ABSTRACT

ARGÜELLES-RAMOS, MIREILLE. Effects of Phyzyme XP and Avizyme 1502 on the Performance of Broiler Breeders and their Progeny. (Under the direction of Dr. John T Brake).

Four experiments were conducted to develop a better understanding of the effect of the inclusion of a phytase enzyme product (Phyzyme XP; PXP) and a carbohydrase/protease cocktail (Avizyme 1502; XAP), alone or in combination, on the productive performance of broiler breeders (broiler parent stock) and their broiler progeny as affected by mineral and nutrient availability to both parent and progeny, maternal effects on the progeny, and interactions with other feed ingredients. The inclusion of 550 units of phytase (FTU) per kg of broiler breeder layer feed increased the absolute difference in fecal moisture (FM) by 10% after been fed for 14 d. It was hypothesized that the cause was either an inappropriate calcium:non-phytate phosphorus ratio (Ca:NPP) as no Ca matrix value was assigned to PXP (Manuscript I) or a hygroscopic effect. Also, the same inclusion level caused the birds to excrete less di- and tri-valent cations and more P possibly due to a lower P requirement of the breeder strain used. Inclusion of PXP and XAP to marginally nutrient deficient broiler breeder layer diets negatively affected reproductive performance and survivability (Manuscript II) probably as a result of heat stress feed management practices and/or protease-protease inhibitor imbalances in the gut. However, when appropriate preventive management measures were taken no negative effect of PXP and XAP was observed on performance or survivability (Manuscript III). Overall, no added benefit on breeder performance was observed when enzymes were included in the breeder diet. Conversely,

feeding the parental flock with diets that included PXP and XAP resulted in an improved AdjFCR of the progeny at 49 d of age (Manuscript III). Inclusion of PXP and XAP in the broiler diets increased male body weight and tended to improve feed efficiency at 41 d of age (Manuscript II). On the other hand, XAP in the presence of 0.43% Cl tended to improved feed efficiency and body weight at 35 d of age versus the enzyme fed with 0.28% Cl (0.35% $NaHCO_3$) (Manuscript IV). This benefit disappeared by 41 d of age probably due to down-regulation of endogenous digestive enzymes. The data suggested that XAP worked better in low dietary electrolyte balance (or high chlorine content) diets.

In summary, the addition of PXP and XAP to the feed of the broiler breeder parental flock or the progeny did not negatively affect broiler breeder or broiler progeny performance when good heat stress management was practiced. It was concluded that the addition of PXP and XAP to broiler breeder and broiler diets was a potentially safe option to reduce feed cost and inorganic P inclusion in feed, when potential interactions with feed ingredients were taken in consideration.

Effects of Phyzyme XP and Avizyme 1502 on the Performance of Broiler Breeders and their Progeny

by
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DEDICATION

To my beloved husband, Laris José Soto-Toledo.

BIOGRAPHY

Mireille Argüelles-Ramos is the second of four siblings of Miriam Ramos-Crespo and Mariano Argüelles-Negrón, and was born and raised in Puerto Rico. In 1998, after obtaining her diploma from the Luis Muñoz-Rivera High School, she decided to apply to the University of Puerto Rico-Mayagüez Campus from where she recieved her degree in Animal Science in 2002. During the last year as undergraduate student, she had an opportunity to work in animal nutrition research and participate in different scientific meetings. These experiences changed her mind about studying veterinary medicine after graduation, and she decided to pursue a MS in Animal Science under the supervision of Dr. Héctor Santiago-Anadón at the same institution. Her research was focused evaluating the effect of the inclusion of phytase enzyme in broiler diets on productive performance and bone integrity. After she received her MS in 2005, she worked for ADM Alliance Nutrition of Puerto Rico as an Animal Nutritionist for two years. Mireille and her husband then decided to move to the USA to pursue a PhD in Poultry Science with a co-major in Physiology under the direction of Dr. John T. Brake. As part of her dissertation research, she worked to determine the effect of the inclusion of feed enzymes on the performance of broiler breeders and their progeny. She has experience in broilers, broiler breeders, incubation, feed milling, and processing. During her doctoral experience, she presented seven papers at different scientific meetings and has received awards at three of these meetings.

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INTRODUCTION

Phosphorus (P) has been found to be the second most abundant mineral in the body, not only for humans but for all domestic animals. Its abundance and ubiquitous presence in the entire body has become just one example of what the term "essential" could mean, especially as this mineral has been reported to influence metabolic, structural, and reproductive functions. For decades scientist have worked to establish minimum dietary requirements for domestic animals in order to promote optimum health and growth that later will be converted to profits for animal producers. The ninth and most recent edition (1994) of the Nutrient Requirements of Poultry from the NRC established that the minimum requirement of non-phytate phosphorus (NPP) was 0.45% and 0.35% for broilers from 0-3 wk and 3-6 wk, respectively. For broiler breeders, 0.45% NPP diets during the rearing phase was recommended. Intensive selection in domestic avian species during the last decade (Rauw et al., 1998) has required advancements in nutrition and physiology to evolve as rapidly as those in genetics and genomics (Burt, 2002). This situation has made industry and academia constantly reevaluate the nutritional requirements necessary to support genetically induced improvements in performance, while taking in consideration animal welfare and the environment.

Nevertheless, P content of animal feeds in the USA has remained high due to the predominant use of corn and soybean meal in animal diets. Furthermore, most of the P in cereals and legumes has been shown to be present in the phytate form, or inositol hexakisphosphate, where six phosphate groups were attached by phosphoester bonds to an inositol ring that has been reported to not be fully available to non-ruminants (O'Dell et al.,

1972; Maenz and Classen, 1998). Only 21% of the total P in corn and 35% in soybean meal (NRC, 1994) were reported to be readily available for absorption, while the remainder was excreted in the feces due to the lack of sufficient endogenous intestinal phytase. As a result, nutritionists have been compelled to include significant amounts of expensive, but highly bioavailable, inorganic sources such as dicalcium phosphate and defluorinated phosphate, among others, in poultry diets. This has been a successful means to maintain performance of animals, but has had detrimental effects on the environment.

Certainly, a high nitrogen (N) and mineral content has made manure an economically affordable and widely used fertilizer, as an alternative to the inorganic versions that have volatile raw material prices. However, some disadvantages in terms of nutrient balance became evident when manure was applied to crops since the N to P ratio has tended to be around 2:1 to 4:1. Most crops have been found to require ratios of around 8:1. Moreover, manure application to land has been based primarily upon the N requirement of plants, resulting in P accumulation in soils. After application, excess P and other nutrients have been shown to reach lakes and reservoirs by "surface runoff" and "subsurface flow" (Sharpley, 1999) promoting the eutrophication process if care was not exercised.

Strategies that had previously been considered to economically reduce the impact on the environment included genetic selection of animals with lower P requirement (Punna and Ronald, 1999), reduced P safety margins during feed formulation, and inclusion of phytase enzyme in feed to improve availability of phytate P. Additionally, carbohydrases and protease cocktails (CPC) have become another strategy intended to increase nutrient availability from grains and grain by-products. The integration of these strategies could

help ameliorate the direct and indirect detrimental effects of concentrated animal husbandry operations on the environment and sustain the profitability of these organizations.

An important aspect that could be affected by the addition of phytase to the diet was a change in the calcium to NPP ratio (Ca:NPP). Typically the homeostatic mechanism of both minerals have been shown to be closely related (Boron and Boulpaep, 2003). Researchers have reported that an inappropriate Ca:NPP ratio could negatively affect water intake (Leeson and Summers, 1987) and water retention (Guo et al., 2008; Enting et al., 2009), which could result in an increased fecal and litter moisture in poultry (Bedford et al., 2007). These reports suggested that mineral content and/or phytase activity in feed must be manipulated carefully in order to avoid litter quality problems that could have detrimental effects on animal welfare (Francesch and Brufau, 2003). Consequently, the short-term inclusion of phytase at a half or full recommended dose in low NPP broiler breeder diets on fecal characteristics was investigated in the present research.

Beyond the positive effects of the inclusion of enzymes in feed on profitability, it has been documented that there were possible negative maternal effects on the progeny when enzyme cocktails were fed to the parent flock. Brake et al. (2003) observed that inclusion of a fungal phytase cocktail with protease and carbohydrase side activities (Wu et al., 2004) to broiler breeders reduced their broiler progeny body weight as a consequence of a reduction in broiler progeny feed consumption. However, it was unknown if the negative effect observed in the broiler progeny was due to the phytase or to the side activities present in the CPC. Moreover, it was unknown if the CPC inclusion level could be causally related to the decreased performance previously observed. A digestibility trial conducted by

Argüelles-Ramos et al. (2010) revealed that the ileal digestibility of most of the essential amino acids could be reduced by high inclusion levels of protease when broiler diets containing 10% DDGSwere fed. This effect may be due to excessive intestinal tissue remodeling as a result of protease-protease inhibitors imbalance (Antalis et al., 2007). Therefore, two studies were conducted in the present research to examine the maternal effect of the inclusion of phytase and CPC enzymes at different levels when the progeny was fed similar diets.

As discussed previously, adequate inclusion of phytase has made possible the significant reduction of inorganic P in monogastric diets without compromising performance, livability, or carcass traits (Ingram et al., 2001; Adeola et al., 2004; Beudeker et al., 2005). In addition to the ability of phytase to increase the availability of P via degradation of the anti-nutritive phytate molecule, this enzyme has been shown to increase availability of other minerals, amino acids, and metabolizable energy (Selle and Ravindran, 2007). In addition to the improvement in nutrient digestibility, researchers have postulated that exogenous enzyme supplementation as CPC could reduce the energy expenditure of the animal by decreasing the synthesis of endogenous enzymes (Mahagna et al., 1995). However, most of the trials performed to assess enzyme effectiveness did not collect data beyond 21 d of age. Thus, the present research examined broilers grown to older ages.

Sodium chloride (NaCl) addition to the diet has typically supplied the sodium Na and Cl requirements of birds. It has been demonstrated that Na and Cl in conjunction with potassium (K) are very important for intestinal nutrient absorption (Boron and Boulpaep, 2003). A deficiency or excess of these minerals in the diet, as well as their imbalance,

could affect the efficiency of the digestive process due to induced changes in the pH of the digesta. Even when diets have been properly formulated to meet all the requirements of the bird, anti-nutritive components in feedstuffs could reduce availability of essential nutrients. It had been speculated that phytate has the capacity to reduce ileal availability of Na by attracting the mineral to the lumen as demonstrated by Ravindran et al. (2006). Due to the potential influence of phytate and phytase on Na availability, researchers have suggested that the electrolyte balance (DEB = mEq Na^+ + mEq K^+ – mEq Cl^-) could interact with the efficacy of the phytase enzyme. Ravindran et al. (2008) observed that addition of phytase to diets with moderate DEB levels improved broiler performance and availability of nutrients, while no real benefit of the phytase enzyme was observed at a high DEB level. However, it was unknown if the interaction between phytase and DEB was due to the DEB *per se*, or to the different Na levels in the diets. The present study examined certain aspects of this relationship.

It has been reported that carbohydrases alone and CPC could improve digestibility of minerals and amino acids (Hew et al., 1998; Kim et al., 2005; Olukosi et al., 2007; Selle et al., 2009). Absorption of these nutrients could be closely related not only to the optimal requirement of Na, but also to an optimum ratio between Na, K, and Cl (DEB), as most of the absorption processes of sugars, amino acids, and some minerals, have been shown to indirectly depend upon the electrochemical difference between the digesta and the intracellular space of absorptive cells in the intestine (Boron and Boulpaep, 2003). Consequently, the possible interaction of the inclusion levels of NaCl and enzyme efficacy was studied in the present research.

LITERATURE REVIEW

Calcium (Ca) and phosphorus (P) are essential minerals for birds due to the variety of functions in which they are involved and their ubiquitous distribution among the organism. They are the most abundant minerals in the body and are present as part of body structural components, messengers in transduction pathways, enzyme cofactors, component of nucleic acids and high energy compounds, among others. Due to the variety of functions that Ca and P perform and their evident homeostatic interrelationship adequate daily intake is crucial to meet the physiological demands for maintenance, growth, and reproduction. However, to have the correct levels and proportion of Ca and non-phytate P (NPP) in the diet could be complex if we take into consideration the variety of feedstuffs, mineral supplements, and enzyme products available, and how they can contribute to or affect the availability of these minerals to the bird. Moreover, environmental concerns related to high total P content in diets and the increase in mineral supplement prices after the 2008 phosphate crisis have to be taken into consideration by nutritionists and feed formulators to keep balanced the nutritional, environmental, and economic aspects of feed production.

Phosphorus and Environmental Concerns

Phosphorus content in poultry feeds in the USA tends to be high due to the use of corn and soybean meal, considering the high P content in these ingredients and levels of inclusion in diets. However, most of the P in cereals and legumes is present in the phytate form, which

is not available for non-ruminants. Only about one-third of the total P in corn and soybean meal are available for absorption (NRC, 1994; Ravindran, 1996), while the rest is excreted in the feces due to the lack of sufficient endogenous phytase. Therefore, inorganic sources such as dicalcium phosphate, defluorinated phosphate, and others have been used as mineral supplements in animal feeds. This means that if the USA has currently in production about 50 million broiler breeders per year, then these birds could be consuming over 6.6 million pounds of inorganic P in a year.

On the other hand, the high content of N and P in chicken manure and litter makes them excellent sources of crop fertilizer. It is known that manure N:P ratio tends to be around 2:1 to 4:1, while required ratio for must crops is about 8:1. Since manure application is based on the N requirement of the plant, a P buildup in the soil has been unavoidable (Kellogg et al., 2000). After application, excess P and nutrients reach lakes and reservoirs by "surface runoff" and "subsurface flow" (Sharpley, 1999) promoting the eutrophication process which is considered a major environmental concern worldwide.

Impact of Phosphate Costs in Agriculture

For many years China has been the major phosphate rock producer with more than 45 million tons per year (2007), followed by the United States and the invaded Moroccan territory of Western Sahara, with more than 29 and 27 million tons, respectively (Jasinski, 2010). Despite the large production of China and the USA, most of it remains for domestic use, leaving Western Sahara as the major global exporter, followed by China.

In 2008 record high prices for feed grade P were observed driven by an increase in the cost of raw material used to produce this mineral supplement, phosphate rock. In 2007 phosphate rock cost was around US$51.10/ton, reflecting a 68% increase from 2006. By 2008 the price increased to US$113.00/ton, representing an increase of 270% from the price in 2006 (Jasinski, 2010).

The record increase in phosphate rock price in 2008 was a result of numerous social and economic factors. During the last decades world population has been increasing at a considerable rate. The expected population by 2050 is 9 billion, which is a 33% increase from 1999 (US Census Bureau, 2010). This is causing a soaring demand for food and fuels. Major sources of food for human and animal consumption such as corn, soybean, and wheat require considerable amounts of phosphate fertilizers to achieve high yielding and profitable harvests. On the other hand, fossil fuels high prices, oil shortages, and environmental issues related to fossil fuel use have promoted the production of ethanol from corn and other crops (Anonymous, 2009a).

Another important factor was the shortage observed at the beginning of 2008 due to winter atmospheric disturbance in key provinces in China (Yunnan, Guizhou, and Sichuan) causing delays in production. Then in May, two important events significantly contributed to the shortage, and subsequent price increase: China raised the special export tariff for phosphate by 100% and then the 8.0 earthquake in Sichuan (Anonymous, 2009b).

Other factors that contributed to increased phosphate prices were:

1. Relatively low carryover stocks (phosphate stock available:total use ratio) in 2006-2007, contributing to the shortage.
2. Depreciation of U.S. dollar.
3. Increase in soft commodity (corn, soy, wheat) prices, driven first by fundamental commodities (which are created within households by using time and market goods and services; Dewar, 2009), followed by speculation.
4. Feedback loop, in which higher downstream prices promoted higher raw material prices and vice versa (Huang et al., 2009; Roberson, 2009).

Looking for Cost-Effective Solutions

Reduction of safety margins and inclusion of phytase

Strategies have been explored to reduce the environmental impact of agricultural operations and to reduce feed costs. One way is by reducing the safety margins used by nutritionist during the feed formulation process. It is known that a reduction in inorganic P in the diet has a positive correlation with mineral content in the feces (Powers and Angel, 2008). Usually this approach is used in conjunction with the inclusion of phytase in feed. Phytase enzyme is a phosphatase capable of releasing the phosphate groups forming the phytate molecule or inositol hexakisphosphate by phosphoester bond hydrolysis. The phytate molecule can be considered as an anti-nutritional compound with a high chelation capacity for divalent cations such as Ca, Zn, Cu, Mg, and others (Reddy et al., 1989). When phosphate groups are release due to the action of the enzyme, other minerals, amino acids,

and nutritional compounds associated with the phytate molecule, can be released and become available for absorption. Many phytase products have been developed since its discovery, and enzyme effectiveness will depend upon numerous factors as source of the enzyme, species fed, age, sex, type of animal operation, and management practices, among others. On average, it is accepted that 500 FTU/kg of feed is equivalent to 0.1% of NPP, depending upon the product used. Calcium and amino acid matrix values are available for some phytase products but these values vary by product and consistency of the results obtained is questionable. Some research trials have shown that the use of phytase at recommended levels could be a cost-effective, safe option to reduce the inclusion of inorganic phosphate in broiler breeder diets without affecting the number of chicks per hen housed (Plumstead et al., 2007) or egg production (Berry et al., 2003). These observations contrasted with Bhanja et al. (2005) who did not found any beneficial effect on performance of adding 500 FTY/kg when broiler breeders where fed diets with 0.18% NPP. These results coincided with findings made by Brake et al. (2003) in which broiler breeders fed diets with 0.1% NPP and no added phytase that survived hot weather conditions had normal reproductive performance. They suggested that this could possibly be due to the endogenous phytase produced by the bird, a higher than expected P availability from feedstuffs, or a lower Ca:P ratio, which means less Ca-phytate complexes formed, increasing the availability of phytate molecules for digestion. However, more research is necessary to determine the appropriate level of phytase in feed and the effect in breeder and progeny performance.

Inclusion of distiller's dried grains with solubles in feed

Another modality by which to reduce feed cost is the use of by-products such as distillers dried grains with solubles, which are sources of NPP. With DDGS prices between $75 and 110/ton the inclusion rate could be up to 20%, and will depend of the price of other ingredients such as corn soybean meal, fat, dicalcium phosphate, and supplemental crystalline amino acids (Noll, 2005), and ingredient or nutrient restrictions applied to the formula that account for the digestibility of DDGS

However, reductions in feed cost will not necessarily result in more profits for a broiler company, since bird performance could be aversely affected. In 2004 Lumpkins et al., performed two experiments to evaluate the use of DDGS from modern ethanol plants in broiler diets. They concluded that inclusion rates for starter diets should not exceed 6% and 12 to 15% for grower and finisher diets. Furthermore, Wang et al. (2007a,b) observed that inclusion levels between 15 and 20% (during all feed phases) can be used, but dressing percentage and breast meat yield could be affected. It has been recommendable to purchase high quality DDGS and perform extensive proximal and amino acid analyses. In that way the use of standardized matrix values and digestibility coefficients (Waldroup et al., 2007) would be more reliable.

Concerns about the use of DDGS are not limited to their nutritional aspect. Manufacturing issues are present from the receiving of the ingredient at the feed mill to the pelleting process. Distiller's dried grains are sprayed with the solubles (50% DM), sent to the dryer, and then shipped. Because of the lack of storage space at the ethanol plants, DDGS are

shipped when moisture equilibrium between the solubles (15-18%) and solids (10-11%) have not been acheived. This will cause hygroscopic particles to agglomerate resulting in serious flowability problems. Moreover, no anti-caking agents have been approved for this ingredient at the time of this writting

Another serious problem arises during the pelleting process. The physical and chemical characteristics of DDGS are very important to determination of the slip and flow resisting forces. The high moisture, fat, and protein in DDGS will cause an increase in the slip-resisting force that will prevent the material from compressing, while the high fiber content and small particle size will reduce extrusion. These effects are caused by the poor moisture absorption of DDGS due to its low bulk density. Also, moisture above 4% can cause the protein to become "gummy" and plasticize at temperature above 63ºC (Koch, 2006) as would be used during the pelleting process. Additionally, the oil content in the DDGS tends to coat hydrophobic particles and thus disrupt particle-particle interactions, and low starch content of the ingredient reduces particle binding (Behnke, 2007). For these reasons, high inclusion levels of DDGS tend to increase energy cost and reduce pellet quality (Koch, 2007). Other issues are the high nutrient variability and the potential concentration of mycotoxins from contaminated corn.

Inclusion of carbohydrase/protease cocktails

Carbohydrases and protease cocktails (CPC) are another strategy intended to increase nutrient availability from grains and by-products. The purpose of carbohydrases (such as xylanase) in the diet has been to digest cell walls to reduce viscosity of the digesta and

expose macronutrients to further digestion by endogenous enzymes. This mechanism has been frequently observed in wheat-based, rather than in corn-soybean meal based diets, as their structural and chemical compositions differed in many ways that favored the effectiveness of the enzymes. It has been suggested that an important amount of the starch in corn was embedded in protein matrixes, which limited the access of important enzymes such as amylase (Brown, 1996).

Many researchers have reported the benefits of use carbohydrase/protease cocktails (CPC) in broilers. Cowieson and Ravindran (2008a) found that supplement energy and amino acid deficient diets with a xylanase, amylase, and protease cocktail (XAP) improved body weight gain and feed efficiency to that of the control treatment level when compared to the non-supplemented nutritionally deficient diet. These authors also investigated how the inclusion rate of the XAP cocktail could affect broiler productive performance (Cowieson and Ravindran, 2008b). They concluded that an inclusion rate of 500 g/MT (minimum activity of 300 U xylanase, 400 U amylase, and 4000 U protease) improved body weight gain, feed efficiency, and digestible energy, when compared to half of that dose.

Other researchers had investigated the effect of the inclusion of XAP in combination with phytase on broiler chick performance (Cowieson and Adeola, 2005). They reported that the addition of XAP at the same enzyme activity mentioned above alone or in combination with 1000 U of phytase/kg improved feed efficiency, while combination of XAP and phytase improved body weight gain and ileal digestible energy. In two other experiments conducted by Cowieson et al (2006a,b) it was found that addition of XAP and phytase to

energy, NPP, Ca and amino acid deficient broiler diets significantly improved nutrient digestibility of corn-soybean meal based diets. Conversely, Tiwari et al. (2010) found that the addition of XAP to corn-soybean meal based diets did not improve broiler performance by itself. However, when XAP and phytase were combined they observed an additive effect on growth performance, while no effects were observed on ileal digestible energy. An age x diet interaction was found that revealed that positive effects from enzyme addition on nutrient retention were more evident during early stages of life. These results were in agreement with finding of Olukosi et al. (2007) in a similar experiment whenbroilers were fed diets with XAP and phytase alone or in combination.

In addition to the improvement in nutrient digestibility, researchers have postulated that exogenous enzyme supplementation could reduce the energy expenditure of the animal by decreasing the synthesis of endogenous enzymes (Mahgna et al., 1995). However, most of the trials performed to assess enzyme effectiveness have not collected data beyond 21 d of age.

The integration of these strategies could help ameliorate the direct and indirect detrimental effects of poultry operations and sustain the profitability of this economic activity. However, nutritionists and formulators have to be aware how the use of enzymes such as phytase, and use of by-products can change the mineral profile of the diet, principally Ca and P content, and of particular importance for broiler breeders, the Ca:P ratio.

Calcium-Phosphorus Homeostasis and Phytase

An appropriate Ca:P ratio in the diet determines the proper absorption of these minerals at the level of the small intestine, and it is not unexpected to observe that the Ca:P ratio in blood is tightly controlled by homeostatic mechanisms.

Calcium absorption is possible by two different pathways: paracellular (passive diffusion) in which absorption of the mineral will depend upon its concentration in the digesta, and transcellular (active transport). In the latter, Ca enters the enterocyte via Ca channels and down its electrochemical gradient. Then, calbindin serves as Ca transporter through the cell. This step is rate limiting and is controlled by 1,25-dihydroxycholecaciferol (calcitriol), which promotes the synthesis of calbindin via its nuclear receptor. Finally, Ca is transported to the basolateral membrane to be extruded against an electrochemical gradient by a Ca-ATPase pump (Boron and Boulpaep, 2003; Bronner, 2003). On the other hand, P is transported across the apical membrane via the sodium/phosphate co-transporter (Na/Pi), and then extruded across the basolateral membrane (unknown mechanism; Boron and Boulpaep, 2003; Marks et al., 2006).

After absorption of Ca and P, plasma levels and physiological status will determine the rate of reabsorption and excretion mainly by the kidneys (mostly for P; Leske and Coon 2002) and endogenous excretion via the intestine (mostly for Ca; Hurwitz and Bar, 1971). When Ca levels in blood are low, parathyroid hormone (PTH) is released increasing bone resorption, reabsorption of Ca in the kidneys, and stimulating the synthesis of calcitriol.

However, high levels of PTH will increase renal P excretion. In contrast, calcitriol increases intestinal absorption and renal reabsorption of Ca and P.

When a high Ca:P ratio feed is ingested (assuming P in its available form), most of the absorption of Ca will be via the paracellular pathway due to the gradient created between the digesta and the interstitial fluid (Bronner, 2009). Phosphorus will be absorbed via Na/Pi, but it is known that high Ca intake can cause a reduction in P absorption (Hurwitz and Bar, 1965). This could be a result of the change in epithelial permeability for P affecting paracellular absorption. Also, if P extrusion across the basolateral membrane is energy dependent (ATPase pump: Kikuchi and Ghishan, 1987), then excess Ca cations could disrupt the electrochemical gradient necessary for the pump to work at normal rate, reducing P absorption. However, Hurwitz and Bar (1971) hypothesized that the relationship between Ca and P is due to "chemical association in the intestinal lumen rather than from interaction at the absorption site".

In a high blood Ca:P ratio scenario, an activation of homeostatic mechanisms would be expected, resulting in increased renal excretion of Ca, and P conservation, especially in the proximal tubule. Furthermore , water reabsorption could be affected by high Ca intake. Nakahama et al. (1996) observed that high Ca intake could decrease the concentration of renal organic osmolytes such as myo-inositol and sorbitol, reducing the water reabsorption capacity of kidney. If homeostatic mechanisms are insufficient to reduce Ca:P ratio, then hypercalcemia can develop. Chronic hypercalcemia could severely affect nephrotic integrity and urine concentrating capacity, thus increasing water excretion.

For laying birds (i.e.. broiler breeders) Ca and P homeostasis is particularly important due to the high Ca requirement for proper eggshell formation and maintenance of bone integrity. The uterus has a high capacity to transport and deposit calcium with rates of 100 mg/hr and greater (Hurwitz and Bar, 1969). However, if the rate at which Ca can be moved from the intestinal lumen to the uterus during the eggshell formation period is insufficient , then bone Ca mobilization via parathyroid hormone action will be the backup mechanism to fulfill Ca demands (Taylor, 1961). During bone Ca mobilization other minerals such as P are mobilized to the blood as part of the solubilization of hydroxyapatite crystals. Muller et al. (1964) estimated that about 1 g of Ca and 0.5 g P were mobilized during the 18 hr eggshell formation phase. The increased P concentration in plasma will cause a decrease in P reabsorption and increased secretion, which could explain observations made by Common (1932, 1933, and 1936).

Taking into consideration the high cost of inclusion of inorganic phosphate in diets we could think that this homeostatic mechanism in broiler breeders is a way to lose money. It has been suggested that an increase in Ca consumption during the eggshell formation period would increase Ca concentration in the intestinal lumen, reducing the Ca contribution from bone and, consequently, P excretion. Coon and Manangi (2008) studied the influence of calcium particle size (limestone) in the excretion of P. The 6-wk trial involved 31-wk-old Cobb 700 hens fed two different particle size limestone (300 vs. 4570 micron) in their diets. These authors observed that feeding large particle size limestone reduced the P excretion by 1.83 mg P/g DM excreta while tibia ash was improved by 3.22%.

Calcium and Phosphorus Requirements of Poultry

Conscious of the indispensability of these minerals, the scientific community has devoted decades of research to establish the minimum requirements for different species of livestock with the purpose to ensure proper growth and development whilemaximizing production efficiency and profits. For broiler breeders, the standard recommendations have been 0.9 – 1.0% Ca and 0.45 – 0.50% NNP for starters and growers (about 2:1 Ca:P ratio), and 4 g of Ca/bird/day and 350 mg of NPP/bird/day at peak egg production (NRC, 1994) (11:1 Ca:P ratio) that could be supplied by a 2.50% Ca and 0.23% NNP layer diet with a feed allocation of 155 g at peak. However, researchers and breeding companies have not been necessarily consistent in their Ca and P inclusion recommendations especially for layer feeds, whose Ca:P ratios can range from 10:1 to 6:1, probably because of the variability of genotypes, feedings programs, management practices, production goals, and personal preferences as shown in Table 1.

As discussed above, providing the Ca and NPP requirements at the properCa:P ratio is critical for proper growth and reproductive performance but despite the importance of an appropriate feeding program and balanced nutrition during rearing and laying periods, and their subsequent impact on production and chick quality, not much research had been conducted in this area.

Ekmay and Coon (2010) studied the effect of different levels of NPP on performance of broiler breeders and their progeny. They found that a consumption of 360 mg NPP/d at peak production (0.25% NPP in the diet) was sufficient to maintain productive and

reproductive performance, bone integrity, and progeny weight at hatching. In a similar experiment, Bhanja et al. (2007) found that consumption of 192 mg NPP/d (0.12% NPP in the diet) was sufficient to maintain performance of caged broiler breeders and subsequent progeny performance.

