), who observed FCR in turkeys improved by 7.6 %. Similarly, other researchers have reported improved FCR in broilers with dietary phytase supplementation (Dilger *et al.*, 2004; Cowieson and Adeola, 2005; Cowieson *et al.*, 2006).

Tibia and toe ash content generally agrees with the literature after adjustment to about 30 % dry matter content (Yalcin *et al.*, 2001). Both tibia and toe ash followed a quadratic function with increasing phytase supplementation to NC diets with maximal ash content at 1000 FTU/kg diet. Dilger *et al.* (2004) also reported a quadratic response on tibia ash content to graded levels of phytase when fed to broilers from 8 to 22 d of age. Similarly,

Cowieson *et al.* (2006) reported a significant increase in toe ash values of female broilers with phytase supplementation.

Similar to the response in tibia and toe ash, AME<sub>n</sub> followed a quadratic function with increasing phytase supplementation to the NC diets with maximal AME<sub>n</sub> at 1000 FTU/kg. Pirgozliev *et al.* (2007) also reported a significant quadratic relationship among dietary phytase activity and AME<sub>n</sub> in low NPP diets fed to turkeys and broilers. Although Hughes *et al.* (2009) observed that dietary phytase supplementation increased AME<sub>n</sub> in laying hens fed diets low in NPP content, they did not observe significant phytase supplementation level effects on AME<sub>n</sub>.

Fecal and ileal mineral content is dependent on diet composition. Other than for P, there is little data in the literature about the mineral composition of excreta and ileal contents in poultry. Yan *et al.* (2001) reported that P excretion was reduced when commercial male chicks were fed low levels of P with increasing supplementation of phytase. This agrees with the data presented in Table 14, where P retention was increased as phytase supplementation increased.

Fecal digestibility values are more accurately defined as body retention. The differences in mineral digestibility values between those determined from fecal and ileal samples are due to the digestive function of the hind-gut microflora, mineral reabsorption that occurs in the ceca, and mineral excretion from the kidneys. The current research data agrees with a previous report by Cowieson *et al.* (2006), who observed dietary phytase supplementation improved the retention of P, K, Na, Fe, Mg, S, and Cu in broilers. In

contrast to the current results, however, Cowieson *et al.* (2006) also reported that phytase supplementation had no significant effect on Ca retention, whereas we observed fecal Ca retention increased by a quadratic function as dietary phytase level increased. These discrepancies may be due to differences among species.

Ileal digestibility of most minerals improved as the level of dietary phytase supplementation increased. P digestibility, which has great influence on environmental and economic sustainability of poultry production, increased quadratically as dietary phytase addition increased, with maximum ileal P digestibility achieved at 1000 FTU/kg. Dilger *et al.* (2004) reported both significant linear and quadratic improvements in ileal P digestibility in broilers fed increasing levels of dietary phytase, but they did not find any improvement in ileal Ca digestibility with increasing dietary phytase levels as observed in this study with turkeys.

Based on the results of this experiment, the experimental phytase product significantly improved the growth response of turkey poult and this response was associated with improvements in feed intake and nutrient bioavailability. Optimum dose of dietary phytase supplementation for body weight gain, feed intake, AME<sub>n</sub>, and digestibility of most of the minerals measured was identified to be about 1000 FTU/kg of feed.

## 6.6 TABLES

Table 1: Room temperature schedule.

Day	Temperature (°F)	Temperature (°C)
1 - 7	85 – 90	29.5 - 32.0
7 - 10	80 – 85	26.5 - 29.5
11 - 14	75 – 80	24.0 - 26.5
15 - 28	70 – 75	21.0 - 26.5

Table 2: Dietary treatment, enzyme identification and incorporation rate.

Diet	Description
A	Positive control <sup>1</sup>
B	Negative control <sup>1</sup>
C	Negative control + 250 FTU Phytase
D	Negative control + 500 FTU Phytase
E	Negative control + 1000 FTU Phytase
F	Negative control + 2000 FTU Phytase

<sup>1</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

Table 3: Composition and nutrient content of diets fed to turkeys.

Ingredients	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
	(%)					
Soybean meal (48 % CP)	47.24	46.96	46.96	46.96	46.96	46.96
Corn	44.73	46.49	46.49	46.49	46.49	46.49
Limestone	1.40	1.57	1.57	1.57	1.57	1.57
Celite™ (insoluble ash)	1.50	1.50	1.50	1.50	1.50	1.50
Dicalcium phosphate (18.5 %)	2.42	1.34	1.34	1.34	1.34	1.34
Soy oil	1.50	0.92	0.92	0.92	0.92	0.92
Salt (NaCl)	0.31	0.32	0.32	0.32	0.32	0.32
D,L-methionine	0.22	0.22	0.22	0.22	0.22	0.22
Choline chloride (60 %)	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix <sup>1</sup>	0.15	0.15	0.15	0.15	0.15	0.15
L-lysine HCl	0.12	0.12	0.12	0.12	0.12	0.12
Mineral premix <sup>2</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>3</sup>	0.10	0.10	0.10	0.10	0.10	0.10
Phytase (5000 FTU/g)	0	0	0.005	0.010	0.020	0.040
Water diluent	0.20	0.20	0.195	0.190	0.180	0.160
<b>Calculated analysis</b>						
Crude protein, %	26.00	26.00	26.00	26.00	26.00	26.00
Metabolizable energy, kcal/kg	2850	2850	2850	2850	2850	2850
Calcium, %	1.50	1.35	1.35	1.35	1.35	1.35
Phosphorus, %	0.905	0.707	0.707	0.707	0.707	0.707
Available P, %	0.60	0.40	0.40	0.40	0.40	0.40
Fat, %	3.59	3.07	3.07	3.07	3.07	3.07
Fiber, %	2.53	2.55	2.55	2.55	2.55	2.55
Methionine, %	0.63	0.63	0.63	0.63	0.63	0.63
Cysteine, %	0.42	0.42	0.42	0.42	0.42	0.42
Methionine + cysteine, %	1.05	1.05	1.05	1.05	1.05	1.05
Lysine, %	1.60	1.60	1.60	1.60	1.60	1.60
Arginine, %	1.82	1.82	1.82	1.82	1.82	1.82
Sodium, %	0.17	0.17	0.17	0.17	0.17	0.17
Potassium, %	1.05	1.05	1.05	1.05	1.05	1.05
Chloride, %	0.25	0.26	0.26	0.26	0.26	0.26
<b>Chemical analysis (dry matter basis)<sup>4</sup></b>						
Dry matter, %	89.9	88.6	88.6	88.6	88.6	88.6
Crude protein, %	26.76	27.85	27.85	27.85	27.85	27.85
Gross energy, kcal/kg	3913	3928	3928	3928	3928	3928
Crude fat, %	4.51	2.76	2.76	2.76	2.76	2.76
Ash, %	8.78	8.19	8.19	8.19	8.19	8.19
Acid detergent fiber, %	5.48	5.01	5.01	5.01	5.01	5.01
Neutral detergent fiber, %	8.51	8.84	8.84	8.84	8.84	8.84
Calcium, %	1.66	1.36	1.37	1.35	1.36	1.34
Phosphorus, %	0.92	0.73	0.75	0.76	0.74	0.74
Sulfur, %	0.33	0.32	0.32	0.30	0.34	0.34
Magnesium, %	0.20	0.19	0.20	0.18	0.20	0.20
Sodium, %	0.14	0.14	0.13	0.13	0.13	0.15
Potassium, %	1.02	1.09	1.10	1.07	1.11	1.06
Copper, ppm	15	14	15	13	14	12
Iron, ppm	504	379	374	370	397	375
Manganese, ppm	133	108	96	101	119	109
Zinc, ppm	148	128	122	125	132	125

