EFFECTS OF AMYLASE SUPPLEMENTATION UPON THE GROWTH, ENDOGENOUS AMYLASE ACTIVITY, AND INTESTINAL MORPHOLOGY OF MALE TURKEY POULTS

by

Casey Warren Ritz

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in
Poultry Management and Applied Nutrition

APPROVED:

R. M. Hulet, Chairman

J. H. Wolford

D. M. Denbow

J. A. Marcy

L. M. Potter

April, 1993

Blacksburg, Virginia

EFFECTS OF AMYLASE SUPPLEMENTATION UPON GROWTH, ENDOGENOUS AMYLASE ACTIVITY, AND INTESTINAL MORPHOLOGY OF MALE TURKEY POULTS

by

Casey W. Ritz

R. M. Hulet, Chairman

Poultry Management and Applied Nutrition

(ABSTRACT)

A series of experiments was conducted to evaluate the effects of amylase supplementation upon the endogenous amylase levels and growth performance of male turkey poults. In the first experiment, a multi-enzyme supplement containing amylase and an additional protease supplement were added to diets of low (24%) and high (28%) protein content. Enzyme supplements significantly improved performance of birds on the low, but not on the high protein diet. Even with the improvement from enzyme addition, the performance of poults fed the low protein diet was not equal to poults fed the high protein diet.

The second experiment was designed to evaluate the effect of varying levels of amylase and xylanase on bird performance, and to determine optimal level of enzyme inclusion in the basal corn-soybean meal diet. Feed utilization or growth of birds did not differ between the amylase diets, however 200 units of amylase per kilogram of feed produced numerically optimal growth and feed efficiency values. Xylanase inclusion

greater than 160 units per kilogram of feed appeared to be detrimental to bird growth and feed efficiency.

In the third experiment, endogenous amylase levels were measured to determine if supplemental amylase produced a quantitative, qualitative, or inhibitory effect upon the endogenous amylase activity. Amylase supplementation was found to be additive to the endogenous levels and did not inhibit endogenous activity. Amylase supplemented diets decreased body weight and feed efficiency loss due to weighing and handling stress on the birds when compared to the control and xylanase diets. Villi lengths within the jejunal and ileal sections of the small intestine were longer during the first three weeks for amylase supplemented birds when compared to intestinal villi of either the control or xylanase fed poults.

The fourth experiment was conducted to compare the effect on growth and feed utilization of the serial addition of various enzyme preparations to corn-soybean poult diets. In retrospect, due to the serial application of the supplements, assessment of the benefits of a given supplement within a composite application was futile. Amylase, however, was present within the composite supplements fed to poults with the highest growth performance values.

These experiments suggest that the addition of amylase to conventional corn-soybean meal turkey poult starter diets can be effective in improving growth and feed utilization during

the first few weeks. The addition of amylase supplements to basal diets did not inhibit the poult endogenous amylase activity levels and were associated with increased intestinal villus length corresponding to increased utilization and absorption of nutrients. Xylanase appeared to be a detrimental enzyme additive to corn-soybean meal diets fed to male poults.

ACKNOWLEDGEMENTS

I would like to begin by thanking my committee, Drs. R. M. Hulet, J. H. Wolford, D. M. Denbow, J. A. Marcy, and L. M. Potter for their time, helpful criticism and interest in my academic development.

Special thanks go to Dr. Hulet for his guidance with my studies and his example of a caring educator and professional, and for a friendship not often found between a student and his advisor.

Many thanks go to Barbara Self for her help in the lab and at the computer.

To Dr. Vernon Felts go thanks for his friendship at the office and companionship in the woods and for introducing me to the art of turkey hunting and the Southern deer hunt!

To the many who assisted me, the Turkey Research Center staff, Michelle Weisbarth, Mary Rupe and Eric Vaughn go thanks for their assistance.

To my parents Robert and Myrna Ritz, for the strong foundation and family ties that have influenced the direction of my life and ambitions, thank you for everything.

Most importantly, to my wife Sharlee and son Andy for their love, support and patience, thank you for helping me reach this goal by giving unselfishly of yourselves.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The concept of adding enzyme supplements to animal feeds is well known. Research in this field of nutrition has been carried out for decades in an attempt to enhance feed digestibility and nutrient availability, yet enzyme supplementation and application could still be considered a young field. Much of the earlier research was not systematic in its approach, using crude enzyme preparations of little known activity or consequence to the feed and animal systems to which they were applied. As a consequence, such work produced unsatisfactory or inconsistent results, thus giving enzyme supplementation a negative connotation.

Recent resurgence of interest in the use of enzyme supplements has resulted from improvements in enzyme preparation and application. Whereas suitable sources of low-cost enzyme preparations were initially derived from markets other than the animal feed trade, such as the food, beverage, and detergent industries, enzymes are now specifically prepared for use in commercial animal feeds.

Concern for the environment has sparked the need for improvements in waste disposal and reduction in nutrient pollution. Concern for the environment, coupled with the emphasis on efficient feed utilization, has increased interest in the use of enzyme supplements as commercial feed additives.

The clearest evidence of gained benefits from enzyme preparations came from studies involving relatively simple problems, such as when feed utilization was affected by anti-nutritional factors (ANF) of a single compound or a class of compounds that could be destroyed by the action of a single enzyme (Chesson, 1987). Now, with enzyme preparations specifically formulated for the feed industry, improvements are being observed in the application of supplements, and the consistency of results from research is more reliable and reproducible.

Most of the research conducted with enzyme supplementation has been performed using high fiber diets. Such diets typically contained grains such as barley, oats, wheat, and rye which had lowered nutritive value due to the high fiber content. It has been found that enzyme preparations increase the digestibility of the fiber, thus allowing for greater flexibility in feed formulation by allowing the use of low cost, high fiber ingredients. Only a limited number of studies have investigated the effects of enzyme supplementation using conventional corn-soybean meal diets in turkeys, and the results from these studies have been inconsistent.

The effect of supplemental enzymes upon the activity of endogenous amylase in poultry is unknown. With the increasing potential for use of enzyme supplements in the poultry

industry, work is needed to determine the response of endogenous amylase to supplemental enzyme preparations, and to assess if such supplements are additive or suppressive of endogenous amylase activity and the growth potential of the male poult.

REVIEW OF LITERATURE

Enzyme Supplementation

Digestibility and Nutrient Availability

The principle objective of adding enzyme preparations to poultry feed is to improve the digestibility, availability, and utilization of feed, particularly of poorer quality, high fiber feeds (Leeson and Summers, 1991). Poor nutrient digestibility with corresponding poor growth and feed conversion in poults and chicks has been the primary problem of utilizing barley (Rotter *et al.*, 1989), wheat (Pettersson and Aman, 1988), and rye (Moran *et al.*, 1969; Fengler and Marquardt, 1988) as grain sources when compared to corn. Increased digesta viscosity is generally considered to be the reason for reduced digestibility. Increased viscosity is due to highly branched non-starch polysaccharides found within the endosperm cell walls of these grains (Antoniou and Marquardt, 1981; Saini and Henry, 1989) that cause gelatinization of digesta. Enzyme supplements have been shown to increase the nutritive value and reduce the viscous characteristics of barley (Fry *et al.*, 1958; Willingham *et al.*, 1960; Herstad and McNab, 1975; White *et al.*, 1983), wheat and rye (Pettersson and Aman, 1989; Graham *et al.*, 1993).

Pretreatment of grains, prior to the addition of enzyme supplements, has been shown to enhance the action of the enzymes. Fry *et al.* (1957,1958) reported that water treatment of either barley, rye or wheat significantly increased nutrient digestibility and resulted in a significant improvement in poult growth. They also found that enzyme supplementation of diets containing barley significantly improved growth and feed utilization of the poults, but did not improve the performance when added to corn or wheat diets. Climatic factors associated with the geographic area where the grain is grown has been shown to influence the feeding value of the grains and response to water treatment and enzyme supplementation (Willingham *et al.*, 1960; Moran *et al.*, 1969).

Potter *et al.* (1965) reported that metabolizable energy (ME) of barley was improved 18-20% by addition of fungal enzymes or water treatment with the improvement attributed to increased digestibility of the protein, fat, nitrogen-free extract, and fiber fractions. Burnett (1962) reported that water treatment did not improve the nutritional value of corn or wheat.

Autoclaving and pelleting have been used as pretreatment procedures prior to enzyme supplementation. Moran and McGinnis (1968) and Moran *et al.* (1969) found that autoclaving did not enhance the digestibility of barley or growth response to enzyme supplements. In contrast, other authors have found

that autoclaving and pelleting prior to enzyme supplementation enhanced the growth and feed utilization of birds fed enzyme supplements (Rexen, 1981; Bedford *et al.*, 1991). Furthermore, gamma irradiation has been reported to considerably improve the digestibility of rye (MacAuliffe *et al.*, 1979; Patel *et al.*, 1980; Campbell *et al.*, 1983) when used as a pretreatment procedure.

Feed Formulation

Improving the digestibility and nutrient availability of poultry feeds with appropriate enzyme preparations affords the poultry industry the opportunity for greater flexibility and accuracy in feed formulations by allowing for: 1) the use of cheaper raw materials without losing performance, 2) less inclusion of processed (cooked, micronized, flaked, etc.) raw materials, 3) improved performance of standard diets (Inborr, 1990; Graham, 1992). In many areas, grains such as barley, rye, oats and wheat, are available at competitive prices compared to corn and give an opportunity to formulate for cost savings without losing performance through the use of enzyme supplementation.

Pretreatment of feed components either by heating or other procedures already mentioned can be expensive when used to improve nutrient availability. Enzyme supplementation can alleviate the need for excessive pretreatment, and, in

particular, heat treatment (Inborr, 1990). However, to be effective, enzymes must be able to withstand the feed manufacturing processes, the pH within the stomach, and the proteolytic activity of other enzymes associated with the small intestine (Chesson, 1987; Classen, 1992).

Studies indicate that potentially digestible nutrients pass through the small intestine without being utilized (Classen *et al.*, 1988; Pettersson and Aman, 1989). This lack of absorption of such nutrients may be due to the type of grain used in feed formulation or the inherently low enzyme production found in some young animals (Leeson and Summers, 1991; Hotten, 1992). Because of these factors, two additional areas for potential feed enzyme application have been identified: 1) supplementing host endogenous enzyme production, and 2) removing anti-nutritional factors (Classen *et al.*, 1991).

Endogenous Application

Nutritionists have questioned whether or not the addition of enzyme supplements to animal starter diets will inhibit the endogenous enzyme production. There is no evidence that such would be the case, however. There is evidence that increased amounts of degradable nutrients will stimulate the production of endogenous digestive enzymes (Nitsan *et al.*, 1974; Owsley *et al.*, 1986; Sell *et al.*, 1989; Brannon, 1990). Rate of

passage through the digestive tract (Nitsan and Madar, 1978) and amount of feed consumed (Nitsan *et al.*, 1974, 1991, 1991a) have been found to alter endogenous enzymes. The amount of nutrients available for digestion and absorption is potentially increased by adding enzymes that will degrade carbohydrate, protein or fiber of starter feeds (Leong *et al.*, 1962; Potter *et al.*, 1965; Pettersson *et al.*, 1990; Rotter *et al.*, 1990), and in turn production of digestive enzymes may be stimulated (Classen *et al.*, 1991; Graham, 1992). Since feed enzymes are of fungal or bacterial origin, the enzyme structure and environmental requirements would be different from that of the host endogenous enzymes (Inborr, 1990). As a result, the supplemental enzymes would not likely produce a negative feedback response, as found with endogenous digestive enzyme regulation (Nitsan and Madar, 1978).

It should be mentioned that most enzymes added to feed are natural proteins and are already found within the digestive systems of animals, produced by the animal itself or the microflora endemic to the gastrointestinal tract or present in the feed. For these reasons, enzyme supplements should not leave harmful residues in the carcass or require a withdrawal period prior to slaughter (Graham, 1992).

Anti-nutritional Factors/Non-starch Polysaccharides

Research, primarily conducted with broilers, has demonstrated the action of supplemental enzymes upon the degradation of viscous non-starch polysaccharide (NSP arabinoxylans, pectins, and beta-glucans), grain endosperm cell wall polysaccharides, and specific anti-nutritional factors (ANF oligosaccharides, lignin, protein inhibitors). The enzymes that break down these complex molecules do so by maximizing the NSP's potential as an energy source while minimizing ANF effects (Hotten, 1992). Until they are degraded NSP and ANF can block digestion and utilization of important nutrients, e.g. protein and starch, as they are an integral part of plant cell wall matrixes and can enhance gel formation involved with an increase in digesta viscosity (Burnett, 1966; Chubb, 1983; Pettersson and Aman, 1989; Rotter *et al.*, 1989; Choct and Annison, 1990; Annison and Choct, 1991; Graham *et al.*, 1993). Leong *et al.* (1962) suggested that fiber *per se* is not a factor in the response to enzyme supplementation but is related to the type of raw nutrients that are present in the feed. They found that enzymes did not improve growth when added to high fiber diets that did not contain barley.

Many researchers have summarized that considerable reduction in digesta viscosity (manifested as sticky droppings) and improvement in growth rate, feed intake and

feed efficiency can be realized by incorporating enzyme supplements into poultry feeds. Digesta viscosity is primarily due to the presence of high molecular weight fractions of NSP. The movement of digesta relative to viscosity and molecular weight and the corresponding growth and feed efficiency is illustrated in Figure 1. Weight gain and feed conversion can be related to digesta viscosity, with higher viscosity giving a linear reduction in bird performance (White *et al.*, 1983; Bedford *et al.*, 1991; Graham, 1992). Many NSP and ANF fractions have been isolated and identified, and enzymes capable of degrading them have been formulated (Cantor *et al.*, 1989; Rotter *et al.*, 1989; Graham, 1991). The reduction of viscosity allows for enhanced movement of the enzymes and nutrients and subsequently improves the rate and extent of digestion and absorption within the small intestine.

Some anti-nutritive factors are feed components that cannot be digested by endogenous enzymes, such as lignin. The presence of this kind of ANF can lead to digestive disorders. Addition of enzyme supplements can degrade such ANF and release more nutrients available for digestion while at the same time reducing the likelihood of digestive upsets (Graham, 1992). Additionally, ANF may impair interactions between nutrient and digestive components, complex with enzymes to reduce activity, increase secretion of water, protein, lipids and electrolytes, and produce physiological and morphological

changes in the intestines (Guenter, 1992).