On the other hand, the addition of phytase could affect the content of P in the diet. Berry et al. (2003) found that the content of NPP in the diet could be reduced to 0.10% when a fungal phytase was added at a rate of 300 FTU/kg without affecting egg production or egg characteristics while improving livability. However, hatchability was negatively affected by the combination. Plumstead et al. (2007) studied the effect of the inclusion of a fungal phytase cocktail on the performance of broiler breeders when added to NPP deficient diets. On a cumulative basis, birds fed the lowest level of NPP (0.22%) plus the phytase cocktail were more efficient and had the highest hen-day production. Additionally, even when fertility was reduced with NPP levels between 0.40% and 0.22% (0.27 and 0.09% NPP, respectively), no negative effect was observed on the number of chicks produced per hen housed.

Brake et al. (2003) studied the effects of the inclusion of enzyme cocktails in broiler breeder feed on progeny performance. From 22 to 64 wk of age broiler breeders were fed one of four diets consisting of two levels of NPP (0.13 or 0.40%) and two levels of a fungal phytase cocktail (0 or 500 FTU/kg feed) with side activities.

The side activities included in this cocktail were protease, alpha-amylase, xylanase, cellulose, and beta-glucanase (Wu et al., 2004). Then, they fed the same enzyme product to

the broiler breeder's progeny. No interactions were observed between breeder and broiler diets, and the breeder diet effect on broiler performance was across all broiler diets. These investigators found that broilers from breeders fed the phytase cocktail were significantly smaller ($P<0.05$) at 35 and 42 d of age than broilers from breeders fed no enzyme cocktail. This effect was explained by the reduced feed consumption (FC; $P<0.10$) from 22 to 35 d of age for broilers from breeders fed the phytase cocktail.

Table 1. Recommended Ca and NPP levels for different broiler breeder strains (2,800 kcal metabolizable energy/kg feed)[1].

Strain	Phase				
	Starter	**Grower**	**Pre-Breeder**	**Breeder 1**	**Breeder 2**
	Ca, NPP (%)				
Arbor Acres	1.00, 0.45	0.90, 0.42	1.20, 0.35	3.00, 0.35	3.30, 0.32
Ross 308	1.00, 0.45	0.90, 0.42	-	3.00, 0.35	-
Ross 708	1.00, 0.45	0.90, 0.35	1.20, 0.35	3.00, 0.35	-
Cobb 500 SF & 700	1.00, 0.45	1.07, 0.44	1.45, 0.44	2.93, 0.44	3.13, 0.39
Hubbard Classic	1.10, 0.5	1.10, 0.45	1.40, 0.40	3.20, 0.40	3.50, 0.37

[1] Hubbard, LLC, 2004; Aviagen, Inc., 2007ab, and 2009; Cobb-Vantress, Inc., 2008ab.

The question that was raised after this experimental series was if the negative effect observed in the progeny was related to the phytase content of the diet. To evaluate this Argüelles-Ramos et al. (2009; 2010) performed two series of experiments (ES1 and ES2) in

which they fed four different broiler breeder diets from 22-64 wk using pure phytase (Phyzyme XP – PXP) and/or a multi-enzyme cocktail containing xylanase, alpha-amylase and subtilisin, a serine-protease (Avizyme 1502 – XAP). In ES1 the dietary treatments were a positive control (PC), a negative control (NC), a NC+PXP, and a NC+PXP+XAP. The PC diet contained 2900 kcal ME/kg and all NC diets were 80 kcal ME/kg. In ES2 the dietary treatments consisted of a PC, a NC, NC+PXP+XAP (full dose XAP) and NC+PXP+ ½XAP (half dose XAP). The PC diet was fed to provide 441 kcal /day at peak egg production, versus 413 kcal/d for all NC treatments. Then, the progeny from ES1 and ES2 breeders were fed similar diets, respectively. Breeder performance overall was not statistically different among treatments.

On the other hand, no interactions between breeder diet and broiler diet were found for either of the ES experiments. For ES1 broiler progeny FC was significantly less from 1-16 d, which coincided with the transient reduction in feed consumption observed by Brake et al. (2003). However, there were no significant differences in body weight (BW) or adjusted feed conversion ratio (AdjFCR). For ES2 broiler progeny, enzyme addition numerically improved AdjFCR from 0-49 d compared with the NC ($P < 0.10$).

Broiler breeders are complex animals with relatively long life cycles that require a well-planned management scheme that includes, among other things, an appropriately balanced nutrition to ensure proper reproductive and progeny performance. It is the responsibility of nutritionists and formulators to know well the nutritional profile of the ingredients used in feeds for an accurate formulation, in this particular case attention to the details of the Ca

and P ratio, and always have in mind the delicate balance between nutrition, environment, and profit.

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MANUSCRIPT I. Effect of the Inclusion of Phytase in Low Non-phytate Phosphorus Broiler Breeder Diets on Fecal Moisture

ABSTRACT

A 21 d experiment was conducted to determine the effect of inclusion of phytase in low non-phytate phosphorus (NPP) broiler breeder diets on fecal moisture (FM) content. A total of forty-eight 30-wk-old Heritage 32 females were fed a standard broiler breeder layer diet (2.7% Ca, 0.37% NPP) a week before experimental diets were applied and fecal collections were performed for determination of FM. Birds were then grouped into 3 blocks (high, average, and low) based on FM content at d 0. From d 1 to 14 of the experimental period birds were fed one of 4 treatment diets that consisted of an Adequate P (AdqP) with 0.50% NPP, a Marginal P (MarP) with 0.25% NPP, a MarP with 275 FTU/kg (MarP+PXP), and a MarP with 550 FTU/kg (MarP+2PXP). A randomized complete block design with 12 replications per treatment and 4 replications per block was used. Egg production, egg quality characteristics, blood and fecal mineral concentration, fecal liquid portion (LP) produced, and FM were determined.

There were no significant treatment effects on egg production or egg quality characteristics. Fecal LP production tended to increase for all treatments with change from the standard broiler breeder diet to the treatment diets (d 0 to 7). However, there was an increase in

daily house temperature during this period, which could have affected LP production. After 7 d of adaptation to treatment diets (d 1 - 7) the LP production decreased for all treatments. At d 7 FM was decreased for all treatments. On the other hand, FM at d 14 was similar to that at d 0 for all treatments, with the exception of the MarP+2PXP treatment, which had significantly greater FM ($P<0.01$). These results indicated that the inclusion of phytase in low NPP broiler breeder diets increased FM content if the phytase dosage was sufficient.

INTRODUCTION

The poor availability of phosphorus (P) in feedstuffs such as corn and soybean meal (NRC, 1994) has made the addition of highly bioavailable inorganic sources of the macromineral P essential. The low bioavailability of the mineral in common feedstuffs has been shown to be a result of two factors. First, most of the P present in ingredients of plant origin was in the form of phytate, or inositol hexakisphosphate, where six phosphate groups were attached by phosphoester bonds to an inositol ring. Second, poultry species and swine have been shown to lack the capacity to produce adequate phytase to optimally utilize the P from these organic sources (Maenz and Classen, 1998). This situation has compelled nutritionists to include significant amounts of expensive but highly bioavailable inorganic sources such as dicalcium phosphate and defluorinated phosphate, among others, in poultry diets. This has been a successful approach to maintain productive performance of animals, but has had detrimental effects on the environment.

Certainly, the high nitrogen (N) and mineral content has made manure an economic and widely used fertilizer, as an option to the inorganic versions that have volatile raw material prices. However, some disadvantages in terms of nutrient balance were evident when manure was applied to crops since the N to P ratio has tended to be around 2:1 to 4:1, while most crops required ratios of around 8:1. Moreover, manure application to land has been based upon the N requirement of plants, resulting in P accumulation in soils. After application, excess P and other nutrients can reach lakes and reservoirs by “surface runoff”

and “subsurface flow” (Sharpley, 1999) promoting the eutrophication process if care was not exercised.

Strategies that had previously been contemplated to economically reduce the impact on the environment included genetic selection of animals with lower P requirement (Punna and Ronald, 1999), reduced P safety margins during feed formulation, and inclusion of phytase enzyme in feed to improve availability of phytate P. All of these strategies alone or together have resulted in reduced inorganic P addition to diets, and a more efficient use of P contained in feedstuffs. However, it has been suggested that addition of phytase to the diet not only affected P availability, but altered the calcium to non-phytate P ratio (Ca:NPP). Researchers had reported that an inappropriate Ca:NPP ratio could negatively affect water intake (Leeson and Summers, 1987) and water retention (Guo et al., 2008; Enting et al., 2009), which could result in an increased fecal and litter moisture in poultry (Bedford et al., 2007). These facts suggested that mineral content and/or phytase activity in feed had to be manipulated in order to avoid litter quality problems that could have detrimental effects on animal welfare (Francesch and Brufau, 2003).

The objective of the present study was to determine the effect of short-term inclusion of phytase at half or full recommended inclusion level in a low NPP broiler breeder layer diet on fecal moisture (FM), mineral excretion, egg production, and egg quality traits.

MATERIALS AND METHODS

Broiler Breeder Management and Data Collection

Forty-eight 30-wk-old Heritage 32 females (genetically selected for lower NPP requirement) were individually placed in 0.46 m x 0.33 m x 0.41 m (l x w x h) cages, with an empty cage between birds to avoid cross-contamination of feed or fecal samples collected. After 7 d feeding a standard broiler breeder layer diet (2.7% Ca and 0.37% NPP), birds were classified by FM content into three blocks (high, average, and low FM) to insure balanced individual bird variation among treatment. Four birds per combination were assigned per subsequent dietary treatment.

The birds were fed a standard broiler breeder diet with a mechanical feeder. Thereafter, individual PVC feeders were used to provide the dietary treatments with minimal cross-contamination between cages.

Drinking water was analyzed for mineral content at the NCDA&CS Agronomic Division Plant/Waste/Solution Section with levels of Ca, P, K, Na, Cl, and Mg being 15.10, 0.29, 3.93, 8.62, 10.30, and 3.85 ppm, respectively.

The daylength in the house was maintained at 16 hours and house temperature was maintained between 60º F and 80º F using curtains and/or heaters with fans.

Cages were fitted with individual aluminum pans to collect feces and a 250 mL beaker to collect the liquid portion (LP) of feces that drained from the feces daily (Figure 4). The aluminum fecal collection pans were located with enough distance from the drinker to

avoid water contamination of the fecal material. The LP volume was measured separately from the remaining fecal material with a graduated cylinder on a daily basis.

At d 0 (pre-treatment collection), d 7, and d 14 of the experimental period, the fecal LP volume per bird was measured, mixed with their respective feather and feed-free solid feces, and then homogenized in an identified individual plastic bag. A sub-sample was placed in a collection pan and dried to a constant weight in an oven at 176º F for determination of dry matter. A second sub-sample was sent to a USDA-ARS laboratory for mineral analysis. The determination of the absolute change in percentage fecal moisture (Δ%FM) over various periods of time while consuming the four experimental diets was calculated as follow:

$$\Delta\ \%FM = \%FM\ d\ 7\ or\ d\ 14 - \%FM\ d\ 0$$

where negative values indicated drier feces with respect to d 0 FM base value.

Egg production was recorded daily from a week before dietary treatments, d 7 to d14, and during the last week of the trial, two eggs per hen were collected. Egg weight, shell weight and thickness, as well as yolk weight, albumen weight and height were determined on the two eggs per hen. Blood samples from each bird were collected on d 14 for determination of blood calcium and inorganic phosphorus using colorimetric methods.

Broiler Breeder Dietary Treatments

A standard broiler grower diet (0.9% Ca, 0.45% NPP) was fed to 28 wk of age. Then each bird was fed 154 g of feed/d of a standard broiler breeder diet (2.7% Ca, 0.37% NPP) for one week before the experimental period. From d 1 to d 14, the 3.0% Ca and varied NPP dietary treatments were fed.

A total of 500 lb of basal premx was prepared and 125 lb of each of 4 dietary treatments was prepared using the basal premix and adding the required amounts of dicalcium phosphate, limestone, filler, and phytase (only diets MarP+PXP and MarP+2PXP) as appropriate for each dietary treatment. The dietary treatments (Table 2) consisted of an adequate P (AdqP), a marginal P (MarP), a marginal P plus 275 FTU/kg of feed (MarP+PXP), and a marginal P plus 550 FTU/kg of feed (MarP+2PXP). The AdqP diet contained 0.50% NPP, in contrast to the other three diets that contained 0.25% NPP. All diets were offered in mash form and were formulated to contain 2900 kcal ME/kg of feed, 14.20% crude protein, and 3.00% Ca, in comparison with the standard breeder diet, which had 2.7% Ca (Table 1).

The phytase enzyme product was Phyzyme XP (Danisco Animal Nutrition, Marlborough, UK), an *Escherichia coli*-derived phytase.

Statistical Methods

A randomized complete block design with 12 replications (birds) per dietary treatment and 4 replications (birds) per FM block (low, average, and high LP producers) was used. The general lineal model of SAS (SAS Institute, 2008) was used to analyze FM, egg production,

and egg quality variables. The mixed model of SAS (SAS Institute, 2008) was used to analyze LP data. Means were partitioned by LSMEANS, and were considered statistically different at $P<0.05$, while differences at $P<0.10$ were considered to be numerical trends.

RESULTS

No effect of dietary treatment was observed on the average hen-day production for a week before the experimental period (pre-treatment period), d 7 and d 14, with averages of 80.58, 56.06, and 74.16%, respectively. A covariance analysis revealed that even when no effect of dietary treatment was found , age (or time of sampling) influenced egg production ($P<0.01$). On the other hand, egg characteristics such as egg weight, yolk weight, albumen weight and height, and shell weight and thickness were not affected by dietary treatment (Table 3). In addition, there were no effects of the dietary treatments on the proportion of the components of the egg with respect to egg weight (data not shown).

No differences in the production of LP volume as an effect of the dietary treatments were observed (Figure 1). However, covariance analyses revealed that temperature and/or day of sampling significantly ($P<0.01$) influenced the response. Figure 2 shows the effect of dietary treatment on the difference in percentage FM at d 7 with respect to the FM at d 0. No differences in FM at d 7 were observed, with an average absolute reduction in FM of 14.35%. At d 14 birds fed the MarP+2PXP diet exhibited greater FM with respect to d 0 as compared to the other three dietary treatments ($P<0.01$).

Table 4 shows the effect of dietary treatments on the concentration of minerals in blood and feces at d 14 of the experimental period. No differences were observed for blood Ca and P, or fecal sodium (Na) and postassium (K). However, the fecal concentration of Ca was higher and P lower in birds fed the MarP and MarP+PXP diets. Birds fed the MarP+2PXP diet were intermediate for the concentration of fecal Ca, while birds fed the AdqP were intermediate for fecal P. Feces from hens fed the MarP+2PXP were lower in manganese (Mn) and zinc (Zn) than the birds fed the AdqP and MarP diets, while the content of Al for the MarP+2PXP group was less than the rest of the dietary treatments. In addition, the concentration of Fe was lowest for the MarP+2PXP fed birds, followed by MarP, and MarP+PXP birds, with the AdqP birds having the highest concentration of Fe in their feces. Magnesium concentration was lower for birds fed the higher concentration of phytase, followed by the MarP fed birds ($P<0.01$), while the AdqP and MarP+PXP fed birds excreted the most Mg.

DISCUSSION

The objective of the present study was to determine the effect of short-term inclusion of phytase in low NPP broiler breeder diets on percentage FM, reproductive performance, and egg quality variables. As shown in Figure 1, LP production was not affected by dietary treatment. However, it was evident that there was an increase in LP volume after the change to the experimental diets, which coincided with an increase in the environmental

temperature. Even though it was logical that an increase in temperature could increase water consumption and, consequently, water excretion, it cannot be ignored that at the same time the birds were changed from a 2.7% to 3.0% Ca level in the diets. Further, FM at this time was actually less (Figure 2), which indicated that changes in water holding capacity of the feces probably occurred. Previous research in commercial layers has demonstrated that increased dietary Ca could cause a temporary increase in water intake and FM as observed by Leeson and Summers (1987).

In general, feces had 14.35% less absolute FM at d 7 when compared with the initial FM at d 0. At d 14 feces had similar moisture as for d 0, except for the MarP+2PXP treatment that had approximately 10% greater absolute FM when fecal LP volume at d 0, d 7 and d 14 was compared. A clear relationship between LP volume and FM was not evident, since higher LP volume values did not result in higher FM. This could be explained by a difference in water holding capacity of the feces as other researchers have reported. Kalmar et al. (2007) observed that African gray parrots fed fine or coarse particle size pelleted feed produced similar FM (~72%) but excreta consistency was significantly affected. They attributed this effect to the presence of non-starch polysaccharides and microbial action upon them, which have been reported to influence water-holding capacity of feces.

Another factor that could have affected FM was the fact that no Ca matrix value was assigned to the phytase, resulting in slightly different Ca:NPP ratios. Previous research had shown that an inaccurate Ca:NPP ratio could result in wet litter especially when phytase

was used (Pos et al., 2003). However, this difference was small and the greatest LP was observed in the AdqP treatment.

Furthermore, Smith et al. (2000) performed a series of experiments to assess the effect of feed mineral concentration on water intake and FM of commercial laying hens. They concluded that increasing dietary NPP, but not Ca, led to a significant linear increase in water intake and consequently to an increase in FM. However, they did not evaluate the influence of the Ca:NPP ratio *per se* on the response. Examination of the data of the P experiment in this previous research revealed that the increase in NPP in the diets from 0.30 to 2.00% NPP (or Ca:NPP ratio of ~13 to 2) made the birds produce feces with a linear increase in FM values between 71.1 and 80.8%. The calcium portion of the experiment evaluated Ca levels between 3.0 and 5.0% (or Ca:NPP ratios between ~7 and 11) without finding a significant linear relationship between the Ca level and FM. Due to the close homeostatic relationship between Ca and P it may have been more appropriate to conclude that Ca increases did not affect FM when Ca:NPP ratio was between ~7 and 11. This was confirmed by the similarity in the FM values of the P experiment when the birds were fed diets with Ca:NPP ratios between ~13 and 5. When birds were fed a lower Ca:NPP ratio diet (4 and 2) they produced wetter feces, but those ratios were not tested in the Ca experiment.

No differences in blood Ca or P were observed among dietary treatments in the present experiment, which suggested that the mineral levels in the feed and water were reasonable and homeostatic mechanisms were sufficient to maintain similar mineral levels in blood

independent of the dietary treatment. This could explain the lack of effect observed on egg quality variables during this short-term experiment.

However, mineral excretion was affected by dietary treatment. Birds fed 2Xpase excreted less di- and trivalent cations (Al, Fe, Mg, Mn, and Zn) and excreted more P. Table 4 clearly shows the similarity in the response of the MarP and MarP+PXP birds in terms of fecal Ca and P content, which suggested a similar P availability or that a Ca:NPP ratio of around 12 caused an increased Ca and reduced P excretion. The AdqP fed birds (Ca:NPP ratio ~ 6) had an intermediate P excretion, followed by the MarP+2PXP birds. Taking in consideration that all diets (except AdqP) had the same content of total P, it could be suggested that P absorption of MarP+2PXP birds was not as effective as for the MarP and MarP+PXP birds. This could be due to the lower P requirement of this mineral by the Heritage 32 birds (Sasikala-Appukuttan et al., 2010). On the other hand, a significant phytase effect was demonstrated by the reduced excretion of other cations evaluated. It has been previously proposed that phosphate release from the phytate molecule was accompanied by the release of chelated cations (Sebastian et al., 1998) as was evident in the present research. In addition, the release of potentially hygroscopic (Castro-Freitas, 2005; Hori et al., 2001) organic macromolecules such as starch and proteins (Selle et al., 2000) that could contribute to increased FM has also been observed. Moreover, the apparent higher availability and absorption of certain cations due to inclusion of phytase in feed could negatively affect the availability of P (Sheikh et al. 1989) or disrupt absorptive mechanisms (Berner, 1976).

Based on the present results, it was concluded that under the conditions of this experiment the addition of phytase at 550 U/kg to an NPP deficient broiler breeder layer diet could result in an increased FM, when no matrix value for Ca was given to the phytase. It was important to remember that a variety of ions and molecules with different electrochemical properties were clearly released during phytase action on its substrate. A better understanding of the chemical and physical properties of feed, digesta, and fecal material could help to better elucidate what could be the physiological factors leading to this response.

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TABLE I. 1. Ingredient composition and calculated analysis for broiler breeder diets fed a week before the experimental period.

Ingredients	Standard Broiler Breeder Diet
	(%)
Corn	68.90
Soybean meal, 48% CP	19.50
Limestone	5.95
Wheat Bran	0.06
Poultry fat	2.50
Dicalcium phosphate	1.79
Salt	0.50
Premixes[1]	0.68
L-Threonine	0.02
DL-Methionine	0.10
	100.00
Calculated nutrients[2]	
ME, kcal/kg	2900
Crude protein, %	14.50
Lysine, %	0.78
Methionine + Cystine, %	0.62
Threonine, %	0.56
Calcium, %	2.70
Non-phytate phosphorus, %	0.37
Sodium, %	0.20

[1]Premixes provided the following (per kg of diet): vitamin A, 13,200 IU; vitamin D_3, 4,000 IU; vitamin E, 66 IU; vitamin B_{12}, 39.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; D-pantothenate, 22 mg; menadione (K_3), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; D-biotin, 252 µg; selenium (as Na_2SeO_3), 0.30 mg; manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride, 1,200 mg; coccidiostat, 500 mg.

[2]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

TABLE I. 2. Ingredient composition and calculated analysis for broiler breeder diets fed during d 1 to 14 of the experimental period.

Ingredients	Dietary Treatments[1]			
	AdqP	MarP	MarP+PXP	MarP+2PXP
	(%)			
Corn	67.04	67.04	67.04	67.04
Soybean meal, 48% CP	19.03	19.03	19.03	19.03
Limestone	6.28	7.18	7.18	7.18
Filler (vermiculite)	2.59	2.39	2.38	2.36
Poultry fat	2.11	2.11	2.11	2.11
Dicalcium phosphate	1.73	1.03	1.03	1.03
Salt	0.50	0.50	0.50	0.50
Premixes[2]	0.65	0.65	0.65	0.65
L-Threonine	0.04	0.04	0.04	0.04
DL-Methionine	0.03	0.03	0.03	0.03
Phytase[3]	0.00	0.00	0.014	0.028
	100.00	100.00	100.00	100.00
Calculated nutrients[4]				
ME, kcal/kg	2900	2900	2900	2900
Crude protein, %	14.20	14.20	14.20	14.20
Lysine, %	0.50	0.75	0.75	0.75
Methionine + Cystine, %	0.63	0.63	0.63	0.63
Threonine, %	0.56	0.56	0.56	0.56
Calcium, %	3.00	3.00	3.00	3.00
Non-phytate phosphorus, %	0.75	0.25	0.25[5]	0.25[5]
Sodium, %	0.20	0.20	0.20	0.20

[1]The four dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 275 FTU/kg feed, and MarP+2PXP = MarP plus 550 FTU/kg feed. Dietary treatments were fed from 7 to 14 d of the experimental period.

[2]Premixes provided the following (per kg of diet): vitamin A, 13,200 IU; vitamin D_3, 4,000 IU; vitamin E, 66 IU; vitamin B_{12}, 39.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; D-pantothenate, 22 mg; menadione (K_3), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; D-biotin, 252 µg; selenium (as Na_2SeO_3), 0.30 mg; manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride, 1,200 mg; coccidiostat, 500 mg.

[3]Phytase was added to MarP+PXP and MarP+2PXP diets at 275 and 550 FTU/kg, respectively.

[4]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

[5]Phytase contribution of 0.12% non-phytate phosphorus.

TABLE I. 3 Effect of inclusion of phytase in low non-phytate phosphorus broiler breeder diets on egg quality variables.

	Dietary Treatments[1]					
Variable	AdqP	MarP	MarP+PXP	MarP+2PXP	SEM[2]	P-value
Egg Weight, g	54.66	54.80	56.75	55.91	1.10	NS
Yolk Weight, g	15.72	15.94	15.67	15.53	0.44	NS
Albumen Weight, g	33.52	33.59	35.67	35.06	0.76	NS
Albumen Height, mm	7.81	8.40	9.04	7.93	0.30	NS
Shell Weight, g	5.42	5.28	5.42	5.32	0.13	NS
Shell Thickness, mm	0.40	0.39	0.39	0.39	<0.01	NS

[1]Thefour dietary treatments consisted of AdqP = adequate P, MarP = marginal P, 1XPae = MarP plus 275 FTU/kg feed, and MarP+2PXP = MarP plus 550 FTU/kg feed. Dietary treatments were fed from 1 to 14 d of the experimental period.
[2]SEM for n=12 hens per diet.

TABLE I. 4. Effect of inclusion of phytase in low non-phytate phosphorus broiler breeder diets on blood mineral concentration and fecal mineral excretion.

		Dietary Treatments[1]					
Mineral	Source	AdqP	MarP	MarP+PXP	MarP+2PXP	SEM[2]	P-value
	Blood	(mg/dL)					
Calcium		25.03	27.95	27.28	28.01	2.02	NS
Phosphorus		4.63	4.92	4.70	4.26	0.34	NS
	Feces	(mg/kg DM)					
Calcium		64,907^{B}	83,042^{A}	82,160^{A}	73,820^{AB}	3974	<0.01
Phosphorus		17,355^{ab}	13,342^{b}	13,599^{b}	21,048^{a}	2128	0.05
Sodium		282	296	263	339	26	NS
Potassium		25,562	24,030	25,080	26,187	730	NS
Aluminum		6,550^{A}	5,678^{A}	5,785^{A}	4,499^{B}	296	<0.01
Iron		7,636^{A}	6,131^{BC}	6,916^{AB}	5,452^{C}	329	<0.01
Magnesium		13,781^{A}	12,668^{AB}	12,930^{A}	10,985^{B}	459	<0.01
Manganese		715	680	625	524	29	<0.01
Zinc		512	519	478	437	18	<0.05

[a, b] Means across columns lacking a common superscript are significantly different at $P < 0.05$.

[A, B] Means across columns lacking a common superscript are significantly different at $P < 0.01$.

[1]The four dietary treatments consisted of AdqP = adequate P, MarP = marginal P, 1XPae = MarP plus 275 FTU/kg feed, and MarP+2PXP = MarP plus 550 FTU/kg feed. Dietary treatments were fed from 1 to 14 d of the experimental period.

[2]Pooled standard error of the mean for n=12 hens per diet.

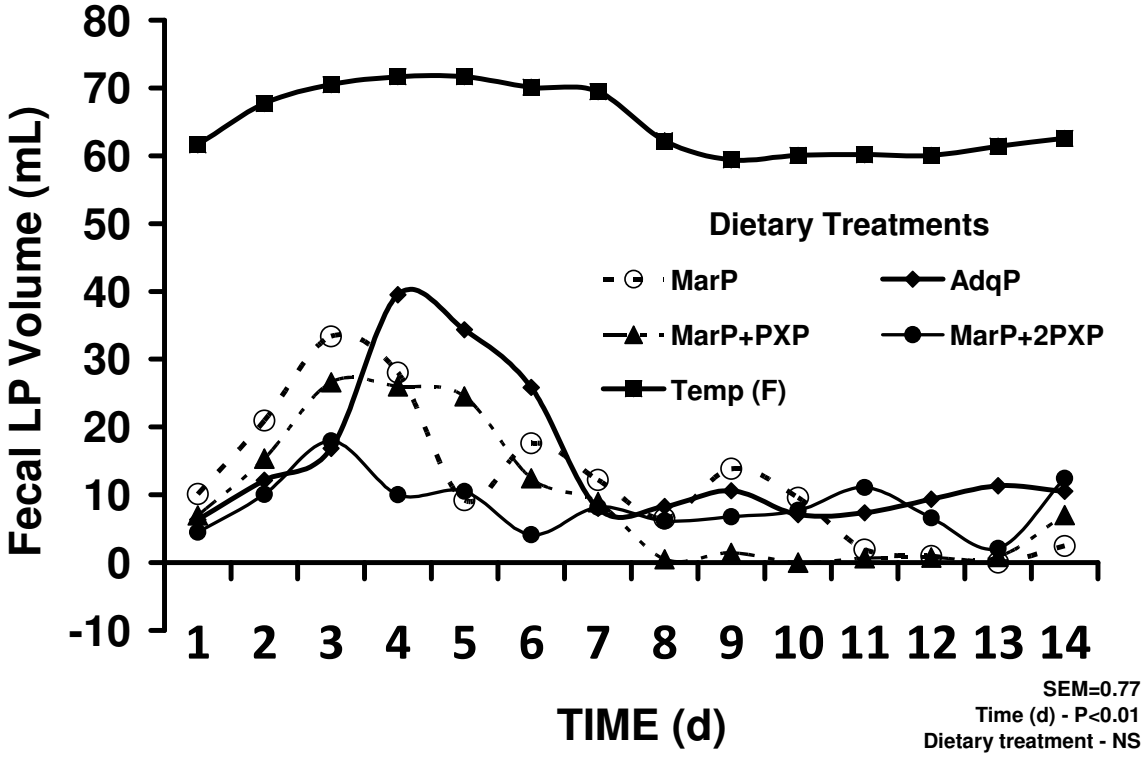

FIGURE I. 1. Daily fecal liquid portion (LP) volume produced during treatment (7 to 14 d) period. The four dietary treatments consisted of AdqP = adequate P (diamond), MarP = marginal P (circle), MarP+PXP = MarP plus 275 FTU/kg feed (triangle), and MarP+2PXP = MarP plus 550 FTU/kg feed (dot). The SEM represents the pooled standard error of the mean for n=48 birds.