Table 3 (continued)

<sup>1</sup>NaSeO<sub>3</sub> premix provided 0.3 mg Se/kg of complete feed.

<sup>2</sup>Each kilogram of mineral premix (.1% inclusion) supplied the following per kg of complete feed: 60 mg Zn as ZnSO<sub>4</sub>·H<sub>2</sub>O; 60 mg Mn as MnSO<sub>4</sub>·H<sub>2</sub>O; 40 mg Fe as FeSO<sub>4</sub>·H<sub>2</sub>O; 5 mg Cu as CuSO<sub>4</sub>; 1.25 mg I as Ca(IO<sub>3</sub>)<sub>2</sub>; 1 mg Co as CoSO<sub>4</sub>.

<sup>3</sup>Each kilogram of vitamin premix (.1% inclusion) supplied the following per kg of complete feed: vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; alpha-tocopherol, 66 IU; niacin, 110 mg; pantothenic acid, 22 mg; riboflavin, 13.2 mg; pyridoxine, 8 mg; menadione, 4 mg; folic acid, 2.2 mg; thiamin, 4 mg; biotin, 0.253 mg; vitamin B<sub>12</sub>, 0.04 mg; ethoxyquin, 100 mg.

<sup>4</sup>Chemical analysis: (1) Crude protein used combustion nitrogen analyzer, AOAC 990.03, (2) Gross energy used bomb calorimeter (IKA Calorimeter System C5000 control, IKA<sup>®</sup> Werke Labortechnik, Staufen, Germany), (3) Crude fat used Soxtec ethyl ether extraction method, (4) Ash used muffle oven method, (5) Acid detergent fiber and neutral detergent fiber used Ankom Fiber System, (6) Minerals used CEM microwave digestion and Inductively Coupled Plasma (ICP) 5300 System.

Table 4: Post-pellet enzyme application formulation for experimental diets.

Ingredient	Experimental Diets					
	A	B	C	D	E	F
Positive control, kg <sup>1</sup>	100	-	-	-	-	-
Negative control, kg <sup>1</sup>	-	100	100	100	100	100
Phytase 5000 FTU/g, g <sup>2</sup>	0	0	5.0	10	20	40
Water diluent, g <sup>1</sup>	200	200	195	190	180	160

<sup>1</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>2</sup>The phytase and water diluent was blended together and thoroughly mixed before spray application on the outside of the pellets in a horizontal double ribbon mixer. The enzyme was sprayed during the 2 minute mixing cycle using a hand sprayer that delivered 2-5 micron size droplets. The sprayer weight was tarred before enzyme spray application and subsequently weighed to assure the correct amount of liquid enzyme-water blend (200 g) was applied.

Table 5: Effect of phytase supplementation on body weight of tom poult<sup>1</sup>.

Dietary treatment	7 d	14 d	21 d	28 d
	(g)			
Positive control <sup>2</sup>	139.4	285.0 <sup>d</sup>	529.4	859.0 <sup>d</sup>
Negative control <sup>2</sup>	144.8	302.1 <sup>c</sup>	536.3	872.7 <sup>cd</sup>
Negative control + 250 FTU phytase	138.9	303.6 <sup>c</sup>	543.8	888.9 <sup>c</sup>
Negative control + 500 FTU phytase	146.8	315.6 <sup>b</sup>	555.5	905.8 <sup>b</sup>
Negative control + 1000 FTU phytase	151.6	332.2 <sup>a</sup>	564.4	903.1 <sup>b</sup>
Negative control + 2000 FTU phytase	146.3	325.0 <sup>a</sup>	564.9	945.8 <sup>a</sup>
SEM(39) <sup>3</sup>	4.7	7.6	19.8	16.7
<i>P</i> -value of treatment	0.4084	<b>0.0010</b>	0.7360	<b>0.0145</b>
<i>Regression analysis of neg. control diets</i>		<i>P</i> -value		
Linear	0.4665	<b>0.0205</b>	0.3561	<b>0.0200</b>
Quadratic	0.3682	0.0859	0.5822	0.9737
Intercept		306.1		878.5
Linear coefficient		0.0128		0.0331

<sup>a-c</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(39) = Standard error of the mean with 39 degrees of freedom.

Table 6: Effect of phytase supplementation on average daily gain (ADG) of tom poult<sup>1</sup>.