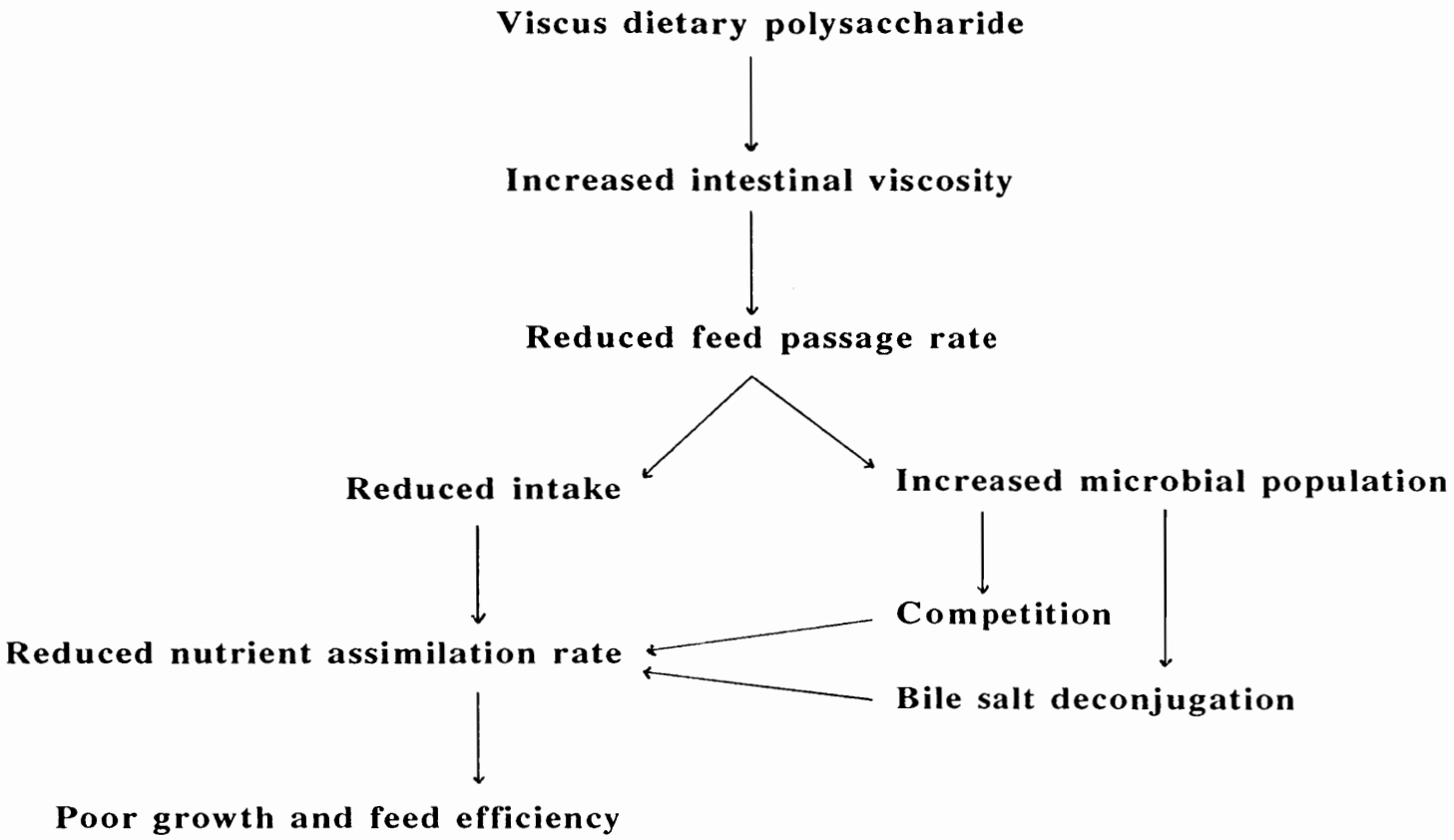

Figure 1. Flow chart of viscosity factors leading to poor growth and feed efficiency. Modified from Bedford (1992).

Age Interaction

Much of the work with enzyme supplements has been associated with young birds since researchers have found little response by adult birds to enzyme supplementation. Indeed, age has been clearly demonstrated to be an influencing factor of enzyme activity within poultry (Krogdahl and Sell, 1989; Pubols, 1991; Nitsan *et al.*, 1991a; Sell *et al.*, 1991). Clickner and Follwell (1926) and Miles *et al.* (1989) observed no increase in egg production from laying hens fed enzyme supplements. Berg (1961) found that pullets did not respond to enzyme supplemented barley diets after 29 weeks of age, and Harper *et al.* (1982) found that the addition of enzyme supplements to either corn or wheat based diets did not enhance egg production of turkey breeders.

Young birds, particularly those under stress (Graham, 1992) may not produce an adequate quantity or array of digestive enzymes to accommodate feed intake (Leeson and Summers, 1991; Hotten, 1992). For this reason, enzyme supplementation may only be of benefit to younger birds (Herstad and McNab, 1975; Rexen, 1981; Reese *et al.*, 1983).

Practical Considerations

There are a number of practical considerations to consider when evaluating enzyme supplementation in feed. Determining the primary problem-causing substrate within a

feed and the subsequent enzyme preparation based on that substrate should be evaluated. Until recently, most enzyme preparations were crude, primarily being composites of multiple enzymes derived from the food, beverage and detergent industries. Amylase was the predominant enzyme in these preparations (Chesson, 1987). Many such preparations used in earlier studies were so divergent in composition that accurate data analysis between studies was ineffective. Willingham *et al.* (1960) and Leong *et al.* (1961) found, however, that highly purified preparations were less effective than crude enzyme supplements.

Today, with the improvements in enzyme preparation and specificity of enzyme-substrate interactions, more and more enzyme products are targeted at specific nutrients within individual feed ingredients. Additionally, optimal enzyme activity within the gut may be better realized if a mixture of enzymes of both fungal and bacterial origin are included in the enzyme preparation (Chesson, 1987).

The procedures used to determine enzyme activity and the protocol used in those determinations are of concern. Unfortunately, the number of enzyme assay procedures are nearly as numerous as there are enzyme manufacturing companies, making it nearly impossible to directly compare the results from different laboratories. In addition, low enzyme concentration and relatively poor recovery result in

inaccurate activity measurements in feeds and high variation between measurements (Rodeheaver and Wyatt, 1984). New methods based on liquid chromatography, gel filtration, electrophoresis and immunological reactions will improve the accuracy and speed of these measurements in the future (Classen *et al.*, 1991).

Handling characteristics and dispersability are a factor when considering enzyme supplementation. Generally, it has been found that enzyme addition to the feed, either in dry form or as a spray (Collier and Hardy, 1986), are the best methods of application. The addition of enzymes to the drinking water is no more, and often less, effective than direct incorporation into the feed (Hesselman, 1983; Potter *et al.*, 1991).

Enzyme stability during storage, feed manufacturing and digestion are of importance in order to ensure a usable product. In order for enzyme supplements to be effective, they must withstand various temperatures, pH, and endogenous gastrointestinal proteolytic activity.

Enzyme supplementation has other potential benefits worthy of consideration. Graham (1992) suggested that reduction of excreta moisture content by enzyme supplements is often reflected in lower water consumption and this leads to less litter problems and fewer carcass downgrades. Additionally, by reducing fermentation in the hind-gut, enzyme

supplements can reduce digestive upsets and the coefficient of variation in live weights among birds. With the reduction in excreta water and improved feed conversion, feed enzymes may also make a significant contribution to reducing poultry manure production (Graham, 1991).

Amylase Supplementation

Supplements

Amylase is used as an enzyme supplement because starch is the predominant energy source within poultry feeds, and the capacity of the host animal's own starch degrading system may be deficient (Chesson, 1987). Experiments using crude enzyme supplements composed of fungal or bacterial amylase are difficult to assess due to inconsistency in results. Amylase supplementation to diets containing grains other than barley has resulted in improvements in body weight gain and feed efficiency, although the results are at times conflicting and inconsistent. Gleaves and Dewan (1970), Parkany-Gyarfas (1975), and Parkany-Gyarfas and Toth (1978) reported enhanced body weight gain and feed efficiency of layers, turkeys, and chickens, respectively, when amylase supplements were added to corn-based diets. In contrast, Hastings (1946), Fry *et al.* (1958), Anderson *et al.* (1961), Moran and McGinnis (1965, 1966, 1968), Moss *et al.* (1977), and Reese *et al.* (1983) were

unable to show any benefit of amylase addition to corn-based diets.

Research comparing purified amylase supplements to crude supplements indicated that the purified supplements did not have the same efficacy (Willingham *et al.*, 1960). It is likely that the crude amylase supplements contained other enzyme activities, such as beta-glucanase and pectinase, that were responsible for the observed effects. Inconsistencies in the growth response of poultry to amylase supplementation could be interpreted in terms of unrecognized variations in the levels of important "contaminating" enzyme activities (Chesson, 1987).

Endogenous Amylase Activity

Moran (1982) reported that one-day old chicks have the capacity to endogenously produce adequate levels of amylase. Age has been correlated with enzyme activity in turkeys (Krogdahl and Sell, 1989; Sell *et al.*, 1989) and in chickens (Nitsan *et al.* 1991, 1991a). Such studies were based upon activity in relation to body size, not adequacy of the activity levels to obtain maximum nutrient utilization. There is need for further evaluation of the effects of dietary amylase supplements upon endogenous amylase activity.

Discrepancy exists as to the presence of amylase within the saliva and crop of poultry. Jerrett and Goodge (1973)

reported that none of the salivary glands of the chicken or turkey displayed significant amylase activity. Amylase activity and starch digestion is found within the crop (Shaw, 1913; Soedermo *et al.*, 1961; Rodeheaver and Wyatt, 1986), though it is not likely that the crop mucosa secretes amylase; rather, the low activity that is present is more likely of food origin than salivary (Farner, 1960; Bhattacharya and Ghose, 1971; Nitsan and Madar, 1978; Phillips and Fuller, 1983) or pancreatic origin (Bird, 1971). Bolton (1965) reported the hydrolyzation of starch within the crop as an indicator of amylase activity.

The pancreas is the main source of amylase within poultry (Moran, 1982). Until recently, there have been contrasting reports as to the presence of secondary endogenous amylase sources. Kokas *et al.* (1967) reported the presence of small intestinal mucosal cell amylase, but Lehrner and Malacinski (1976) reported that there was no evidence to support that claim. Lehrner and Malacinski (1976) reported that secreted pancreatic amylase is capable of generating each amylase type identified in the small intestine lumenal contents and mucosal cell extracts, and implied that amylase extracted from the intestinal cells merely represented strongly absorbed pancreatic amylase. However, Ariyoshi *et al.* (1964) found that starch digestion only decreased by 30% when chickens were depancreatized, indicating the presence of a secondary source

of endogenous amylase. Since these earlier studies, it has been established that the mucosal cells of the small intestine do indeed secrete amylase for feed digestion (Moog, 1981; Osman, 1982; Granner, 1985; Caspary, 1992).

Starch Digestion

Starch is typically the largest single nutrient in feed that provides the greatest proportion of metabolizable energy, and amylase is the only enzyme elaborated by fowl that digests starch (Moran 1982, 1985). Starch is composed of two structural components, a linear fraction (amylose) and a branched fraction (amylopectin), and is digested by amylase into maltose, dextrins, and oligosaccharides, which are converted by maltase and other disaccharidases into glucose. Burnett (1962) found that amylase supplementation overcame growth decreases caused by heat-damaged starch. French (1973) and Moran (1982, 1985) have published excellent reviews of the amylolytic action of amylase upon starch and the incorporation of energy release from the starch degradation.

Small Intestine Morphology

The digestive system of birds is proportionally shorter than that found in mammals (Browne, 1922). Much of this relative decrease is within the intestinal region and would

suggest that birds have less area for digestion and absorption. Additionally, shorter gut length would suggest shorter retention time of feed within the gut and decreased efficiency in nutrient recovery from feed components. The microstructure of the avian intestine has structural components to help compensate for the lack of intestinal length.

The avian intestine lacks not only the length but also the intense folding associated with digestive surface area as found within mammals. However, the mucosal cell layer contains many structures called villi, which contain epithelial cells on their surface, each of which has extensive projections called microvilli (Moog, 1981; Turk, 1982). The microvilli brush border not only acts to increase surface area but also secretes enzymes for nutrient digestion and absorption (Caspary, 1992).

The small intestine is divided into three sections from the proximal to the distal end termed duodenum, jejunum and ileum, respectively. The villi become shorter and broader as the intestine extends towards the ceca, being a function of the digestion and absorption associated with each section. Between the villi are the *crypts of Lieberkuhn* where cell division rapidly occurs. These cells differentiate as they move up the villus becoming either principal or goblet cells (Turk, 1982). The life cycle of these cells is quite

rapid. Imondi and Bird (1966) and Fernando and McCraw (1973) reported life cycles of 48 to 96 hours under normal conditions. The *crypts of Lieberkuhn* also secrete the enzymes enterokinase, which activates trypsinogen, and secretes some amylase (Frandson, 1981). Mature crypt cells are capable of digestion-absorption activities and dominate the villus shortly after the chick hatches, when yolk sac reserves are depleted (Moran, 1985).

The rapid turnover rate of villus epithelium in turn would permit changes in villus length. Dobesh and Clemens (1988) found that gut microstructure and subsequent absorptive-secretory processes adapt to diet selection in dogs. If the absorptive surface area changes to meet dietary conditions in birds, there would be a necessity for rapid turnover of villus epithelium that could result in changes of villus length. Moran (1985) reported that fowl adjust to changes in diet, particularly dietary starch, by altering the amount of amylase endogenously released and by varying the intestinal surface area.

There are indications that villi have a "critical length" determined by nutrient retrieval and cost of maintenance (Moran, 1985). Cameron and Cleffmann (1964) and Michael and Hodges (1973) showed that feed restriction produced a reduction in villi length without impairment of nutrient utilization. Villi lengthening occurred with *ad libitum*

feeding to meet increased growth requirements and in response to competition with microflora (Cook and Bird, 1973) and parasitization (Fernando and McCraw, 1973; Humphrey and Turk, 1974). Enzyme supplementation affects upon intestinal microstructure have not been reported.

LITERATURE CITED

Anderson, J. O., D. C. Dobson, and R. K. Wagstaff, 1961. Studies on the value of hulless barley in chick diets and means of increasing this value. Poultry Sci. 40:1571-1584.

Annison, G., and M. Choct, 1991. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. World's Poult. Sci. J. 47:232-241.