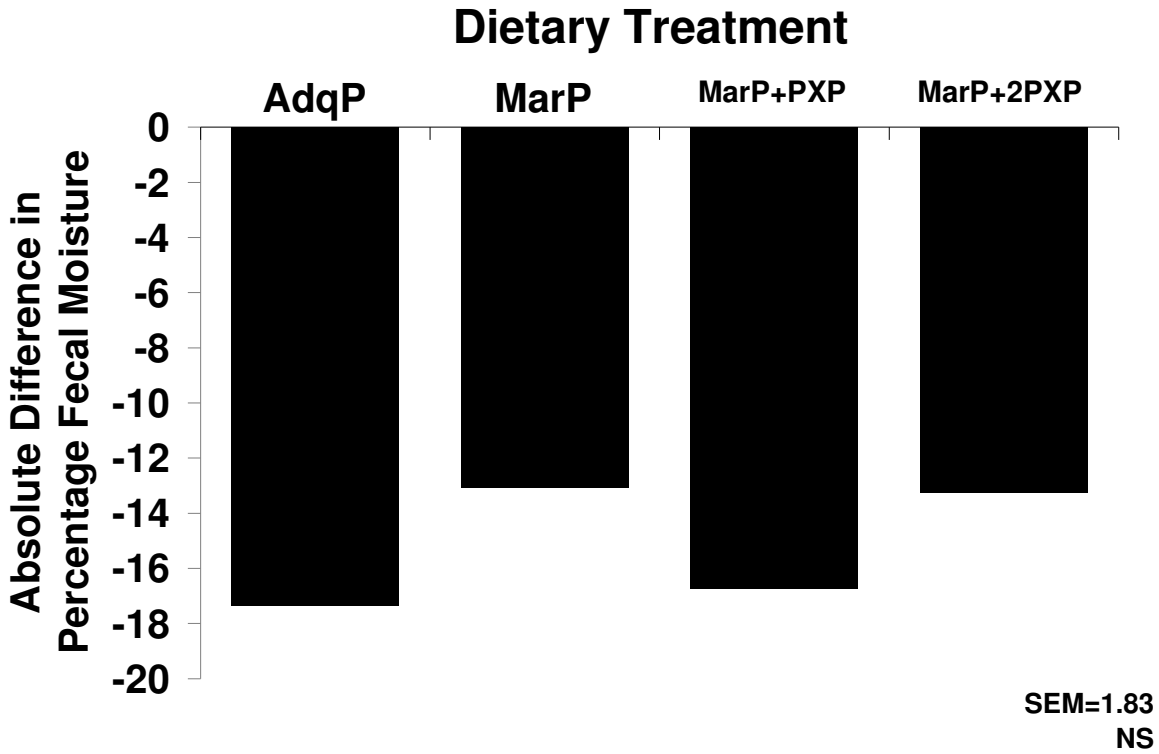

FIGURE I. 2. Effect of inclusion of phytase in low non-phytate phosphorus broiler breeder diets on the absolute change in percentage fecal moisture (1 to 7 d) after 7 d in dietary treatments. The four dietary treatments consisted of AdqP = adequate P, MarP = marginal P, 1XPae = MarP plus 275 FTU/kg feed, and MarP+2PXP = MarP plus 550 FTU/kg feed. Dietary treatments were fed from 1 – 7 d of the experimental period. SEM represents pooled standard error of the mean for n=48 birds.

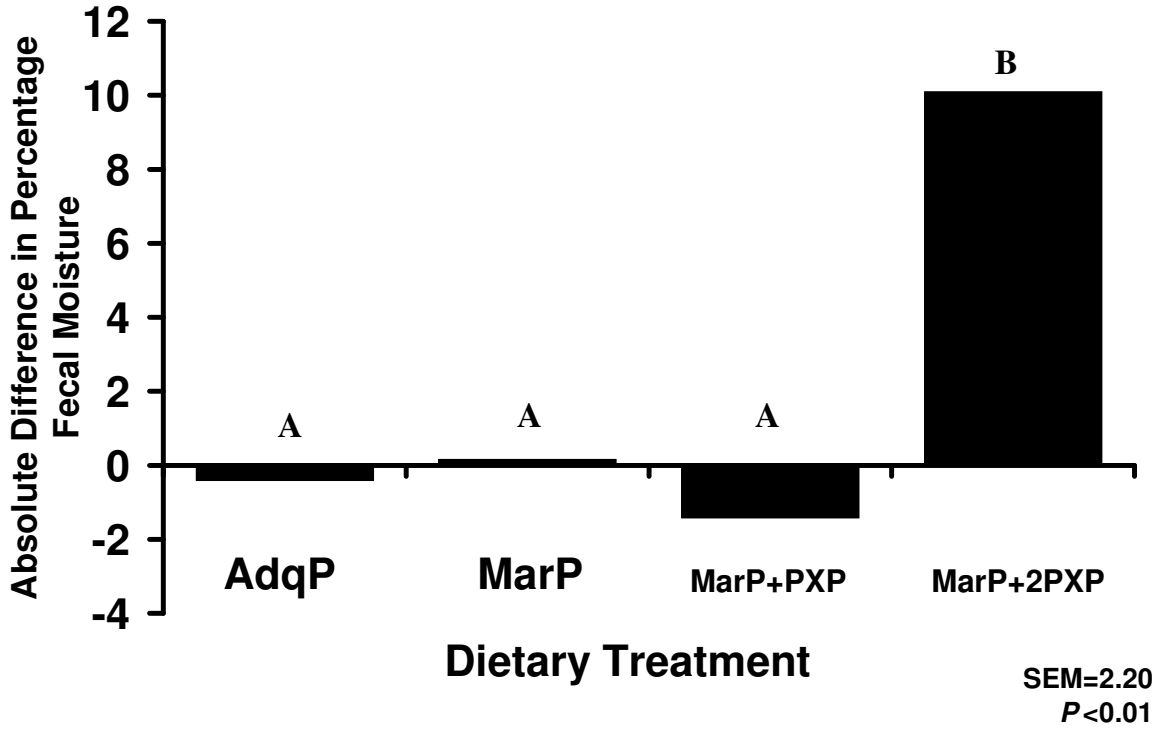

FIGURE I. 3. Effect of inclusion of phytase in low non-phytate phosphorus broiler breeder diets on the absolute change in percentage fecal moisture (1 to 14 d) after 14 d on dietary treatments. Th four dietary treatments consisted of AdqP = adequate P, MarP = marginal P, 1XPae = MarP plus 275 FTU/kg feed, and MarP+2PXP = MarP plus 550 FTU/kg feed. Dietary treatments were fed from 1 – 14 d of the experimental period. SEM represents pooled standard error of the mean for n=48 birds. Means across columns lacking a common superscript (A,B) are significantly different at the $P < 0.01$.

FIGURE I. 4. Aluminum feces collecting tray with hanging beaker to collect liquid portion (LP) of the feces. The trays were placed with enough distance from the drinkers and feeders, and an empty cage between birds was left to avoid sample and treatment cross-contamination.

MANUSCRIPT II. Effects of Phyzyme XP and Avizyme 1502 on the performance of broiler breeders and their progeny

ABSTRACT

Previous research has suggested that there may be negative vertical effects of multi-enzyme cocktails fed to broiler breeders on broiler progeny performance when the progeny also received the same enzyme(s) in the feed. Thus, a study was conducted to evaluate the use of phytase (Phyzyme XP; PXP; Dansico Animal Nutrition) with or without a combination of xylanase, amylase, and protease (XAP) enzymes (Avizyme 1502; Danisco Animal Nutrition) in broiler breeder diets on the performance of broiler breeders and their progeny when the progeny also received similar diets. There were 1104 21-wk-old Ross 708SF females and 128 Ross 344 males allocated to 4 treatments with 4 replicate pens each. Treatments were an Adequate Phosphorus (AdqP), Marginal P (MarP), MarP+PXP, and MarP+PXP+XAP. The AdqP diet contained 2900 kcal ME/kg and all three MarP diets were 80 kcal ME/kg lower. Diets were formulated with corn, soybean meal, and 20% distillers dried grains with solubles (DDGS). Phytase was added at 500 FTU/kg to replace 0.12% non-phytate phosphorus (NPP) and 0.10% calcium (Ca). Eggs and any mortality were collected and recorded twice daily. Fertility and hatchability were determined weekly. All variables were evaluated for the cumulative experimental period and for 10 4-wk-periods from 24 to 64 wk of age. Egg production variables were not affected by breeder dietary

treatments for the cumulative production period. However, AdqP breeders exhibited better overall fertility than their counterparts ($P<0.01$). Also, MarP+PXP+XAP breeders exhibited an inferior productive performance ($P<0.05$) during periods 6, 7, and 10. Similar dietary treatments were applied to broiler progeny produced at 40 wk of breeder age to complete a 4 X 4 design. There were 2304 chicks (278 males and 278 females per breeder treatment) distributed among 96 mixed sex pens. Broiler BW and feed consumption (FC) were measured at 0, 16, 35, and 41 d, and any mortality was collected and weighed twice daily. At 42 d one male per pen was killed to evaluate carcass traits. There were no significant main effects or interactions for broiler BW, adjusted feed conversion ratio (AdjFCR), or carcass traits due to the breeder diets. However, MarP+PXP and MarP+PXP+XAP broiler dietary treatments had an overall positive effect on broiler performance. Based on the data collected, inclusion of PXP and XAP in nutritionally marginal broiler breeder diets did not improve cumulative egg production or fertility. Furthermore, XAP addition negatively affected breeder livability. On the other hand, addition of PXP and XAP to broiler diets improved performance, particularly in males. Nevertheless, an additive effect of PXP and XAP enzymes was not evident. Finally, the lack of interactions and breeder diet effects on overall broiler performance indicated no negative vertical effect of parental treatment on progeny response to the dietary enzymes evaluated in this study.

INTRODUCTION

Most of the P in cereals and legumes has been shown to be present in the phytate form, which has been reported to be not fully available to non-ruminants (O'Dell et al., 1972). Only 21% of the total P in corn and 35% in soybean meal (NRC, 1994) were reported to be available for absorption, while the remainder was excreted in the feces due to the lack of sufficient endogenous intestinal phytase. Therefore, inorganic sources of P have been generally used as dietary mineral supplements to meet animal nutritional requirements.

The high content of nitrogen (N) and P in chicken manure and litter makes them excellent sources for use as a crop fertilizer. However, excess P and other nutrients can reach lakes and reservoirs by "surface runoff" and "subsurface flow" (Sharpley, 1999), which will promote the eutrophication process. Strategies have been explored to reduce such environmental impacts of agricultural operations as well as to reduce feed costs. One strategy has been to reduce safety margins during the feed formulation process as it has been shown that a reduction in inorganic P in the diet has a positive correlation with mineral content in the feces (Power and Angel, 2008). This approach has been used in conjunction with the inclusion of phytase in feed. Phytase enzymes have been reported to release the P groups that comprise the phytate molecule, an anti-nutritional compound with a high chelation capacity for divalent cations such as Zn, Fe, Mn, Ca, Cu, Mg, and others. Another modality to reduce feed cost has been the increased use of by-products such as distillers dried grains with solubles (DGGS) and poultry by-product meal. Carbohydrases

and protease cocktails have also been employed to increase nutrient availability from grains and by-products.

Brake et al. (2003) studied the effect of the inclusion of enzyme cocktails in broiler breeder feed on progeny performance. From 22 to 64 wk of age broiler breeders were fed one of four diets consisting of two levels of available P (NPP) (0.13 or 0.40%) and two levels of a fungal phytase cocktail (0 or 500 FTU/kg feed) with enzyme side activities that included protease, alpha-amylase, xylanase, cellulase, and beta-glucanase (Wu et al., 2004). The same enzyme product was then fed to the broiler breeder progeny. No interactions were observed between breeder and broiler diets, but the breeder diet effect on broiler performance was apparent across all broiler diets. These authors reported that broilers from breeders fed the phytase cocktail were significantly smaller ($P<0.05$) at 35 and 42 d of age relative to broilers from breeders fed no enzyme cocktail. This effect was explained by a reduced feed consumption (FC) ($P<0.10$) from 22 to 35 d for broilers from breeders fed the phytase cocktail. However, it was unknown if the negative effect observed in the broiler progeny was due to the phytase or to the side activities present in the enzyme cocktail. The objective of this study was to delineate this question by studying the effects of inclusion of PXP alone or in combination with XAP on the performance of broiler breeders and their progeny when the progeny also received similar diets.

MATERIALS AND METHODS

Rearing and Laying Phase Management

There were 1200 1-d-old female Ross 708SF and 288 male Ross 344 broiler breeders placed in an enclosed fan-ventilated house with 16 14.30 m^2 female pens and 16 4.56 m^2 male pens. From 0 to 21 d all female pens had 4 tube feeders, then 3 feeders to 12 wk of age, and then 4 feeders through the end of the experiment, while males had one tube feeder per pen at all times. Two bell type drinkers per female pen were used, while males had one per pen. The litter material used was wood shavings and general litter management in the pullet and breeder houses had a focus to develop natural immunity to coccidia. The lighting program consisted of 23 h per day to 7 d, and then 8 h day to 21 wk of age. At 21 wk of age the day length was increased to 14 h, then 15 h at 23 wk, 15.5 h at 5% production, and finally 16 h at 50% production.

At 21 wk of age, 64 females and 7 males were moved to each of 16 pens in a breeder house. Each of the 16 pens comprised 15.9 m^2 with two thirds plastic slats and one third pine shavings litter. There were a total of four tube female feeders with restriction grills to exclude males (45 x 58 mm holes) and one male feeder in each pen. Each pen had two bell drinkers and two double and four single nests. Eggs were collected and recorded twice daily before being stored prior to incubation.

Pullet and Breeder Diets

Day-old chicks (females and males) were fed a 17% crude protein (CP) mash starter feed to 42 d of age followed by a 15% CP mash feed to 25 wk of age. A concave feeding program

(Walsh and Brake, 2007) was used to deliver a cumulative FC at 21 wk of age of 8825 g (~25,600 kcal) for females and 11,305 g (~32,800 kcal) for males. At 25 wk of age breeders began to be fed one of 4 different dietary treatments that consisted of an adequate P control (AdqP), a marginal P control (MarP), a MarP plus 500 U of phytase (Phyzyme XP TPT; Dansico Animal Nutrition; MarP+PXP), and a MarP+PXP plus 0.10% of a xylanase, amylase, and protease cocktail (Avizyme 1502; Danisco Animal Nutrition; MarP+PXP+XAP) (Table 1). The AdqP diet was calculated to contain 2900 kcal ME/kg of feed, while the three MarP diets (MarPs) were 80 kcal ME/kg less. The energy allocation at peak production was 450 kcal ME/d (152 g feed /b/d) for the AdqP diet, which was on the low end of recommended ME requirements so that a reduction in performance of the MarP breeders that had a ME allocation of 438 kcal/d at the same FC was expected. All the treatment diets were corn-soybean based with 20% DDGS, while calculated to be be isonitrogenous with similar amino acid levels and balance.

The phytase enzyme product was Phyzyme XP TPT (Danisco Animal Nutrition, Marlborough, UK), an *Escherichia coli*-derived phytase, added at 500 FTU/kg. The enzyme cocktail, Avizyme 1502 (Danisco Animal Nutrition, Malborough, UK), was used at the manufacturer's recommended level to provide 300 U of xylanase/kg from *Trichoderma longibrachiatum*, 4000 U of protease from *Bacillus subtilis* and 400 U of amylase from *Bacillus amyloliquofaciens*.

The Ca and NPP content of AdqP and MarP diets were 2.8% and 0.37%, respectively. The MarP+PXP and MarP+PXP+XAP diets had 2.7% Ca and 0.25% NPP, taking into

consideration the inclusion of PXP (500 FTU/kg) to be equivalent to 0.12% NPP and 0.10% Ca.

Broiler Progeny Management

After 21 d of incubation, chicks were hatched, feather sexed, and separately maintained by breeder pen at all times until placement. A total of 24 birds (12 females and 12 males) per pen were permanently identified with neck tags, and placed in each of 96, 2.23 m^2 pens. During the first week, 3 supplemental feeder trays were placed in every pen, then reduced to 2 until 10 d, and finally the final feeder tray was removed at 14 d. All pen floors were covered with wood shavings, and each pen had 1 tube feeder and 1 bell-type drinker, plus a plastic drinker font during the first 7 d of age. The lighting program to 7 d consisted of 23 h of light, then 21 h to 21 d of age. After 21 d natural light at natural day length entered the house through translucent curtains to control excessive growth and improve livability.

Broiler Dietary Treatment

The 4 broiler dietary treatments tested were AdqP, MarP, MarP+PXP, MarP+PXP+XAP (Tables 2, 3, and 4). The AdqP diets were calculated to contain 3070, 3150, and 3200 kcal ME/kg for the starter, grower, and finisher diets, respectively. All MarP diets were designed to be 150 kcal ME/kg lower for each diet type. Broilers were provided feed for *ad libitum* consumption with starter feed offered as crumbles, while grower and finisher feeds were provided in pellet form. All the treatment diets were corn-soybean based with 10% corn DDGS, while being isonitrogenous with similar amino acid levels and balances.

Data Collection

*Broiler Breeders***.** All males and females were group weighed at placement and individually at 20 wk of age. After moving to the laying house, all males and 20 randomly selected females per pen were weighed at 24, 30, 36, 42, 50, 58, and 64 wk of age. Nest and floor eggs were collected and recorded twice daily. Nest eggs were collected and identified by pen, separated from floor eggs, and stored at 62º F for incubation purposes. Sixty eggs per pen were set every week to determine fertility and hatchability. After hatching was completed, eggs that did not hatch were examined macroscopically to determine fertility and/or time of embryonic mortality. Egg samples were collected at 28, 46, 53, 59, and 64 wk of age for egg quality assessment. Egg weight, yolk weight, albumen weight and height, and egg shell weight were determined in order to calculate egg component percentages based on the total egg weight.

*Broiler Progeny***.** Group BW by sex, and FC were measured at 0, 16, 35, and 41 d, and mortality was recorded daily for adjusted feed conversion ratio (AdjFCR) calculations. At 41 d one male per pen was selected for processing and determination of carcass traits.

Statistical Methods

Broiler Breeders. A one-way ANOVA using a completely randomized block design with 4 replicate pens per dietary treatment was employed. Variables such as egg production and fertility data were determined and analyzed on a weekly basis and by periods from 24 to 64 wk of age. There was a total of 10, 4-wk periods, except for period 10 that comprised 5 wk. Data were examined for normality of distributions and homogeneity of variance, and data were transformed when necessary. The general lineal model of SAS Institute (2008) was

used to analyze variables of egg production and BW and differences among means were partitioned by LSMEANS. The glimmix procedure of SAS Institute (2008) was used to analyze fertility data where each individual egg was taken as a binomial event, either fertile or infertile with an appropriate contrast used for means comparison. The lifetest procedure of SAS was used for survival analysis. Means were considered to be statistically different when $P \leq 0.05$, while means different at $P \leq 0.10$ were considered to be numerical trends.

*Broiler Progeny***.** A randomized complete block design with a 4 x 4 factorial arrangement was used (broiler breeder diet x broiler diet). For variables where no significant interactions were found, the data was analyzed a second time by one-way ANOVA to delineate the treatment main effects on the variable studied, with a total of 24 replicate pens per treatment. The general lineal model of SAS was used to analyze live performance and carcass traits. Variable means were partitioned by LSMEANS and statements of statistical significance were based on $P<0.05$ unless otherwise indicated.

RESULTS

Broiler Breeders

Samples showed no significant differences among treatments in BW samples at 24, 30, 38, 42, 50, or 58 wk of age (Table 5). However, when all birds were weighed at 64 wk AdqP and MarP+PXP birds were significantly heavier than MarP+PXP+XAP birds (Table 5). Hens fed the MarP diet were intermediate between the MarP+PXP and MarP+PXP+XAP

hens. No significant differences among dietary treatments were observed for male BW (Table 5).

With respect to the production variables eggs per hen housed (EHH), hen-day production (HDP), and feed per egg (FEGG) from 24 to 64 wk, no statistical differences were found among breeder treatments, with averages of 158.8 eggs, 55.4%, and 310 g of feed/egg, respectively (Table 6).

However, when these data were evaluated by production period differences were observed during periods 4 ($P<0.10$), 6 and 7 ($P<0.01$), and 10 ($P<0.05$) where birds fed the MarP+PXP+XAP produced fewer EHH (Figure 1) and exhibited lower HDP (Figure 2) than birds fed the three other treatments. During period 4 the MarP+PXP+XAP birds laid 3.5 EHH at a 50.4% HDP rate, while the AdqP, MarP, and MarP+PXP birds laid 4.2, 3.8, and 3.8 EHH, at 59.9, 54.0, and 54.7% HDP, respectively. During period 6 birds receiving the MarP+PXP+XAP diet produced 3.6 EHH and exhibited HDP of 51.7%. AdqP, MarP, and MarP+PXP birds laid 4.0, 3.9, and 3.9 EHH with HDP rates of 57.1, 55.3, and 55.4%, respectively. During period 7, MarP+PXP+XAP hens laid an average of 3.8 EHH and had a HDP of 54.0%. In contrast, the AdqP, MarP, and MarP+PXP hens laid 4.0, 4.1, and 4.1 EHH, while the HDP was 57.5, 58.2, and 58.4%, respectively. Finally, during period 10 EHH decreased to 3.0 and HDP to 42.5% for breeders fed the MarP+PXP+XAP diet. Also, a decrease in EHH was observed for the AdqP, MarP, and MarP+PXP breeders (3.3, 3.4, and 3.3), as well as HDP rates (46.9, 47.9, and 46.7%, respectively). The survival analysis (Figure 3) demonstrated that females fed AdqP, MarP, and MarP+PXP diets had longer

survival times than MarP+PXP+XAP females (P<0.01), while no statistical differences were observed among males.

Broiler breeders fed the AdqP diet exhibited significantly (P<0.01) better fertility from 24 to 64 wk than hens fed the other three dietary treatments (97.5% versus 95.5%). Hatchability variables were not statistically different for the cumulative production phase (24 to 64 wk of age) with averages of 3.0%, 2.2%, 90.0%, and 93.8% for early dead, late dead, total hatchability, and hatchability of fertile eggs, respectively.

Numerically better fertility (P<0.10) was observed for AdqP birds during periods 6 (97.7%) and 7 (98.3%), while the remaining treatments averaged 96.9% and 96.1% during periods 6 and 7, respectively (Figure 4). During period 8, AdqP birds had a statistically higher (P<0.01) fertility of 98.6% compared to the remainder of the dietary treatments that had an average fertility of 93.4%. No significant differences were detected in early dead but trends (P<0.10) were observed during periods 4 and 10 where breeders fed MarP and MarP+PXP diets, respectively, exhibited greater percentage early dead than did the other treatments. During period 5 the percentage late dead was higher (P<0.05) for MarP+PXP birds (2.6%) when compared with AdqP and MarP+PXP+XAP birds (1.2 and 0.4%, respectively). The MarP birds were intermediate with 1.4% late dead. Hatchability rates were statistically different during periods 6 and 9. During period 6 breeders fed the AdqP and MarP+PXP+XAP diets had higher fertility (93.8 and 93.7%, respectively) than breeders fed the MarP+PXP diet (90.5%), while MarP breeders (92.8%) were intermediate. During period 9 a change was observed in which MarP+PXP birds had higher hatchability (92.1%) than MarP (87.5%) and MarP+PXP+XAP (86.0%) with AdqP birds intermediate (91.5%)

between MarP and MarP+PXP, but different from the MarP+PXP+XAP diet. Hatchability of fertile eggs was not different, although there was a trend (P<0.10) for the MarP (96.0%) breeders to be higher than MarP+PXP+XAP breeders (92.8%), with AdqP and MarP+PXP breeders intermediate (94.1 and 95.2%, respectively).

No statistical differences were found concerning egg components at 28, 46, 53, 59, and 64 wk of age. Overall means for egg, yolk, albumen, and shell weight were 63.0, 19.1, 37.1, and 5.4 g, respectively, while the average albumen height was 7.3 mm.

Broiler Progeny

No significant interactions were observed between breeder diet and broiler diet for any productive performance and carcass trait variables. Therefore, for the purpose of brevity, only main effects will be reported.

Breeder Diet Effect

Female BW at hatching (Table 7) was greater (P<0.05) for broilers from breeders fed the MarP diet. However, at 16 d broilers from breeders fed the MarP+PXP diet were heavier (P<0.01; 553 g) than MarP broilers (548 g), with broilers from breeders fed the AdqP diet intermediate (557 g). Finally, broilers from MarP+PXP+XAP breeders were smaller at 16 d (533.5 g) than all their counterparts. At 35 d, statistical differences had disappeared, leaving only a trend similar to that observed at 16 d.

Broiler males from breeders fed the MarP+PXP+XAP diet were smaller (P<0.05) at hatching than male broilers from breeders fed AdqP and MarP diets, with MarP+PXP broilers intermediate. At 16 d males broilers from breeders fed MarP+PXP+XAP diet were smaller than the male progeny from the other three breeder dietary treatments. No

statistical differences in broiler BW were found at 35 or 41 d, with averages of 2164 g and 2661 g, respectively.

A transient reduction in FC was detected at 16 d where progeny from MarP+PXP+XAP breeders consumed less feed ($P<0.01$) than the other treatments. However, this difference disappeared by 35 d.

No differences in the percentage dressed carcass, drums, *Pectoralis major*, *Pectoralis minor*, thighs, or wings were detected (Table 8). However, the percentage fat pad in the MarP progeny was numerically higher.

Broiler Diet Effect

There were no significant differences among broiler dietary treatments for female BW (Table 9) at 0 and 41 d with averages of 39 and 2181 g, respectively. However, at 16 d females fed the MarP+PXP diet were heavier ($P < 0.01$; 561 g) than MarP broilers (548 g) with MarP+PXP+XAP intermediate (554 g). Furthermore, AdqP females were the smallest with an average BW of 535 g. No statistical differences were found at 35 d but a similar trend for female BW as at 16 d was observed.

Although there were no differences in male BW at hatching, by 16 d males fed the MarP+PXP diet (591 g) and the MarP+PXP+XAP diet (570 g) were heavier ($P<0.01$) than their counterparts. Although statistical differences in male BW disappeared by 35 d, a trend similar to that observed at 16 d remained to 41 d.

On the other hand, FC at 16 d was greater ($P<0.01$) for MarP+PXP and MarP+PXP+XAP broilers (763 and 758 g, respectively). By 35 d, FC by AdqP broilers was less ($P<0.05$; 2571 g) than for MarP (2620 g) and MarP+PXP+XAP broilers (2640 g), while MarP+PXP

broilers were intermediate (2603 g). However, by 41 d AdqP broilers had consumed more feed ($P<0.05$; 1241 g) than birds fed the two enzyme treatments, with MarP broilers (1186 g) intermediate.

Broilers that received the AdqP diet had poorer AdjFCR ($P<0.05$; 1.45) by 16 d compared to their counterparts. Nevertheless, no differences were observed after 35 d although a trend detected at 41 d suggested that AdqP and MarP broilers had poorer AdjFCR than AdqP+PXP+XAP broilers.

No differences among treatments were observed in female or male broiler mortality. Female mortality averaged 1.04, 2.00, and 2.69% at 16 d, 35 d, and 41 d, respectively. Overall male mortality was 1.13, 2.69, and 5.64% at the same ages.

Broiler carcass traits (Table 10) were not significantly affected by breeder dietary treatments, apart from a trend observed where broilers from breeders fed MarP+PXP had less percentage fat pad than broilers from the other treatments.

DISCUSSION

In theory, the MarP breeders were expected to have the poorest performance due to the reduction of 80 kcal ME/kg of feed (-12 kcal/day at peak egg production), and the AdqP and MarP+PXP+XAP to have been similar due to the added ME contribution of XAP. However, no difference in egg production was observed between the AdqP and MarP diets, which suggested that the ME requirement to maintain egg production could be less than 450

kcal ME/d using our particular feeding program under these circumstances. However, productive performance observed in this experiment was lower than that obtained by Plumstead et al. (2007) that used a 2925 kcal ME/kg diet throughout life. These differences may be explained by reduced ME for starter, grower, and layer diets (all diets: -25 kcal ME; MarP layer diets: -105 kcal ME), while using the same feeding program for all the treatments. These changes produced a smaller daily energy allocation for all treatments during the growing phase and for MarP birds during the laying period, when compared with the allocation used by Plumstead et al. (2007). This could have limited nutrient reserves required for adequate persistency during the laying period. Another possible reason for an overall reduced performance could be the high inclusion rate of DDGS in the present study. Lumpkins et al. (2005) observed a reduction in eggs per hen housed when low-density diets with 15% DDGS were fed.