Dietary treatment	0 – 7 d	7 – 14 d	14 – 21 d	21 – 28 d
	(g/bird / day)			
Positive control <sup>2</sup>	12.18	20.40 <sup>d</sup>	34.36	47.04
Negative control <sup>2</sup>	12.87	21.83 <sup>c</sup>	34.02	46.79
Negative control + 250 FTU phytase	12.25	22.64 <sup>b</sup>	34.31	49.40
Negative control + 500 FTU phytase	13.18	23.63 <sup>a</sup>	33.77	50.05
Negative control + 1000 FTU phytase	14.31	23.86 <sup>a</sup>	33.98	48.40
Negative control + 2000 FTU phytase	12.95	24.11 <sup>a</sup>	35.67	51.31
SEM(39) <sup>3</sup>	0.66	0.62	1.69	1.63
<i>P</i> -value of treatment	0.2561	<b>0.0008</b>	0.9751	0.3489
<i>Regression analysis of neg. control diets</i>	<b><i>P</i>-value</b>			
Linear	0.5603	<b>0.0065</b>	0.5196	0.2277
Quadratic	0.1765	0.0855	0.6838	0.9173
Intercept		21.93		
Linear coefficient		3.26x10 <sup>-3</sup>		

<sup>a-c</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 8 replicate cages. Average daily gain includes mortality.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(39) = Standard error of the mean with 39 degrees of freedom.

Table 7: Effect of phytase supplementation on feed intake (g/bird per d) of tom poult<sup>1</sup>.

Dietary treatment	0 – 7 d	7 – 14 d	14 – 21 d	21 – 28 d
	(g/bird / day)			
Positive control <sup>2</sup>	14.06	24.58 <sup>c</sup>	40.72	89.62
Negative control <sup>2</sup>	13.74	24.03 <sup>c</sup>	45.93	98.86
Negative control + 250 FTU phytase	13.21	25.79 <sup>b</sup>	42.10	87.90
Negative control + 500 FTU phytase	14.66	27.02 <sup>a</sup>	41.40	84.23
Negative control + 1000 FTU phytase	14.31	27.20 <sup>a</sup>	41.30	85.36
Negative control + 2000 FTU phytase	14.07	26.92 <sup>a</sup>	45.15	92.10
SEM(39) <sup>3</sup>	0.53	0.77	2.43	3.98
<i>P</i> -value of treatment	0.4963	<b>0.0175</b>	0.5403	0.1382
<i>Regression analysis of neg. control diets</i>		<i>P</i> -value		
Linear	0.5157	<b>0.0396</b>	0.8204	0.6894
Quadratic	0.3258	<b>0.0318</b>	0.1152	<b>0.0168</b>
Intercept		24.35		96.26
Linear coefficient		5.38x10 <sup>-3</sup>		-2.56x10 <sup>-2</sup>
Quadratic coefficient		-2.07x10 <sup>-6</sup>		1.19x10 <sup>-5</sup>

<sup>a-d</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 8 replicate cages. Average daily feed intake includes mortality.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(39) = Standard error of the mean with 39 degrees of freedom.

Table 8: Effect of phytase supplementation on periodic feed conversion ratio (FCR) of tom poult<sup>1</sup>.

Dietary treatment	0 - 7 d	7 - 14 d	14 - 21 d	21 - 28 d
	(g:g)			
Positive control <sup>2</sup>	1.22	1.21	1.19	1.94
Negative control <sup>2</sup>	1.18	1.10	1.35	2.13
Negative control + 250 FTU phytase	1.27	1.12	1.24	1.83
Negative control + 500 FTU phytase	1.17	1.17	1.21	1.69
Negative control + 1000 FTU phytase	1.11	1.12	1.34	1.80
Negative control + 2000 FTU phytase	1.25	1.13	1.28	1.89
SEM(39) <sup>3</sup>	0.08	0.04	0.09	0.13
<i>P</i> -value of treatment	0.7240	0.2796	0.7368	0.2890
<i>Regression analysis of neg. control diets</i>	<b><i>P</i>-value</b>			
Linear	0.8048	0.7585	0.8262	0.5949
Quadratic	0.3762	0.3378	0.8247	0.0944

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(39) = Standard error of the mean with 39 degrees of freedom.

Table 9: Effect of phytase supplementation on cumulative feed conversion ratio (FCR) of tom poult<sup>1</sup>.

Dietary treatment	14 d	21 d	28 d
	(g:g)		
Positive control <sup>2</sup>	1.21	1.19	1.49
Negative control <sup>2</sup>	1.12	1.24	1.59
Negative control + 250 FTU phytase	1.16	1.20	1.45
Negative control + 500 FTU phytase	1.17	1.19	1.39
Negative control + 1000 FTU phytase	1.12	1.18	1.41
Negative control + 2000 FTU phytase	1.16	1.23	1.50
SEM(39) <sup>3</sup>	0.03	0.05	0.07
<i>P</i> -value of treatment	0.2956	0.9455	0.4568
<i>Regression analysis of neg. control diets</i>	<b><i>P</i>-value</b>		
Linear	0.5699	0.9352	0.7384
Quadratic	0.8066	0.3762	0.1004

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(39) = Standard error of the mean with 39 degrees of freedom.

Table 10: Effect of phytase supplementation on periodic mortality (%) of tom poult<sup>1</sup>.

Dietary treatment	0 – 7 d	7 – 14 d	14 – 21 d	21 – 28 d
	(%)			
Positive control <sup>2</sup>	4.13	0.00	1.34	0.00
Negative control <sup>2</sup>	8.63	0.00	1.63	0.00
Negative control + 250 FTU phytase	8.63	0.00	0.00	0.00
Negative control + 500 FTU phytase	4.13	3.00	0.00	0.00
Negative control + 1000 FTU phytase	8.25	0.00	0.00	0.00
Negative control + 2000 FTU phytase	8.62	4.13	0.00	0.00
SEM(39) <sup>3</sup>	0.07	0.04	0.03	0.00
<i>P</i> -value of treatment	0.6015	0.2514	0.5700	1.0000
<i>Regression analysis of neg. control diets</i>	<b><i>P</i>-value<sup>4</sup></b>			
Linear	0.9855	0.2859	0.2917	1.0000
Quadratic	0.8830	0.9618	0.2261	1.0000

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(39) = Standard error of the mean with 39 degrees of freedom based on the mortality transformed to arcsin  $\sqrt{\%}$ .

<sup>4</sup>*P*-values based on mortality transformed to arcsin  $\sqrt{\%}$ .

Table 11: Effect of phytase supplementation on cumulative mortality (%) of tom poult<sup>1</sup>.