Antoniou, T., and R. R. Marquardt, 1981. Influence of rye pentosans on the growth of chicks. Poultry Sci. 60:1898-1904.

Ariyoshi, S., T. Koike, F. Furuta, K. Ozone, Y. Matsumura, M. K. Dimick, W. L. Hunterg, W. Wang, and S. Lepkovsky, 1964. The digestion of protein, fat and starch in the depancreatized chicken. Poultry Sci. 43:232-238.

Bedford, M. R., 1992. Viscosity overview. Symposium on feed enzymes presented at the 81st Poultry Science Association annual meeting, Fayetteville, Arkansas.

Bedford, M. R., H. L. Classen, and G. L. Campbell, 1991. The effect of pelleting, salt, and pentosanase on the viscosity of intestinal contents and the performance of broilers fed rye. Poultry Sci. 70:1571-1577.

Berg, L. R., 1961. Effect of adding enzymes to barley diets at different ages of pullets on laying house performance. Poultry Sci. 40:34-39.

Bhattacharya, S., and K.C. Ghose, 1971. Influence of food on the amylase system in birds. Comp. Biochem. Physiol. 40:317-320.

Bird, F. H., 1971. Distribution of trypsin and alpha-amylase activities in the duodenum of the domestic fowl. Br. Poult. Sci. 12:373-378.

Bolton, W., 1965. Digestion in the crop of the fowl. Br. Poult. Sci. 6:97-102.

Brannon, P. M., 1990. Adaptation of the exocrine pancreas to diet. Annu. Rev. Nutr. 10:88-105.

Browne, T. G., 1922. Some observations on the digestive system of the fowl. J. Comp. Pathol. Ther. 35:12-32.

Burnett, G. S., 1962. The effect of damaged starch, amylolytic enzymes, and proteolytic enzymes on the utilization of cereals in chickens. Br. Poult. Sci. 3:89-103.

Burnett, G. S., 1966. Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. Br. Poult. Sci. 7:55-75.

Cameron, I. L., and G. Cleffmann, 1964. Initiation of mitosis in relation to the cell cycle following feeding of starved chickens. J. Cell Biol. 21:169-174.

Campbell, G. L., H. L. Classen, R. D. Reichert, and L. D. Campbell, 1983. Improvement of the nutritive value of rye for broiler chickens by gamma irradiation-induced viscosity reduction. Br. Poult. Sci. 24:205-212.

Cantor, A. H., A. J. Pescatore, T. H. Johnson, and W. K. Pfaff, 1989. Influence of beta-glucanase allzyme on performance of broiler chicks fed barley-based diets. Proceedings of Alltech's 5th Annual Symposium, Nicholasville, Kentucky.

Caspary, W.F., 1992. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr. 55:299S-308S.

Chesson, A., 1987. Supplementary enzymes to improve the utilization of pig and poultry diets. Pages 71-90 *in:* Recent Advances in Animal Nutrition. W. Haresign ed. Butterworths.

Choct, M., and G. Annison, 1990. Anti-nutritive activity of wheat pentosans. Br. Poult. Sci. 31:809-819.

Chubb, L. G., 1983. Anti-nutritive factors in animal feedstuffs. Pages 21-35 *in:* Recent Advances in Animal Nutrition. W. Harsign ed. Butterworths

Classen H. L., 1992. Enzyme overview. Symposium on feed enzymes presented at the 81st Poultry Science Association annual meeting, Fayetteville, Arkansas.

Classen. H., G. Campbell, and J. Grootwassink, 1988. Improved feeding value of Saskatchewan-grown barley for broiler chickens with dietary enzyme supplementation. Can. J. Anim. Sci. 68:1253-1259.

Classen, H. L., H. Graham, J. Inborr, and M. R. Bedford, 1991. Growing interest in feed enzymes to lead to new products. Feedstuffs 63 (4):1-5.

Clickner, F. H., and E. H. Follwell, 1926. Application of "protozyme" (*Aspergillus orizae*) to poultry feeding. Poultry Sci. 5:241-247.

Collier, B., and B. Hardy, 1986. The use of enzymes in pig and poultry feeds. The Feed Compounder 6:28-30.

Cook, R. H., and F. H. Bird, 1973. Duodenal villus area and epithelial cellular migration in conventional and germ-free chicks. Poultry Sci. 52:2276-2280.

Dobesh, G. D., and E. T. Clemens, 1988. Nutritional impact on the canine colonic microstructure and function. Nutr. Res. 8:625-633.

Farner, D. S., 1960. Digestion and the digestive system. Pages 411-467 *in:* Biology and Comparative Physiology of Birds. A.J. Marshall, ed. Vol. I Academic Press, New York.

Fengler, I. A., and R. R. Marquardt, 1988. Water-soluble pentosans from rye: II. Effects on rate of dialysis and on the retention of nutrients by the chick. Cereal Chem. 65:298-302.

Fernando, M. A., and B. M. McCraw, 1973. Mucosal morphology and cellular renewal in the intestine of chickens following a single infection of *Eimeria acervulina*. J. Parasitol. 59:493-501.

Frandson, R. D., 1981. Anatomy of the digestive system. Page 325 *in:* Anatomy and Physiology of Farm Animals. 3rd edition, Lea and Febiger, UK.

French, D., 1973. Chemical and physical properties of starch. J. Anim. Sci. 37:1048-1061.

Fry, R. E., J. B. Allred, L. S. Jensen, and J. McGinnis, 1957. Influence of water-treatment on nutritional value of barley. Proc. Soc. Exp. Biol. Med. 95:249-251.

Fry, R. E., J. B. Allred, L. S. Jensen, and J. McGinnis, 1958. Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. Poultry Sci. 37:372-375.

Gleaves, E. W., and S. Dewan, 1970. Influence of a fungal enzyme in corn and milo layer rations. Poultry Sci. 49:596-598.

Graham, H., 1991. Enzymes in monogastric feeds. Int. Milling Flour and Feed July, 1991 pages 33-36.

Graham, H., 1992. Review of recent experimental work on the application of feed enzymes to wheat and barley based turkey diets. Turkeys October, pages 26-27.

Graham, H., M. Bedford, and M. Choct, 1993. High gut viscosity can reduce poultry performance. Feedstuffs February 1, pages 14-15.

Granner, D. K., 1985. Hormones of the pancreas and gastrointestinal tract. Pages 587-609 *in:* Harper's Review of Biochemistry 20th ed. Martin, Mayes, Rodwell, Granner (eds) Lange Medical Publications, Los Altos, CA.

Guenter W., 1992. Anti-nutritional factors. Symposium on feed enzymes presented at the 81st Poultry Science Association annual meeting, Fayetteville, Arkansas.

Harper, J. A., G. H. Arscott, W. B. Roush, and M. P. Goeger, 1982. The comparative feeding value of corn and wheat with and without an enzyme supplement in turkey breeder rations. Poultry Sci. 61:1183-1187.

Hastings, W. H., 1946. Enzyme supplements to poultry feeds. Poultry Sci. 25:584-586.

Herstad, O., and J. M. McNab, 1975. The effect of heat treatment and enzyme supplementation on the nutritive value of barley for broiler chicks. Br. Poult. Sci. 16:1-8.

Hesselman, K., 1983. Effects of beta-glucanase supplementation to barley based diets for broiler chickens. Report 112. Sveriges Lantbruksuniversitet, Uppsala.

Hotten, P., 1992. Enzymes as a feed additive. Feed Mix May pages 9-12.

Humphrey, C. D., and D. E. Turk, 1974. The ultrastructure of chick intestinal absorptive cells during *Eimeria acervulina* infection. Poultry Sci. 53:1001-1008.

Imondi, A. R., and F. H. Bird, 1966. The turnover of intestinal epithelium in the chick. Poultry Sci. 45:142-147.

Inborr, J., 1990. Practical application of feed enzymes. Feed Compounder 10:41-49.

Jerrett, S. A., and W. R. Goodge, 1973. Evidence for amylase in avian salivary glands. J. Morph. 139:27-46.

Kokas, E., J. L. Phillips, and W. D. Brunson, 1967. The secretory activity of the duodenum in chickens. Comp. Biochem. Physiol. 22:81-90.

Krogdahl, A., and J. L. Sell, 1989. Influence of age on lipase, amylase, and protease activities in pancreatic tissue and intestinal contents of young turkeys. Poultry Sci. 68:1561-1568.

Leeson, S., and J. D. Summers, 1991. Ingredient evaluation. Page 47 *in:* Commercial Poultry Nutrition. University Books, Guelph, Ontario.

Lehrner, L. M., and G. M. Malacinski, 1976. Analysis of the structural forms of alpha-amylase present in chicken (*Gallus domesticus*) pancreatic duct juice and intestinal lumen. Comp. Biochem. Physiol. 53:61-66.

Leong, K. C., L. S. Jensen, and J. McGinnis, 1959. Effect of water treatment and enzyme supplementation on the metabolizable energy of barley. Poultry Sci. 41:36-39.

Leong, K. C., L. S. Jensen, and J. McGinnis, 1961. Influence of fiber content of diet on the chick growth response to enzyme supplements. Poultry Sci. 40:615-619.

MacAuliffe, T., D. Zaviezo, and J. McGinnis, 1979. Effect of gamma irradiation, fractionation, and penicillin supplementation on the rachitogenic activity of rye for chicks. Poultry Sci. 58:329-332.

Michael, E., and R. D. Hodges, 1973. Histochemical changes in the fowl small intestine associated with enhanced absorption after feed restriction. Histochemie 36:39-49.

Miles, R. D., J. E. Marion, R. D. Barnett, N. Ruiz, and R. H. Harms, 1989. Response of laying hens to various grains and enzyme supplementation. Florida Scientist 52:1-5.

Moog, F., 1981. The lining of the small intestine. Sci. Amer. 245:154-176.

Moran, E. T. Jr., 1982. Starch digestion in fowl. Poultry Sci. 61:1257-1267.

Moran, E. T. Jr., 1985. Digestion and absorption of carbohydrates in fowl and events through perinatal development. J. Nutr. 115:665-674.

Moran, E. T. Jr., and J. McGinnis, 1965. The effect of cereal grain and energy level of the diet on the response of turkey poults to enzyme and antibiotic supplements. Poultry Sci. 44:1253-1261.

Moran, E. T. Jr., and J. McGinnis, 1966. A comparison of corn and barley for the developing turkey and the effect of antibiotic and enzyme supplementation. Poultry Sci. 45:636-639.

Moran, E. T. Jr., and J. McGinnis, 1968. Growth of chicks and turkey poults fed western barley and corn grain-based rations: effects of autoclaving on supplemental enzyme requirement and asymmetry of antibiotic response between grains. Poultry Sci. 47:152-158.

Moran, E. T. Jr., S. P. Lall, and J. D. Summers, 1969. The feeding value of rye for the growing chick: effect of enzyme supplements, antibiotics, autoclaving and geographical area of production. Poultry Sci. 48:939-949.

Moss, B. R., A. F. Beeckler, C. W. Newman, and A. M. El-Negoumy, 1977. Enzyme supplementation of broiler rations. Poultry Sci. 56:1741.

Nitsan, Z., and Z. Madar, 1978. The level and origin of amylase (EC 3.2.1.1) in the digestive tract of chicks receiving trypsin inhibitors in their diet. Br. J. Nutr. 40:235-241.

Nitsan, Z., Y. Dror, I. Nir, and N. Shapira, 1974. The effects of force-feeding on enzymes of the liver, kidney, pancreas and digestive tract of chicks. Br. J. Nutr. 32:241-247.

Nitsan, Z., G. Ben-Avraham, Z. Zoref, and I. Nir, 1991a. Growth and development of the digestive organs and some enzymes after hatching in broiler chickens. Br. Poult. Sci. 32:515-523.

Nitsan, Z., E. A. Dunnington, and P. B. Siegel, 1991. Organ growth and digestive enzyme levels to fifteen days of age in lines of chickens differing in body weight. Poultry Sci. 70:2040-2048.

Osman, A. M., 1982. Amylase in chicken intestine and pancreas. Comp. Biochem. Physiol. 73:571-574.

Owsley, W. F., D. E. Orr Jr., and L. F. Tribble, 1986. Effects of age and diet on the development of the pancreas and the synthesis and secretion of pancreatic enzymes in the young pig. J. Anim. Sci. 63:497-504.

Parkany-Gyarfas, A., 1975. Utilization of alpha-amylase-supplemented diet in turkey feeding tests. Acta Alimentaria 4:23-35.

Parkany-Gyarfas, A., and M. Toth, 1978. Feed utilization efficiency of enzyme-containing feeds in livestock raising. Part I. Alpha-amylase-containing feeds in the raising of broiler chickens. Acta Alimentaria 7:111-120.

Patel, N. H., M. S. Jami, and J. McGinnis, 1980. Effect of gamma irradiation, penicillin, and/or pectic enzyme on chick growth depression and fecal stickiness caused by rye, citrus pectin, and guar gum. Poultry Sci. 59:2105-2110.

Pettersson, D., and P. Aman, 1988. Effects of enzyme supplementation of diets based on wheat, rye or triticale on their productive value for broiler chickens. Anim. Feed. Sci. Technol. 20:313-324.

Pettersson, D., and P. Aman, 1989. Enzyme supplementation of a poultry diet containing rye and wheat. Brit. J. Nutr. 62:139-149.

Pettersson, D., H. Graham, and P. Aman, 1990. Enzyme supplementation of low or high crude protein concentration diets for broiler chickens. Anim. Prod. 51:399-404.

Phillips S. M., and R. Fuller, 1983. The activities of amylase and a trypsin-like protease in the gut contents of germ-free and conventional chickens. Br. Poult. Sci. 24:115-121.

Potter, L. M., R. M. Hulet, and C. W. Ritz, 1991. Effects of added enzyme supplements to diets of turkeys. Poultry Sci. 70 (Suppl 1):176.(Abstr)

Potter, L. M., M. W. Stutz, and L. D. Matterson, 1965. Metabolizable energy and digestibility coefficients of barley for chicks as influenced by water treatment or by presence of fungal enzyme. Poultry Sci. 44:565-573.

Pubols, M. H., 1991. Ratio of digestive enzymes in chick pancreas. Poultry Sci. 70:337-342.

Reese, G. L., R. S. Karg, B. I. Fancher, and P. W. Waldroup, 1983. The use of supplemental enzymes in the diet of broiler chickens. Nutr. Rep. Int. 28:919-929.

Rexen, B., 1981. Use of enzymes for improvement of feed. Anim. Feed Sci. Technol. 6:105-114.