No differences were observed in production variables at peak lay (period 2). However, during periods 4 to 7 and 10 statistical differences in EHH, HDP, and female mortality were evident and negatively affected in the MarP+PXP+XAP breeders. Period 4 observations coincided with high summer temperatures, necessitating female feed reductions that quantitatively affected the coccidiostat dosage given in the feed, followed by a coccidiosis outbreak that was controlled by administering the medication in the drinking water. Despite the re-establishment of normal feed allocation following the hot weather that kept coccidia under control, the MarP+PXP+XAP birds never exhibited consistent production during the experimental period, possibly as a long term effect of the disease and/or dietary treatment effect. Despite all birds having the same cumulative nutrition to

photostimulation, other factors such as heat stress (Niu et al., 2009; Quinteiro et al., 2010; Sohail et al., 2010;) and reduced nutrient allocations due to decreased feed allocation (females only) (Hangalapura et al., 2005) could modulate immune function, especially in a high nutrient demand situation as with laying birds. Additionally, it was possible that the protease in the feed could have disrupted the protease-protease inhibitor balance in the gut, promoting an excessive remodeling of the lumen that affected nutrient absorption (Antalis et al., 2007). Also, it has been reported that many microorganisms such as coccidia use proteases as their mechanism to invade host cells (Feng et al., 2007). Thus, a combination of events could have logically occurred.

On the other hand, the AdqP birds exhibited better cumulative fertility from 22 to 64 wk, and during periods 6, 7, and 8. Disregarding any enzyme effect, greater energy allocation was one possible explanation for this effect on fertility. Results clearly showed that fertility was more sensitive to energy allocation than was egg production, but no consistent differences or trends were detected in hatchability (Table 6) or egg components. Similarly, Romero-Sanchez et al. (2008) reported that reduced breeder male daily nutrient allocation negatively affected fertility. No cumulative differences in hatchability were observed and there were no consistent effects on hatchability during the different periods, apart from periods 6 and 9. Consistent with the present data, Bahnja et al. (2005) fed broiler breeders two level of NPP (0.18% and 0.30%) and two levels of phytase (0 and 500 FTU/kg) without any enzyme effect on performance. Further, Berry et al. (2003) found that the addition of phytase at 300 FTU/kg to NPP deficient diets had a negative effect on hatchability but no other performance variables were affected.

Broiler breeder energy allocation affected female progeny BW at hatching, while no effect was observed for male progeny other than a reduced chick weight for the MarP+PXP+XAP progeny. These results were in agreement with a preliminary experiment conducted in our laboratory where chicks from the same breeder flock (30 wk of age) were grown to 42 d. In the previous experiment, the MarP female progeny had a BW at hatching of 40 g, while their counterparts averaged 39 g ($P \leq 0.01$). Moreover, the MarP male progeny weighed 41 g versus 39 g ($P \leq 0.05$) from the other three treatments. Previous research conducted in mammals has shown that maternal nutrient restriction can negatively affect muscle fiber number and composition, and change lipid deposition due to down-regulation of enzymes involved in fatty acid oxidation (such as carnitine palmitoyltransferase – 1; CPT-1) in the offspring (Zhu et al., 2006). Also, Rao et al. (2009) found that maternal nutrient restriction can affect offspring growth pre and post hatching. However, to our knowledge the effect of marginal energy levels on productive performance of broiler breeder strains and their progeny had not been previously assessed at the time of the present research. We can infer that these broiler breeder dietary effects on female and male broiler BW were transient, as a result of a significantly lower FC to 16 d. By 41 d no significant differences in BW, FC, and AdjFCR remained. Despite the absence of differences in most carcass traits, progeny from MarP breeders accumulated more abdominal fat ($P < 0.06$) at 42 d than did MarP+PXP progeny. Others have reported that breeder nutrition affected progeny development and performance (Spratt and Leeson, 1987; Kidd et al., 2005) and that maternal nutrient restriction down-regulated enzymes important for energy metabolism (Zhu et al., 2006), which might predispose higher fat deposition.

Greater broiler BW for the MarP+PXP and MarP+PXP+XAP males and MarP+PXP females was observed at 16 d as a consequence of a greater FC. By 41 d the MarP+PXP+XAP males demonstrated a numerically greater BW as compared to the AdqP and MarP males. Due to their slower growth rate the AdqP broilers birds had poorer AdjFCR at 16 d than did the rest of the treatments. By 41 d the MarP+PXP+XAP broilers had numerically better AdjFCR than did AdqP and MarP broilers. These results were in agreement with those of Woyengo et al. (2010) who found that the addition of phytase at 600 FTU/kg plus a multicarbohydrase cocktail to corn-soybean meal-canola based diets improved broiler BW gain and feed conversion at 21 d. Also, Dilger et al. (2004) observed increased BW gain, FC, and improvements in feed efficiency when broilers were fed different levels of microbial phytase. Moreover, Cowieson and Adeola (2005) added different levels of PXP and XAP alone or in combination to broiler diets with marginal levels of ME and concluded that the addition of PXP and XAP individually improved productive performance while there could be a positive additive effect when fed in combination.

Based on the data collected, inclusion of PXP and XAP in nutritionally marginal broiler breeder diets did not improve cumulative production, fertility, or hatchability variables. Furthermore, XAP addition appeared to negatively affect survival in females. On the other hand, the addition of PXP and XAP to broiler diets improved performance, especially in males. However, an additive effect of enzymes in broiler diets was not clearly apparent. Finally, the lack of an interaction of breeder and broiler diets or breeder diet effect on

cumulative broiler performance indicated that there was no negative vertical effect of parental treatment on progeny response to the dietary enzymes evaluated in this study.

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TABLE II.1. Ingredient composition and calculated analysis for broiler breeder layer dietary treatments (25 to 64 wk of age).

Ingredients	Dietary Treatment[1]			
	AdqP	MarP	MarP+PXP	MarP+PXP +XAP
	(%)			
Corn	53.20	55.25	55.25	55.25
Soybean meal (48% CP)	10.00	9.79	9.79	9.79
Corn DDGS	20.00	20.00	20.00	20.00
Wheat bran	5.50	5.50	5.50	5.50
Poultry fat	2.24	0.40	0.40	0.40
Limestone	6.52	6.53	6.65	6.65
Dicalcium phosphate	1.05	1.04	0.37	0.37
Filler (vermiculite)	0.20	0.20	0.74	0.64
Sodium chloride	0.45	0.45	0.45	0.45
Premixes[2]	0.67	0.68	0.68	0.68
Phytase premix[3]	0.00	0.00	0.01	0.01
Avizyme 1502[4]	0.00	0.00	0.00	0.10
L-Lysine	0.11	0.11	0.11	0.11
DL-Methionine	0.02	0.01	0.01	0.01
L-Threonine	0.04	0.04	0.04	0.04
	100.00	100.00	100.00	100.00
Calculated nutrients[5]				
ME, kcal/kg	2902	2820	2820	2820
Crude protein, %	15.16	15.21	15.21	15.21
Lysine, %	0.75	0.75	0.75	0.75
Methionine + Cysteine, %	0.63	0.63	0.63	0.63
Threonine, %	0.56	0.56	0.56	0.56
Calcium, %	2.80	2.80	2.80[4]	2.80[4]
Non-phytate phosphorus, %	0.40	0.40	0.40[4]	0.40[4]
Sodium, %	0.20	0.20	0.20	0.20

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.

[2]Premixes provided the following (per kg of diet): vitamin A, 13,200 IU; vitamin D_3, 4,000 IU; vitamin E, 66 IU; vitamin B_{12}, 39.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; D-pantothenate, 22 mg; menadione (K_3), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; D-biotin, 252 µg; selenium (as Na_2SeO_3), 0.30 mg; manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride, 1,200 mg; coccidiostat, 500 mg.

[3]Phyzyme XP TPT (Danisco Animal Nutrition, Marlborough, UK), an Escherichia coli-derived phytase, was added at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

[4]Avizyme 1502 (Danisco Animal Nutrition, Malborough, UK), is composed of xylanase (300 U/kg), *Trichoderma longibrachiatum*, protease (4000 U/kg), *Bacillus subtilis* and amylase (400 U/kg), and *Bacillus amyloliquofaciens*.

[5]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

TABLE II.2. Ingredient composition and calculated analysis for broiler progeny dietary treatments for the starter period (1 to16 d of age).

Ingredients	Dietary Treatment[1]			
	AdqP	MarP	MarP+PXP	MarP+PXP +XAP
	(%)			
Corn	52.17	56.52	56.52	56.52
Soybean meal (48% CP)	29.50	28.65	28.65	28.65
Corn DDGS	10.00	10.00	10.00	10.00
Poultry Fat	3.71	0.20	0.20	0.20
Limestone	1.45	1.46	1.58	1.58
Dicalcium phosphate	1.68	1.67	0.99	0.99
Filler (sand)	0.10	0.10	0.63	0.63
Sodium chloride	0.47	0.47	0.47	0.47
Premixes[2]	0.63	0.63	0.63	0.63
Phytase premix[3]	0.00	0.00	0.03	0.03
Avizyme 1502[4]	0.00	0.00	0.00	0.10
L-Lysine	0.13	0.14	0.14	0.14
DL-Methionine	0.15	0.15	0.15	0.15
L-Threonine	0.01	0.01	0.01	0.01
	100.00	100.00	100.00	100.00
Calculated nutrients[5]				
ME, kcal/kg	3070	2917	2917	2917
Crude protein, %	22.00	22.00	22.00	22.00
Lysine, %	1.24	1.24	1.24	1.240
Methionine + Cystine, %	0.91	0.91	0.91	0.91
Threonine, %	0.79	0.79	0.79	0.79
Calcium, %	1.00	1.00	1.00[4]	1.00[4]
Non-phytate phosphorus, %	0.45	0.45	0.45[4]	0.45[4]
Sodium, %	0.20	0.20	0.20	0.20
Calcium, analyzed, %	0.92	0.80	0.80	0.79
Total phosphorus, analyzed, %	0.72	0.72	0.64	0.62

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.

[2]Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D_3, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K_3), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na_2SeO_3), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

[3]Phyzyme XP TPT (Danisco Animal Nutrition, Marlborough, UK), an Escherichia coli-derived phytase, was added at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

[4]Avizyme 1502 (Danisco Animal Nutrition, Malborough, UK), is composed of xylanase (300 U/kg), *Trichoderma longibrachiatum*, protease (4000 U/kg), *Bacillus subtilis* and amylase (400 U/kg), and *Bacillus amyloliquofaciens*.

[5]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

TABLE II. 3. Ingredient composition and calculated analysis for broiler progeny dietary treatments for the grower period (17 to 35 d of age).

Ingredients	Dietary Treatment[1]			
	AdqP	MarP	MarP+PXP	MarP+PXP +XAP
	(%)			
Corn	57.48	61.81	61.81	61.81
Soybean meal (48% CP)	24.30	23.44	23.44	23.44
Corn DDGS	10.00	10.00	10.00	10.00
Poultry Fat	4.11	0.62	0.62	0.62
Limestone	1.29	1.30	1.40	1.40
Dicalcium phosphate	1.32	1.31	0.63	0.63
Filler (sand)	0.08	0.11	0.67	0.56
Sodium chloride	0.48	0.47	0.46	0.47
Premixes[2]	0.62	0.62	0.62	0.62
Phytase premix[3]	0.00	0.00	0.03	0.03
Avizyme 1502[4]	0.00	0.00	0.00	0.10
L-Lysine	0.17	0.18	0.18	0.18
DL-Methionine	0.13	0.12	0.12	0.12
L-Threonine	0.02	0.02	0.02	0.02
	100.00	100.00	100.00	100.00
Calculated nutrients[5]				
ME, kcal/kg	3156	3004	3004	3004
Crude protein, %	20.00	20.00	20.00	20.00
Lysine, %	1.13	1.13	1.13	1.13
Methionine + Cystine, %	0.83	0.83	0.83	0.83
Threonine, %	0.72	0.72	0.72	0.72
Calcium, %	0.85	0.85	0.85[4]	0.85[4]
Non-phytate phosphorus, %	0.38	0.38	0.38[4]	0.38[4]
Sodium, %	0.20	0.20	0.20	0.20
Calcium, analyzed, %	0.94	0.86	0.80	0.78
Total phosphorus, analyzed, %	0.67	0.66	0.52	0.53

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.

[2]Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D_3, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K_3), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na_2SeO_3), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

[3]Phyzyme XP TPT (Danisco Animal Nutrition, Marlborough, UK), an Escherichia coli-derived phytase, was added at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

[4]Avizyme 1502 (Danisco Animal Nutrition, Malborough, UK), is composed of xylanase (300 U/kg), *Trichoderma longibrachiatum*, protease (4000 U/kg), *Bacillus subtilis* and amylase (400 U/kg), and *Bacillus amyloliquofaciens*.

[5]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

TABLE II. 4. Ingredient composition and calculated analysis for broiler progeny dietary treatments for the finisher period (36 to 42 d of age).

.	Dietary Treatment[1]			
	AdqP	MarP	MarP+PXP	MarP+PXP +XAP
	(%)			
Corn	62.85	66.77	66.77	66.77
Soybean meal (48% CP)	19.24	18.75	18.75	18.75
Corn DDGS	10.00	10.00	10.00	10.00
Poultry Fat	3.84	0.43	0.43	0.43
Limestone	1.26	1.27	1.37	1.37
Dicalcium phosphate	1.19	1.18	0.49	0.49
Filler (sand)	0.09	0.08	0.64	0.54
Sodium chloride	0.48	0.48	0.48	0.48
Premixes[2]	0.62	0.62	0.62	0.62
Phytase premix[3]	0.00	0.00	0.03	0.03
Avizyme 1502[4]	0.00	0.00	0.00	0.10
L-Lysine	0.24	0.24	0.24	0.24
DL-Methionine	0.15	0.14	0.14	0.14
L-Threonine	0.04	0.04	0.04	0.04
	100.00	100.00	100.00	100.00
Calculated nutrients[5]				
ME, kcal/kg	3196	3045	3045	3045
Crude protein, %	18.15	18.27	18.27	18.27
Lysine, %	1.04	1.04	1.04	1.04
Methionine + Cystine, %	0.80	0.80	0.80	0.80
Threonine, %	0.67	0.67	0.67	0.67
Calcium, %	0.80	0.80	0.80[4]	0.80[4]
Non-phytate phosphorus, %	0.35	0.35	0.35[4]	0.35[4]
Sodium, %	0.20	0.20	0.20	0.20
Calcium, analyzed, %	0.90	0.78	0.75	0.75
Total phosphorus, analyzed, %	0.65	0.62	0.50	0.50

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.

[2]Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D_3, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K_3), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na_2SeO_3), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

[3]Phyzyme XP TPT (Danisco Animal Nutrition, Marlborough, UK), an Escherichia coli-derived phytase, was added at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

[4]Avizyme 1502 (Danisco Animal Nutrition, Malborough, UK), is composed of xylanase (300 U/kg), *Trichoderma longibrachiatum*, protease (4000 U/kg), *Bacillus subtilis* and amylase (400 U/kg), and *Bacillus amyloliquofaciens*.

[5]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

TABLE II. 5. Effect of broiler breeder layer dietary treatment on broiler breeder female and male body weight during the laying phase (28 to 64 wk of age).

		Dietary Treatments[1]					
Age	Sex	AdqP	MarP	MarP+PXP	MarP+PXP+XAP	SEM	P-value
(wk)	Females			(g)			
24		2673	2688	2605	2.620	0.037[2]	NS
30		3109	3095	3092	3.057	0.035[2]	NS
38		3305	3217	3214	3.110	0.053[2]	NS
42		3416	3346	3378	3.417	0.034[2]	NS
50		3453	3478	3463	3.357	0.052[2]	NS
58		3631	3509	3525	3.472	0.050[2]	NS
64		3707[a]	3549[bc]	3683[ab]	3.528[c]	0.041[3]	<0.05
	Males						
24		3.580	3.528	3.453	3.458	0.054[3]	NS
30		3.783	3.643	3.680	3.605	0.082[3]	NS
38		4.245	3.808	3.790	3.698	0.205[3]	NS
42		4.068	3.880	3.960	3.863	0.113[3]	NS
50		4.450	4.135	4.318	4.193	0.120[3]	NS
58		4.600	4.413	4.403	4.315	0.111[3]	NS
64		4.783	4.555	4.550	4.575	0.142[3]	NS

[a, b, c]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$).

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.

[2] SEM for n=4 pens with 20 hens weighed per pen.

[3] SEM for n=4 pens with all females or males weighed per pen.

TABLE II. 6. Effect of broiler breeder layer dietary treatment on egg production, fertility, and hatchability during the laying phase (25 to 64 wk of age).

Variable	Treatments[1]					
	AdqP	MarP	MarP+PXP	MarP+PXP +XAP	SEM	P-value
Eggs per hen housed, n	162.83	160.46	159.55	152.21	4.02	NS
Feed per egg, g	254	261	261	258	6	NS
Hen-day production, %	59.01	57.49	57.81	57.54	1.13	NS
Fertility[2], %	97.53^{A}	95.53^{B}	95.71^{B}	95.39^{B}	-	< 0.01
Fertile hatchability, %	93.75	94.06	93.88	93.62	0.65	NS
Hatchability, %	90.37	88.87	89.74	87.93	0.73	NS
Early dead, %	3.03	3.16	3.27	3.47	0.23	NS
Late dead, %	3.21	2.72	2.85	2.92	0.34	NS

[A, B]Means across rows lacking a common superscript are significantly different ($P < 0.01$).

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.

[2]Standard error was not available with glimmix procedure due to categorical data.

TABLE II. 7. Effect of breeder layer dietary treatment on female and male broiler progeny performance (40 wk of age breeder flock).

Variable	Age (d)	n	Treatment[1] AdqP	MarP	MarP+PXP	MarP+PXP +XAP	SEM[2]	P-value
Female BW, g	0	24	39.2^{b}	39.7^{a}	39.2^{b}	38.9^{b}	0.2	<0.05
	16	24	557^{AB}	548^{B}	559^{A}	534^{C}	4	< 0.01
	35	24	1814^{x}	1793^{xy}	1816^{x}	1781^{y}	11	<0.10
	41	24	2176	2172	2201	2173	15	NS
Male BW, g	0	24	39.9^{a}	40.0^{a}	39.7^{ab}	39.3^{b}	0.2	<0.05
	16	24	583^{a}	585^{a}	592^{a}	568^{b}	5	<0.05
	35	24	2170	2187	2166	2134	16	NS
	41	24	2666	2689	2641	2646.	22	NS
Feed consumption, g	0-16	24	754^{A}	755^{A}	760^{A}	734^{B}	5	< 0.01
	17-35	24	2631	2607	2613	2582	16	NS
	36-41	24	1171	1190	1172	1195	25	NS
Adjusted feed conversion ratio, g:g	0-16	24	1.42	1.44	1.41	1.44	0.01	NS
	0-35	24	1.73	1.72	1.72	1.72	0.01	NS
	0-41	24	1.90	1.90	1.90	1.90	0.01	NS

$^{x, y}$Means across rows lacking a common superscript are significantly different at the $P < 0.10$.
$^{a, b}$Means across rows lacking a common superscript are significantly different at the $P < 0.05$.
$^{A, B, C}$Means across rows lacking a common superscript are significantly different at the $P < 0.01$.
[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.
[2]SEM for n=24 mixed sex pens.

TABLE II. 8. Effect of broiler breeder dietary treatment on male broiler progeny carcass traits as a percentage of male BW at 41 d of age (40 wk of age breeder flock).

Variable	N	Treatments[1]				SEM[2]	P-value
		AdqP	MarP	MarP+PXP	MarP+PXP +XAP		
		(%)					
Breast skin	24	1.76	2.06	1.87	1.80	0.10	NS
Dressed carcass	24	73.65	73.25	74.16	73.11	0.38	NS
Fat pad	24	1.26^{xy}	1.44^{x}	1.19^{y}	1.35^{xy}	0.07	<0.10
Drums	24	10.33	10.17	10.45	10.20	0.13	NS
Thighs	24	12.97	13.14	13.02	13.01	0.24	NS
Wings	24	7.37	7.35	7.63	7.27	0.10	NS
Pectoralis major	24	18.92	18.61	18.51	18.30	0.32	NS
Pectoralis minor	24	4.22	4.04	4.14	3.99	0.09	NS

[x-y]Means across rows lacking a common superscript are significantly different ($P < 0.10$)

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.

[2] SEM for n=24 pens with a sub-sampling of 1 male broiler per pen.

TABLE II. 9. Effect of broiler dietary treatment on female and male broiler performance (40 wk of age breeder flock).

Variable	Age (d)	n	Treatment[1] AdqP	MarP	MarP+PXP	MarP+PXP + XAP	SEM[2]	P-value
Female body weight, g	0	24	39.5	39.2	39.1	39.0	0.2	*NS*
	16	24	535^{C}	548^{B}	561^{A}	554^{AB}	4	<0.01
	35	24	1791^{y}	1786^{y}	1824^{x}	1804^{xy}	11	<0.10
	41	24	2180	2169	2190	2183	15	*NS*
Male body weight, g	0	24	39.6	39.71	39.6	40.1	0.2	*NS*
	16	24	563^{B}	573^{B}	591^{A}	597^{A}	5	<0.01
	35	24	2064	2153	2165	2194	16	*NS*
	41	24	2641^{y}	2637^{y}	2653^{xy}	2712^{x}	22	<0.10
Feed consumption, g	0-16	24	741^{B}	742^{B}	763^{A}	758^{A}	5	<0.01
	17-35	24	2571^{b}	2620^{a}	2603^{ab}	2640^{a}	16	<0.05
	36-41	24	1240.6^{a}	1185.7^{ab}	1168.3^{b}	1133^{b}	25	<0.05
Adjusted feed conversion ratio, g:g	0-16	24	1.45^{a}	1.43^{b}	1.42^{b}	1.42^{b}	0.01	<0.05
	0-35	24	1.71	1.73	1.71	1.73	0.01	*NS*
	0-41	24	1.91^{x}	1.91^{x}	1.89^{xy}	1.88^{y}	0.01	0.10

$^{x, y}$Means across rows lacking a common superscript are significantly different at the $P < 0.10$.
$^{a, b}$Means across rows lacking a common superscript are significantly different at the $P < 0.05$.
$^{A, B, C}$Means across rows lacking a common superscript are significantly different at the $P < 0.01$.
[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.
[2]SEM for n=24 mixed sex pens.

TABLE II. 10. Effect of broiler dietary treatment on male broiler carcass traits as a percentage of live BW at 41 d of age (40 wk of age breeder flock).

Variable	N	Treatments[1]				SEM[2]	P-value
		AdqP	MarP	MarP+PXP	MarP+PXP + XAP		
		(%)					
Dressed carcass	24	73.48	73.91	73.56	73.21	0.38	NS
Breast skin	24	1.97	1.74	1.96	1.82	0.10	NS
Fat pad	24	1.42	1.23	1.23	1.38	0.07	NS
Drums	24	10.21	10.34	10.39	10.21	0.13	NS
Thighs	24	12.74	13.00	13.34	13.05	0.24	NS
Wings	24	7.45	7.45	7.45	7.28	0.10	NS
Pectoralis major	24	18.40	19.05	18.36	18.53	0.31	NS
Pectoralis minor	24	4.01	4.17	4.16	4.07	0.09	NS

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502.

[2] SEM for n=24 pens with a sub-sampling of 1 male broiler per pen.

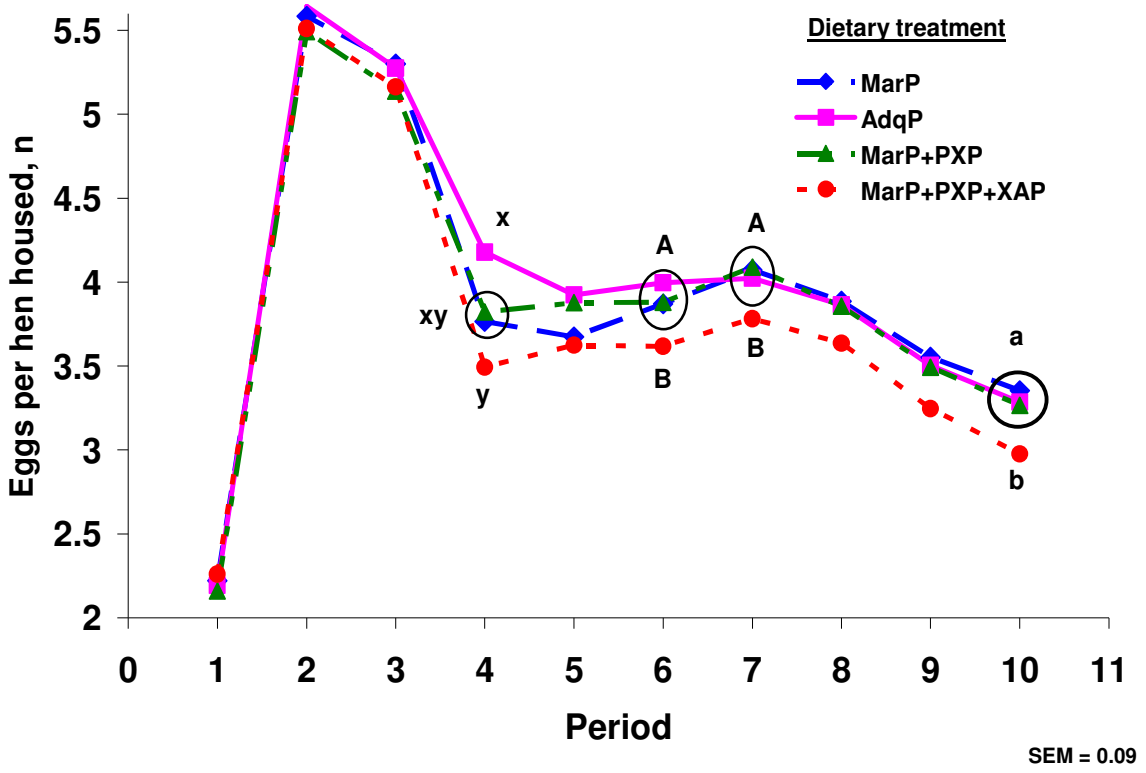

FIGURE II. 1. Effect of breeder layer dietary treatment on weekly eggs per hen housed for successive production periods from 24 to 64 wk of age. Period means inside a circle share the same superscript(s). The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502. Period: 1 = 24 to 27 wk; 2 =28 to 31 wk; 3 = 32 to 35 wk; 4 = 36 to 39 wk; 5 = 40 to 43 wk; 6 = 44 to 47 wk; 7 = 48 to 51 wk; 8 = 52 to 55 wk; 9 = 56 to 59 wk; 10 = 60 to 64 wk. The SEM is the pooled standard error of the mean. Means across rows lacking a common superscript (x,y) are significantly different at the $P < 0.10$. Means across rows lacking a common superscript (a,b) are significantly different at the $P < 0.05$. Means across rows lacking a common superscript (A,B) are significantly different at the $P < 0.01$.

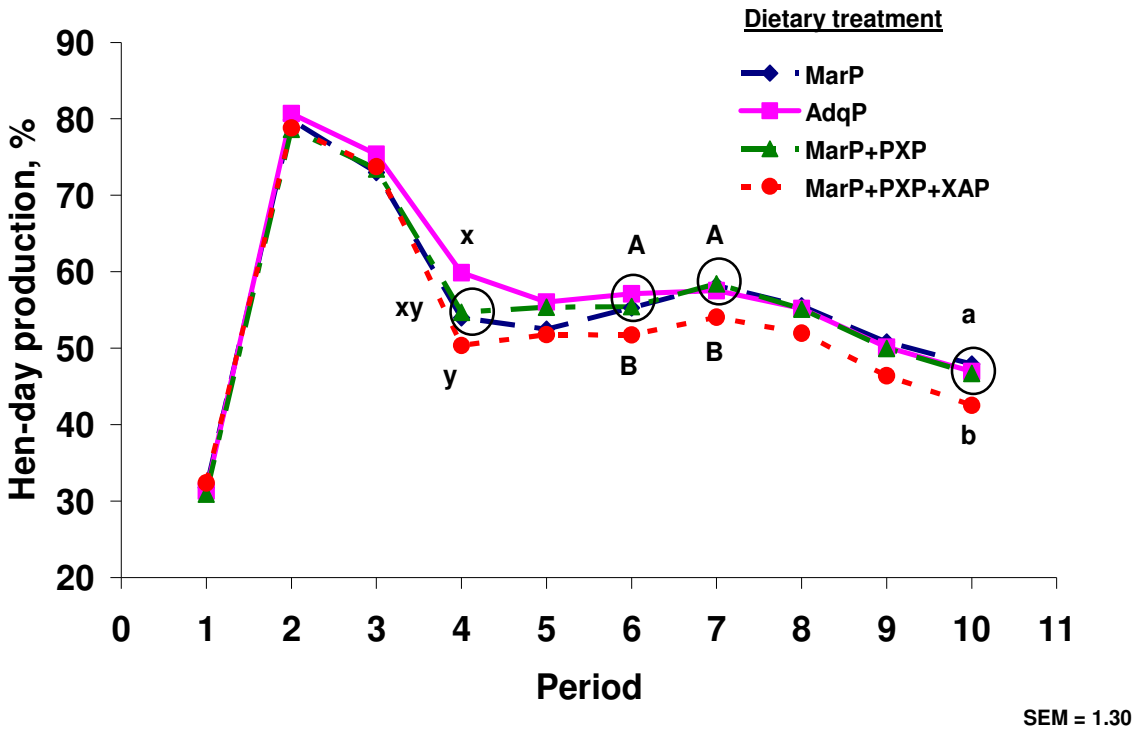

FIGURE II. 2. Effect of breeder layer dietary treatment on hen-day production for successive production periods from 24 to 64 wk of age. Period means inside a circle share the same superscript(s). The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502. Period: 1 = 24 to 27 wk; 2 =28 to 31 wk; 3 = 32 to 35 wk; 4 = 36 to 39 wk; 5 = 40 to 43 wk; 6 = 44 to 47 wk; 7 = 48 to 51 wk; 8 = 52 to 55 wk; 9 = 56 to 59 wk; 10 = 60 to 64 wk. The SEM is the pooled standard error of the mean. Means across rows lacking a common superscript (x,y) are significantly different at the $P < 0.10$. Means across rows lacking a common superscript (a,b) are significantly different at the $P < 0.05$. Means across rows lacking a common superscript (A,B) are significantly different at the $P < 0.01$.