Dietary treatment	14 d	21 d	28 d
	(%)		
Positive control <sup>2</sup>	3.75	5.00	5.00
Negative control <sup>2</sup>	7.50	8.75	8.75
Negative control + 250 FTU phytase	7.50	7.50	7.50
Negative control + 500 FTU phytase	6.25	6.25	6.25
Negative control + 1000 FTU phytase	7.50	7.50	7.50
Negative control + 2000 FTU phytase	10.00	10.00	10.00
SEM(39) <sup>3</sup>	0.07	0.07	0.07
<i>P</i> -value of treatment	0.7823	0.9296	0.9296
<i>Regression analysis of neg. control diets</i>	<b><i>P</i>-value<sup>4</sup></b>		
Linear	0.6375	0.7328	0.7328
Quadratic	0.8830	0.7761	0.7761

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(39) = Standard error of the mean with 39 degrees of freedom based on the mortality transformed to arcsin  $\sqrt{\%}$ .

<sup>4</sup>*P*-values based on mortality transformed to arcsin  $\sqrt{\%}$ .

Table 12: Effect of phytase supplementation on tibia and toe ash (as is, %) of 28 d old tom poult<sup>1</sup>.

Dietary treatment	Tibia	Toe
	(%)	
Positive control <sup>2</sup>	15.6210 <sup>a</sup>	5.4036 <sup>a</sup>
Negative control <sup>2</sup>	13.7186 <sup>e</sup>	5.0764 <sup>d</sup>
Negative control +250 FTU phytase	14.5914 <sup>d</sup>	4.8846 <sup>e</sup>
Negative control + 500 FTU phytase	14.7709 <sup>c</sup>	5.1690 <sup>c</sup>
Negative control + 1000 FTU phytase	15.1000 <sup>b</sup>	5.2904 <sup>b</sup>
Negative control + 2000 FTU phytase	14.4815 <sup>d</sup>	5.1543 <sup>c</sup>
SEM(39) <sup>3</sup>	0.2934	0.0877
<i>P</i> -value of treatment	<b>0.0018</b>	<b>0.0041</b>
<i>Regression analysis of neg. control diets</i>	<i>P</i> -value	
Linear	0.2351	0.1418
Quadratic	<b>0.0062</b>	0.1306
Intercept	13.85	
Linear coefficient	2.36x10 <sup>-3</sup>	
Quadratic coefficient	-1.03x10 <sup>-6</sup>	

<sup>a-e</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(39) = Standard error of the mean with 39 degrees of freedom.

Table 13: Effect of phytase supplementation on apparent metabolizable energy (AME<sub>n</sub>), apparent nitrogen retention (ANR), and digestible ash (Dash) as determined from fecal samples including an indigestible acid insoluble ash marker<sup>1</sup>.

Dietary treatment	AME <sub>n</sub> <sup>2</sup>	ANR <sup>3</sup>	Dash <sup>4</sup>
	(%)		
Positive control <sup>5</sup>	2743 <sup>b</sup>	55.75 <sup>d</sup>	20.19 <sup>c</sup>
Negative control <sup>5</sup>	2704 <sup>c</sup>	53.78 <sup>e</sup>	18.67 <sup>c</sup>
Negative control + 250 FTU phytase	2611 <sup>c</sup>	52.74 <sup>f</sup>	32.01 <sup>b</sup>
Negative control + 500 FTU phytase	2742 <sup>b</sup>	63.55 <sup>a</sup>	35.42 <sup>a</sup>
Negative control + 1000 FTU phytase	2783 <sup>a</sup>	60.43 <sup>b</sup>	35.62 <sup>a</sup>
Negative control + 2000 FTU phytase	2634 <sup>d</sup>	58.67 <sup>c</sup>	34.73 <sup>a</sup>
SEM(39) <sup>6</sup>	26	0.97	1.55
<i>P</i> -value of treatment	<b>0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
<i>Regression analysis of neg. control diets</i>		<b><i>P</i>-value</b>	
Linear	0.5380	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
Quadratic	<b>0.0038</b>	<b>0.0002</b>	<b>&lt;0.0001</b>
Intercept	2652	52.99	21.97
Linear coefficient	0.21	1.55x10 <sup>-2</sup>	2.79x10 <sup>-2</sup>
Quadratic coefficient	-1.08x10 <sup>-4</sup>	-6.37x10 <sup>-6</sup>	-1.09x10 <sup>-5</sup>

<sup>a-f</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>AME<sub>n</sub> = Apparent metabolizable energy adjusted for nitrogen (kcal/kg).

<sup>3</sup>ANR = Apparent nitrogen retention.

<sup>4</sup>Dash = Digestible ash.

<sup>5</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>6</sup>SEM(39) = Standard error of the mean with 39 degrees of freedom.

Table 14: Effect of phytase supplementation on fecal content of Ca, P, S, Mg, Na, K, Cu, Fe, Mn, and Zn (dry matter basis) of tom poults<sup>1</sup>.