Rodeheaver, D. P., and R. D. Wyatt, 1984. Evaluation of analytical techniques for quantification of poultry alpha-amylase. Poultry Sci. 63:1855-1860.

Rodeheaver, D. P., and R. D. Wyatt, 1986. Distribution of alpha-amylase activity in selected broiler tissues. Poultry Sci. 65:325-329.

Rotter, B., M. Neskar, R. Marquardt, and W. Guenter, 1989. Effects of different enzyme preparations on the nutritional value of barley in chicken diets. Nutr. Rep. Int. 39:107-120.

Rotter, B. A., O. D. Friesen, W. Guenter, and R. R. Marquardt, 1990. Influence of enzyme supplementation on the bioavailable energy of barley. Poultry Sci. 69:1174-1181.

Saini, J. S., and R. J. Henry, 1989. Fractionation and evaluation of pentosans: comparisons with wheat and rye. Cereal Chem. 66:11-14.

Sell, J. L., O. Koldovsky, and B. L. Reid, 1989. Intestinal disaccharidases of young turkeys: temporal development and influence of diet composition. Poultry Sci. 68:265-277.

Sell, J. L., C. R. Angel, F. S. Piquer, E. G. Mallarino, and H. A. Al-Batshan, 1991. Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. Poultry Sci. 70:1200-1205.

Shaw, T. P., 1913. Digestion in the chick. Am. J. Physiol. 31:439-446.

Soedermo, D., M. R. Kare, and R. H. Wasserman, 1961. Observations on the removal of sugar from the mouth and crop of the chicken. Poultry Sci. 40:123-128.

Turk, D. E., 1982. The anatomy of the avian digestive tract as related to feed utilization. Poultry Sci. 61:1225-1244.

White, W. B., H. R. Bird, M. L. Sunde, J. A. Marlett, N. A. Prentice, and W. C. Burger, 1983. Viscosity of β-D-glucan as a factor in the enzymatic improvement of barley for chicks. Poultry Sci. 62:853-862.

Willingham, H. E., K. C. Leong, L. S. Jensen, and J. McGinnis, 1960. Influence of geographical area of production on response of different barley samples to enzyme supplements or water treatment. Poultry Sci. 39:103-108.

CHAPTER 1

ENDOGENOUS AMYLASE LEVELS AND RESPONSE TO SUPPLEMENTAL FEED ENZYMES AND HANDLING IN MALE TURKEYS FROM HATCH TO EIGHT WEEKS OF AGE

Endogenous Amylase Levels and Response to Supplemental Feed Enzymes and Handling in Male Turkeys From Hatch to Eight Weeks of Age

ABSTRACT Endogenous amylase levels in the pancreas, small intestine, and crop were measured in Nicholas male poults fed diets with and without supplemental amylase from 0 to 8 weeks of age. Eight birds from each of three diets (control, 200 activity units amylase/g supplement, 320 activity units xylanase/g supplement) were sacrificed every three days to determine the amylase and xylanase activity within the pancreas, small intestine, and crop. Pancreatic organ weight was not affected by diet, indicating an absence of dietary amylase effect upon pancreatic tissue growth. Pancreatic amylase activity levels within those birds supplemented with amylase were maintained after a handling stress while amylase activity within the control and xylanase fed birds decreased after a handling stress. Intestinal chyme amylase activity was found to increase with dietary amylase supplementation throughout most the sampling periods over the control, indicating an additive effect of dietary amylase upon endogenous amylase activity. Increased amylase activity may potentially produce improved digestive capabilities during early growth, facilitating a more rapid and complete conversion from the lipid-based diet of the hatchling to the typical protein/carbohydrate-based diet of market turkeys, and

may facilitate increased weight gain and feed utilization. Amylase supplementation may also buffer endogenous amylase levels during stress, decreasing gastrointestinal stress response. Xylanase supplementation within the feed does not appear to affect endogenous amylase activity.

INTRODUCTION

Amylase is an important pancreatic enzyme required for food digestion. Amylase analogues of plant origin are commonly used in enzyme supplement preparations. Krogdahl and Sell (1989) showed that endogenous levels of amylase reach peak activity at 21 days of age in turkey poults fed a reference diet containing 12% sucrose or experimental diets containing 12% fat. They determined that age of the turkey influences enzyme activity levels within the pancreas and small intestine. The influence of supplemental enzymes upon the activity of endogenous amylase in turkeys or chickens is unknown. With the increase in interest in the use of enzyme supplements in poultry diets, work is needed to determine the response of endogenous digestive enzymes to the addition of supplemental enzyme preparations, and to assess if such supplements exert effects upon endogenous enzyme activity.

Xylan is a major component of hemicellulose and can be enzymatically hydrolyzed to xylose by xylanase. Xylanase is of fungal or bacterial origin (Deschamps and Huet, 1985; Biswas *et al.*, 1990) and is not an enzyme endogenous to avian or mammalian species. Xylanase must, therefore, be a supplement in the diet of the animal if any nutritional benefit from xylan degradation is to be derived.

This experiment was, therefore, conducted to determine (1) the levels of endogenous amylase within male poults (0 to

8 weeks of age) fed a traditional corn-soybean meal diet and (2) the effects of amylase and xylanase supplementation upon endogenous amylase levels found within growing poults.

MATERIALS AND METHODS

Three dietary treatments were used in this study; a control and two experimental diets containing two enzyme preparations: Avizyme TK0492-1® containing predominantly amylase (EC 3.2.1.1) and Avizyme TK0492-2® containing predominantly xylanase (EC 3.2.1.8), as provided in enzyme cocktail supplements (Table 1) supplied by FinnFeeds International LTD. Each supplement was added to a conventional corn-soybean meal turkey starter basal (Table 2) at an inclusion rate of 1.1 g supplement/kg feed. Each diet was fed to 152 Nicholas male poults obtained from a commercial hatchery and randomly allocated into Petersime brooder batteries within a windowless, environmentally-controlled facility. Feed and water were available *ad libitum,* and all diets were fed in mash form. At three weeks of age, the poults were transferred to developer batteries in a similarly controlled environment and reared until eight weeks of age.

Enzyme Assay Procedures

Pancreas, intestinal chyme, and crop samples were obtained by sacrificing eight randomly selected birds from each diet every three days for an eight-week period for use in determining the influence of the enzyme supplements on endogenous amylase activity. At one day of age, 24 poults (8 from each diet) were killed by cervical dislocation whereupon

the crop content, intestinal chyme and pancreas were removed. Thereafter, similar samples were taken from each of 24 birds every three days (Day 1 to 55).

The small intestine was excised beginning at the distal end of the ventriculus and extending to the ileocecal junction and uncoiled by cutting the mesentery away from the tract. Intestinal chyme was collected by gently massaging the organ to obtain a homogeneous sample of chyme from each of the three sections of the small intestine. Care was taken to remove blood and excess tissue from each sample to minimize interference with the amylase assay and quantification of amylase activity within a given sample. Samples were frozen immediately in liquid N_2 and stored at -20 °C.

Amylase activity was determined using the method of Bernfeld (1955) as modified by Gertler and Nitsan (1970). To prevent possible enzyme degradation, samples and supernatants were kept on ice throughout the preparation procedure. Samples were diluted 5-10x with distilled water based on sample weight, homogenized with a polytron[1] or stomacher[2] and centrifuged at 16,000 g for 20 minutes. The supernatant was diluted an additional 10-4000x depending upon the origin of the sample, with pancreas samples requiring greater dilution

---

[1]Brinkman Polytron, Kinematica GmBH, Luzern, Switzerland.

[2]Stomacher Lab-Blender 400, Tekmar Co., Cincinnati, OH 45222.

in order to spectrophotometrically quantify the amylase activity present within each sample. Amylase activity was quantified following the procedure described by O'Sullivan *et al.* (1992) wherein an activity unit was defined spectrophotometrically[3] as a change in absorbance at 540 nm due to liberation of reducing groups from a solution of 1% starch after three minutes incubation at 37 C with pH 6.9 in excess 3,5-dinitro-salicylic acid.

Xylanase activity was determined using a procedure described by Biely *et al.* (1988). Replicate aliquots of supernatant (0.5 ml) were added to a xylan substrate dyed with Remazol Brilliant Blue (Sigma, M-5019), dissolved in acetate buffer (11.5mg/ml) and incubated for 4 hours at 37 C. The reaction was stopped with 96% ethanol to precipitate the unhydrolyzed RBB-xylan substrate. Following a 30-minute standing period to obtain thermal equilibrium of samples, the duplicates were centrifuged at 4,000 g for 10 minutes and the resulting decanted supernatant absorbance was read at 590 nm against a respective substrate blank to quantify xylanase activity.

Correct dilution for each sample was determined when spectrophotometric optical density (OD) readings were read within a confidence range of 1×10^{-1} and 9×10^{-1} OD units.

---

[3]Spectronic 1001, Bausch and Lomb, Rochester, NY 14603.

Optical density units for duplicates A and B for each sample (used to determine mean sample activity) were kept within ± 2 X 10^{-1} OD units of each other to insure an acceptable range of confidence for a given sample.

Luna and Luna (1992) reported that amylase is susceptible to structural breakdown during sample preparation. Therefore, three sample preparation methods were compared to determine if the method influenced amylase breakdown within pancreatic tissue samples. Eight replicate samples were analyzed for each method. The first method entailed homogenizing the tissue prior to centrifugation, and vortexing supernatant-substrate combinations throughout the assay. The second method involved mincing the sample, stomaching it prior to centrifugation, and vortexing the supernatant and substrate combinations throughout the assay. The final procedure involved mincing and stomaching of the sample as in the second method, then replacing the vortex procedure with inversion of the test tubes to mix the supernatant-substrate combinations during the assay.

Analysis of variance and Duncan's multiple range test procedures were used for data analysis and mean comparisons (SAS, 1985). Significance was determined at the $P<.05$ level.

RESULTS AND DISCUSSION

Sample Preparation Comparison

Recorded pancreatic amylase activity was significantly higher with the use of the homogenizer compared to the methods that did not utilize homogenization (Table 3). These results suggest that the homogenization of pancreatic tissue liberates amylase and increased the amount of amylase available to react with the substrate, and does not appear to cause destruction of the enzyme prior to complexing with the substrate. Based on these results, the method of sample preparation utilizing homogenization was followed to obtain the results reported in the remainder of this chapter and in Appendix I.

Enzyme Activities

Differences among pancreatic weights by dietary treatment within any of the sampling periods were not significant (Figure 1), as similarly observed by Krogdahl and Sell (1989). This lack of weight and treatment interaction indicates the absence of a dietary enzyme influence upon pancreatic tissue growth. Body weights of birds sacrificed were not taken. This precludes the possibility of evaluating organ weight as a percentage of body weight as reported by Krogdahl and Sell (1989). Variation in amylase and xylanase activity between the sample sets at any given time period was likely affected by sample size, consistency of the sample material at the time

of collection, collection time postprandial, and assay sensitivity.

Mortality was 1.5% for the eight-week period, excluding those birds sacrificed for the assay sample collection, and was not significantly influenced by diet, enzyme supplement, or their interaction ($P<.05$).

The enzyme activity measured in the control fed birds is considered to be the endogenous amylase levels of male poults fed a corn-soybean meal diet. Pancreatic amylase activity corresponding to the respective dietary treatments are found in Figure 2 (also Appendix IV-Figure 1). Amylase activity in the pancreas of poults fed the amylase supplemented diet was significantly higher on Days 10, 25, 43, and 46 [and numerically higher on Days 16, 37, 40, and 55].

Age and metabolic need have been correlated to enzyme activity in turkeys (Krogdahl and Sell, 1989; Sell *et al.*, 1989, 1991). Pancreatic amylase activity appeared to increase in a linear fashion with age and continued to follow this progressive rise throughout the 8 weeks. This continued increase in activity appears to be in contrast to that reported by Krogdahl and Sell (1989) where a stabilization in pancreatic amylase was observed after 21 days. Moran (1985) reported that one-day old chicks have the capacity to produce adequate levels of amylase to facilitate digestion. The results of this study would indicate that amylase activity

continues to increase with age to maintain adequate activity levels for digestion.

The amylase activity found in the intestinal chyme (Figure 3, Appendix IV-Figure 2) is of interest because the additive and/or beneficial effects of enzyme supplementation is most likely to be observed there due to the dietary origin of the supplements. Though not significantly different from the remaining diets, the amylase supplemented diet resulted in higher levels of amylase activity within the intestinal chyme at several sampling periods. Higher amylase levels within the chyme may indicate that there may be an additive effect of dietary amylase upon endogenous amylase activity within the intestine, allowing for greater ingredient degradation and absorption. Since the supplemental amylase is of plant origin and the endogenous amylase is of animal origin, the physical structure of the supplemental amylase should be sufficiently different (Inborr, 1990) so that the increased amylase activity within the chyme would not induce a negative feedback inhibition response in the pancreas, as described by Nitsan and Madar (1978). This additive effect of supplemental amylase upon the endogenous amylase activity may potentially improved digestive capabilities during the first weeks of growth in the digestive tract, as suggested by Leeson and Summers (1991) and Hotten (1992). In addition, the supplemental amylase may facilitate a more rapid and complete

conversion from the lipid-based diet of the hatchling to the typical protein/carbohydrate-based diet of market turkeys. Potentially increasing digestive capabilities may benefit growth performance during brooding, as shown by the body weight and feed utilization values obtained with amylase supplementation reported in Chapter 2.

Amylase activity in the crop (Figure 4) was not found to be affected by diet. The presence of amylase in the crop indicates the beginning of starch digestion and sugar absorption, as previously suggested by Shaw (1913), Soedermo *et al.* (1961), Bolton (1965), and Rodeheaver and Wyatt (1986). It is likely, however, that most of the amylase activity in the crop is of feed origin (Nitsan and Madar, 1978) or due to regurgitation or reflux activity of the intestine and pancreas (Bird, 1971). While the salivary glands and crop mucosa do synthesize amylase, their contribution of amylase for the total digestive process is very limited (Jerrett and Goodge, 1973).

Xylanase activity was similar within the crop and intestine of birds fed the xylanase supplemented diet (Figure 5). Xylanase activity was not determined for the amylase diet because of the low levels of xylanase added within the diet and the sensitivity limits of the assay.