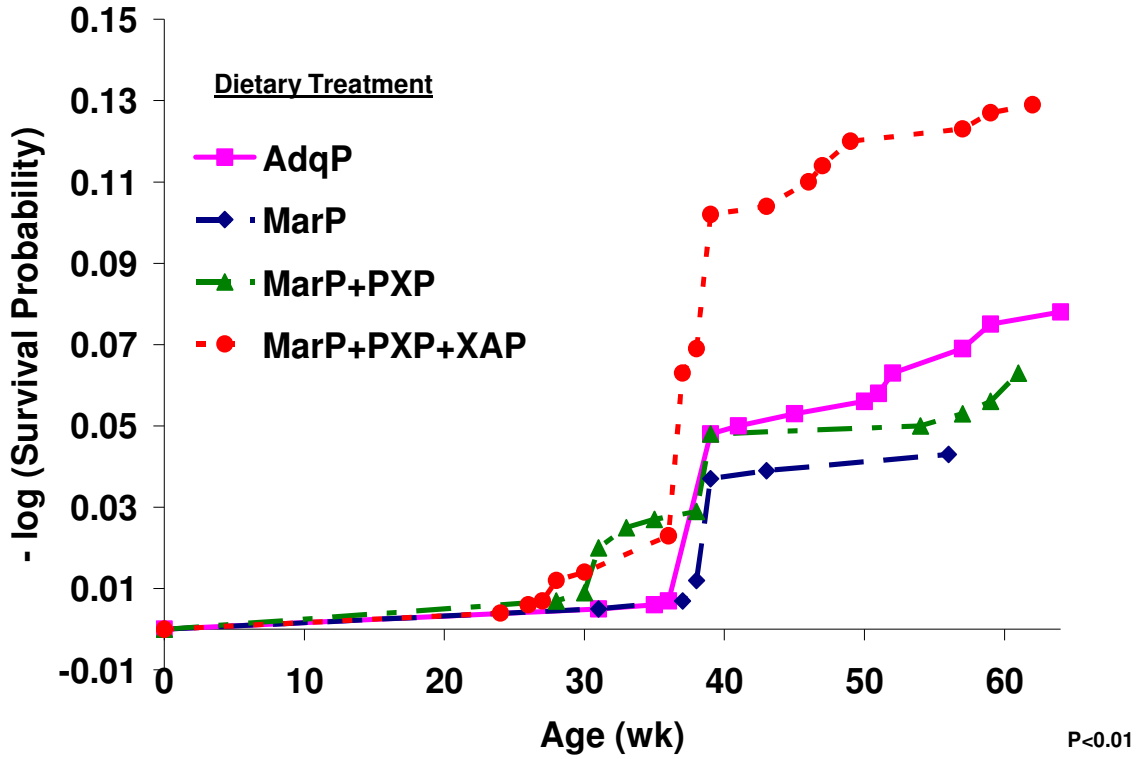

FIGURE II. 3. Effect of breeder layer dietary treatment on female survival for successive production periods from 24 to 64 wk of age. The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502. The P-value for test of equality (log-rank) over dietary treatment.

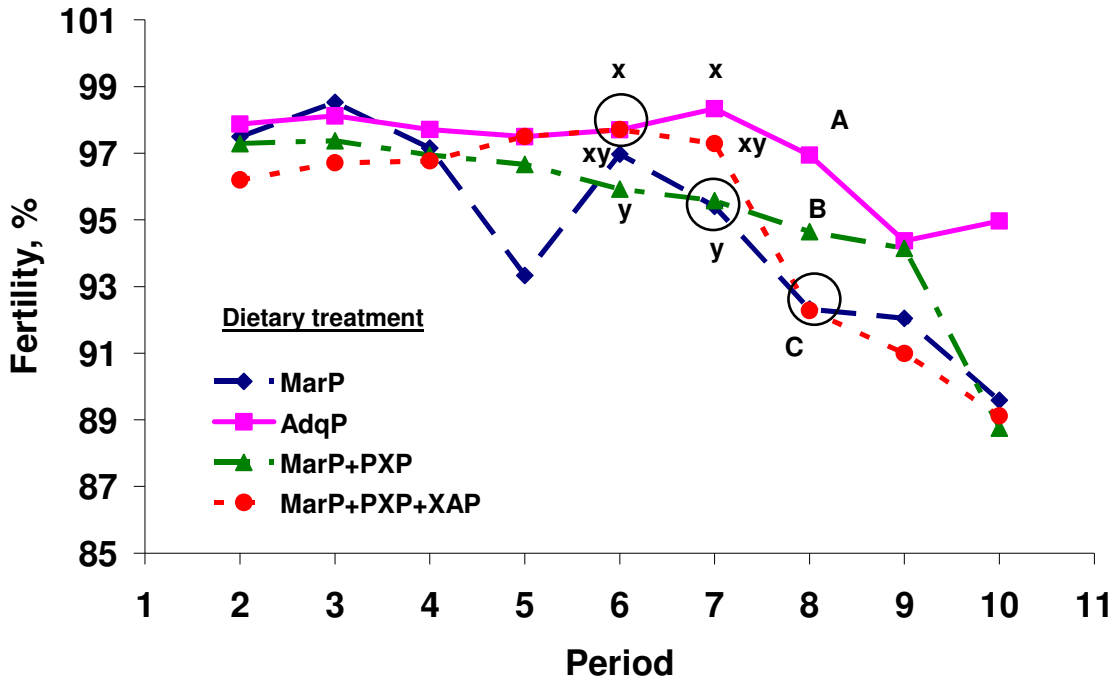

FIGURE II. 4. Effect of breeder layer dietary treatment on fertility for different production periods (24-64 wk of age). Period means inside a circle share the same superscript(s). The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP plus 0.1% inclusion of Avizyme 1502. Period: 1 = 24 to 27 wk; 2 =28 to 31 wk; 3 = 32 to 35 wk; 4 = 36 to 39 wk; 5 = 40 to 43 wk; 6 = 44 to 47 wk; 7 = 48 to 51 wk; 8 = 52 to 55 wk; 9 = 56 to 59 wk; 10 = 60 to 64 wk. The standard error is not available with glimmix procedure due to categorical data. Means across rows lacking a common superscript (x,y) are significantly different at the $P < 0.10$. Means across rows lacking a common superscript (A,B) are significantly different at the $P < 0.01$.

MANUSCRIPT III. Effects of Phyzyme XP and Avizyme 1502 in the Presence of Marginal Energy and Limited Protein on the Performance of Broiler Breeders and their Progeny

ABSTRACT

Previous research has suggested that there may be negative vertical effects of multi-enzyme cocktails fed to broiler breeders on breeder and broiler progeny performance when the progeny also received the same enzyme in the feed. To assess this, a broiler breeder study was conducted to evaluate the use of phytase (PXP) in combination with the full dose or half of the recommended dose of a combination of xylanase, amylase, and protease (XAP) enzymes. There were 928 21-wk-old Ross 708SF females and 128 Ross 344 malesallocated to 4 treatments with 4 replicate pens each. Treatment diets (2850 kcal/kg) were an Adequate Phosphorus (AdqP), Marginal P (MarP), MarP+PXP+XAP (full dose), and MarP+PXP+½XAP (half dose). The AdqP diet was fed to reach 441 kcal/d at peak egg production versus 411 kcal/d for all the MarP treatments. Eggs and mortality were collected and recorded twice daily, and BW determined every 6 wk. Similar dietary treatments were applied to broiler progeny at 31 wk of breeder age. There were 1728 male chicks (432 per breeder treatment) distributed among 96 pens. BW and feed consumption (FC) were measured at 0, 14, 21, 35, 42, and 49 d, and mortality was weighed daily. No adverse effect

was found on livability and fertility traits when the full dose of the enzyme was fed. No overall negative effects were found with the addition of the enzymes, but egg production and feed efficiency were not improved when compared to the MarP. No interactions between breeder and broiler treatment were detected. At 49 d, broilers from breeders fed the MarP+PXP+½XAP diet were numerically heavier and had improved feed conversion ($P<0.10$) as compared to broilers breeders fed the MarP diet. In conclusion, overall productive and reproductive performance were not negatively affected by a reduced energy and protein allocation or by addition of PXP and full dose of XAP, when appropriate management during heat stress episodes was practiced. Also, no negative vertical effect of parental treatment on progeny response to the dietary enzymes evaluated in this study was observed. Finally, nutritional status of the parental breeder flock, broiler progeny age, and energy content of the broiler diet could be important aspects to be considered when broiler progeny feed is formulated.

INTRODUCTION

Strategies have been explored to reduce the environmental impact of animal agricultural operations as well as to reduce feed costs. One way to approach these problems has been the inclusion of phytase in feeds, which reduced the contribution of inorganic sources of non-phytate phosphorus (NPP) in the diet. Additionally, carbohydrases and protease cocktails have become another strategy intended to increase nutrient availability from grains and grain by-products. The integration of these strategies could help ameliorate the direct and indirect detrimental effects of animal husbandry operations and sustain the profitability of these economic activities. However, previous research has shown that the inclusion of carbohydrase/protease (CPC) enzyme cocktails at the dose recommended by the manufacturer in breeder feeds could negatively affect livability, reproductive performance and have effects on early progeny productive performance (Manuscript II). It has been well documented that maternal nutrition could affect progeny intestinal function (Rebel et al., 2006) and have profound effects on the endocrine control of appetite and modulation of metabolic pathways (Bautista et al., 2008; Vickers et al., 2000) with possible detrimental effects on progeny livability (Virden et al., 2003) and health. As a consequence of maternal nutrient restriction during mid-gestation of ewes, Zhu et al. (2006) observed poor muscular fiber development, and higher accumulation of pelvic fat and intramuscular triglycerides.

Few studies have been conducted looking at the carryover effect of the inclusion of enzymes in broiler breeder diets on breeder and progeny performance. Moreover, it was unknown if

the enzyme cocktail dosage could be related to decreased performance previously observed. The objective of these experiments was to determine the effect of the inclusion of phytase and a carbohydrase-protease cocktail (CPC) at two different levels in broiler breeder feeds on breeder and broiler progeny performance when these also received the same enzymes in their feed.

MATERIALS AND METHODS

Growing Pullet and Breeder Laying Management

There were 1280 1-d-old Ross 708SF females and 288 Ross 344 males placed in a litter-floor blackout growing house. The birds were grown sex-separate thru 21 wk of age, when 928 females and 112 males were moved to a curtain-sided breeder house. The breeder house had 16 floor pens (17.52 m^2) with two-thirds slat and one-third litter. Each pen had 3 automatic drinkers, 3 tube feeders with male exclusion grills for females, and one tube feeder for males. The photoperiod was increased to 14 h at 21 wk of age and increased from 14 h to 15 h at 23 wk, then to 15.5 h at 25 wk, and 16 h at 28 wk. Natural light entered the house through open or translucent curtains during day light hours.

At 21 wk of age 64 average females and 7 average males per pen were moved to a breeder house. The house consisted of 16, 15.9 m^2 pens, with two-thirds plastic slats and one-third litter. There were a total of four tube female feeders with restriction grills to exclude males (45 x 58 mm holes), and one male feeder in each pen. Each pen had two bell drinkers and

two double and four single nests. Egg production was collected and recorded twice daily. Eggs were identified by pen and stored in a cooler at 62°F. Sixty eggs per pen were set every week to determine hatchability. After hatching at 21 d, eggs that did not hatch were subject to macroscopic embryo diagnosis to determine fertility and/or period of embryonic mortality. Mortality and eggs were collected twice daily. Group BW of all females per pen and individual BW for all males per pen was measured every 6 wk to monitor BW gain and make adjustments to the feeding program if necessary. At 30 wk of age, 180 eggs per treatment were set and incubated under standard conditions to produce broiler progeny.

Growing Pullet and Laying Breeder Diets

During the rearing period the birds were fed a standard starter feed (17% CP mash) from 1 to 42 d of age followed by a standard grower feed (15.5% CP mash) from 42 d (6 wk) to 25 wk of age (about 5% egg production). The estimated cumulative nutrition with the concave feeding programs employed at 21 wk of age was ~1800 g CP and 32,800 kcal ME, and ~ 1350 g of CP and 25,600 kcal ME for males and females, respectively.

At 25 wk of age, the birds were changed to 1 of 4 dietary treatments (Table 1), consisting of an adequate Pcontrol (AdqP), marginal P control (MarP), a MarP with the addition of Phyzyme XP (PXP; 500 FTU/kg feed) plus full dose of Avizyme 1502 (XAP), and a MarP with the addition of PXP and half dose of XAP (½XAP). All laying diets were calculated to contain 2850 kcal ME/kg and 14% CP. The AdqP diet was fed to provide 441 kcal/day at peak egg production versus 411 kcal/day for all MarP diets with the intention to create a protein and energy deficiency in birds fed any of the MarP diets. Calcium and NPP levels

were adjusted in the AdqP diet to equalize their consumption with the MarP diets, while 0.1% Ca and 0.12% NPP was removed from the two enzyme dietary treatments to account for the contribution of 500 FTU/kg feed of PXP.

All the treatment diets were corn-soybean based with 3% corn distiller's dried grains with solubles (DDGS), and were formulated to be isonitrogenous and with similar amino acid levels.

Broiler Progeny Management

At 30 wk of broiler breeder flock age, 180 eggs per treatment were set and incubated under standard conditions to produce broiler progeny. During incubation and sexing, eggs and chicks, respectively, were placed in baskets identified by broiler breeder pen.

There were 1728 day-old Ross 344 x 708 males placed in 96 floor pens (2.23 m^2). Each pen had one tube feeder and one automatic drinker. During the first week, 3 feeder trays were placed in each pen, then reduced to 2 until 10 d, and finally to 1 until 14 d. All pen floors were covered with wood shavings, and had 1 plastic supplemental drinker during the first 7 d of age. The lighting program consisted of 23 h of light during the first week, followed by 21 h during the second and third wk, and then natural light only after the third wk to the end of the experimental period. Natural light entered the house through curtains during day light hours. Brooding temperature was 97ºF at receiving, and then decreased 1º F per day to 7 d. During the second wk the temperature was decreased to 85ºF, and then to 80ºF by the third week. After that period temperature was controlled by mechanical ventilation as necessary to maintain a 75 to 80º F environment.

Mortality was collected and weighed twice daily. Pen BW and FC were determined at 1, 14, 35, 42, and 49 d of age, and adjusted feed conversion ratio (AdjFCR) was calculated per pen.

Broiler Dietary Treatments

In a manner similar to the broilers breeders, four broiler dietary treatments were fed including an AdqP, MarP, MarP+PXP+XAP (full dose), and MarP+PXP+½XAP (half dose) (Tables 2, 3, and 4). Metabolizable energy for the AdqP diet series was 2950, 3036, 3076, while MarP diets has 80, 86, and 150 kcal ME less for the starter, grower, and finisher diet series, respectively. Birds were fed *ad libitum* their respective starter diet from 1 to 14 d (22% CP crumble), followed by a grower diet (20% CP pellet) from 15 to 35 d, and finally a finisher diet (18% CP pellet) from 35 to 49 d. All of the treatment diets were corn-soybean based with 5% corn distiller's dried grains with solubles (DDGS), and 3.69% poultry by-product meal, and were isonitrogenous with similar amino acid levels.

The phytase enzyme product was Phyzyme XP TPT (Danisco Animal Nutrition, Marlborough, UK), an *Escherichia coli*-derived phytase, added at 500 FTU/kg. The enzyme cocktail, Avizyme 1502 (Danisco Animal Nutrition, Malborough, UK), was used at the manufacturer's recommended level to provide 300 U of xylanase/kg from *Trichoderma longibrachiatum*, 4000 U of protease from *Bacillus subtilis* and 400 U of amylase from *Bacillus amyloliquofaciens*.

Data Collection

Broiler Breeders

All males and females were group weighed at placement and individually at 20 wk of age. All males and females per pen were weighed at 24, 30, 36, 42, 50, 59, and 64 wk of age during the breeder laying phase. Nest and floor eggs were collected and recorded twice daily. Nest eggs were identified by pen but separated from floor eggs and stored for incubation purposes. Floor eggs were not set. A total of 60 eggs per pen were set weekly under standard incubation conditions. After hatching at 21 d, eggs that did not hatch were subjected to macroscopic embryo diagnosis to determine fertility and/or period of embryonic mortality.

Broiler Progeny

Group BW by sex, and FC were measured at 1, 14, 35, 42, and 49 d and mortality was weighed and recorded daily for AdjFCR calculations. At 41 d one average male per pen was selected for processing and determination of carcass traits.

Statistical Methods

Broiler Breeders

A one-way ANOVA using a completely randomized block design with 4 replicate pens per dietary treatment was used. Data concerning variables such as egg production and fertility data were determined and analyzed on a weekly basis and period basis from 24 to 64 wk of age. There were a total of 10, 4-wk periods, except for a 5-wk period 10. Data were checked for normality of distributions and homogeneity of variance and data were properly

transformed as necessary. The general lineal model of SAS Institute (2008) was used to analyze egg production and BW variables and means were partitioned by LSMEANS. The glimmix procedure of SAS Institute (2008) was used to analyze fertility data where each individual egg was taken as a binomial event, either fertile or infertile and appropriate contrasts were used for means comparison. The lifetest procedure of SAS was used for survival analysis. Means were considered statistically different when $P<0.05$, while $P<0.10$ were consider to be numerical trends.

Broiler Progeny

A randomized complete block design in a 4 x 4 factorial arrangement was used (broiler breeder diet x broiler diet). For variables where no significant interactions were found, the data was analyzed as one-way ANOVA to discern the main effects of the variable studied, with a total of 24 replicate pens per treatment. The general lineal model of SAS was used to analyze live performance and carcass traits. Variable means were partitioned by LSMEANS and were statistically different when $P \leq 0.05$, with $P \leq 0.10$ considered to be numerical trends.

RESULTS

Broiler Breeders

Table 5 shows the effect of breeder dietary treatment on female and male BW at 24, 30, 36, 42, 50, 59, and 64 wk of age. There were no significant differences among treatments for female BW at 24 and 30 wk. However, from 36 to 50 wk, and at 64 wk AdqP birds were

heavier than the remaining treatments (P<0.01). At 24 wk MarP males were heavier (P<0.05) than AdqP and MarP+PXP+½XAP males, while MarP+PXP+XAP males were intermediate. No differences were observed from 30 to 58 wk, but at 64 wk a similar trend as that noted above at 24 wk was observed.

No differences were detected with regards to cumulative eggs per hen housed (EHH), hen-day production (HDP), or feed per egg produced (FEgg; Table 6), and the survival analysis showed no difference among treatments (female survival time mean = 61.46 wk; male survival time mean = 49.77 wk).

During periods 3 and 6 HDP and EHH were greater (P<0.05) for AdqP birds (Figures 1 and 2). During periods 5 and 10 AdqP hens produced more eggs than MarP+PXP+XAP and ½XAP hens, with MarP hens intermediate. The same trend was apparent during period 8.

Cumulative fertility was greater (P<0.01) for MarP and MarP+PXP+XAP breeders than for AdqP breeders (Table 6). Breeders fed the MarP+PXP+½XAP diet produced the lowest fertility. A similar trend was evident for cumulative hatchability. No statistical differences were found for percentage fertile hatchability, early dead, or late dead embryo mortality.

On the other hand, during period 5 the MarP and MarP+PXP+XAP birds exhibited greater fertility than the ones fed the AdqP diet, while birds fed the half dose XAP diet were intermediate (Figure 3). By period 6, AdqP birds exhibited the poorest fertility, while the same was true for MarP+PXP+½XAP breeders during period 7. During period 10 a similar trend was followed, but the MarP+PXP+½XAP birds were only different for MarP birds. No differences in hatchability (Figure 4) were detected until the final production period where

the MarP dietary treatment was superior to AdqP and MarP+PXP+½XAP ($P<0.05$) treatments while birds fed the MarP+PXP+XAP diet were intermediate.

Broiler Progeny

Breeder Diet Effects

There were no significant interactions ($P>0.05$) between breeder and broiler diets with regards to the variables studied (data not shown for brevity). Tables 7 and 8 show the main effects of breeder and broiler dietary treatments on broiler performance.

Table 7 shows the effect of breeder dietary treatment on broiler progeny performance in terms of BW, FC, and AdjFCR. A trend was observed at hatching in which broilers from breeders fed the MarP diet were slightly heavier than progeny from birds fed the other two MarP diets. At 21 and 35 d MarP progeny were significantly heavier ($P<0.05$) when compared to AdqP and MarP+PXP+XAP progeny, with MarP+PXP+½XAP progeny intermediate. By 42 d a similar trend was found ($P<0.10$) but no differences among treatments were found at 49 d.

The FC was greater for MarP birds from 15 to 21 d ($P<0.01$) when compared with the other three dietary treatments preceded by a similar numerical differences from hatching to 14 d of age ($P<0.10$). Comparable observations were found from 22 to 35 d ($P<0.05$) but during this time the MarP+PXP+½XAP broilers exhibited intermediate FC. After 36 d no differences were found among the treatments. On a cumulative basis (0-49 d), the MarP progeny consumed more feed ($P<0.10$) than broilers from breeders fed either AdqP or MarP+PXP+XAP diets, with the MarP+PXP+½XAP diets intermediate.

Cumulative AdjFCR was numerically different from 0 to 35 and 0 to 49 d. During both periods MarP broiler progeny had the poorest AdjFCR, followed by MarP+PXP+XAP broilers with the AdqP and MarP+PXP+½XAP (0-49 d) broiler most efficient. From 0 to 35 d the MarP+PXP+½XAP broilers had an intermediate AdjFCR.

No statistical differences in hen mortality (mean = 7.46%) were detected among treatments for the entire production period.

Broiler Diet Effects

Table 8 shows the effect of broiler dietary treatments on BW, FC, and AdjFCR. No differences were observed among treatments on BW at hatching. By 14 d broilers fed the MarP+PXP+½XAP diet were heavier ($P<0.01$) than AdqP broilers followed by the MarP broilers. Broilers fed the MarP+PXP+XAP diet were intermediate. At 21 d broilers consuming feed at both levels of XAP cocktail had greater BW than did the MarP control broilers, while birds fed AdqP diets were not different from the other three treatments. After 22 d of age no statistical differences in BW were detected.

On the other hand, differences in FC were observed from 0 to 14 d and from 43 to 49 d ($P< 0.05$). At 14 d MarP broilers had consumed less feed than their counterparts, while from 43 to 49 d the AdqP broilers had a lower FC than did broilers fed XAP diets. During this period the MarP broilers exhibited intermediate FC. Cumulative FC (0-49 d; $P<0.05$) was greater for birds fed MarP+PXP+½XAP diets when compared to AdqP and MarP treatments.

Cumulative AdjFCR was improved for birds fed either enzyme treatment ($P<0.05$) at 14 d, followed by MarP birds while broilers receiving the AdqP dietary treatment exhibited the

poorest AdjFCR. After 15 d no significant differences were observed among treatments in AdjFCR.

DISCUSSION

Differences in female breeder BW began to be observed at 36 wk of age as AdqP females became heavier than the remainder of the treatments. This difference in BW was probably due to greater feed allocation for the AdqP breeders and, consequently, energy allocation (AdqP: 155 g feed, 441 kcal/d at peak versus MarP: 145 g feed, 413 kcal/d). Also, after 33 wk of age birds were fed NaCl reduced diets (0.5% vs. 0.35%). It had been shown that Na+ was an essential mineral necessary for the absorption of simple sugars, amino acids, water, and influenced absorption of other minerals (Boron and Boulpaep, 2005). However, the minimum consumption during the laying period was 200 mg Na/bird/day (for MarP birds), which was greater than the suggested minimum requirement of Na of 150 mg (NRC, 1994). Statistical and numerical differences were observed for male BW at 24 (before being placed on dietary treatments) and 64 wk, respectively. After the dietary treatments were started (25 wk of age) no differences were found. It was important to remember that feed (ME) allocation for males was the same for all treatments and the only difference was the dietary enzyme content and a small variation in Ca and NPP content between the AdqP and the three MarP diets. A trend at 64 wk indicated that enzyme dosageaffected male BW. It should be noted that males were given a consistent increase in feed throughout the experiment.

No differences were observed for cumulative EHH, HDP, and FEgg. However, observations during various periods suggested that AdqP hens laid more eggs than birds fed enzymes during periods 5, 8, and 10, and more than the three MarP dietary treatments during periods 3 and 6. It was important to mention that a reduction in egg production was expected during period 3 (32 – 35 wk), particularly for the three MarP treatments, due to a reduction in female FC as part of the reduced feed management program during hot weather that was employed to minimize female mortality during heat stress. Based on these observations it was possible to state that energy and protein allocation had the most important roles in determination of egg production rate and that enzyme additions did not successfully compensate for basic nutrient allocation reductions.

No differences were observed in the survival analysis for females or males, which contrasted with what we observed in Manuscript II, where females fed the XAP diet exhibited a lower survival. Most of the mortality occurred during a heat stress episode where coccidiostat consumption was reduced quantitatively as a consequence of decreased feed allocations. In the present experiment, preventive measures were taken to avoid the prior situation by administering the coccidistat in the drinking water. Furthermore, Manuscript II birds were exposed to the dietary treatments for 11 wk previous to the heat stress episode, while the breeders of the present experiment consumed the treatments diets for only 5 wk before high temperatures arrived.

An unusually high male mortality was observed at 39 wk of age for one of MarP+PXP+½XAP pens reducing male numbers from 7 to 2, and then to 1 at 44 wk of age.

At 46 wk (period 6) a male intra-spiking was performed to redistribute and equalize the number of males per pen/treatment for all treatments. For that reason fertility data from weeks 40 to 46 for that particular pen was excluded from the analysis, understanding that fertility was probably affected most by the male:female ratio and not by the dietary treatment. Differences in fertility were observed during periods 5, 6, 7, and 10. During period 5, fertility was higher for treatments MarP and MarP+PXP+XAP followed by MarP+PXP+½XAP, while the AdqP treatment had the poorest performance. Period 6 exhibited a similar trend, where all three MarP dietary treatments produced better fertility than AdqP. However, during period 7 (after intra-spiking) the MarP+PXP+½XAP treatment had the lowest fertility when compared to the other two MarP dietary treatments with the AdqP treatment being intermediate. We observed a similar trend during period 10, but only the MarP group was superior to MarP+PXP+½XAP group.

One reason to explain the reduction in fertility during this period for the MarP+PXP+½XAP treatment could be the reduction of the male:female ratio in 3 out of 4 pens from 3.7 to 5.7 males per 100 females, while the remainder of the pens had an average of 8.2 males per 100 females, which was a typical recommendation for breeders from 20 to 30 wk of age, and 6 to 7 males per 100 females later in the laying period (Aviagen Group, 2000; Hubbard, LLC, 2004; Cobb-Vantress, Inc., 2008).

Broiler Progeny

Breeder Diet Effect

Broilers from breeders fed the MarP breeder diet tended to be heavier ($P<0.10$) at hatching than chicks from breeders fed the MarP+PXP+XAP and MarP+PXP+½XAP diets, while the broilers from breeders fed the AdqP diet were not different from the other three treatments. A similar trend remained through the experimental period to 42 d but disappeared by 49 d when no significant differences were observed among treatments with an average 49 d BW of 3695 g per bird. This observation was consistent with another broiler breeder-broiler experimental series performed in our laboratory (Manuscript II) in which broilers from severely energy restricted breeders were significantly heavier at hatching but not at the end of the broiler experimental period.

As mentioned previously, from 0 to 14, 15 to 21, and 22 to 35 d of age broilers from breeders fed the MarP diet consumed more feed ($P<0.10$) than did AdqP broilers. The same was true for broilers from MarP+PXP+XAP breeders from 15 to 21 and 22 to 35 d. No differences in FC were observed from 36 to 42 d and 42 to 49 d. Still, FC from 0 to 49 d was higher ($P<0.10$) for broilers from MarP breeders, if compared to broilers from AdqP and MarP+PXP+XAP breeders. Progeny from MarP+PXP+½XAP breeders were similar to the other treatments. These findings were in agreement with the transient reduction in FC that Brake et al. (2003) and Argüelles-Ramos et al. (2009) observed in broiler progeny during the first two wk of age when breeders were fed enzyme cocktails.

It was important to mention that breeders fed the three MarP diets in Manuscript II were subject to a smaller energy restriction, while in the present experiment birds fed the three MarP diets had a greater energy restriction plus protein restriction that could explain the difference in response between the two experiments. Previous research conducted in mice (Sellayah et al., 2008) indicated that progeny appetite and metabolism could be modulated by maternal protein consumption during gestation. Moreover, Vickers et al. (2000) found that severe maternal nutritional restrictions (30% of daily requirement) during rat embryo development could lead to progeny hyperphagia, and combined with high energy consumption early in life could have magnified metabolic irregularities such as increased plasma insulin and leptin, high blood pressure, and obesity. In contrast to our findings, Vickers observed a reduction in BW at birth as a result of maternal nutrient restriction. In our experiment, only energy and protein were restricted in a marginal way, which may not have been sufficient to adversely affect BW at hatching. On the other hand, BW at hatching for chicks in this experiment was lower than for chicks from Manuscript II, but by 42 d the latter were smaller. We can not be sure if this was an effect of breeder flock age (40 versus 31 wk of age), breeder nutrient allocation, or a combination of both.