Dietary treatment	Ca	P	S	Mg	Na	K	Cu	Fe	Mn	Zn
	(%)				(ppm)					
Positive control <sup>2</sup>	3.18 <sup>a</sup>	1.51 <sup>a</sup>	0.44	0.50	0.21 <sup>ab</sup>	2.35 <sup>c</sup>	44	737 <sup>a</sup>	217 <sup>a</sup>	425 <sup>a</sup>
Negative control <sup>2</sup>	2.98 <sup>b</sup>	1.51 <sup>a</sup>	0.43	0.46	0.22 <sup>a</sup>	2.37 <sup>c</sup>	36	510 <sup>b</sup>	164 <sup>bc</sup>	323 <sup>bc</sup>
Negative control + 250 FTU phytase	2.25 <sup>d</sup>	1.05 <sup>b</sup>	0.45	0.48	0.19 <sup>cd</sup>	2.58 <sup>b</sup>	36	476 <sup>c</sup>	158 <sup>cd</sup>	311 <sup>c</sup>
Negative control + 500 FTU phytase	2.68 <sup>c</sup>	0.95 <sup>c</sup>	0.42	0.42	0.18 <sup>d</sup>	2.21 <sup>d</sup>	38	494 <sup>bc</sup>	168 <sup>b</sup>	322 <sup>bc</sup>
Negative control + 1000 FTU phytase	2.31 <sup>d</sup>	0.99 <sup>c</sup>	0.46	0.46	0.20 <sup>bc</sup>	2.51 <sup>b</sup>	37	490 <sup>bc</sup>	167 <sup>b</sup>	322 <sup>bc</sup>
Negative control + 2000 FTU phytase	2.07 <sup>e</sup>	1.06 <sup>b</sup>	0.49	0.52	0.23 <sup>a</sup>	2.94 <sup>a</sup>	40	497 <sup>bc</sup>	172 <sup>b</sup>	335 <sup>b</sup>
SEM(36) <sup>3</sup>	0.10	0.05	0.02	0.02	0.01	0.12	2	22	8	17
<i>P</i> -value of treatment	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.2790	0.1422	<b>0.0106</b>	<b>0.0042</b>	0.0602	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0006</b>
<i>Regression of neg. control diets</i>	<b><i>P</i>-value</b>									
Linear	<b>0.0002</b>	0.3803	<b>0.0348</b>	0.0686	0.1174	<b>0.0045</b>	0.1250	0.9836	0.7570	0.3955
Quadratic	0.1897	<b>0.0095</b>	0.6268	0.1010	<b>0.0111</b>	0.1770	0.7954	0.5788	0.9686	0.7138
Intercept	2.69	1.13	0.43		0.21	2.32				
Linear coefficient	-3.37x10 <sup>-4</sup>	-3.14x10 <sup>-4</sup>	3.04x10 <sup>-5</sup>		-6.19x10 <sup>-5</sup>	1.19x10 <sup>-5</sup>				
Quad. coefficient		1.41x10 <sup>-7</sup>			3.59x10 <sup>-8</sup>					

<sup>a-f</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(36) = Standard error of the mean with 36 degrees of freedom.

Table 15: Effect of phytase supplementation on ileal content of Ca, P, S, Mg, Na, K, Cu, Fe, Mn, Zn and Al (dry matter basis) of tom poult<sup>1</sup>.

Dietary treatment	Ca	P	K	Na	Mg	S	Cu	Fe	Mn	Zn	Al
	(g/kg)				(mg/kg)						
Positive control <sup>2</sup>	33.20 <sup>a</sup>	13.37 <sup>a</sup>	11.06	8.95 <sup>b</sup>	2015 <sup>b</sup>	1929	16.76	588 <sup>a</sup>	173 <sup>a</sup>	181 <sup>a</sup>	245 <sup>a</sup>
Negative control <sup>2</sup>	31.36 <sup>a</sup>	11.76 <sup>b</sup>	11.62	10.14 <sup>a</sup>	2074 <sup>b</sup>	1882	16.20	433 <sup>b</sup>	157 <sup>b</sup>	162 <sup>b</sup>	189 <sup>b</sup>
Negative control + 250 FTU phytase	22.75 <sup>b</sup>	10.42 <sup>c</sup>	12.73	10.07 <sup>a</sup>	2221 <sup>a</sup>	2012	14.93	389 <sup>bc</sup>	138 <sup>cd</sup>	144 <sup>cd</sup>	180 <sup>b</sup>
Negative control + 500 FTU phytase	30.70 <sup>a</sup>	9.55 <sup>d</sup>	11.37	8.60 <sup>b</sup>	1770 <sup>d</sup>	1993	14.97	436 <sup>b</sup>	148 <sup>bc</sup>	156 <sup>bc</sup>	172 <sup>bc</sup>
Negative control + 1000 FTU phytase	19.59 <sup>c</sup>	7.57 <sup>e</sup>	12.12	8.61 <sup>b</sup>	1860 <sup>c</sup>	1949	13.76	363 <sup>c</sup>	132 <sup>d</sup>	134 <sup>de</sup>	170 <sup>bc</sup>
Negative control + 2000 FTU phytase	17.87 <sup>c</sup>	5.46 <sup>f</sup>	11.54	8.79 <sup>b</sup>	1761 <sup>d</sup>	1836	14.70	347 <sup>c</sup>	121 <sup>d</sup>	122 <sup>e</sup>	160 <sup>c</sup>
SEM(39) <sup>3</sup>	2.58	0.45	0.49	0.44	186	145	1.50	47	16	15	22
<i>P</i> -value of treat.	<b>0.0002</b>	<b>&lt;0.0001</b>	0.2254	<b>0.0374</b>	<b>0.0057</b>	0.5343	0.0882	<b>&lt;0.0001</b>	<b>0.0007</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
<i>Regression of neg. control diets</i>	<b><i>P</i>-value</b>										
Linear	<b>0.0013</b>	<b>&lt;0.0001</b>	0.5782	0.0565	<b>0.0090</b>	0.3598	0.2309	<b>0.0071</b>	<b>0.0065</b>	<b>0.0022</b>	<b>0.0414</b>
Quadratic	0.4513	<b>0.0277</b>	0.6590	0.0757	0.2473	0.3020	0.1017	0.7307	0.6618	0.7096	0.5376
Intercept	29.05	11.73			2135			429	153	159	187
Lin. Coefficient	-6.14x10 <sup>-3</sup>	-5.10x10 <sup>-3</sup>			-0.454			-6.02x10 <sup>-2</sup>	-2.45x10 <sup>-2</sup>	-2.65x10 <sup>-2</sup>	-2.67x10 <sup>-2</sup>
Quad. coefficient		9.80x10 <sup>-7</sup>									

<sup>a-f</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>3</sup>SEM(39) = Standard error of the mean with 36 degrees of freedom.

Table 16: Effect of phytase supplementation on fecal digestibility (%) of dry matter of Ca, P, S, Mg, Na, K, Cu, Fe, Mn and Zn of tom poult<sup>1</sup>.