Stress Response

The handling and transfer of the birds for purposes of weighing or randomization has been documented as being stressful (Bowen and Washburn, 1984). Gross and Siegel (1983) and Zulkifli *et al*. (1993) reported that stress indicators, namely heterophil/lymphocyte ratios taken from laying hens, increase within 24 hours after handling and placement in a new cage environment. Sapolsky (1992) noted that the gastrointestinal response plays a critical role in adaptation. The gastrointestinal response includes inhibition of digestion via the autonomic nervous system through decreased blood flow to the gastrointestinal tract and inhibition of hormone output, namely cholecystokinin (CCK). Cholecystokinin stimulates pancreatic enzyme secretion and, when inhibited, results in decreased digestive enzyme secretion (Granner, 1985). The gastrointestinal adaptation response may result in decreased growth and feed efficiency for a period of time after stress, depending upon the magnitude and duration of the stress period (Sapolsky, 1992).

In this study, poults fed the control and xylanase diets had decreased pancreatic amylase activity 1 to 3 days after weighing or transport, while the amylase diet activity was depressed only after the final weighing and randomization period (Table 4, Appendix IV-Table 1). The data in Table 1 (Appendix IV) and Table 4 indicate the change in amylase

activity within the pancreas and intestine before and after handling and transport.

Stress from handling, food and water deprivation, heat, or threat to homeostasis increases serum glucocorticoid levels (Beuving, 1980; Munck and Guyre, 1986). Since glucocorticoid hormones inhibit release of most cytokines (Sapolsky, 1992), decreased CCK levels may have been involved in the decreased activity of pancreatic amylase corresponding to birds fed Diets 1 and 3. By six days post-weighing, the amylase activity of the samples taken from birds fed Diets 1 and 3 were again increasing, indicating a return to normal amylase levels. Since birds fed the amylase supplemented diet did not have a drop in pancreatic amylase activity as observed in birds fed Diets 1 and 3, amylase supplementation may be reducing the response to stress and thereby diminishing possible stress-induced growth stasis or decreased feed efficiency. The response of birds fed the amylase supplemented diet suggests that amylase was still being secreted at sufficient levels by the pancreas during the periods of stress, or that the amylase in the diet was perhaps assisting in the maintenance of amylase synthesis either by the pancreas, the intestinal mucosa or both.

Dietary amylase appears to supplement endogenous amylase activity levels within the male turkey poult. The increase in total amylase activity associated with the amylase

supplemented diet may ameliorate inhibitory response to stress manifested as decreased growth and feed efficiency. If found to be consistent through further experimentation, this reduction in stress response from amylase supplementation potentially has application to the poultry industry through improved growth, greater feed efficiency, and reduced digestive response to stress or disease.

ACKNOWLEDGEMENTS

Enzyme supplements were formulated by Cultor LTD. Technology Center, 02460 Kantvik, Finland, and provided by FinnFeeds International LTD., Market House, Ailesbury Court, High Street, Marlborough, Wiltshire, SN8 1AA, U.K.

REFERENCES

Bernfeld, P., 1955. Amylases, α and β. Pages 149-155 *in:* Methods in Enzymology, Vol. 1. S. P. Colowick and N. O. Kaplan, ed. Academic Press, New York, NY.

Beuving, G., 1980. Corticosteroids in laying hens. Pages 65-84 *in:* The laying hen and its environment. R. Moss (ed) Martinus, Nijhoff, Boston.

Bhattacharya, S., and K. C. Ghose, 1971. Influence of food on the amylase system in birds. Comp. Biochem. Physiol. 40:317-320.

Biely, P., D. Mislovicova, and R. Toman, 1988. Remazol brilliant blue-xylan: a soluble chromogenic substrate for xylanases. Methods in Enzymology 160:536-541.

Bird, F. H., 1971. Distribution of trypsin and alpha-amylase activities in the duodenum of the domestic fowl. Br. Poult. Sci. 12:373-378.

Biswas, S. R., S. C. Jana, A. K. Mishra, and G. Nanda, 1990. Production, purification, and characterization of xylanase from a hyperxylanolytic mutant of *Aspergillus ochraceus*. Biotech. and Bioeng. 35:244-251.

Bolton, W., 1965. Digestion in the crop of the fowl. Br. Poult. Sci. 6:97-102.

Bowen, S. J., and K. W. Washburn, 1984. Preconditioning to heat stress by a nontemperature stressor. Poultry Sci. 63:917-919.

Deschamps, F. and M. C. Huet, 1985. Xylanase production in solid-state fermentation: and study of its properties. Appl. Microbiol. Biotech. 22:177-180.

Fry, R. E., J. B. Allred, L. S. Jensen, and J. McGinnis, 1958. Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. Poultry Sci. 37:372-375.

Gertler, A., and Z. Nitsan, 1970. The effect of trypsin inhibitors on pancreatopeptidase E, trypsin, chymotrypsin and amylase in the pancreas and intestinal tract of chicks receiving raw and heated soya-bean diets. Br. J. Nutr. 24:893-904.

Granner, D. K., 1985. Hormones of the pancreas and gastrointestinal tract. Pages 587-609 *in:* Harper's Review of Biochemistry 20th ed. Martin, Mayes, Rodwell, Granner (eds) Lange Medical Publications, Los Altos, CA.

Gross, W. B., and H. S. Siegel, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases 27:972-979.

Hotten, P., 1992. Enzymes as a feed additive. Feed Mix May 1992.

Inborr, J., 1990. Practical application of feed enzymes. Feed Compounder 10:41-49.

Jerrett, S. A., and W. R. Goodge, 1973. Evidence for amylase in avian salivary glands. J. Morph. 139:27-46.

Krogdahl, A., and J. L. Sell, 1989. Influence of age on lipase, amylase, and protease activities in pancreatic tissue and intestinal contents of young turkeys. Poultry Sci. 68:1561-1568.

Leeson, S., and J. D. Summers, 1991. Ingredient evaluation. Page 47 *in:* Commercial Poultry Nutrition. University Books, Guelph, Ontario.

Luna, L. G., and D. E. Luna, 1992. Methods for the use of hyaluronidase amylase and neuraminidase (sialidase). Histo-Logic® 22:292-297. (Technical bulletin for histotechology produced by Miles Laboratories).

Moran, E. T. Jr, 1982. Starch digestion in fowl. Poultry Sci. 61:1257-1267.

Moran, E. T. Jr., 1985. Digestion and absorption of carbohydrates in fowl and events through perinatal development. J. Nutr. 115:665-674.

Munck, A., and P. M. Guyre, 1986. Glucocorticoid physiology, pharmacology and stress. Pages 81-96 *in:* Advances in Experimental Medicine and Biology. Chrousos, Loriaux, Lipsett (eds). Plenum Press, NY.

Nitsan, Z., and Z. Madar, 1978. The level and origin of amylase (EC 3.2.1.1) in the digestive tract of chicks receiving trypsin inhibitors in their diet. Br. J. Nutr. 40:235-241.

Osman, A. M., 1982. Amylase in chicken intestine and pancreas. Comp. Biochem. Physiol. 73:571-574.

O'Sullivan, N. P., E. A. Dunnington, A. S. Larsen, and P. B. Siegel, 1992. Correlated responses in lines of chickens divergently selected for fifty-six-day body weight. 3. Digestive enzymes. Poultry Sci. 71:610-617.

Rodeheaver, D. P., and R. D. Wyatt, 1984. Evaluation of analytical techniques for quantification of poultry α-amylase. Poultry Sci. 63:1855-1860.

Rodeheaver, D. P., and R. D. Wyatt, 1986. Distribution of α-amylase activity in selected broiler tissues. Poultry Sci. 65:325-329.

Sapolsky, R. M., 1992. Neuroendocrinology of the stress-response. Pages 287-324 *in:* Behavioral Endocrinology. J.B. Becker, S.M. Breedlove, and D. Crews, ed. MIT press, Cambridge, Mass.

SAS Institute, 1985. SAS® User's Guide: Statistics. Version 5 Edition. SAS Institute Inc., Cary, N.C.

Sell, J. L., O. Koldovsky, and B. L. Reid, 1989. Intestinal disaccharidases of young turkeys: temporal development and influence of diet composition. Poultry Sci. 68:265-277.

Sell, J. L., C. R. Angel, F. S. Piquer, E. G. Mallarino, and H. A. Al-Batshan, 1991. Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. Poultry Sci. 70:1200-1205.

Shaw, T. P., 1913. Digestion in the chick. Am. J. Physiol. 31:439-446.

Soedermo, D., M. R. Kare, and R. H. Wasserman, 1961. Observations on the removal of sugar from the mouth and crop of the chicken. Poultry Sci. 40:123-128.

Zulkifli, I., E. A. Dunnington, W. B. Gross, A. S. Larsen, A. Martin, and P. B. Siegel, 1993. Responses of dwarf and normal chickens to feed restriction, *Eimeria tenella* infection, and sheep red blood cell antigen. *In press*.

Table 1. Enzyme activity of dietary supplements

Diet	Amylase	Xylanase	Protease	Pectinase
	Activity units/g supplement			
1 (Control)	-	-	-	-
2 (Amylase)	200	80	100	750
3 (Xylanase)	1	320	100	750

Supplement inclusion rate = 1.1g/kg diet.
Activity unit = micromole substrate hydrolyzed/min/g.

Table 2. Basal diet composition

Ingredient	g/kg diet
Ground yellow corn	459.7
Stabilized fat[1]	20.0
Dehulled soybean meal	435.0
Menhaden fish meal	25.0
Meat and bone meal	25.0
Defluorinated phosphate	22.5
Ground limestone	2.5
Iodized salt	4.0
DL-Methionine	2.8
Trace mineral mix[2]	1.0
Vitamin and additive mix[3]	2.5
Total	1000.0
Crude protein (%)	28
Metabolizable energy (kcal/kg diet)	2960

[1]Commercial animal-vegetable blend.

[2]The trace mineral mix provided (per g mix): 0.45 mg cobalt, 5 mg copper, 2 mg iodine, 120 mg manganese, 120 mg zinc, and 40 mg iron, with calcium carbonate as a diluent.

[3]The vitamin and additive mix provided (per g mix): 1764 IU vitamin A, 661.5 ICU vitamin D_3, 1.1 IU vitamin E, 0.88 mg riboflavin, 1.76 mg d-calcium pantothenate, 8.82 mg niacin, 74.97 mg choline chloride, 0.002 mg vitamin B_{12}, 0.22 mg thiamine mononitrate, 0.220 mg pyridoxine-HCl, 0.02 mg d-biotin, 0.04 mg selenium, 198 mg methionine, 0.22 mg folic acid, 1.06 mg menadione sodium bisulfite complex, and 25 mg ethoxyquin (as a preservative).

Table 3. Comparison of amylase activity measurements as influenced by pancreatic sample preparation method

Procedure[1]	Activity units[2]
Homogenize/Vortex	14,347[a]
Stomach/Vortex	9,067[b]
Stomach/Invert	9,201[b]
Pooled SEM = 558	

Means with different superscripts are significantly different ($P<.05$).
[1]Eight replicate pancreas samples were analyzed for each procedure.
[2]Activity unit = micromole substrate hydrolyzed/min/g.

Table 4. Amylase activity response to handling stress

Stressor	Date	Sampling date	Diet[1]	Relative change[2]	
				Pancreas	Intestine
Weigh	Day 8	Day 10	1	-	+
			2	+	+
			3	-	o
Weigh, move	Day 22	Day 25	1	-	o
			2	+	o
			3	-	o
Weigh, randomize	Day 36	Day 37	1	-	o
			2	+	o
			3	-	o
Weigh, randomize	Day 50	Day 52	1	-	+
			2	-	+
			3	-	+

[1] Diet 1 = control
2 = amylase supplementation
3 = xylanase supplementation

[2]Relative change in activity determined by using 2X ANOVA coefficient of variation differential between mean activity prior to and after stress.
+ = increase in activity
- = decrease in activity
o = no change

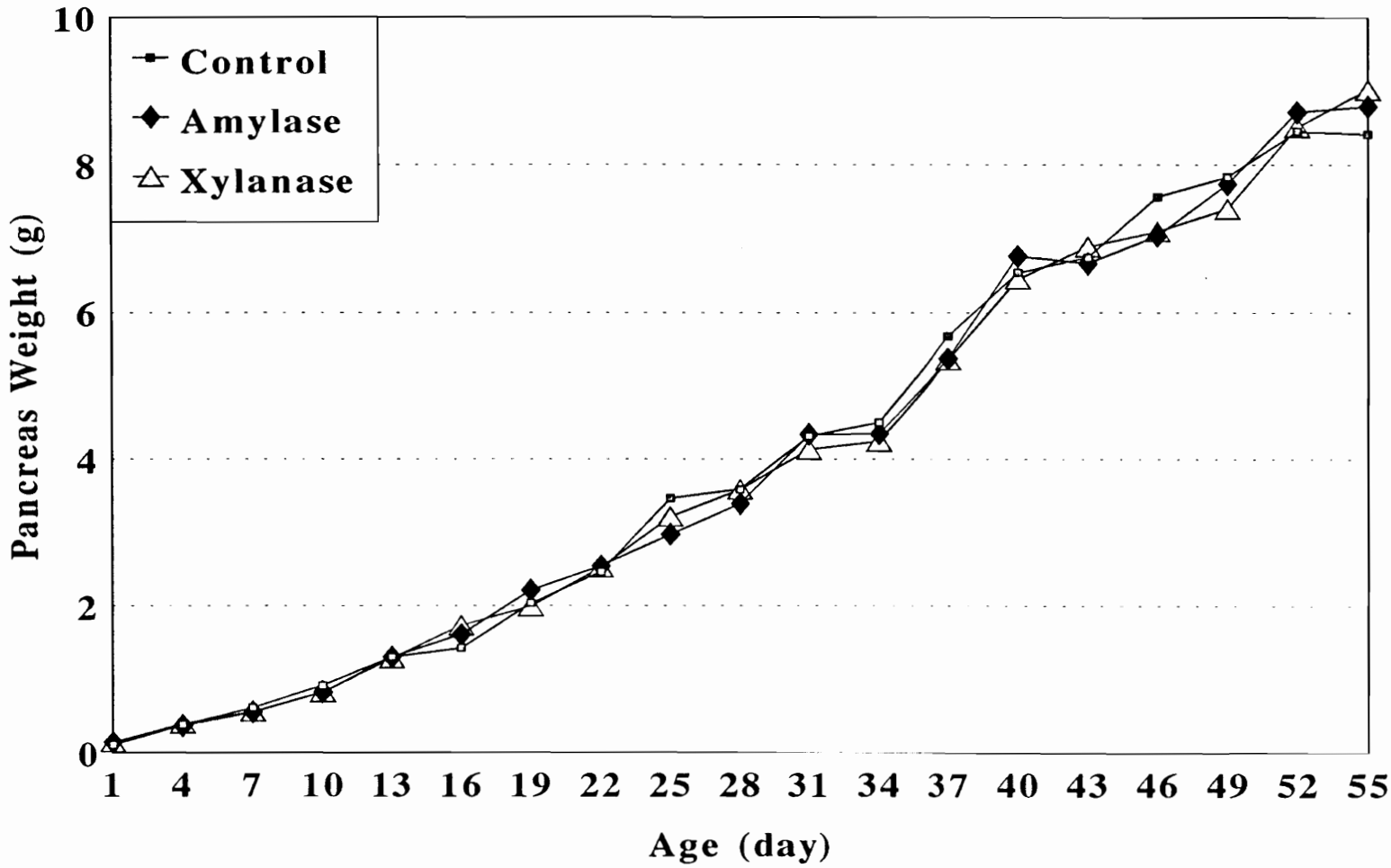

Figure 1. Pancreas weight of male turkeys 0 to 55 days of age fed either control, amylase, or xylanase supplemented diets. Each point represents n = 8.