From 0 to 14, 0 to 21, and 0 to 42 d of broiler age, no differences were observed among breeder dietary treatment with respect to broiler AdjFCR. However, at 35 d broilers from breeders fed the AdqP diet exhibited better AdjFCR ($P<0.10$) than broilers from MarP breeders, while MarP+PXP+XAP and MarP+PXP+½XAP were intermediate. At 49 d broilers from AdqP and MarP+PXP+½XAP breeders exhibited better AdjFCR ($P<0.10$)

when compared to broilers from MarP breeders. The MarP+PXP+XAP broilers were not different from the other treatments. However, in this experiment the consistently high feed intake of MarP broilers resulted in a poorer AdjFCR.

Broiler Diet Effect

Broiler BW was significantly higher for broilers fed MarP+PXP+XAP and MarP+PXP+½XAP diets at 14 d ($P<0.01$). At 21 d ($P<0.05$) the same trend was observed probably as a carryover effect. However, no significant differences in BW were observed among treatments at 35, 42, and 49 d, in contrast with observations made by Café et al. (2002) where inclusion of a similar enzyme cocktail (CPC) consistently increased BW when a corn-soybean meal based diet was fed to broilers.

At 14 d a transient reduction in FC ($P<0.05$) was observed in broilers fed the MarP diet, which explained the reduced BW at 14 d experienced by this group. By 49 d FC of MarP+PXP+XAP and MarP+PXP+½XAP broilers was higher ($P<0.05$) than that of the AdqP birds, while MarP broilers were intermediate. The lack of effect after the third week of age could be due to the ME difference between the AdqP and the three MarP diets diets. Based upon our observations, it was possible to suggest that there was an optimum ME level when PXP and XAP enzymes were used in combination that changed with age of bird, considering the changes in digestive physiology in a growing bird. For the starter period the ME difference between the AdqP and the three MarP dietary treatments was 80 kcal ME/kg. By 14 d this difference was enough to reduce FC in MarP broilers, while addition of PXP and XAP enzymes to the feed improved FC to the AdqP level. However, broilers fed enzyme

treatments grew faster and were more efficient, which was interpreted to mean that a 2870 kcal ME/kg starter diet supplemented with PXP and XAP enzymes could produce better performance at 14 d than an unsupplemented 2950 kcal diet. Conversely, a difference of 152 kcal/kg (AdqP 3036 kcal vs. the three MarP diets 2884 kcal) in the grower feed was not adequate to produce differences among treatments at the end of this period. With respect to the finisher feed, the differences in ME between AdqP and the three MarP diets was 151 kcal (3076 vs. 2925 kcal), which caused an increase in FC from 43 to 49 d, but did not improve BW or feed efficiency, probably due to the low ME level of the three MarP diets, and relative lack of time to develop a response.

The AdjFCR was better ($P<0.05$) in broilers fed MarP+PXP+XAP and MarP+PXP+½XAP diets at 14 d as compared with broilers fed AdqP, which was similar with our observations in Manuscript II at 16 d. However, no differences in AdjFCR among treatments were observed during the remainder of the experimental period. Differences in broiler performance between the experiments also could be an effect of the overall nutritional status of the breeder flocks. In general, male progeny from Manuscript II (and from a preliminary broiler study in our laboratory with the same breeder flock) were heavier at hatching, also consumed less feed, and were smaller at 42 d, when compared with the males of this experiment at the same age. As mentioned previously, nutrient restriction during mammalian embryo development affected initial BW and FC of progeny.

In conclusion, overall productive and reproductive performance were not negatively affected by a reduced energy and protein allocation or by addition of PXP and full dose of XAP, when

appropriate management during heat stress episodes was practiced. Also, no negative vertical effect of parental treatment on progeny response to the dietary enzymes evaluated in this study was observed. Finally, nutritional status of the parental breeder flock, broiler progeny age, and energy content of the broiler diet were shown to be important aspects to be considered when broiler progeny feed was formulated.

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TABLE III. 1. Ingredient composition and calculated analysis for broiler breeder layer dietary treatments (25 – 64 wk of age).

Ingredients	Dietary Treatment[1]			
	AdqP	MarP	MarP+PXP +XAP	MarP+PXP +½XAP
			%	
Corn	68.95	68.01	68.95	68.95
Soybean meal (48% CP)	14.05	14.22	14.05	14.05
Corn DDGS	3.00	3.00	3.00	3.00
Wheat bran	3.00	3.00	3.00	3.00
Poultry Fat	2.10	2.44	2.10	2.10
Limestone	5.89	6.27	6.44	6.44
Dicalcium phosphate	1.54	1.69	0.95	0.95
Filler (sand)	0.24	0.14	0.17	0.22
Sodium chloride[2]	0.50	0.50	0.50	0.50
Premixes[3]	0.60	0.60	0.60	0.60
Phytase premix	0.00	0.00	0.01	0.01
Avizyme 1502	0.00	0.00	0.10	0.05
L-Lysine	0.03	0.03	0.03	0.03
DL-Methionine	0.07	0.07	0.07	0.07
L-Threonine	0.03	0.03	0.03	0.03
	100.00	100.00	100.00	100.00
Calculated nutrients[4]				
ME, kcal/kg	2850	2850	2850	2850
Crude protein, %	14.00	14.00	14.00	14.00
Lysine, %	0.70	0.70	0.70	0.70
Methionine + Cysteine, %	0.58	0.58	0.58	0.58
Threonine, %	0.50	0.50	0.50	0.50
Calcium, %	2.62	2.80	2.70[5]	2.70[5]
Non-phytate phosphorus, %	0.35	0.37	0.25[5]	0.25[5]
Sodium, %	0.20	0.20	0.20	0.20
Calcium, analyzed, %	2.85	2.77	3.01	2.87
Total phosphorus, analyzed, %	0.68	0.68	0.56	0.59

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP+½XAP plus 0.1% inclusion of Avizyme 1502.

[2] Sodium chloride content was reduced to 0.35% from 33 wk of age reducing the content of sodium to 0.15%. Proper adjustments to the content of filler (sand) were made.

[3]Premixes provided the following (per kg of diet): vitamin A, 13,200 IU; vitamin D_3, 4,000 IU; vitamin E, 66 IU; vitamin B_{12}, 39.6 µg; riboflavin, 13.2 mg; niacin, 110 mg; D-pantothenate, 22 mg; menadione (K_3), 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg; D-biotin, 252 µg; selenium (as Na_2SeO_3), 0.30 mg; manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg; choline chloride, 1,200 mg; coccidiostat, 500 mg.

[4]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

[5]Phyzyme XP was added at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

TABLE III. 2. Ingredient composition and calculated analysis for broiler progeny dietary treatments during the starter period (0 – 14 d of age).

Ingredients	Dietary Treatment[1]			
	AdqP	MarP	MarP+PXP+ XAP	MarP+PXP +½XAP
			%	
Corn	55.17	57.33	57.33	57.33
Soybean meal (48% CP)	27.27	26.88	26.88	26.88
Corn DDGS	5.00	5.00	5.00	5.00
Poultry Fat	4.08	2.30	2.30	2.30
Poultry by-product meal	3.69	3.69	3.69	3.69
Limestone	1.25	1.26	1.42	1.42
Dicalcium phosphate	2.11	2.10	1.36	1.36
Filler (sand)	0.08	0.10	0.55	0.60
Sodium chloride	0.50	0.50	0.50	0.50
Premixes[2]	0.60	0.60	0.60	0.60
Phytase premix	0.00	0.00	0.03	0.03
Avizyme 1502	0.00	0.00	0.10	0.05
L-Lysine	0.09	0.09	0.09	0.09
DL-Methionine	0.12	0.12	0.12	0.12
L-Threonine	0.04	0.03	0.03	0.03
	100.00	100.00	100.00	100.00
Calculated nutrients[3]				
ME, kcal/kg	2950	2870	2870	2870
Crude protein, %	22.00	22.00	22.00	22.00
Lysine, %	1.22	1.22	1.22	1.22
Methionine + Cysteine, %	0.85	0.85	0.85	0.85
Threonine, %	0.80	0.80	0.80	0.80
Calcium, %	1.00	1.00	0.90[4]	0.90[4]
Non-phytate phosphorus, %	0.45	0.45	0.33[4]	0.33[4]
Sodium, %	0.21	0.21	0.21	0.21
Calcium, analyzed, %	1.07	0.99	0.96	1.01
Total phosphorus, analyzed, %	0.76	0.75	0.64	0.68

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP+½XAP plus 0.1% inclusion of Avizyme 1502.

[2]Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D_3, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K_3), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na_2SeO_3), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

[3]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

[4] Phyzyme XP was added to MarP+PXP and MarP+PXP+XAP diets at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

TABLE III. 3. Ingredient composition and calculated analysis for broiler progeny dietary treatments during the grower period (14 – 35 d of age).

Ingredients	Dietary Treatment[1]			
	AdqP	MarP	MarP+PXP +XAP	MarP+PXP +½XAP
		%		
Corn	60.26	64.37	64.27	64.41
Soybean meal (48% CP)	22.05	21.31	21.33	21.30
Corn DDGS	5.00	5.00	5.00	5.00
Poultry by-product meal	3.69	3.69	3.69	3.69
Poultry Fat	4.60	1.21	1.25	1.19
Limestone	1.11	1.11	1.28	1.28
Dicalcium phosphate	1.72	1.71	0.97	0.97
Filler (sand)	0.08	0.10	0.58	0.58
Sodium chloride	0.50	0.50	0.50	0.50
Premixes[2]	0.60	0.60	0.60	0.60
Phytase premix	0.00	0.00	0.03	0.03
Avizyme 1502	0.00	0.00	0.10	0.05
L-Lysine	0.16	0.17	0.17	0.17
DL-Methionine	0.15	0.15	0.15	0.15
L-Threonine	0.08	0.08	0.08	0.08
	100.00	100.00	100.00	100.00
Calculated nutrients[3]				
ME, kcal/kg	3036	2884	2884	2884
Crude protein, %	20.00	20.00	20.00	20.00
Lysine, %	1.13	1.13	1.13	1.13
Methionine + Cysteine, %	0.82	0.82	0.82	0.82
Threonine, %	0.76	0.76	0.76	0.76
Calcium, %	0.85	0.85	0.75[4]	0.75[4]
Non-phytate phosphorus, %	0.38	0.38	0.26[4]	0.26[4]
Sodium, %	0.21	0.21	0.21	0.21
Calcium, analyzed, %	0.89	0.86	0.88	0.91
Total phosphorus, analyzed, %	0.70	0.67	0.62	0.64

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP+½XAP plus 0.1% inclusion of Avizyme 1502.

[2]Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D_3, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K_3), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na_2SeO_3), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

[3]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

[4] Phyzyme XP was added to MarP+PXP and MarP+PXP+XAP diets at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

TABLE III. 4. Ingredient composition and calculated analysis for broiler progeny dietary treatments during the finisher period (36 - 49 d of age).

Ingredients	Dietary Treatment[1]			
	AdqP	MarP	MarP+PXP+ XAP	MarP+PXP +½XAP
		%		
Corn	65.30	69.39	69.33	69.44
Soybean meal (48% CP)	17.22	16.48	16.50	16.48
Corn DDGS	5.00	5.00	5.00	5.00
Poultry by-product meal	3.69	3.69	3.69	3.69
Poultry Fat	4.47	1.10	1.12	1.08
Limestone	1.08	1.10	1.26	1.26
Dicalcium phosphate	1.57	1.57	0.82	0.82
Filler (sand)	0.11	0.09	0.67	0.57
Sodium chloride	0.50	0.50	0.50	0.50
Premixes[2]	0.60	0.60	0.50	0.60
Phytase premix	0.00	0.00	0.03	0.03
Avizyme 1502	0.00	0.00	0.10	0.05
L-Lysine	0.22	0.24	0.24	0.24
DL-Methionine	0.14	0.14	0.14	0.14
L-Threonine	0.10	0.10	0.10	0.10
	100.00	100.00	100.00	100.00
Calculated nutrients[3]				
ME, kcal/kg	3076	2925	2925	2925
Crude protein, %	18.15	18.15	18.15	18.15
Lysine, %	1.05	1.05	1.05	1.05
Methionine + Cysteine, %	0.77	0.77	0.77	0.77
Threonine, %	0.70	0.70	0.70	0.70
Calcium, %	0.80	0.80	0.70[4]	0.70[4]
Non-phytate phosphorus, %	0.35	0.35	0.23[4]	0.23[4]
Sodium, %	0.21	0.21	0.21	0.21
Calcium, analyzed, %	0.84	0.95	0.82	0.91
Total phosphorus, analyzed, %	0.65	0.71	0.59	0.63

[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP = MarP plus 500 FTU/kg feed (Phyzyme XP), and MarP+PXP+XAP = MarP+PXP+½XAP plus 0.1% inclusion of Avizyme 1502.

[2]Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D_3, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K_3), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na_2SeO_3), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

[3]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

[4] Phyzyme XP was added to MarP+PXP and MarP+PXP+XAP diets at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

TABLE III. 5. Effect of breeder layer dietary treatment on female and male body weight during the laying period (28-64 wk-of-age).

		Dietary Treatments[1]					
Age	Sex	AdqP	MarP	MarP+PXP+XAP	MarP+PXP+ ½XAP	SEM[2]	P-value
(wk)	Females			**(g)**			
24		2787	2787	2784	2777	19	NS
30		3130	3066	3077	3077	24	NS
36		3416^{A}	3227^{B}	3265^{B}	3256^{B}	23	<0.01
42		3723^{A}	3515^{B}	3530^{B}	3492^{B}	17	<0.01
50		3910^{A}	3653^{B}	3653^{B}	3670^{B}	25	<0.01
59		4034^{x}	3806^{xy}	3715^{y}	3722^{y}	79	<0.10
64		4223^{A}	3852^{B}	3765^{B}	3739^{B}	40	<0.01
	Males						
24		3682^{bc}	3796^{a}	3759^{ab}	3661^{c}	26	<0.05
30		3860	3948	3986	3944	74	NS
38		4125	4136	4177	4076	89	NS
42		4134	4314	4177	4241	115	NS
50		4526	4709	4681	4691	113	NS
58		5143	5214	4966	4749	138	NS
64		5346^{x}	5311^{x}	5154^{xy}	4845^{y}	132	NS

$^{x-y}$Means across rows lacking a common uppercase superscript are significantly different ($P < 0.10$).
$^{a-b}$Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$).
$^{A-B}$Means across rows lacking a common uppercase superscript are significantly different ($P < 0.01$).
[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP+XAP = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.10% inclusion of Avizyme 1502 and MarP+PXP+½XAP = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.05% inclusion of Avizyme 1502.
[2] SEM for n=4 pens with all hens or males weighed per pen.

TABLE III. 6. Effect of broiler breeder layer dietary treatment on egg production, fertility, and hatchability during the laying phase (25 to 64 wk of age).

Variable	AdqP	MarP	MarP+PXP +XAP	MarP+PXP +½XAP	SEM	P-value
Eggs per hen house, n	167.64	161.92	159.76	156.74	4.35	NS
Hen-day production, %	58.46	56.49	55.72	54.67	1.51	NS
Feed per egg, g	254	244	248	251	5	NS
Fertility, %	92.78^{B}	96.30^{A}	95.11^{A}	92.94^{B}	-	< 0.01
Fertile hatchability, %	93.38	93.42	93.16	93.61	0.58	NS
Hatchability, %	86.65^{b}	89.99^{a}	88.60^{ab}	87.06^{b}	0.81	< 0.05
Early dead, %	3.77	3.21	3.66	3.66	0.32	NS
Late dead, %	2.01	2.72	2.57	2.10	0.29	NS

[A, B]Means across rows lacking a common superscript are significantly different ($P < 0.01$).
[a, b] Means across rows lacking a common superscript are significantly different ($P < 0.05$).
[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP+XAP = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.10% inclusion of Avizyme 1502, and MarP+PXP+½XAP = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.05% inclusion of Avizyme 1502.
[2]Standard error is not available with glimmix procedure due to categorical data.

TABLE III. 7. Effect of broiler breeder layer dietary treatment on broiler body weight (BW), feed consumption (FC), and adjusted feed conversion ratio (AdjFCR) from 49 d of age.

			Dietary Treatments[1]					
Variable	Age	n	AdqP	MarP	MarP+PXP+ XAP	MarP+PXP ½XAP	SEM[2]	*P*-value
	d							
BW, g	0	24	36.9^{xy}	37.0^{x}	36.6^{y}	36.6^{y}	0.1	<0.10
	14	24	459	471	462	461	4	NS
	21	24	927^{b}	959^{a}	927^{b}	938^{ab}	8	0.01
	35	24	2226^{bc}	2264^{a}	2206^{c}	2246^{ab}	14	<0.05
	42	24	2930^{xy}	2963^{x}	2902^{y}	2946^{xy}	17	<0.10
	49	24	3697	3669	3666	3749	36	NS
FC, g	0-14	24	569^{y}	588^{x}	574^{xy}	578^{xy}	5	<0.10
	15-21	24	689^{B}	721^{A}	695^{B}	701^{B}	6	< 0.01
	22-35	24	2160^{b}	2251^{a}	2172^{b}	2202^{ab}	24	0.05
	36-42	24	1434	1451	1437	1460	11	NS
	43-49	24	1673	1642	1644	1631	15	NS
	0-49	24	6524^{y}	6653^{x}	6512^{y}	6573^{xy}	40	<0.10
AdjFCR, g:g	0-14	24	1.24	1.24	1.24	1.25	0.01	NS
	0-21	24	1.36	1.36	1.37	1.37	0.01	NS
	0-35	24	1.54^{y}	1.57^{x}	1.56^{xy}	1.55^{xy}	0.01	<0.10
	0-42	24	1.65	1.67	1.67	1.67	0.01	NS
	0-49	24	1.76^{y}	1.81^{x}	1.78^{xy}	1.76^{y}	0.01	<0.10

$^{x-y}$Means across rows lacking a common uppercase superscript are significantly different ($P < 0.10$).
$^{a-b}$Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$).
$^{A-B}$Means across rows lacking a common uppercase superscript are significantly different ($P < 0.01$).
[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP+XAP = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.10% inclusion of Avizyme 1502, and MarP+PXP+½XAP = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.05% inclusion of Avizyme 1502.
[2]SEM for n=24 pens with all males per pen.

TABLE III. 8. Effect of broiler dietary treatment on broiler body weight (BW), feed consumption (FC), and adjusted feed conversion ratio (AdjFCR) from 49 d of age.

			Dietary Treatments[1]					
Variable	Age	n	AdqP	MarP	MarP+PXP+ XAP	MarP+PXP +½XAP	SEM[1]	*P*-value
	d							
BW, g	0	24	36.7	36.7	36.7	36.9	0.1	NS
	14	24	460^{BC}	449^{C}	469^{AB}	474^{A}	4	<0.01
	21	24	935^{ab}	920^{b}	946^{a}	949^{a}	8	<0.05
	35	24	2230	2221	2240	2252	14	NS
	42	24	2927	2920	2932	2964	17	NS
	49	24	3680	3702	3684	3714	36	NS
FC, g	0-14	24	582^{a}	562^{b}	581^{a}	585^{a}	5	<0.05
	15-21	24	698	698	700	711	6	NS
	22-35	24	2163	2200	2189	2233	24	NS
	36-42	24	1438	1429	1449	1457	11	NS
	43-49	24	1611^{b}	1639^{ab}	1666^{a}	1675^{a}	15	<0.05
	0-49	24	6491^{b}	6527^{b}	6583^{ab}	6661^{a}	40	<0.05
AdjFCR, g:g	0-14	24	1.26^{a}	1.25^{ab}	1.24^{b}	1.23^{b}	0.01	<0.05
	0-21	24	1.37	1.37	1.36	1.36	0.01	NS
	0-35	24	1.55	1.56	1.55	1.56	0.01	NS
	0-42	24	1.65	1.66	1.67	1.68	0.01	NS
	0-49	24	1.76	1.77	1.79	1.79	0.01	NS

[x-y]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.10$).
[a-b]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$).
[A-B]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.01$).
[1]The dietary treatments consisted of AdqP = adequate P, MarP = marginal P, MarP+PXP+XAP = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.10% inclusion of Avizyme 1502, and MarP+PXP+½XAP = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.05% inclusion of Avizyme 1502.
[2]SEM for n=24 pens with all males per pen.

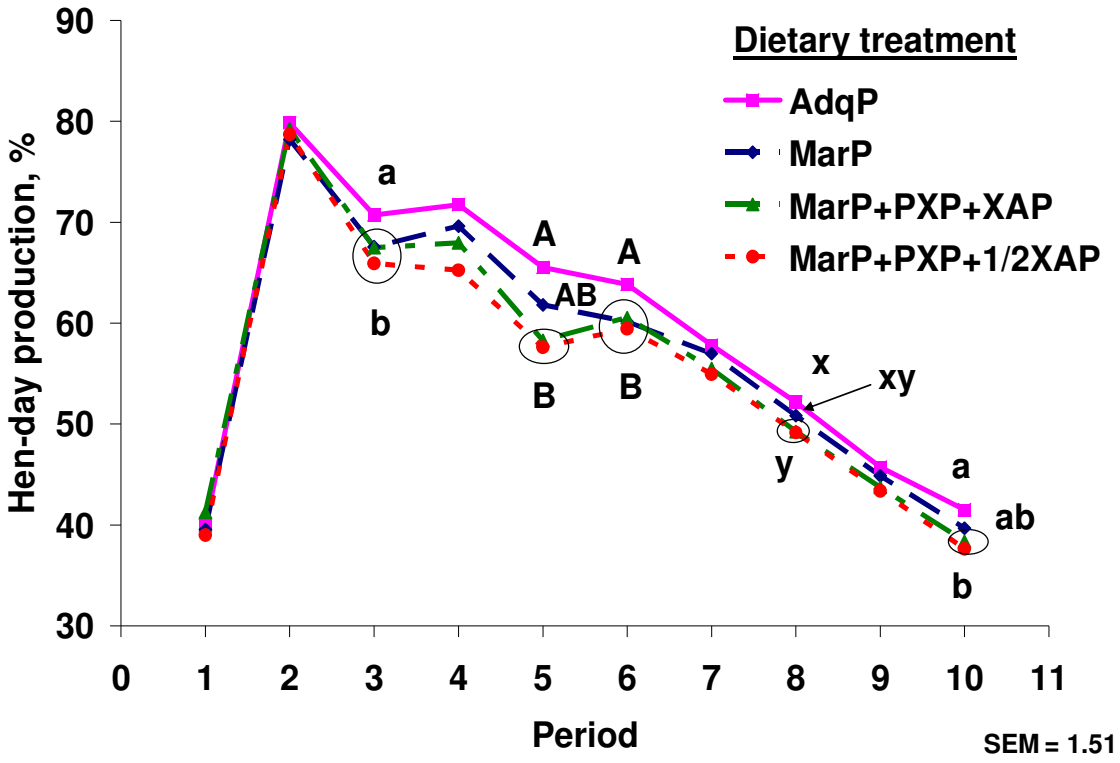

FIGURE III. 1. Effect of broiler breeder layer dietary treatment on hen-day production at different periods during the production phase (24 to 64 wk of age). The dietary treatments consisted of AdqP (diamond) = adequate P, MarP (square) = marginal P, MarP+PXP+XAP (triangle) = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.10% inclusion of Avizyme 1502, and MarP+PXP+½XAP (dot) = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.05% inclusion of Avizyme 1502. Period: 1 = 24 to 27 wk; 2 =28 to 31 wk; 3 = 32 to 35 wk; 4 = 36 to 39 wk; 5 = 40 to 43 wk; 6 = 44 to 47 wk; 7 = 48 to 51 wk; 8 = 52 to 55 wk; 9 = 56 to 59 wk; 10 = 60 to 64 wk. The SEM is the pooled standard error of the mean. Means across rows lacking a common uppercase superscript (x,y) are significantly different ($P<0.10$). Means across rows lacking a common uppercase superscript (a.b) are significantly different ($P<0.05$). Means across rows lacking a common uppercase superscript (A,B) are significantly different ($P<0.01$).

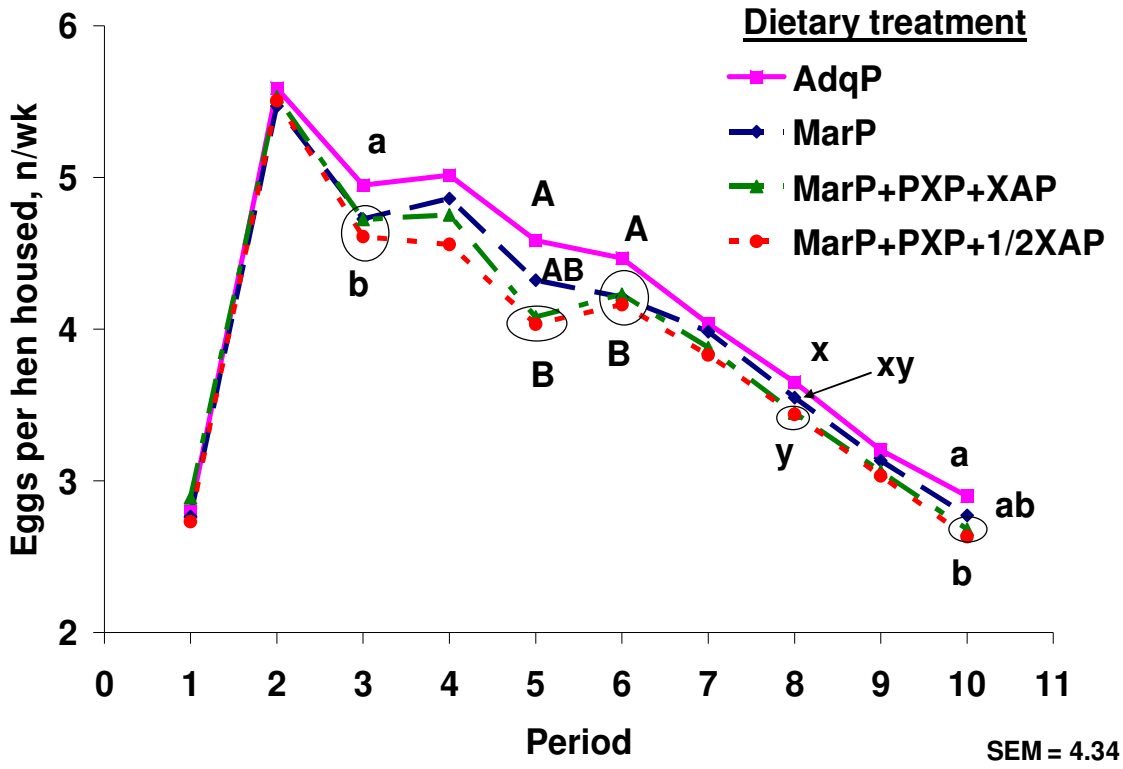

FIGURE III. 2. Effect of breeder layer dietary treatment on egg per hen housed for different production periods (24-64 wk-of-age). The dietary treatments consisted of AdqP (diamond) = adequate P, MarP (square) = marginal P, MarP+PXP+XAP (triangle) = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.10% inclusion of Avizyme 1502, and MarP+PXP+½XAP (dot) = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.05% inclusion of Avizyme 1502. Period: 1 = 24 to 27 wk; 2 =28 to 31 wk; 3 = 32 to 35 wk; 4 = 36 to 39 wk; 5 = 40 to 43 wk; 6 = 44 to 47 wk; 7 = 48 to 51 wk; 8 = 52 to 55 wk; 9 = 56 to 59 wk; 10 = 60 to 64 wk. The SEM is the pooled standard error of the mean. Means across rows lacking a common uppercase superscript (x,y) are significantly different ($P<0.10$). Means across rows lacking a common uppercase superscript (a.b) are significantly different ($P<0.05$). Means across rows lacking a common uppercase superscript (A,B) are significantly different ($P<0.01$).