Dietary treatment	DM <sup>2</sup>	Ca	P	S	Mg	Na	K	Cu	Fe	Zn	Mn
	(%)										
Positive control <sup>3</sup>	80.40 <sup>e</sup>	26.15 <sup>c</sup>	34.86 <sup>e</sup>	47.95 <sup>c</sup>	9.08 <sup>d</sup>	41.59 <sup>e</sup>	10.37 <sup>e</sup>	11.33 <sup>c</sup>	43.85 <sup>d</sup>	7.25 <sup>e</sup>	37.44 <sup>d</sup>
Negative control <sup>3</sup>	80.06 <sup>e</sup>	14.79 <sup>f</sup>	41.80 <sup>d</sup>	48.41 <sup>bc</sup>	13.83 <sup>c</sup>	38.60 <sup>f</sup>	16.54 <sup>d</sup>	5.32 <sup>d</sup>	48.67 <sup>c</sup>	9.58 <sup>d</sup>	41.65 <sup>c</sup>
Negative control + 250 FTU phytase	81.52 <sup>d</sup>	40.13 <sup>c</sup>	50.66 <sup>c</sup>	49.96 <sup>b</sup>	17.82 <sup>bc</sup>	51.19 <sup>c</sup>	19.11 <sup>c</sup>	14.55 <sup>b</sup>	55.44 <sup>b</sup>	15.66 <sup>c</sup>	48.01 <sup>b</sup>
Negative control + 500 FTU phytase	85.69 <sup>a</sup>	37.23 <sup>d</sup>	60.31 <sup>a</sup>	44.29 <sup>d</sup>	33.82 <sup>a</sup>	60.85 <sup>a</sup>	36.96 <sup>a</sup>	14.46 <sup>b</sup>	60.09 <sup>a</sup>	20.81 <sup>b</sup>	52.10 <sup>a</sup>
Negative control + 1000 FTU phytase	84.59 <sup>b</sup>	45.56 <sup>b</sup>	58.71 <sup>a</sup>	57.70 <sup>a</sup>	29.88 <sup>a</sup>	54.89 <sup>b</sup>	30.43 <sup>b</sup>	19.86 <sup>a</sup>	59.36 <sup>a</sup>	23.28 <sup>a</sup>	51.18 <sup>a</sup>
Negative control + 2000 FTU phytase	83.32 <sup>c</sup>	48.43 <sup>a</sup>	53.55 <sup>b</sup>	51.63 <sup>b</sup>	20.93 <sup>b</sup>	44.82 <sup>d</sup>	16.63 <sup>d</sup>	13.89 <sup>b</sup>	56.45 <sup>b</sup>	20.71 <sup>b</sup>	47.02 <sup>b</sup>
SEM <sup>4</sup>	0.70	2.12	1.76	2.05	4.17	2.22	2.07	2.28	1.93	2.06	2.00
Deg. of freedom	39	38	38	38	32	38	34	23	38	24	38
P-value of treat.	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0032</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0253</b>	<b>&lt;0.0001</b>	<b>0.0028</b>	<b>&lt;0.0001</b>
<i>Regression of neg. control diets</i>	<b>P-value</b>										
Linear	<b>0.0289</b>	<b>&lt;0.0001</b>	<b>0.0041</b>	0.1191	0.1113	0.9974	0.9595	0.1580	<b>0.0261</b>	<b>0.0034</b>	0.3423
Quadratic	<b>0.0003</b>	<b>0.0002</b>	<b>&lt;0.0001</b>	0.2133	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0027</b>	<b>0.0001</b>	<b>0.0004</b>	<b>0.0018</b>
Intercept	80.22	20.67	43.42		13.73	41.83	16.26	7.77	50.12	10.09	43.01
Lin. coefficient	9.39x10 <sup>-3</sup>	4.29x10 <sup>-2</sup>	3.22x10 <sup>-2</sup>		3.65x10 <sup>-2</sup>	3.51x10 <sup>-2</sup>	3.66x10 <sup>-2</sup>	2.04x10 <sup>-2</sup>	1.97x10 <sup>-2</sup>	2.36x10 <sup>-2</sup>	1.83x10 <sup>-2</sup>
Quad. coefficient	-3.90x10 <sup>-6</sup>	-1.47x10 <sup>-5</sup>	-1.37x10 <sup>-5</sup>		-1.64x10 <sup>-5</sup>	-1.70x10 <sup>-5</sup>	-1.82x10 <sup>-5</sup>	-8.59x10 <sup>-6</sup>	-8.34x10 <sup>-6</sup>	-9.22x10 <sup>-6</sup>	-8.23x10 <sup>-6</sup>

<sup>a-f</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>DM = Dry matter.

<sup>3</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>4</sup>SEM = Standard error of the mean.

Table 17: Effect of phytase supplementation on ileal digestibility (%) of dry matter of Ca, P, S, Mg, K, Cu, Fe, Zn and Mn of tom poult<sup>1</sup>.