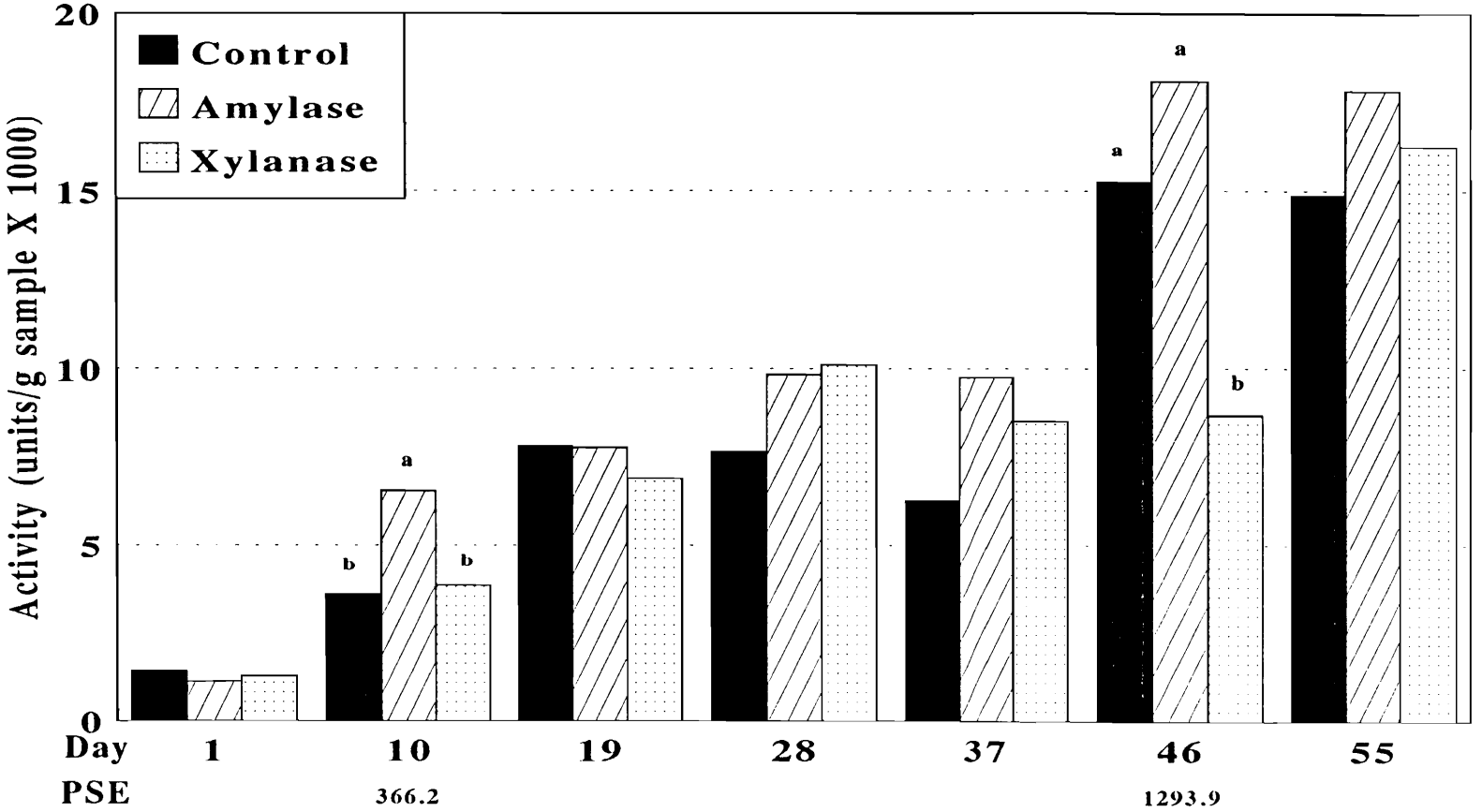

Figure 2. Pancreatic amylase activity of male turkeys 0 to 55 days of age as affected by dietary amylase and xylanase supplementation. Means (n = 8) within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

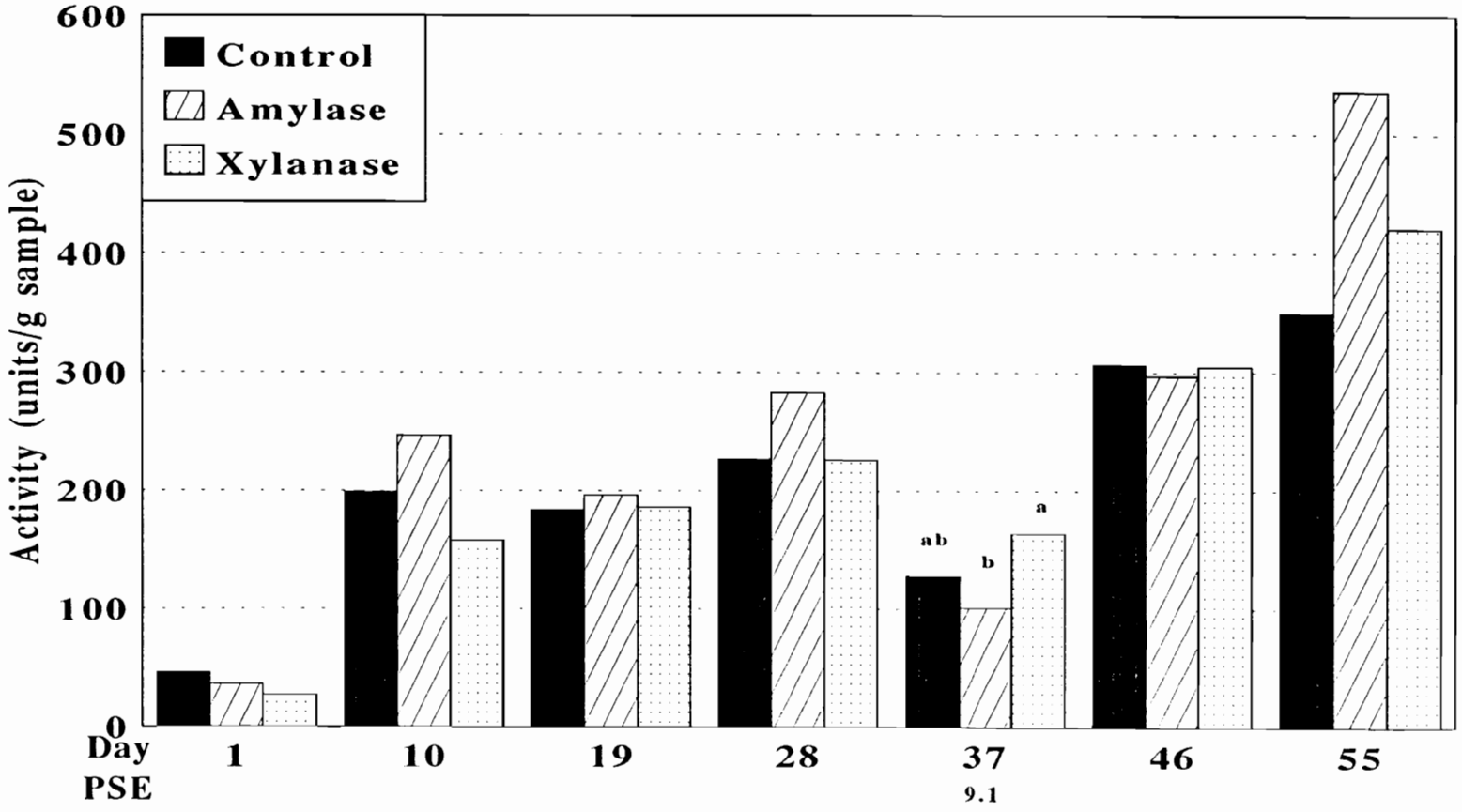

Figure 3. Intestinal amylase activity of male turkeys 0 to 55 days of age as affected by dietary amylase and xylanase supplementation. Means (n = 8) within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

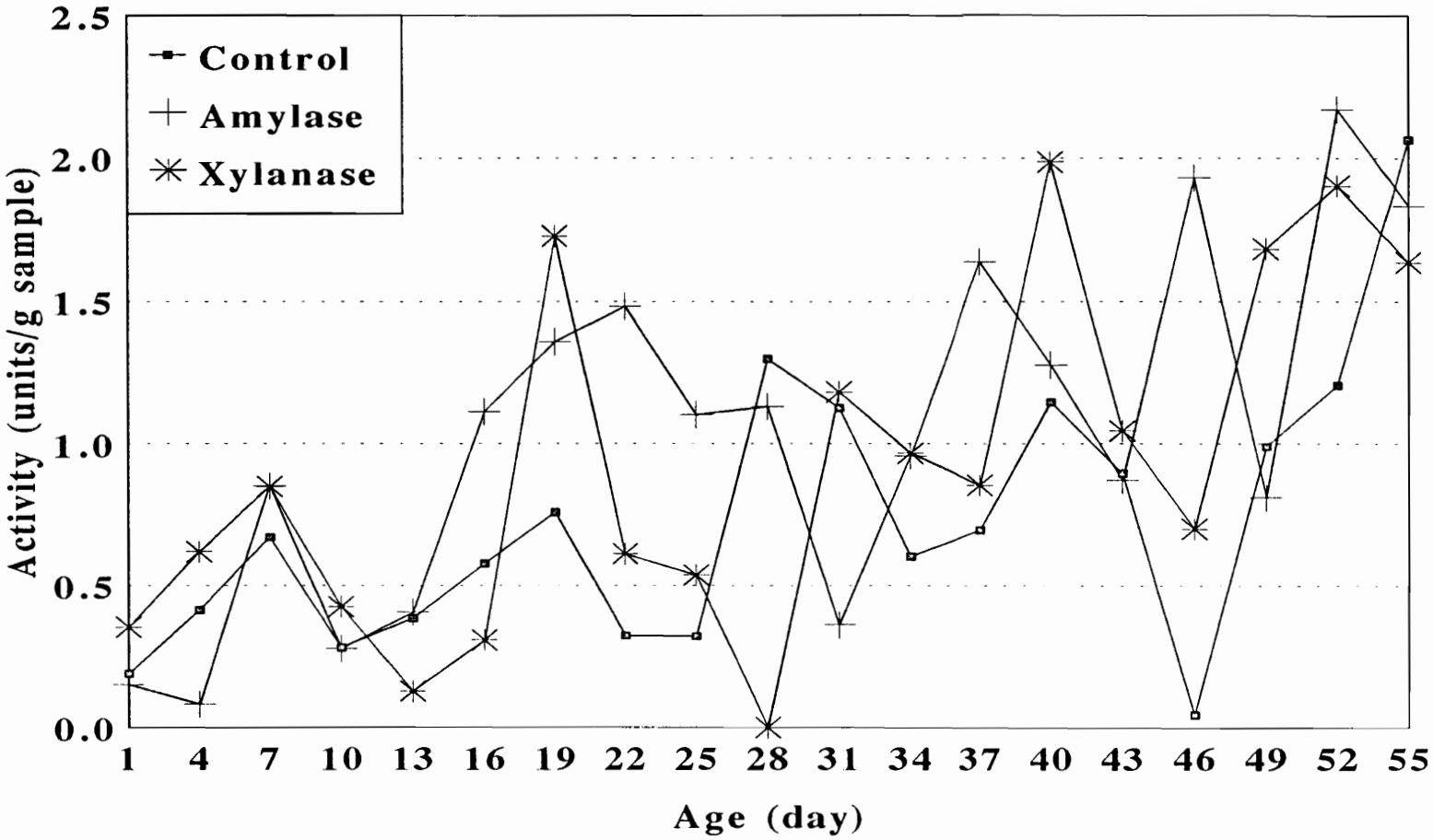

Figure 4. Crop amylase activity of male turkeys 0 to 55 days of age as affected by dietary amylase and xylanase supplementation. Each point represents 8 replicate assays.