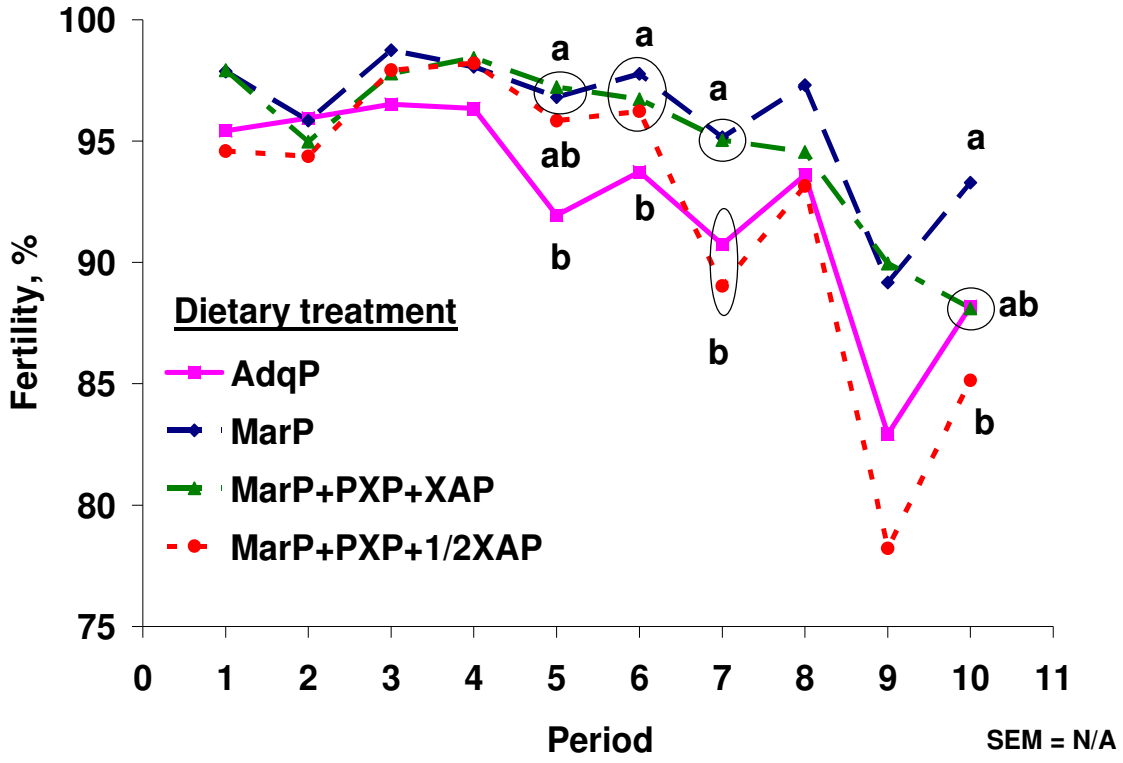

FIGURE III. 3. Effect of breeder layer dietary treatment on fertility for different production periods (24-64 wk-of-age). The dietary treatments consisted of AdqP (diamond) = adequate P, MarP (square) = marginal P, MarP+PXP+XAP (triangle) = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.10% inclusion of Avizyme 1502, and MarP+PXP+½XAP (dot) = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.05% inclusion of Avizyme 1502. Period: 1 = 24 to 27 wk; 2 =28 to 31 wk; 3 = 32 to 35 wk; 4 = 36 to 39 wk; 5 = 40 to 43 wk; 6 = 44 to 47 wk; 7 = 48 to 51 wk; 8 = 52 to 55 wk; 9 = 56 to 59 wk; 10 = 60 to 64 wk. The SEM is the pooled standard error of the mean. Means across rows lacking a common uppercase superscript (a.b) are significantly different ($P<0.05$).

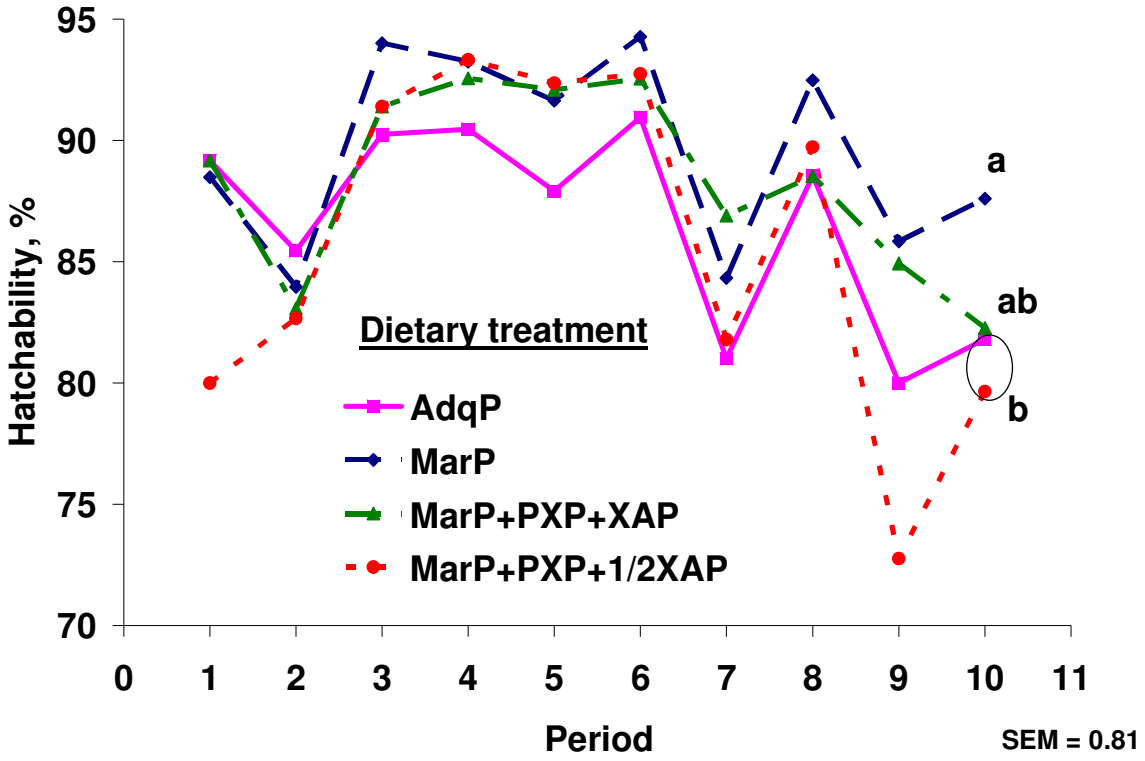

FIGURE III. 4. Effect of breeder layer dietary treatment on hatchability for different production periods (24-64 wk-of-age). The dietary treatments consisted of AdqP (diamond) = adequate P, MarP (square) = marginal P, MarP+PXP+XAP (triangle) = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.10% inclusion of Avizyme 1502, and MarP+PXP+½XAP (dot) = MarP plus 500 FTU/kg feed (Phyzyme XP) and 0.05% inclusion of Avizyme 1502. Period: 1 = 24 to 27 wk; 2 =28 to 31 wk; 3 = 32 to 35 wk; 4 = 36 to 39 wk; 5 = 40 to 43 wk; 6 = 44 to 47 wk; 7 = 48 to 51 wk; 8 = 52 to 55 wk; 9 = 56 to 59 wk; 10 = 60 to 64 wk. The SEM is the pooled standard error of the mean. Means across rows lacking a common uppercase superscript (a.b) are significantly different (P<0.05).

MANUSCRIPT IV. Effects of Dietary Phytase and a Carbohydrase-Protease Cocktail on the Performance of Broilers Fed Different Levels of Chloride

ABSTRACT

Previous research suggested that the beneficial effect of certain enzyme supplements on broiler performance could be influenced by the chloride (Cl) content of the diet. A broiler experiment was conducted to determine the effect of the inclusion of phytase (PXP) or a carbohydrase/protease cocktail (CPC) that contained xylanase, amylase, and protease (XAP) on broilers fed diets that contained two different levels of Cl. A total of 1024 d-old Ross 344 x 708SF males was randomly distributed among 32 pens. There were 8 dietary treatments that consisted of one of two Cl levels: 0.28% or 0.43%, and one of four energy/enzyme combinations: adequate positive control (AdqME), negative control with marginal reduction in metabolizable energy (MarME), MarME+PXP, and MarME+XAP. The AdqME diets were formulated to be 100 kcal ME/kg higher than the MarME diets for the starter and grower feeds used. For 0.28% Cl diets, sodium bicarbonate ($NaHCO_3$) was added to provide Na at a level of 0.21% of complete feed. Group body weight (BW), and feed consumption (FC) were measured at 0, 16, 35, and 42 d, and mortality was recorded daily for adjusted feed conversion ratio (AdjFCR) calculations. Birds fed the MarME and MarME+PXP diets exhibited greater BW ($P<0.05$) at 14 and 35 d of age than did

MarME+XAP birds, but at 35 d birds fed the MarME+XAP diet tended to gain more weight in the presence of 0.43% Cl ($P<0.10$). The FC at 14 d was lower ($P<0.05$) for AdqME and MarME+XAP diets relative to the MarME diet. However, birds fed the MarME+XAP diet consumed less feed at the 0.43% Cl level. No differences in FC were observed beyond 15 d of age. At 14 d of age AdjFCR was improved for broilers fed MarME+XAP when the diet contained 0.43% Cl. However, no differences were observed in AdjFCR for any of the energy/enzyme combinations. Based on these results, it was possible to conclude that Cl levels in the diet could affect dietary enzyme function during early stages of life.

INTRODUCTION

Exogenous enzymes such as phytase, xylanase, amylase, and protease have been extensively scrutinized during the last two decades in studies designed to demonstrate their efficacy. Inclusion of phytase has made possible a significant reduction of inorganic phosphorus in monogastric diets without compromising productive performance, livability, or carcass traits (Ingram et al., 1995; Adeola et al., 2004; Beudeker et al., 2005). Beyond the capacity of phytase to increase the availability of phosphorus via degradation of the anti-nutritive phytate molecule, this enzyme has been demonstrated to increase the availability of other minerals as well asprotein and starch. Previous research showed that addition of phytase to adequate or marginally deficient diets could increase the digestibility coefficients of phosphorus, calcium, amino acids, and metabolizable energy (Selle and Ravindran, 2007).

The purpose of carbohydrases (such as xylanase) in the diet has been to digest cell walls to reduce viscosity of the digesta and expose macronutrients to further digestion by endogenous enzymes. This mechanism has been frequently observed in wheat-based, rather than in corn-soybean meal based diets, as the structural and chemical composition of wheat-based diets differed in many ways that were important for the effectiveness of the enzymes. It has been suggested that an important amount of the starch in corn was embedded in protein matrixes, which limited the access of important enzymes such as amylase (Brown, 1996). To solve these problems carbohydrase/protease cocktails (CPC)

have been developed to assure optimal digestion of all macronutrients. In addition to the improvement in nutrient digestibility, researchers have postulated that exogenous enzyme supplementation could reduce the energy expenditure of the animal by decreasing the synthesis of endogenous enzymes (Mahagna et al., 1995). However, most of the trials performed to assess enzyme effectiveness did not collect data beyond 21 d of age.

On the other hand, the addition of common table salt to the diet has typically been used to supply the sodium (Na) and chlorine (Cl) requirements of birds, which have been estimated by some authors to be 0.28% Na and 0.25% Cl (Oviedo-Rondón et al. 2001), and 0.15% Na and 0.23% Cl (Murakami et al., 2001) for birds from 1 to 21 d and 22 to 42 d of age, respectively. It has been demonstrated that Na and Cl in conjuction with potassium (K) are very important for intestinal nutrient absorption (Boron and Boulpaep, 2003). A deficiency or excess of these minerals in the diet, as well as their imbalance, could affect the efficiency of the digestive process due to induced changes in the pH of the digesta.

Even when diets have been properly formulated to meet all the requirements of the bird, anti-nutritive components in feedstuffs could reduce availability of essential nutrients. Phytate has the capacity to reduce ileal availability of Na by attracting the mineral to the lumen as demonstrated by Ravindran et al. (2006) who suggested that the decrease in pH due to the presence of high concentrations of phytate could stimulate the secretion of $NaHCO_3$, which compromised mineral availability. Due to the influence of phytate and phytase on Na availability, researchers have suggested that the dietary electrolyte balance (DEB; mEq Na^+ + mEq K^+ – mEq Cl^-) could interact with the efficacy of the phytase

enzyme. In a study conducted by Ravindran et al. (2008), investigators observed that addition of phytase to marginally energy-protein deficient diets at low to moderate DEB levels improved broiler performance and availability of nutrients, while no real benefit of the enzyme was observed at a high DEB level. However, the design of the diets did not permit Ravindran et al. (2008) to establish if the interaction between phytase and DEB was due to the DEB *per se*, or to the different Na levels in the diets.

It has been reported that carbohydrases alone and CPC could improve digestibility of minerals and amino acids (Hew et al., 1998; Kim et al., 2005; Olukosi et al., 2007; Selle et al., 2009). Absorption of these nutrients could be closely related not only to the optimal requirement of Na, but also to an optimum ratio between Na, K, and Cl (DEB), as most of the absorption process of sugars, amino acids, and some minerals, have been shown to indirectly depend upon the electrochemical difference between the digesta and the intracellular space of absorptive cells in the intestine (Boron and Boulpaep, 2003).

The objective of this study was to determine if the response to phytase or CPC supplementation on broiler productive performance was affected by different dietary levels of Cl produced by different inclusion levels of NaCl or $NaHCO_3$.

MATERIALS AND METHODS

Broiler Progeny Management and Data Collection

Eggs from a 60-wk-old Ross 344 x 708SF breeder flock were collected and identified by breeding pen for a week and then incubated under standard conditions. Chicks were hatched and maintained separate by breeder pen (16) at all times until placement. After sexing, 32 (2 per breeder pen) males were permanently identified with neck tags and placed in 32, 4.83 m^2 floor pens. During the first week 3 feeder trays were placed in every pen, then reduced to 2 until 10 d, and finally to 1 at 10 d. The final supplemental feeder was removed at 14 d of age. All pen floors were covered with wood shavings, and each pen had 1 tube feeder and 1 bell-type drinker, plus a plastic font drinker during the first 7 d. The lighting program during the first 7 d was 23 h of light, then 21 h to 21 d. After 21 d of age natural light only was used to control excessive growth and improve livability. Total starter feed per bird was equalized to 1.14 kg per live chick at 7 d. Grower feed was then added and fed to the end of the study.

Group body weight (BW), and feed consumption (FC) were measured at 0, 14, 35, and 42 d, and BW gain was calculated by time interval. Mortality was recorded daily for adjusted feed conversion ratio (AdjFCR) calculations.

Broiler Dietary Treatments

A total of 8 broiler dietary treatments were tested with 2 levels of chloride (Cl; 0.43 and 0.28%), and four energy/enzyme combinations: an adequate positive control (AdqME), a

marginal negative control (MarME), a MarME plus phytase enzyme (MarME+PXP), and MarME plus a carbohydrase/protease cocktail (MarME+XAP). The AdqME diet had 2900 and 3000 kcal/kg for the starter and grower feeds, respectively (Tables 1 and 2). All the MarME diets (NCs) were formulated to be 100 kcal/kg lower in ME. To diets with 0.28% Cl, 0.35% $NaHCO_3$ was added to equalize the Na content.

Birds were provided feed for *ad libitum* consumption with starter feed offered as crumbles, while grower feed was in pellet form. All the treatment diets were corn-soybean meal based with 10 – 10.9% corn distiller's dried grains with solubles (DDGS) and 4 – 4.5% poultry by-product meal (PBM) while calculatd to be isonitrogenous with similar amino acid levels. Increases in ME in the AdqME diets were made possible by adding extra poultry fat.

The phytase enzyme product was Phyzyme XP TPT (Danisco Animal Nutrition, Marlborough, UK), an *Escherichia coli*-derived phytase, added at 500 FTU/kg. The CPC cocktail, Avizyme 1502 (XAP; Danisco Animal Nutrition, Malborough, UK), was used at the manufacturer recommended level to provide 300 U of xylanase /kg from *Trichoderma longibrachiatum*, 4000 U of protease from *Bacillus subtilis*, and 400 U of amylase from *Bacillus amyloliquofaciens*.

Statistical Methods

A randomized complete block design with a 4 x 2 factorial arrangement of treatments was used (diet x Cl level), and the general lineal model of SAS (SAS Institute, 2008) was used

to analyze live performance. Variable means were partitioned by LSMEANS and were considered statistically different when $P<0.05$, while $P<0.10$ was considered to be numerical trends.

RESULTS

The effect of dietary treatments on broiler BW, BW gain, FC, and AdjFCR at 0, 14, 35, and 42 d of age is shown in Table 3 At 14 d broilers fed MarME and MarME+PXP diets were heavier ($P<0.05$) than the birds fed the MarME+XAP diets, while the ones fed AdqME diets were intermediate. By 35 and 42 d of age MarME and MarME+PXP groups had higher ($P<0.01$) BW than the other two treatments. At 35 d interactions were observed ($P<0.10$) that suggested that BW (Figure 1) and BW gain (Figure 2) of broilers fed MarME+XAP diets was greater when the diet contained 0.43% Cl. Statistical differences in BW at 42 d were a carryover from the BW gain from 15-35 d ($P<0.01$), as no differences were observed in BW gain from 35 to 42 d.

At 14 d broilers fed the MarME diets consumed more feed ($P<0.05$) than those consuming the AdqME and MarME+XAP dietary treatments. Birds fed the MarME+PXP diet had an intermediate FC. Even when no main effects were found for AdjFCR, interactions were detected at the $P<0.07$ and $P<0.03$ levels for FC (Figure 3) and AdjFCR (Figure 4), respectively, at 14 d. This suggested that feeding MarME+XAP diets with 0.43% Cl decreased FC and AdjFCR when they were compared to the MarME+XAP diet at 0.28%

Cl. No differences in FC were observed after 15 d. No main effects of Cl level were observed for any of the variables and none of the factors studied affected mortality (Table 3).

DISCUSSION

The objective of this study was to determine the effect of the addition of PXP or XAP to corn-soybean meal based diets formulated with two different levels of Cl on broiler live performance to 14, 35, and 42 d of age. In this experiment, we used a marginally low energy level (in comparison with adequate NRC recommendations) and used recommended levels of NPP and Ca for AdqME, MarME, and MarME+XAP (Tables 1 and 2). Only in MarME+PXP diets were NPP and Ca reduced by 0.12% and 0.10%, respectively, expecting PXP to compensate for these reductions. Also, Na and K were expected to be the same for all the diets (0.22% and 0.69%, respectively) as ingredients did not differ among diets, and only Cl was different at 0.43% or 0.28%. As a general observation, average AdjFCR at all ages was slightly higher than expected. It was important to take in consideration that these broilers came from a breeder flock that was fed a marginally energy and protein deficient diet. Previous research showed that maternal nutrient restriction could increase progeny FC (Vickers et al., 2000) and negatively affect FCR (Manuscript II).

In general, MarME and MarME+PXP fed broilers were heavier throughout the entire experimental period when compared to the other two treatments. As expected, broilers fed the MarME+PXP diet maintained performance similar to the MarME diet, which would

suggest the PXP enzyme restored NPP and Ca in the MarME diet to normal levels as reported by other researchers (Kiiskinen et al., 1994; Pirgozliev et al., 2008; Musavi et al., 2009; Picón-Rubio et al., 2009).

On the other hand, broilers fed AdqME and MarME+XAP diets weighed less at all ages. However, the BW gain from 0 to 14 d revealed that only MarME+XAP broilers grew more slowly than MarME and MarME+PXP birds, while birds fed the AdqME diet were intermediate. This response was explained by the reduction in FC during the same time interval when AdqME and MarME+XAP were fed, which might suggest that the XAP enzyme cocktail was effective in increasing available energy of the MarME diet. However, the interaction of diet level with Cl level showed that birds fed the MarME+XAP diet at the 0.28% Cl level consumed more feed than the broilers fed the same diet at the 0.43% Cl level. Nevertheless, the reduction in FC achieved with the XAP supplementation was overcome by the inclusion of $NaHCO_3$. Previous research in different animal species demonstrated that the addition of $NaHCO_3$ increased FC (Cooper et al., 1996; Yoruk et al., 2004a; Yoruk et al., 2004b; Tripathi et al., 2004; Ahmad et al., 2006). However, consistency of this response has been debated by other researchers (Fuentes et al., 1998; Balnave et al., 1999) as multiple factors in the diet and physiology of the animal could have modifying effects.

Despite the differences observed in BW, BW gain , and FC, no main effects of diet or Cl level were observed for AdjFCR at 14 d of age. However, a significant interaction was observed that indicated that birds fed the MarME+XAP diet at the 0.43% Cl level were

more efficient than the ones fed the same diet at the 0.28% Cl level. One might predict that the AdqME and MarME+XAP diets would have had a similar response in terms of FC relative to the differences in Cl levels in the diet. However, it appeared that the energy coming from the addition of XAP (mainly from carbohydrates) was not as effective for regulation of FC in the presence of $NaHCO_3$ as it was for the AdqME diet, which had a higher contribution of energy from fat. Previous research in mammals suggested that fat induced satiety was a more effective means of controlling energy intake than additional carbohydrates (Chapman et al., 1999). Conversely, the extra energy present in the AdqME diet (100 kcal ME more than MarME) and MarME+XAP diet was not necessarily converted to BW by the birds, as happened with the MarME and MarME+PXP birds as a result of higher FC. This response may be somewhat explained by the difference in energy (kcal ME/100g of feed) to lysine (ME:Lys) ratios present in the diets. Previous research in male broilers conducted by Macleod (1997) showed that lower true metabolizable energy to lysine ratios increased FC, BW gain, and carcass energy retention as fat and protein. In our experiment, all diets had the same concentration of lysine (1.26%), and the ME:Lys ratios were around 230 for the AdqME and MarME+XAP diets (ME increase of 73 kcal with respect to the MarME diet), respectively, and 222 for MarME and MarME+PXP diets.

In general, benefits from the addition of XAP to the MarME diet were absent after 14 d. This could be due to the down-regulation and/or reduced excretion of endogenous enzymes due to exogenous enzymes inclusion at early age (Mahagna et al., 1995) that may modulate digestive physiology later in life.

From 15 to 35 d broilers fed the MarME and MarME+PXP diets were heavier than AdqME and MarME+XAP fed birds. However, significant diet x Cl level interactions for BW and BW gain at the $P<0.09$ and $P<0.06$ level, respectively, were observed during this time interval that suggested that BW gain of birds fed the MarME+XAP diet was reduced at the 0.28% Cl level. This could be an indication that the apparent contribution to the dietary ME as a result of the supplementation with XAP cocktail was possible only with the 0.43% Cl level. Calculated dietary electrolyte balance (DEB) for the 0.43% Cl level diets was 150 mEq/kg, contrasting with the 193 mEq/kg of the 0.28% Cl diets. This might have suggested that XAP worked better at lower DEB. Ravindran et al. (2008) studied the effect of DEB and the addition of phytase to marginally deficient non-phytate phosphorus and calcium diets. Four DEB levels that consisted of 150, 225, 300, and 375 mEq/kg (i.e. 0.15, 0.18, 0.35, and 0.52% Na, respectively) and two levels of microbial phytase (0 and 500 FTU/kg) were studied. They found that feed efficiency was improved when phytase was added to lower DEB level diets (150 – 300 mEq), but not when 375 mEq/kg were fed. However, they could not determine if this response was because of the DEB level or due to the differences in Na level of the different diets. They attributed the response at 375 mEq to an excess in Na (0.52%) that negatively affected nutrient absorption and metabolism.

On the other hand, Selle et al. (2009) fed four NPP deficient diets that were supplemented with no enzyme, phytase, xylanase, or a combination of both, and then compared these with an adequate NPP diet. All diets had the same sodium, potassium, and chloride content, or a DEB of 196 mEq/kg, which was lower than the 255 mEq/kg recommended by Oviedo-

Rondón et al. (2001) for young broilers. They observed that addition of enzymes alone or in combination improved feed efficiency and ileal amino acid digestibility. Unfortunately, diets with different DEB values were not evaluated in that experiment.

The lack of statistical difference in FC (after 15 d) coincided with observations made by Cowieson and Ravindran (2008) who fed male broilers from 1 to 21 d two diets that were either a nutritionally adequate (PC) or a marginally energy and amino acid deficient (NC). They supplemented these diets with one of two levels of XAP (supplemented or non-supplemented) to complete a 2 x 2 factorial design. They found that addition of the XAP enzyme cocktail did not change the FC with respect to the non-supplemented diet by 21 d of age. On the other hand, BW gain and AdjFCR were improved by the addition of the enzyme cocktail at the same age, something that was not observed in the present experiment. It was important to point out that ME in our AdqME and MarME diets were 181 and 121 kcal/kg, respectively, less than those of Cowieson and Ravindran. In addition, our diets had around 9% less corn, limiting the availability of substrate upon which the enzymes could work. This supported the idea that the effectiveness of feed enzymes would depend upon many factors in the diet and animal digestive tract (Bedford, 2000).

Based on these results it was possible to conclude that Cl levels (controlled by dietary levels of sodium chloride or sodium bicarbonate) in the diet could affect dietary enzyme function during the early stages of broiler life. In addition, factors such as ingredient composition, and energy and amino acid profile could be important to consider in order to obtain the maximum benefit from the XAP enzyme cocktail.

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TABLE IV. 1. Ingredient composition and calculated analysis for broiler dietary treatments for the starter period.

Ingredients	Basal Dietary Treatment[1]			
	AdqME	MarME	MarME+PXP	MarME+XAP
	(%)			
Corn	48.26	51.04	51.03	50.88
Soybean meal (48% CP)	26.40	25.87	25.86	25.79
Corn DDGS	10.70	10.60	10.80	10.60
Poultry by-product meal	4.40	4.48	4.40	4.55
Poultry Fat	5.56	3.32	3.32	3.39
Sodium chloride[1]	-	-	-	-
Sodium bicarbonate[1]	-	-	-	-
Limestone	0.88	0.88	1.05	0.88
Dicalcium phosphate	2.21	2.22	1.47	2.22
Premixes[2]	0.60	0.60	0.60	0.60
Filler (sand)	0.04	0.05	0.50	0.05
Phytase premix[3]	0.00	0.00	0.03	0.00
Avizyme 1502[4]	0.00	0.00	0.00	0.10
L-Lysine	0.11	0.11	0.11	0.11
DL-Methionine	0.15	0.15	0.15	0.15
L-Threonine	0.09	0.09	0.09	0.09
	100.00	100.00	100.00	100.00
Calculated nutrients[5]				
ME, kcal/kg	2900	2800	2800	2800
Crude protein, %	23.00	23.00	23.00	23.00
Lysine, %	1.26	1.26	1.26	1.26
Methionine + Cysteine, %	0.91	0.91	0.91	0.91
Threonine, %	0.84	0.84	0.84	0.84
Calcium, %	0.90	0.90	0.90[4]	0.90
Non-phytate phosphorus, %	0.45	0.45	0.45[4]	0.45
Sodium, %	0.21	0.21	0.21	0.21
Calcium, analyzed, %	1.13	1.11	0.93	1.11
Total phosphorus, analyzed, %	0.99	0.99	0.87	0.99

[1]The were eight dietary treatments consisted of AdqME = adequate P, MarME = marginal P, MarME+PXP = MarME plus 500 FTU/kg feed (Phyzyme XP), and MarME+XAP = MarME plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate and a content of 0.28% Cl. The 0.5% salt versions had no sodium bicarbonate and a content of 0.43% Cl.

[2]Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D_3, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K_3), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na_2SeO_3), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

[3]Phyzyme XP TPT (Danisco Animal Nutrition, Marlborough, UK), an Escherichia coli-derived phytase, was added at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

[4]Avizyme 1502 (Danisco Animal Nutrition, Malborough, UK), is composed of xylanase (300 U/kg), *Trichoderma longibrachiatum*, protease (4000 U/kg), *Bacillus subtilis* and amylase (400 U/kg), and *Bacillus amyloliquofaciens*.

[5]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

TABLE IV. 2. Ingredient composition and calculated analysis for broiler dietary treatments for the grower period.

Ingredients	Basal Dietary Treatment[1]			
	AdqME	MarME	MarME+PXP	MarME+XAP
	(%)			
Corn	55.89	55.17	55.17	55.17
Soybean meal (48% CP)	18.83	18.96	18.96	18.96
Corn DDGS	10.00	10.00	10.00	10.00
Poultry by-product meal	4.40	4.40	4.40	4.40
Poultry Fat	6.01	5.00	5.00	5.00
Sodium chloride[1]	-	-	-	-
Sodium bicarbonate[1]	-	-	-	-
Limestone	0.92	0.92	1.09	0.92
Dicalcium phosphate	2.02	2.01	1.27	2.01
Premixes[2]	0.60	0.60	0.60	0.60
Filler (sand)	0.10	1.67	2.21	1.57
Phytase premix[3]	0.00	0.00	0.03	0.00
Avizyme 1502[4]	0.00	0.00	0.00	0.10
L-Lysine	0.30	0.30	0.30	0.30
DL-Methionine	0.20	0.20	0.20	0.20
L-Threonine	0.16	0.16	0.16	0.16
	100.00	100.00	100.00	100.00
Calculated nutrients[5]				
ME, kcal/kg	3000	2900	2900	2900
Crude protein, %	20.00	20.00	20.00	20.00
Lysine, %	1.20	1.20	1.20	1.20
Methionine + Cysteine, %	0.86	0.86	0.86	0.86
Threonine, %	0.80	0.80	0.80	0.80
Calcium, %	0.85	0.85	0.85[4]	0.85
Non-phytate phosphorus, %	0.40	0.40	0.40[4]	0.40
Sodium, %	0.21	0.21	0.21	0.21
Calcium, analyzed, %	1.05	1.06	0.96	1.10
Total phosphorus, analyzed, %	0.92	0.93	0.81	0.94

[1]The were eight dietary treatments consisted of AdqME = adequate P, MarME = marginal P, MarME+PXP = MarME plus 500 FTU/kg feed (Phyzyme XP), and MarME+XAP = MarME plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate and a content of 0.28% Cl. The 0.5% salt versions had no sodium bicarbonate and a content of 0.43% Cl.

[2]Premixes provided the following (per kg of diet): vitamin A, 6600 IU; vitamin D_3, 2,000 IU; vitamin E, 33 IU; vitamin B_{12}, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione (K_3), 4 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; D-biotin, 126 µg; selenium (as Na_2SeO_3), 0.15 mg; manganese, 60 mg; zinc, 60 mg; iron, 40 mg; copper, 5 mg; iodine, 1.3 mg; cobalt, 0.5 mg; choline chloride, 600 mg; monensin, 99.21 mg.

[3]Phyzyme XP TPT (Danisco Animal Nutrition, Marlborough, UK), an Escherichia coli-derived phytase, was added at 500 FTU /kg to replace 0.12% non-phytate phosphorus (NPP) and 0.1% calcium (Ca).