Dietary treatment	DM <sup>2</sup>	Ca	P	S	Mg	K	Cu	Fe	Zn	Mn
	(%)									
Positive control <sup>3</sup>	69.01 <sup>d</sup>	22.54 <sup>d</sup>	29.85 <sup>d</sup>	71.81	52.73 <sup>cd</sup>	47.28 <sup>d</sup>	46.69 <sup>e</sup>	45.71 <sup>d</sup>	42.90 <sup>d</sup>	39.72 <sup>d</sup>
Negative control <sup>3</sup>	69.17 <sup>d</sup>	22.06 <sup>d</sup>	27.17 <sup>d</sup>	72.96	51.43 <sup>d</sup>	49.87 <sup>c</sup>	45.88 <sup>e</sup>	46.41 <sup>d</sup>	39.75 <sup>d</sup>	35.48 <sup>e</sup>
Negative control + 250 FTU phytase	74.08 <sup>b</sup>	32.02 <sup>c</sup>	44.93 <sup>c</sup>	75.34	55.44 <sup>c</sup>	53.38 <sup>b</sup>	57.25 <sup>c</sup>	59.04 <sup>b</sup>	54.56 <sup>b</sup>	48.79 <sup>c</sup>
Negative control + 500 FTU phytase	71.67 <sup>c</sup>	38.80 <sup>b</sup>	45.87 <sup>c</sup>	73.02	62.45 <sup>b</sup>	54.24 <sup>b</sup>	54.18 <sup>d</sup>	50.81 <sup>c</sup>	47.82 <sup>c</sup>	41.49 <sup>d</sup>
Negative control + 1000 FTU phytase	75.82 <sup>a</sup>	45.29 <sup>a</sup>	62.78 <sup>b</sup>	77.86	65.07 <sup>ab</sup>	58.31 <sup>a</sup>	63.00 <sup>a</sup>	64.42 <sup>a</sup>	60.63 <sup>a</sup>	54.18 <sup>b</sup>
Negative control + 2000 FTU phytase	75.78 <sup>ab</sup>	49.04 <sup>a</sup>	72.69 <sup>a</sup>	78.82	66.55 <sup>a</sup>	60.05 <sup>a</sup>	60.31 <sup>b</sup>	65.43 <sup>a</sup>	63.45 <sup>a</sup>	57.33 <sup>a</sup>
SEM <sup>4</sup>	1.73	3.82	3.11	3.21	3.71	2.57	2.77	2.72	3.31	2.77
Degrees of freedom	39	28	39	39	39	39	39	39	39	38
<i>P</i> -value of treatment	<b>0.0155</b>	<b>0.0006</b>	<b>&lt;0.0001</b>	0.0827	<b>&lt;0.0001</b>	<b>0.0465</b>	<b>0.0002</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
<i>Regression analysis of neg. control diets</i>	<b><i>P</i>-value</b>									
Linear	<b>0.0054</b>	<b>0.0005</b>	<b>&lt;0.0001</b>	<b>0.0333</b>	<b>&lt;0.0001</b>	<b>0.0055</b>	<b>0.0010</b>	<b>0.0001</b>	<b>&lt;0.0001</b>	<b>0.0002</b>
Quadratic	0.1228	0.0767	<b>0.0010</b>	0.7242	<b>0.0034</b>	0.2992	<b>0.0049</b>	0.1101	0.0998	0.1895
Intercept	69.98	25.18	29.33	72.99	51.48	50.99	47.56	48.02	42.24	37.73
Linear coefficient	8.12x10 <sup>-3</sup>	3.05x10 <sup>-2</sup>	4.51x10 <sup>-2</sup>	4.68x10 <sup>-3</sup>	2.17x10 <sup>-2</sup>	1.11x10 <sup>-2</sup>	2.37x10 <sup>-2</sup>	2.01x10 <sup>-2</sup>	2.45x10 <sup>-2</sup>	2.07x10 <sup>-2</sup>
Quadratic coefficient			-1.17x10 <sup>-5</sup>		-7.11x10 <sup>-6</sup>		8.66x10 <sup>-6</sup>			

<sup>a-f</sup>Means with different superscripts within a column differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 8 replicate cages.

<sup>2</sup>DM = Dry matter.

<sup>3</sup>Negative control diet contains 0.20 % less NPP and 0.15 % less Ca than positive control diet.

<sup>4</sup>SEM = Standard error of the mean.

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## **CHAPTER 7**

### **SUMMARY**

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<sup>1</sup>The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service or the North Carolina Cooperative Extension Service of the products mentioned nor criticism of similar products not mentioned.

Coccidiosis is one of the most economically important diseases in poultry production. It is a common enteric stressor in both chickens and turkeys and is often associated with secondary infections that further challenge mucosal development and performance. Traditionally, pharmaceutical coccidiostats have been used to prevent coccidiosis in poultry. However, increasing consumer and governmental pressures have forced poultry producers to search for more natural alternatives to control coccidiosis.

Intestinal health directly influences growth performance and profitability of poultry production. Many complex factors contribute to bird health including: feed digestibility, nutrient absorption, environmental conditions, disease challenges and stress. Dietary inclusion of co-products from processes geared towards human use is not a new concept for poultry producers. However, relatively high inclusion levels of distillers dried grains with solubles (DDGS), a co-product from corn ethanol production, is a new challenge. DDGS use in poultry diets is limited because of its variability in nutritional value and its poor handling characteristics during transport and feed manufacturing. Furthermore, rising feed ingredient costs in recent years has been an additional challenge for nutritionists. One strategy to overcome these rising feed costs is the dietary inclusion of exogenous enzymes. The first enzyme typically added to poultry diets is phytase, which increases phytic phosphorus bioavailability. The second enzyme typically added to poultry diets is an energy releasing enzyme. Several energy releasing enzymes are available including: xylanase, amylase, and glucanase.

The first hypothesis tested was that dietary inclusion of natural antibiotic alternatives may help alleviate enteric difficulties during a cocci-challenge. Furthermore, energy-releasing dietary exogenous enzymes may have an additional beneficial effect when fed in poultry diets containing DDGS. Five experiments were completed to test these hypotheses.

The first experiment, Chapter 2 of this dissertation, evaluated the effect of oregano essential oil as a natural alternative to a chemical coccidiostat during a cocci-challenge in poult from 1-15 d of age. Two-hundred day-old Nicholas toms were assigned to 20 cages with two levels of coccidia (challenged vs unchallenged) and two dietary levels of oregano essential oil (0 or 300 mg Regano<sup>®</sup>/kg) in a 2 x 2 factorial experimental design. The four treatments were as follows: control (CC), *Eimeria* challenged (EE), oregano essential oil (OEO) and *Eimeria* challenged plus oregano essential oil (E-OEO). Regano<sup>®</sup> (Ralco Nutrition, Inc., Marshall, MN) contains oregano oil in a soluble hemicellulose emulsifier. A stock solution of Regano<sup>®</sup> at 4.7 mL/L was given at 1:128 to drinking water for the first week. Each poult was gavaged at 3 d with either a 1 mL solution containing *E. adenoides* and *E. meleagrimitis* (total of 2500 oocysts/bird) or 1 mL water. On 8 d, proximal ileum samples were taken for scanning electron microscopy (SEM) and for histomorphometrical analysis on 8 and 15 d. Intestinal weight (rIW) between the gizzard and the cloaca relative to body weight (BW) was determined on one bird/cage at 8 and 15 d.

Cocci-challenge decreased both 7 and 14 d poult BW. In addition, cocci-challenge increased rIW at 8 and 15 d. Oregano essential oil supplementation decreased 7 d BW but had

no significant effect on 14 d BW. The rIW results for oregano essential oil supplementation were reflective of the BW. Regano<sup>®</sup> supplementation increased 8 d rIW but had no effect on 15 d rIW. SEM images obtained from ileal villi at 8 d of age revealed that the Regano<sup>®</sup> treatment was associated with increased mucin secretion and villi retraction that was exacerbated by the cocci-challenge, which may be a protective mechanism against the brush boarder irritation or distress. As a result, cocci-challenge compromised the growth of poult likely because of increased maintenance costs to support greater relative intestinal mass and mucosa. The oregano essential oil (Regano<sup>®</sup>) treatment had minimal benefit as a natural coccidiostat for turkey poult.