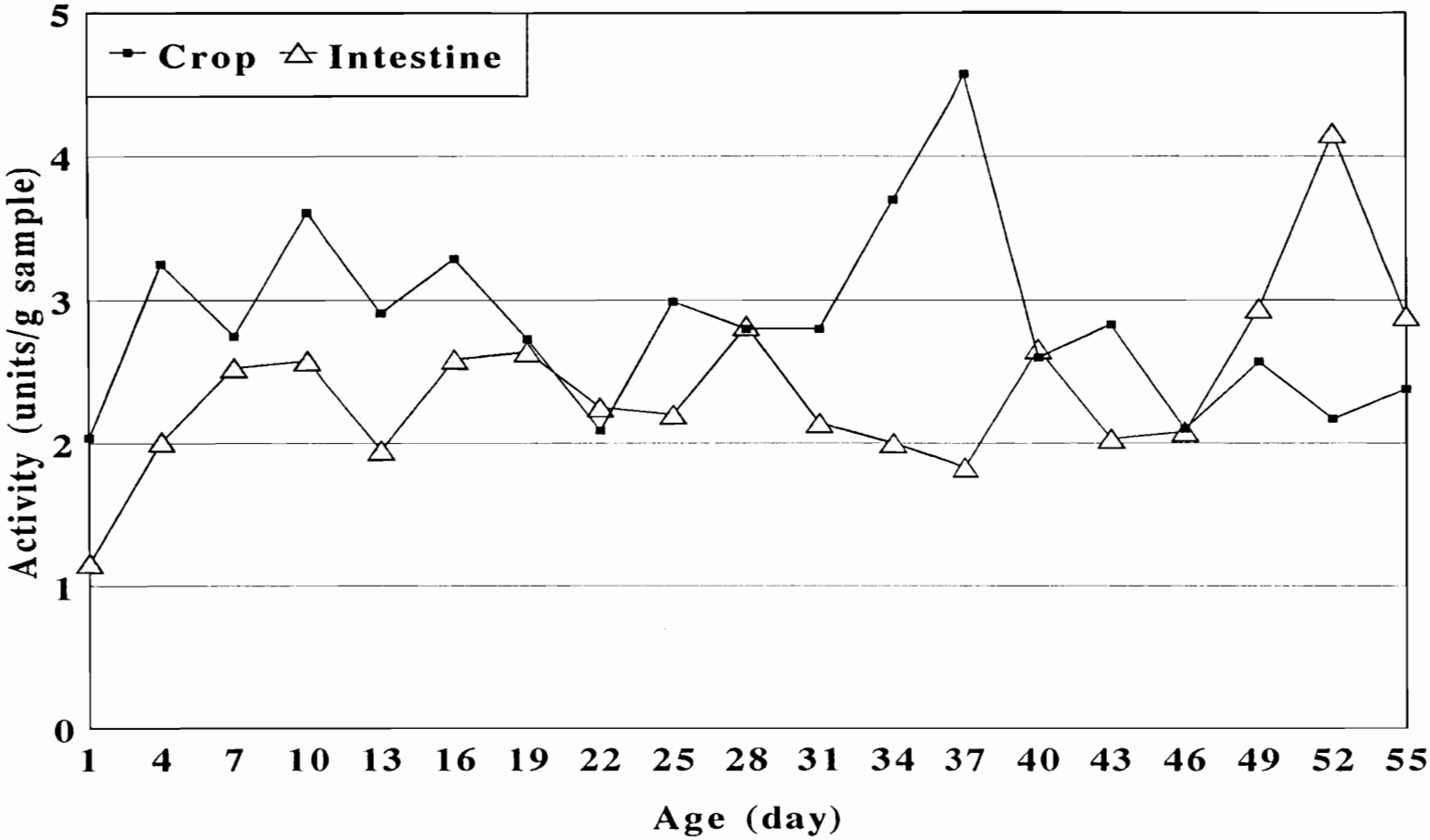

Figure 5. Mean xylanase activity of male turkeys 0 to 55 days of age as affected by dietary xylanase supplementation of 320 activity units per kilogram of feed. Each point represents the mean of replicate assays (n=8).

CHAPTER 2

GROWTH AND INTESTINAL MORPHOLOGY OF MALE TURKEYS AS INFLUENCED BY DIETARY SUPPLEMENTATION OF AMYLASE AND XYLANASE

GROWTH AND INTESTINAL MORPHOLOGY OF MALE TURKEYS AS INFLUENCED BY DIETARY SUPPLEMENTATION OF AMYLASE AND XYLANASE

ABSTRACT Three diets, a control and two diets supplemented with either 200 amylase or 320 xylanase activity units/g supplement, were each fed to 100 male poults (10 replicates of 10 poults per pen) from 0 to 5 weeks of age to observe the effects upon body weight gain, feed intake, and feed efficiency. The amylase supplemented diet significantly increased feed efficiency through the first 2 weeks, and significantly increased body weight gain and feed intake through the first 3 weeks. The growth differential observed with the birds fed the amylase diet was maintained over the control fed birds through the 5-week trial, indicating a positive residual effect from the amylase supplemented diet upon growth. Xylanase supplementation did not improve growth or feed efficiency over the control.

Mean villus length within the jejunum and ileum was significantly increased at 2 and 3 weeks of age by dietary supplementation of amylase when compared to the control and xylanase diets. Mean duodenal villus length was not significantly affected. These findings suggest that the increased growth associated with the amylase diet during 0 to 3 weeks can, in part, be explained by the increase in absorptive surface area, allowing for increased digestion of

available nutrients coupled with increased enzyme activity for carbohydrate degradation from the supplemental enzymes.

INTRODUCTION

Since the 1920's, researchers have observed beneficial effects from enzyme supplements in poultry feeds, particularly those feeds that contain grains with a high fiber component (Hastings, 1946; Moran and McGinnis, 1968; Pettersson and Aman, 1989). Diets containing barley, oats and rye were the focus of attention. In these early studies, enzyme preparations were found to increased the digestibility of the carbohydrate components, rendered nutrients more available for digestion and allowed for greater flexibility in feed formulation by allowing the use of low cost, high fiber-containing diets.

Limited studies have dealt with enzyme supplementation of traditional corn-soybean diets of high nutritional value, high digestibility, and low fiber. The results from these limited studies have generally been inconclusive and inconsistent. Fry *et al.* (1958) found that the enzyme supplementation of poult diets containing corn or wheat did not produce any significant growth or feed utilization responses. Additionally, Fry *et al.* (1958) observed that enzyme supplementation of barley diets was not as effective as unsupplemented corn diets in promoting growth and feed efficiency. In contrast, Parkany-Gyarfas (1975) found improvements in body weight of 3.6% and feed utilization of 4.0% in poults when a corn-soybean meal diet was supplemented

with α-amylase.

The objective of this experiment was to further evaluate the effects of adding enzyme preparations to traditional corn-soybean meal diets for turkey poults by comparing the effects of amylase and xylanase supplementation upon body weight gain, feed consumption, feed efficiency and intestinal histomorphology.

MATERIALS AND METHODS

Three dietary treatments were used in this study; a control diet and two experimental diets in which Avizyme TK0492-1® containing prodominantly amylase (EC 3.2.1.1) or Avizyme TK0492-2® containing predominantly xylanase (EC 3.2.1.8) was added (Table 1). The supplements were provided by FinnFeeds International LTD. The enzyme supplements were added to a conventional corn-soybean meal turkey starter basal (Table 2) at an inclusion rate of 1.1 g supplement/kg feed. Each diet was fed to 100 Nicholas male poults (10 replicates of 10 poults per pen) obtained from a commercial hatchery and randomly allocated into 30 Petersime brooder battery pens within a windowless, environmentally-controlled facility. All birds had free access to feed and water throughout the entire experiment, and all diets were fed in mash form.

Body weight and feed consumption by pen were recorded weekly. Body weight gain, feed consumption and feed efficiency were determined on weekly and cumulative bases.

In order to measure villus length in duodenal, jejunal and ileal sections of the small intestine, tissue samples were taken from 3 randomly selected birds per diet on a weekly basis for an 8-week period. The birds were sacrificed by cervical dislocation whereupon the small intestine was carefully excised. Samples were taken from a similar location

within each intestinal section at every sampling period; the apex of the duodenal loop and 15 cm anterior and posterior from the yolk sac remnant (vitelline diverticulum), considered to be the line of demarkation between the jejunum and ileum (Siddons, 1969; McLelland, 1991). Samples were placed in buffered neutral formalin for 24 hours and then transferred to a 50% ethanol storage solution.

For histopathic slide preparation, the samples were placed in a tissue processor[4], paraffin embedded[5] and subsequently sectioned sagittally with a microtome[6] at 10 microns and mounted on slides. The tissue sections on the slides were stained using Ehrlich's Hematoxylin stain (Sigma HHS-1) and a 1% eosin stain within a dehydration sequence utilizing ethanol and xylene. Two sagittal sections were taken from each tissue sample for villus length quantification. Villus length (from crypt to apex) was measured under a microscope[7] at 10 X magnification using an eyepiece that contained a millimeter scale. Villus length means were derived from an average 12 villus measurements.

---

[4]Histomatic™ Tissue Processor, Fisher Scientific Co., Pittsburgh, PA 15219.

[5]Tissue-Tek II embedding center, Fisher Scientific Co., Pittsburgh, PA 15219.

[6]Reichert-Jung 2040 Autocut microtome, Heidelberg, Germany.

[7]Olympus Microscope BH-2, Olympus Optical Co. LTD, Japan.

Tissue degeneration occurred within some of the slides and resulted in variable mean determination n values (Appendix V, Tables 7, 8, 9).

Analysis of variance and Duncan's multiple range test procedures were used for data analysis and mean comparisons (SAS, 1985). Significance was determined at the $P<.05$ level.

RESULTS AND DISCUSSION

Growth Response

Mortality was low, 1.7%, for the entire experiment and was not significantly influenced by diet, enzyme supplement, or their interaction ($P<.05$). Amylase supplementation significantly increased body weight 2.9% over the control during the third week and 5.3%, 8.6%, and 5.4% over the xylanase diet the first three weeks, respectively (Figure 1). Body weight gain was significantly increased 9.5% and 12.2% over the xylanase diet for the 0 to 1 and 1 to 2 week growth periods, respectively (Figure 2). Feed consumption for the birds on the amylase diet was significantly increased during the third week by 4.2% and 5.3% over the control and xylanase diets, respectively (Figure 3).

Feed efficiency was significantly increased 6.4% over the xylanase diet the first week and 6.5% and 7.9%, respectively over the control and xylanase diets the second week (Figure 4). Cumulative feed efficiency was also improved 5.4% and 7.3% the second week over the control and xylanase diets, respectively, and 3.1% over the control during the third week (Figure 5).

Statistical differences among the diets were not found after the third week in body weight, feed consumption, or feed efficiency; however, there was a tendency towards better weight gain associated with the amylase diet after the third

week. Data from this study would indicate that early growth of the birds can be significantly improved when provided amylase supplementation in the diet at the inclusion rate corresponding to this study.

Xylanase supplementation in the poult diets did not improve body weight gain, feed consumption, or feed efficiency over the control fed birds. The growth performance for birds supplemented with xylanase suggests that the level of inclusion used within this study was not beneficial. Pettersson *et al.* (1990) observed similar lack of growth response associated with xylanase supplements fed to broiler chickens. Bedford *et al.* (1992) reported that xylanase supplementation did not improve the rate of gain, feed intake, or starch and protein digestibility in swine. In the present study, pasted vents were not noticed to be a problem or associated with any given diet. Therefore, increased viscosity of the digesta was not considered as an explanation for the slower growth associated with the xylanase diet. An anti-nutritional factor response or perhaps an immune response may be of consideration to explain the slower growth. However, no analyses or procedures were used to determine any such responses in this study.

Villus Length

Mean villus length within the three sections of the small intestine from 0 to 8 weeks of age are shown in Figures 6, 7 and 8. Photomicrographs of sectional villi from which mean villus length measurements were taken are represented in Appendix V, Figures 1, 2, 3. Duodenal villus length did not follow any consistent pattern of significance over time between any of the diets. This lack of consistency would be expected with regard to amylase since the pancreatic amylase secretions enter the duodenum at the distal end of the duodenum loop (Bird, 1971); and since the jejunum, not the duodenum, is considered to be the primary site of digestive absorption (Osman, 1982; Moran, 1985).

The lengths of the villi within the jejunal and ileal sections of the small intestine of birds receiving the amylase diet were increased during the 0 to 3-week growth period relative to the birds receiving the control and xylanase supplemented diets. The increased villus length suggests an increased absorptive surface area capable of greater absorption of available nutrients (Caspary, 1992). When coupled with increased enzyme activity from supplemental enzymes, greater surface area facilitates carbohydrate degradation and subsequent nutrient absorption. Increased surface area indeed may be the reason for the significantly increased growth from 0 to 3 weeks, resulting in increased

digestion capabilities in the poult as a result of enhanced nutrient absorption.

Moran (1985) reported that birds adjust to changes in diet, particularly dietary starch, by altering the amount of amylase secreted from the pancreas and by altering intestinal surface area. Since the only difference between the treatments were the enzyme supplements, amylase either directly or indirectly influenced the villi length. Mean villus length of birds fed the control and xylanase diets was significantly smaller than that of the amylase diet during the second and third weeks (Figures 7, 8). This decreased length with corresponding decrease in surface area may partially explain the slower growth associated with the control and xylanase diets. Turnover rate of intestinal epithelial tissue was not measured and was assumed to have been normal with a turnover every 48-96 hours (Imondi and Bird, 1966; Fernando and McCraw, 1973). Therefore, supplementing amylase in turkey starter diets has the potential to improve the digestive capabilities of poults by stimulating villus formation/elongation and thereby improving poult performance during the critical first weeks of brooding. In addition, the increased growth resulting from amylase supplementation may have residual effects on growth and feed utilization. Conversely, xylanase supplementation does not appear to improve growth and feed efficiency at the level of inclusion

within this study.

Data from this experiment indicate that improvements in growth and feed efficiency resulting from enzyme supplementation such as amylase may help the poultry industry to improve chick and poult starts. Since the amylase used within feed supplements is primarily from fungal and bacterial sources and has endogenous analogues within avian species, amylase can be considered an alternative feed supplement to improve early poult growth and feed utilization.

ACKNOWLEDGEMENTS

Enzyme supplements were formulated by Cultor LTD. Technology Center, 02460 Kantvik, Finland, and provided by FinnFeeds International LTD., Market House, Ailesbury Court, High Street, Marlborough, Wiltshire, SN8 1AA, U.K.

REFERENCES

Bedford, M. R., J. F. Patience, H. L. Classen, and J. Inborr, 1992. The effect of dietary enzyme supplementation of rye- and barley-based diets on digestion and subsequent performance in weanling pigs. Can. J. Anim. Sci. 72:97-105.

Bird, F. H., 1971. Distribution of trypsin and α-amylase activities in the duodenum of the domestic fowl. Br. Poult. Sci. 12:373-378.

Caspary, W. F., 1992. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr. 55:299S-308S.

Fernando, M. A., and B. M. McCraw, 1973. Mucosal morphology and cellular renewal in the intestine of chickens following a single infection of *Eimeria acervulina*. J. Parasitol. 59:493-501.

Fry, R. E., J. B. Allred, L. S. Jensen, and J. McGinnis, 1958. Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. Poultry Sci. 37:372-375.

Hastings, W. H., 1946. Enzyme supplements to poultry feeds. Poultry Sci. 25:584-586.

Imondi, A. R., and F. H. Bird, 1966. The turnover of intestinal epithelium in the chick. Poultry Sci. 45:142-147.

McLelland, J., 1991. Avian Anatomy. Pages 60-61. W.B. Saunders Co., Philadelphia, PA.

Moran, E. T. Jr., 1985. Digestion and absorption of carbohydrates in fowl and events through perinatal development. J. Nutr. 115:665-674.

Moran, E. T. Jr., and J. McGinnis, 1968. Growth of chicks and turkey poults fed western barley and corn grain-based rations: effects of autoclaving on supplemental enzyme requirement and asymmetry of antibiotic response between grains. Poultry Sci. 47:152-158.