[4]Avizyme 1502 (Danisco Animal Nutrition, Malborough, UK), is composed of xylanase (300 U/kg), *Trichoderma longibrachiatum*, protease (4000 U/kg), *Bacillus subtilis* and amylase (400 U/kg), and *Bacillus amyloliquofaciens*.

[5]Nutrient compositions were calculated from proximate analyses of all ingredients. The final diet composition was confirmed by proximate analyses.

TABLE IV. 3. Effect of dietary treatment on broiler body weight (BW), body weight gain (BWG), feed consumption (FC), and adjusted feed conversion ratio (AdjFCR), and mortality.

Variable	Age	Treatment[1]								Source of variability		
		Diet					Cl level				Cl	Diet x
		AdqME	MarME	MarME+PXP	MarME+XAP	SEM[2]	0.43%	0.28%	SEM[3]	Diet	level	Cl
		(g)								(*P*-value)		
BW	0	45.4	45.6	45.5	45.4	0.3	45.5	45.5	0.2	NS	NS	NS
	14	511^{ab}	521^{a}	520^{a}	506^{b}	4.3	516	514	3	<0.05	NS	NS
	35	2472^{B}	2531^{A}	2533^{A}	2481^{B}	12.7	2506	2502	9	<0.01	NS	<0.10
	42	3285^{B}	3375^{A}	3365^{A}	3294^{B}	21.3	3325	3335	15	<0.01	NS	NS
BWG	0-14	466^{ab}	476^{a}	474^{a}	460^{b}	4.2	470	468	3	<0.05	NS	NS
	15-35	1960^{B}	2009^{A}	2010^{A}	1975^{B}	10.7	1990	1988	8	<0.01	NS	<0.10
	36-42	813	845	832	813	13.6	819	833	10	NS	NS	NS
FC	0-14	670^{b}	710^{a}	693^{ab}	675^{b}	9.1	677	697	6	<0.05	NS	<0.10
	15-35	3276	3388	3396	3464	71.0	3333	3429	50	NS	NS	NS
	36-42	1566	1696	1593	1636	57.6	1615	1631	41	NS	NS	NS
		(g:g)										
AdjFCR	14	1.44	1.50	1.46	1.47	0.20	1.44	1.49	0.01	NS	NS	<0.05
	35	1.63	1.65	1.65	1.71	0.03	1.64	1.68	0.02	NS	NS	NS
	42	1.70	1.75	1.74	1.79	0.03	1.72	1.77	0.02	NS	NS	NS
		(%)										
Mortality	14	0.78	1.56	1.56	1.71	0.65	1.76	0.78	0.46	NS	NS	NS
	35	1.17	2.34	2.73	3.13	1.15	3.32	1.37	0.81	NS	NS	NS
	42	1.17	2.73	4.29	3.51	1.12	3.51	2.34	0.79	NS	NS	NS

[A, B] Means across columns lacking a common superscript are significantly different at the $P < 0.01$. [a, b] Means across columns lacking a common superscript are significantly different at the $P < 0.05$.

[1]The eight dietary treatments consisted of AdqME = adequate P, MarME = marginal P, MarME+PXP = MarME plus 500 FTU/kg feed (Phyzyme XP), and MarME+XAP = MarME plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate and a content of 0.28% Cl. The 0.5% salt versions had no sodium bicarbonate and a content of 0.43% Cl. [2]SEM for n=8 pens. [3]SEM for n=16 pens.

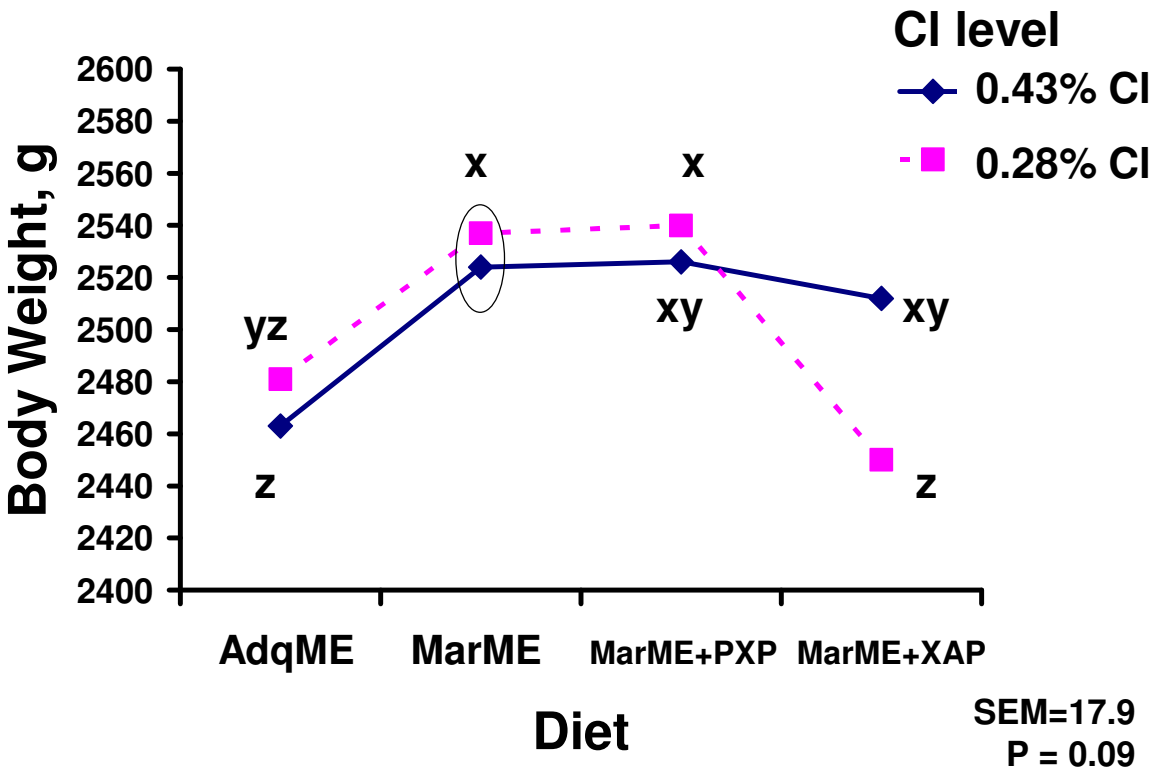

FIGURE IV. 1. Effect of dietary treatment on broiler body weight at 35 d of age. Means inside a circle share the same superscript(s). The eight dietary treatments consisted of AdqME = adequate P, MarME = marginal P, MarME+PXP = MarME plus 500 FTU/kg feed (Phyzyme XP), and MarME+XAP = MarME plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate and a content of 0.28% Cl (square). The 0.5% salt versions had no sodium bicarbonate and a content of 0.43% Cl (diamond). The pooled standard error of the mean is for n=32 pens. Means lacking a common superscript (x, y, z) are significantly different at P<0.10.

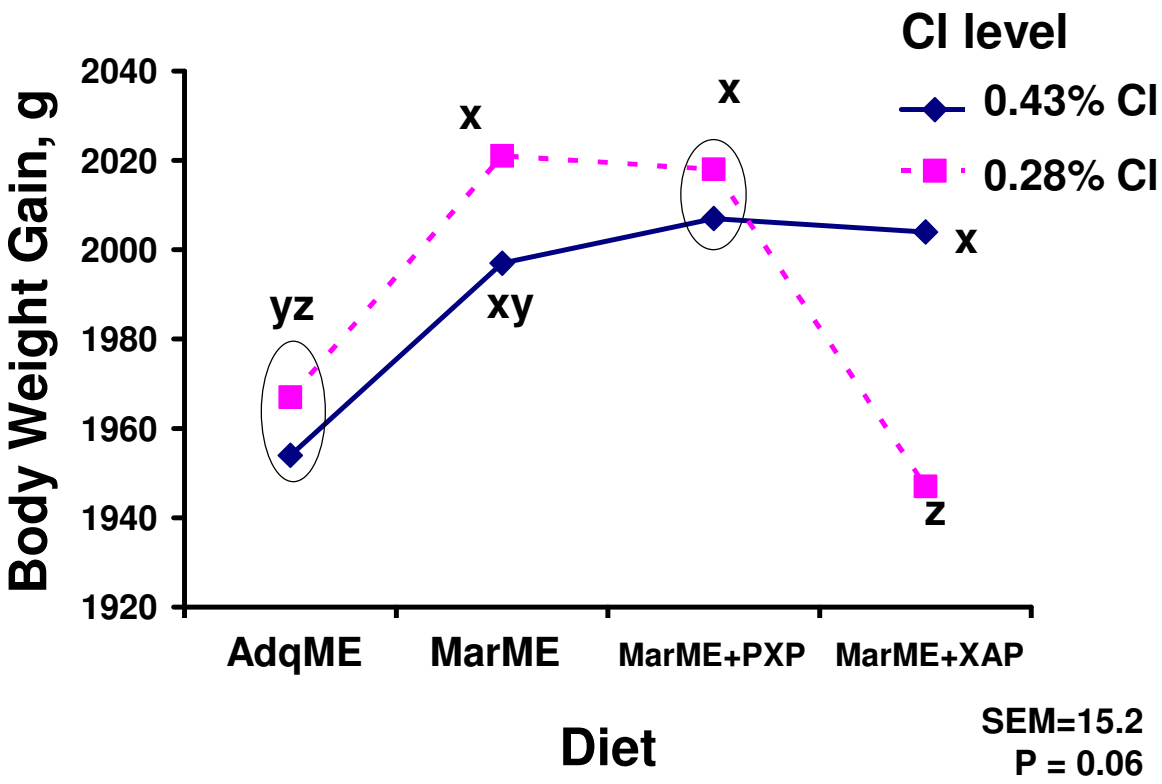

FIGURE IV. 2. Effect of dietary treatment on broiler body weight gain from 14 to 35 d of age. Means inside a circle share the same superscript(s). The eight dietary treatments consisted of AdqME = adequate P, MarME = marginal P, MarME+PXP = MarME plus 500 FTU/kg feed (Phyzyme XP), and MarME+XAP = MarME plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate and a content of 0.28% Cl (square). The 0.5% salt versions had no sodium bicarbonate and a content of 0.43% Cl (diamond). The pooled standard error of the mean is for n=32 pens. Means lacking a common superscript (x, y, z) are significantly different at P<0.10.

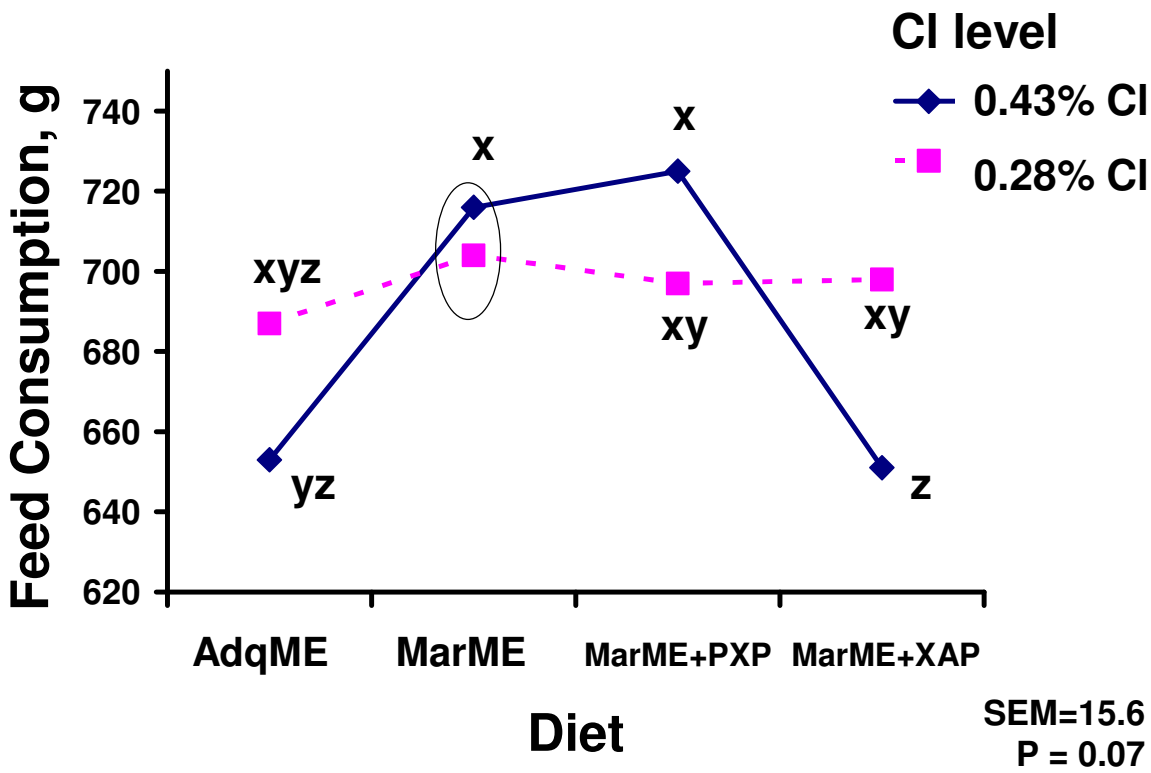

FIGURE IV. 3. Effect of dietary treatment on broiler feed consumption from 0 to 14 d of age. Means inside a circle share the same superscript(s). The eight dietary treatments consisted of AdqME = adequate P, MarME = marginal P, MarME+PXP = MarME plus 500 FTU/kg feed (Phyzyme XP), and MarME+XAP = MarME plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate and a content of 0.28% Cl (square). The 0.5% salt versions had no sodium bicarbonate and a content of 0.43% Cl (diamond). The pooled standard error of the mean is for n=32 pens. Means lacking a common superscript (x, y, z) are significantly different at P<0.10.

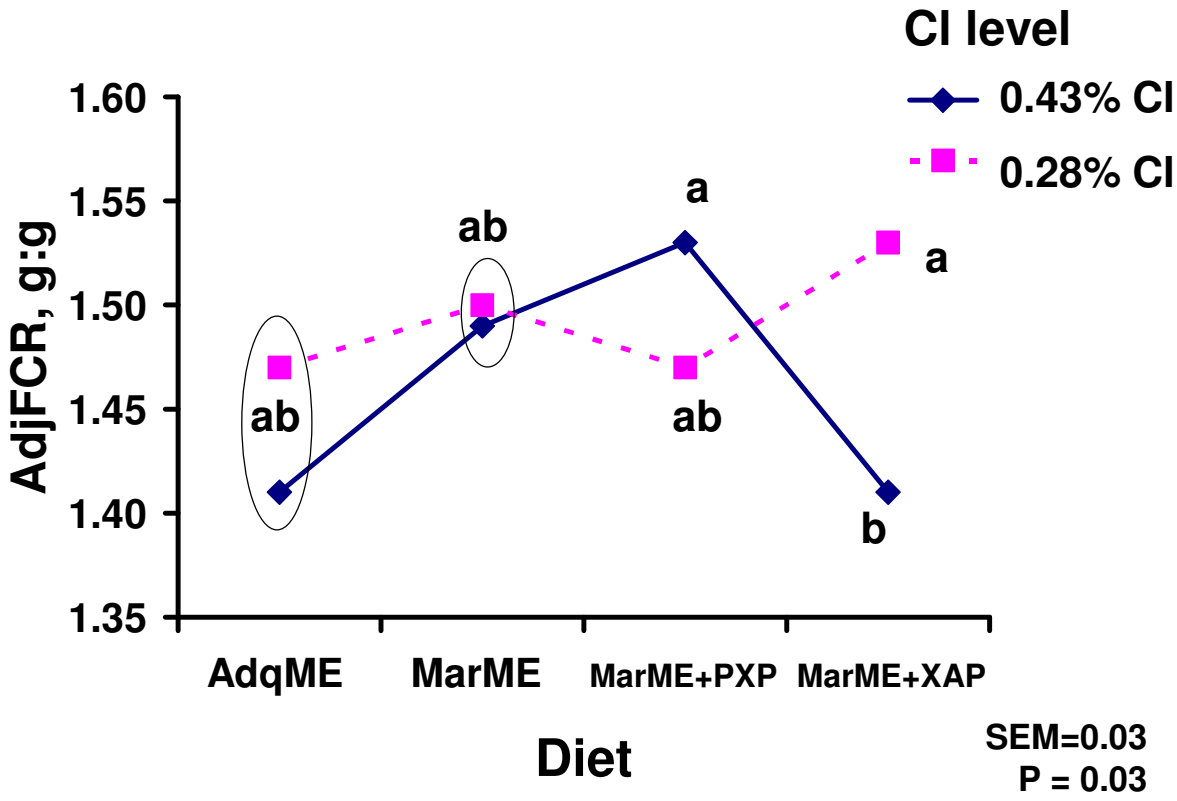

FIGURE IV. 4. Effect of dietary treatment on broiler adjusted feed conversion ratio from 0 to 14 d of age. Means inside a circle share the same superscript(s). The eight dietary treatments consisted of AdqME = adequate P, MarME = marginal P, MarME+PXP = MarME plus 500 FTU/kg feed (Phyzyme XP), and MarME+XAP = MarME plus 0.1% inclusion of Avizyme 1502, with 0.5% or 0.25% NaCl. The 0.25% NaCl versions contained 0.35% sodium bicarbonate and a content of 0.28% Cl (square). The 0.5% salt versions had no sodium bicarbonate and a content of 0.43% Cl (diamond). The pooled standard error of the mean is for n=32 pens. Means lacking a common superscript (a,b) are significantly different at $P<0.05$.

SUMMARY AND CONCLUSIONS

The objective of this research was to develop a better understanding of the effect of the inclusion of a phytase enzyme product (Phyzyme XP; PXP) and a carbohydrase/protease cocktail (Avizyme 1502; XAP) alone or in combination on the productive performance of broiler breeders (broiler parent stock) and their broiler progeny as effected by mineral and nutrient availability to both parent and progeny, maternal effects on the progeny, and interactions with other feed ingredients.

Effect of phytase inclusion in broiler breeder diets on fecal moisture

This experiment revealed that the fecal liquid portion (LP) was not affected by dietary enzyme inclusion or non-phytate phosphorus (NPP) level while the change in Ca level (from 2.7% to 3.0%) and/or a coincident increase in ambient temperature caused an increase in fecal LP, possibly in response to a higher water intake as observed by other researchers. The change from a standard broiler breeder diet to the experimental diets caused hens to reduce the absolute fecal moisture (FM) by 14% following a 7 d adaptation period. However, after 14 d the absolute difference in FM virtually disappeared except when 550 FTU/kg phytase was added to the feed. This phytase inclusion level increased the absolute difference in FM by 10% probably due to an inappropriate Ca:NPP ratio as no Ca matrix value for phytase was assigned in this particular study as would normally be the case in laying diets. Paradoxically, no relationship was established between fecal LP and

FM content as higher LP volume values did not result in higher FM, which suggested a difference in the water holding capacity of the feces, as observed in previous research. No differences in blood Ca or P were observed among dietary treatments in the present experiments, which suggested that the mineral levels in the feed and water were reasonable and homeostatic mechanisms were sufficient to maintain similar mineral levels in blood independent of the dietary treatment.

However, mineral excretion was apparently affected by dietary treatment as the highest inclusion of phytase made birds excrete less di- and trivalent cations and excrete more P, possibly due to the lower P requirement of this mineral by the Heritage 32 birds used in this particular study.

The capacity of phytase to not only release phosphate groups from the phytate molecule but also chelated cations was evident in the present research. In addition, the release of hygroscopic organic macromolecules that could contribute to increased FM had also been observed. Moreover, the apparent higher availability and absorption of certain cations due to inclusion of phytase in feed could negatively affect the availability of P or disrupt absorptive mechanisms.

Based on the present results, it was concluded that under the conditions of this experiment the addition of phytase at 550 U/kg to an NPP deficient broiler breeder layer diet resulted in an increased FM when no matrix value for Ca was given to the phytase.

The reduction in ME at peak egg production in MarP diets did not affect egg production, suggesting that the ME requirement to maintain egg production was less than 450 kcal ME/hen/d using the particular feeding program employed. However, a smaller than usual energy allocation during growing and laying periods and high inclusion of DDGS in feed resulted in lower egg production than observed in previous research, which would have reduced the daily energy requirement.

In general, egg production, as measured by EHH andHDP, and female survivability were negatively affected when PXP and XAP were added in combination to the feed. During a heat stress episode combined with a deliberate female feed restriction (associated quantitative coccidiostat reduction) to control heat stress related mortality, breeders were more likely to die when they received the combination of two enzyme products. The heat stress *per se* and reduced nutrient allocations could have also modulated immune function to affect overall mortality. However, it was possible that the protease (from XAP) in the feed could have disrupted the protease-protease inhibitor balance in the gastrointestinal tract, promoting an excessive remodeling of the lumen that affected nutrient absorption, predisposing birds to coccidiosis. Thus, a combination of deleterious events could have logically occurred.

Results clearly showed what other researchers had observed in that fertility was more sensitive to energy allocation than was egg production, but no consistent differences or trends were detected in hatchability or egg components.

Female and male progeny from the negative control (MarP) fed breeders were heavier at hatching, in agreement with findings from a preliminary experiment with progeny from the same parental flock, probably as a result of the maternal nutrient restriction that can affect muscle development and modulate fatty acid oxidation in the progeny. However, these broiler breeder dietary effects on female and male broiler BW were transient, as a result of a significantly lower FC to 16 d. By 41 d no significant differences in BW, FC, and AdjFCR remained.

Inclusion of PXP alone or in combination with the XAP in broiler diets resulted in greater broiler BW for males and females at 16 d as a consequence of a greater initial FC. By 41 d of age feeding of both enzyme products resulted in greater BW for males, and numerically better AdjFCR.

Based on the data collected, inclusion of PXP and XAP in nutritionally marginal broiler breeder diets did not improve cumulative egg production, fertility, or hatchability. Furthermore, XAP addition appeared to negatively affect survival in females during a heat stress related reduction in feed intake. On the other hand, the addition of PXP and XAP to broiler diets improved performance, especially in males. However, an additive effect of the two enzyme products in broiler diets was not clearly apparent. Finally, the lack of an interaction of breeder and broiler diets or breeder diet effect on cumulative broiler performance indicated that there was no negative vertical effect of parental treatment on progeny response to the dietary enzymes evaluated in this study other than the transient effect on early FC.

Effects of Phyzyme XP and Avizyme 1502 in the presence of marginal energy and limited protein on the performance of broiler breeders and their progeny

Differences in female breeder body weight were observed after 36 wk of age when adequate P (AdqP) females became heavier than the hens in the other treatments. Male feed allocation was the same for all treatments throughout the study, which produced similar BW among the treatments throughout the study although a trend at 64 wk indicated that XAP was affecting male BW.

No treatment effect was observed for cumulative egg production and feed efficiency. However, observations during various periods suggested that AdqP hens laid more eggs, especially during the heat stress episodes when feed restriction was essential to reduce female mortality. Based on these observations, it was possible to state that energy and protein allocation had the most important roles in determination of egg production rate and that enzyme additions did not successfully compensate for basic nutrient allocation reductions used in this experiment.

Survivability contrasted with what we observed in Manuscript II, where females fed the XAP diet exhibited a lower survival. Most of the mortality in the earlier experiment occurred during a heat stress episode where coccidiostat consumption was reduced as a consequence of decreased feed allocation. In the present experiment, preventive measures were taken to avoid the prior situation by administering the coccidiostat in the drinking water. Furthermore, Manuscript I birds were exposed to the dietary treatments for 11 wk previous to the heat stress episode, while the breeders of the present experiment consumed the treatment diets for only 5 wk before high temperatures arrived.

In general, MarP fed breeders had better fertility than breeders fed the AdqP diet and unexpected male mortality in particular pens definitely affected the male:female ratio, which confounded the treatment response to some extent.

As previously observed in another broiler breeder-broiler experimental series performed in our laboratory (Manuscript II), broilers from energy restricted breeders were significantly heavier at hatching but not at the end of the broiler experimental period.

In general, feed consumption tended to be higher to 35 d of age for broilers from breeders fed the MarP and the full dose XAP diets, while no differences were observed subsequent to that age. Nevertheless, cumulative FC was higher for broilers from MarP breeders when compared to progeny from breeders receiving the full dose of XAP, which confirmed previous findings using enzyme cocktails.

It was important to mention that MarP breeders in Manuscript II were subject to a less severe energy restriction, while in the present experiment MarP birds had a greater energy restriction plus protein restriction that could explain the difference in response between the two experiments as maternal nutrient restriction could modulate progeny appetite and metabolism. On the other hand, BW at hatching for chicks in this experiment was lower than for chicks from Manuscript II, but by 42 d of age the latter were smaller. We can not be sure if this was an effect of breeder flock age (40 versus 31 wk of age), breeder nutrient allocation, or a combination of both.

The broiler AdjFCR was not consistently affected by the treatments. At 42 d no differences were observed, which was consistent with previous observations (Manuscript II) when

similar diets were fed to the parental flock. However, at 49 d of age it was observed that breeders fed a full or half dose of XAP produced progeny as efficient as the ones from AdqP breeders.

Broiler BW was significantly higher at 21 d of age when broiler diets were supplemented with a full or half dose of XAP. However, no significant differences in BW were observed among treatments at 35, 42, and 49 d, in contrast to Manuscript II where 42 d broiler BW was improved by the enzyme addition. The lack of effect after the third week of age could be due to the ME difference between the AdqP and MarP diets. Based upon our observations, it was possible to conclude that there was an optimum ME level when PXP and XAP enzymes were used in combination that changed with age of bird, which suggested that changes in digestive physiology in a growing bird should be considered.

The AdjFCR was better in broilers fed full and half dose of XAP at 14 d as compared with broilers fed AdqP, which was similar with our observations in Manuscript II at 16 d. However, no differences in AdjFCR among treatments were observed during the remainder of the experimental period. Differences in broiler performance between the experiments also could be an effect of the overall nutritional status of the broiler breeder flocks. In general, male broiler progeny from Manuscript II (and from a preliminary broiler study in our laboratory with the same breeder flock) were heavier at hatching, also consumed less feed, and were smaller at 42 d, when compared with the males of this experiment at the same age. As mentioned previously, nutrient restriction during mammalian embryo development has been reported to negatively affect initial BW and FC of progeny.

In conclusion, overall productive and reproductive performance were not negatively affected by a reduced energy and protein allocation or by addition of PXP and full dose of XAP, when appropriate management during heat stress episodes was practiced. Also, no negative vertical effect of parental treatment on progeny response to the dietary enzymes evaluated in this study was observed. Finally, nutritional status of the parental breeder flock, broiler progeny age, and energy content of the broiler diet were shown to be important aspects to be considered when broiler progeny feed was formulated.

Effects of dietary phytase and a carbohydrase-protease cocktail on the performance of broilers fed different levels of chloride

As a general observation, average AdjFCR at all ages was slightly higher than expected probably as a consequence of the overall nutritional status of the parental flock which was subject to marginally energy and protein deficient diets at the time of production of the progeny. As observed in previous research, maternal nutrient restriction could increase progeny FC and negatively affect FCR, in agreement with Manuscript III.

The addition of phytase helped maintain performance similar to the MarME diet, which would suggest that the PXP restored NPP and Ca in the MarME diet to normal levels as reported by other researchers.

On the other hand, broilers fed AdqME and MarME+XAP diets weighed less at all ages. Part of the response was explained by the reduction in FC to 14 d of age when AdqME and MarME+XAP were fed. However, the content of Cl ($NaHCO_3$) affected the response to the dietary treatment as birds fed the with XAP in the diet at the 0.28% Cl level consumed

more feed than the broilers fed the same diet at the 0.43% Cl level. This was in agreement with previous research that demonstrated that the addition of $NaHCO_3$ could increase FC. Additionally, supplementation with XAP made broilers more efficient when the diet contained 0.43% Cl. On the other hand, the extra energy present in the AdqME diet (100 kcal ME more than MarME) and presumably in MarME+XAP diets was not necessarily converted to BW by the broilers. In general, benefits from the addition of XAP to the MarME diet were absent after 14 d possibly due to the down-regulation and/or reduced excretion of endogenous enzymes as a result of inclusion of exogenous enzymes at an early age.

At 35 d BW and BW gain were reduced at the 0.28% Cl level, which was an indication that the apparent contribution to the dietary ME as a result of the supplementation with XAP cocktail was possible only at the 0.43% Cl level.

The calculated electrolyte balance (DEB) for the 0.43% Cl level diet was 150 mEq/kg, contrasting with the 193 mEq/kg of the 0.28% Cl diet, with only chlorine different among diets. This might have suggested that XAP worked better at lower DEB. The lack of statistical difference in FC (after 15 d) that confirmed observations made by other researchers that addition of the XAP enzyme cocktail did not change the FC with respect to the non-supplemented diet up to 21 d of age.

Based on these results, it was possible to conclude that Cl levels (or sodium bicarbonate) in the diet could affect dietary enzyme function during the early stages of broiler life. In addition, factors such as ingredient composition, and energy and amino acid profile could

be important to consider in order to obtain the maximum benefit from the XAP enzyme cocktail.

In conclusion, the addition of PXP and XAP to the feed of the broiler breeder parental flock or the progeny did not negatively affect broiler breeder or broiler progeny performance when good heat stress management was practiced. It was concluded that the addition of PXP and XAP to broiler breeder and broiler diets was a potentially safe option to reduce feed cost and inorganic P inclusion in feed, when potential interactions with feed ingredients were taken in consideration.