If oregano essential oil appeared to have negligible benefits as a natural coccidiostat, perhaps a product with multiple natural activities is more beneficial. In the second experiment, Chapter 4, the effects of feeding Natustat<sup>™</sup>, an “all-natural” product containing organic minerals, plant extracts and at least one yeast-derived mannoligosaccharide, was evaluated in the same experimental design as the previous chapter. Natustat<sup>™</sup> was supplemented at 1000 mg/kg of diet. As found in the previous study, cocci-challenge decreased 7 and 14 d BW. In contrast, the cocci-challenge in this study significantly worsened 1 to 14 d FCR. Interestingly, Natustat<sup>™</sup> had no significant effect on poult growth performance. The only significant diet X challenge interaction observed was on the jejunum rIW at 15 d: dietary supplementation of Natustat<sup>™</sup> increased rIW among cocci-challenged poultry but not among the unchallenged birds. Although dietary Natustat<sup>™</sup> supplementation did not improve poult performance during this cocci-challenge, it may improve compensatory absorption of nutrients by promoting jejunum

development after challenge. Additional studies on Natustat™ are necessary to confirm its effect on the intestinal development and growth response after a challenge.

In the third experiment, Chapter 4, the response of enzymes in low energy diets with DDGS inclusion was examined in market tom turkeys. Turkey toms were assigned to eight dietary treatments arranged as a factorial of two dietary inclusion levels of DDGS (0 and 15 %) and four diets: 1) positive control (PC), 2) negative control (NC), 3) NC with phytase (1000 FTU/kg of feed), and 4) NC with phytase and a combination of xylanase, amylase, and protease (XAP) enzymes (Avizyme 1502, Danisco Animal Nutrition). The PC and NC diets differed by 150 kcal metabolizable energy (ME)/kg of diet, 0.15 % available phosphorus and 0.14 % calcium. Turkeys were fed *ad libitum* eight phases of pelleted feed that approximated requirements of digestible nutrients by age. BW and FCR were determined at 23, 43, 57, 85, 98, 119, and 137 d of age. Dietary inclusion at a level of 15 % DDGS reduced pellet mill performance by approximately 25 %, but it had no adverse effects on growth performance when digestibility was properly taken into consideration. Lower dietary fat in the NC diet improved pellet characteristics and resulted in a similar performance to the PC diet with additional 150 kcal ME/kg. No additional benefit of enzyme inclusion in diets with 15 % DDGS was observed.

Chapter 5 reported the response of enzymes in low energy diets with DDGS inclusion fed to broilers until 49 d of age. In this experiment, broilers were fed diets in mash form to separate the feed form effect from a true enzyme response. Ross 708 broilers chicks were randomly distributed among 72 pens (16 males and 16 females/pen), each assigned to one of 12 dietary

treatments arranged as a factorial of 2 dietary energy levels (low and high), 2 dietary DDGS inclusion levels (0 and 10 %), and 3 dietary enzyme treatments (control, Ronozyme<sup>®</sup> WX, Roxazyme<sup>®</sup> G2; DSM Nutritional Products, Inc.). Ronozyme<sup>®</sup> WX is a xylanase product, while Roxazyme<sup>®</sup> G2 contains a broad array of enzymes. The high and low dietary energy treatment levels differed by 200 kcal ME/kg. Broilers were fed 3 phases of mash feed that approximated requirements of digestible nutrients by age. BW and FCR were determined at 14, 35, 42, and 49 d of age. The high-energy diets significantly improved male and female BW and FCR compared to the low-energy diets, which were reduced by 200 kcal ME/kg of diet. DDGS inclusion at 10 % significantly increased 49 d male BW 2.1 %, decreased 1-14 d feed intake 4.9 % but increased overall feed intake 1.7 %. DDGS inclusion also significantly increased cumulative FCR 5 points. Dietary supplementation with Ronozyme<sup>®</sup> WX, which only contained xylanase activity, improved growth performance of broilers fed 10 % DDGS, but dietary supplementation with Roxazyme<sup>®</sup> G2, which contained xylanase and  $\beta$ -glucanase activities, had variable responses. Ronozyme<sup>®</sup> WX supplemented in low-energy diets containing 10 % DDGS restored broiler growth performance to the level of the high-energy control at 49 d. In other words, Ronozyme<sup>®</sup> WX, but not Roxazyme<sup>®</sup> G2, provided a 200 kcal ME uplift.

The final experimental chapter of this dissertation evaluated the efficacy of a new phytase on poult performance over a range of doses. Nicholas toms were assigned to dietary treatments including a positive control (PC), negative control (NC), NC plus 250, 500, 1000 or 2000 phytase units (FTU) /kg of feed. BW and FCR were determined at 7, 14, 21, and 27 or 28 d of

age. NC diet contained 0.2 % less non-phytate phosphorus (NPP) and 0.15 % less Ca than PC diet. Excreta were collected daily from 22 to 24 d of age. Ileal contents, tibias and toes were collect at either 27 or 28 d of age. The results of this study clearly demonstrate that the experimental phytase product significantly improved the growth response of turkey poults and this response was associated with improvements in feed intake and nutrient bioavailability. Optimum dose of dietary phytase supplementation for body weight gain, feed intake, AME<sub>n</sub>, and digestibility of most of the minerals measured was identified to be about 1000 FTU/kg of feed.

In conclusion, this dissertation presents evidence that the products tested as natural alternatives to antibiotic enteric conditioners and coccidiostats are not effective. Therefore, the search for natural alternatives continues. Secondly, dietary supplementation of exogenous energy releasing enzymes does not provide additional energy uplift in diets containing DDGS when compared to traditional corn soy diets. Additional research is necessary to determine the mechanism why these enzymes to not provide an additional uplift in diets with DDGS inclusion. However, dietary enzyme supplementation is an excellent strategy to help reduce the impact of rising ingredient costs and reduce the environmental impact of poultry production. Further research is also necessary to determine the enzyme strategy (amylase, xylanase, glucanase, etc.) that will be employed in poultry diets after phytase supplementation.