Osman, A. M., 1982. Amylase in chicken intestine and pancreas. Comp. Biochem. Physiol. 73:571-574.

Parkany-Gyarfas, A., 1975. Utilization of alpha-amylase-supplemented diet in turkey feeding tests. Acta Alimentaria 4:23-35.

Pettersson, D., and P. Aman, 1989. Enzyme supplementation of a poultry diet containing rye and wheat. Br. J. Nutr. 62:139-149.

Pettersson, D., H. Graham, and P. Aman, 1990. Enzyme supplementation of low or high crude protein concentration diets for broiler chickens. Anim. Prod. 51:399-404.

SAS Institute, 1985. SAS® User's Guide: Statistics. Version 5 Edition. SAS Institute Inc., Cary, NC.

Siddons, R. C., 1969. Intestinal disaccharidases in the chick. Biochem. J. 112:51-59.

Table 1. Enzyme activity within dietary supplements

Diet	Amylase	Xylanase	Protease	Pectinase
	Activity units/g supplement			
1 (Control)	-	-	-	-
2 (Amylase)	200	80	100	750
3 (Xylanase)	1	320	100	750

Supplement inclusion rate = 1.1g/kg diet.
Activity unit = micromole substrate hydrolyzed/min/g.

Table 2. Basal diet composition

Ingredient	g/kg diet
Ground yellow corn	459.7
Stabilized fat[1]	20.0
Dehulled soybean meal	435.0
Menhaden fish meal	25.0
Meat and bone meal	25.0
Defluorinated phosphate	22.5
Ground limestone	2.5
Iodized salt	4.0
DL-Methionine	2.8
Trace mineral mix[2]	1.0
Vitamin and additive mix[3]	2.5
Total	1000.0
Crude protein (%)	28
Metabolizable energy (kcal/kg diet)	2960

[1]Commercial animal-vegetable blend.

[2]The trace mineral mix provided (per g mix): 0.45 mg cobalt, 5 mg copper, 2 mg iodine, 120 mg manganese, 120 mg zinc, and 40 mg iron, with calcium carbonate as a diluent.

[3]The vitamin and additive mix provided (per g mix): 1764 IU vitamin A, 661.5 ICU vitamin D_3, 1.1 IU vitamin E, 0.88 mg riboflavin, 1.76 mg d-calcium pantothenate, 8.82 mg niacin, 74.97 mg choline chloride, 0.002 mg vitamin B_{12}, 0.22 mg thiamine mononitrate, 0.220 mg pyridoxine-HCl, 0.02 mg d-biotin, 0.04 mg selenium, 198 mg methionine, 0.22 mg folic acid, 1.06 mg menadione sodium bisulfite complex, and 25 mg ethoxyquin (as a preservative).

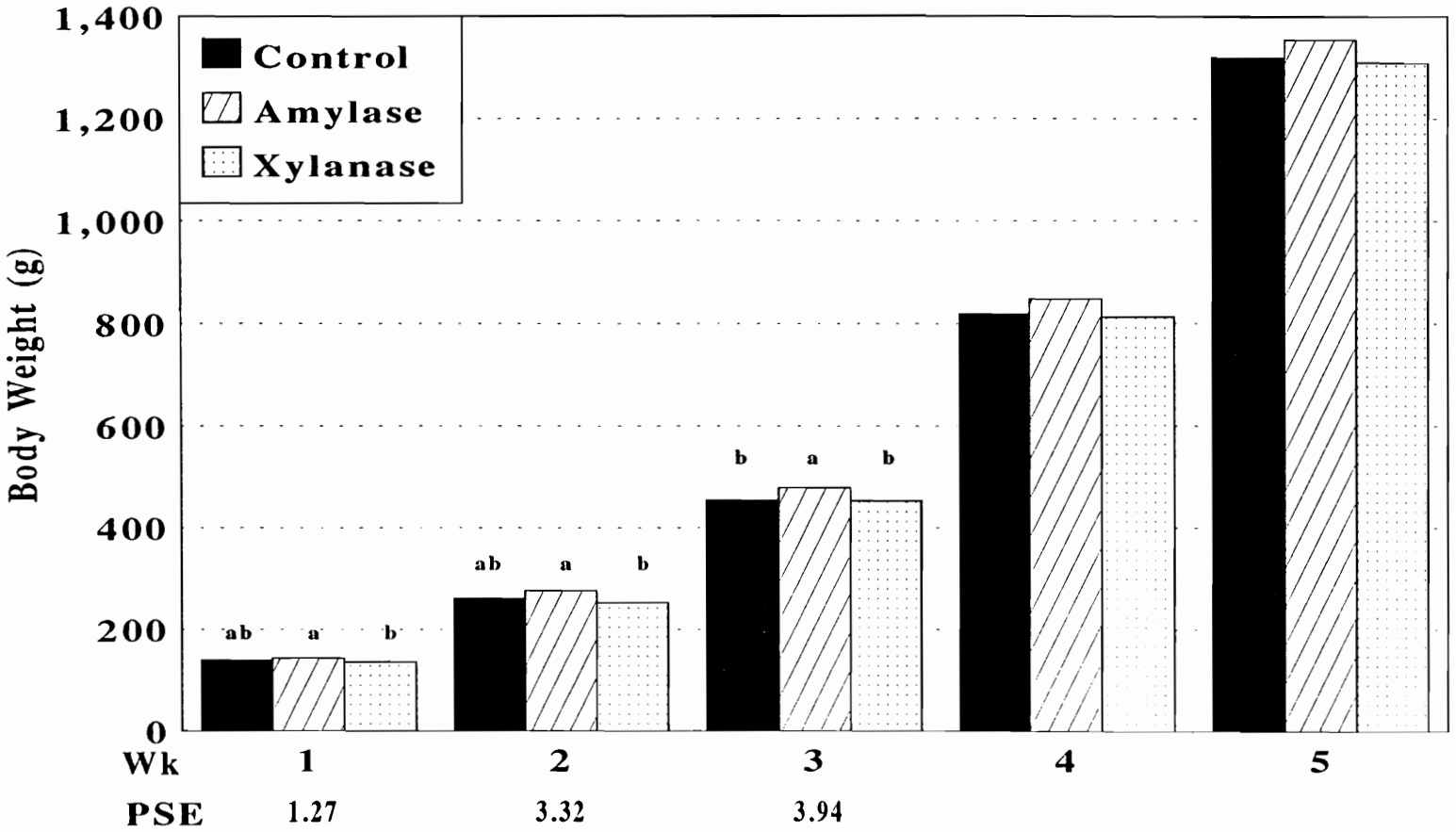

Figure 1. Mean body weight of male turkeys 0 to 5 weeks of age as affected by dietary amylase and xylanase supplementation. Means within an age with different superscripts are significantly different ($P < .05$). PSE, pooled standard error of the mean.

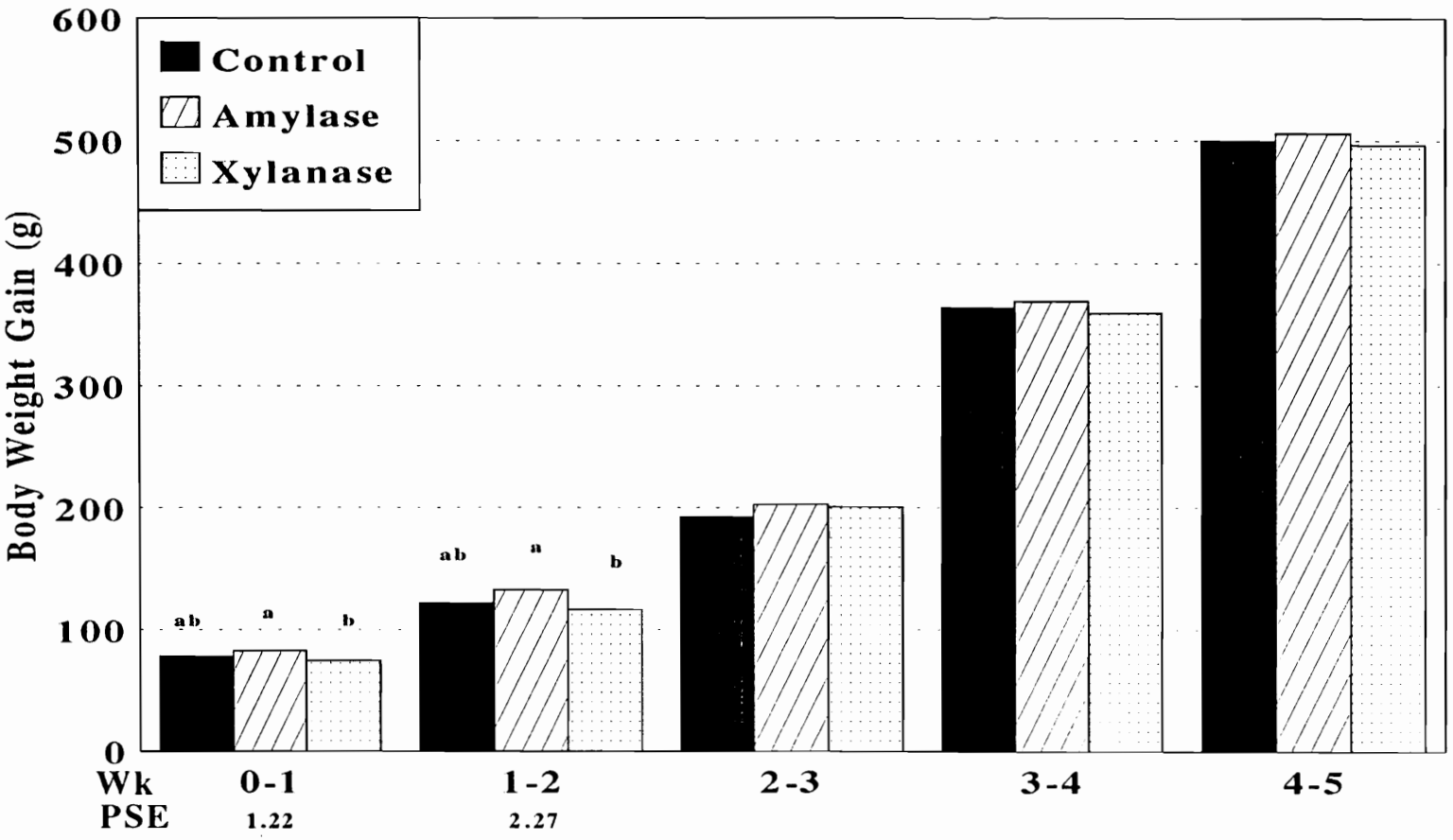

Figure 2. Mean body weight gain of male turkeys 0 to 5 weeks of age as affected by dietary amylase and xylanase supplementation. Means within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

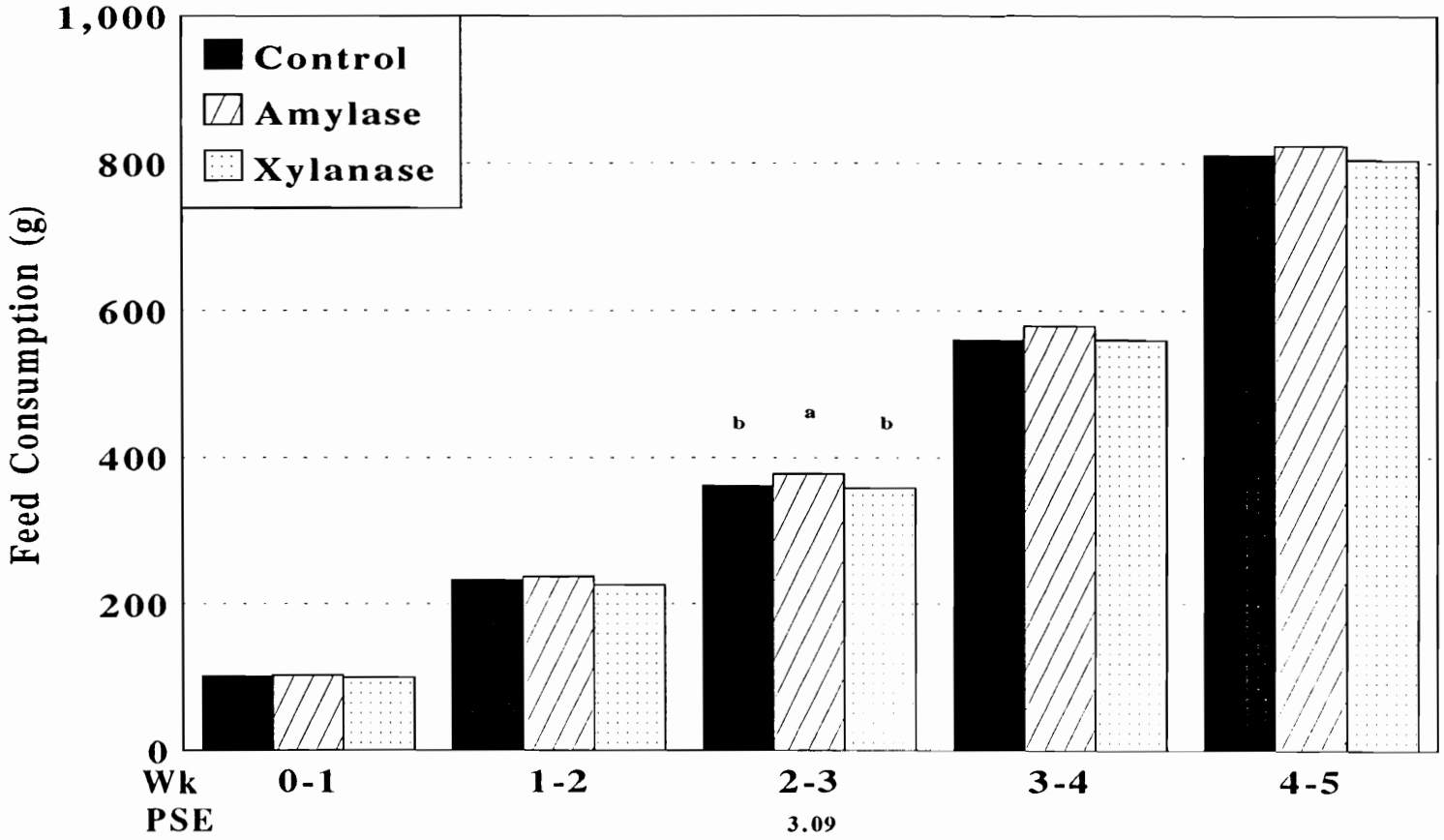

Figure 3. Mean period feed consumption of male turkeys 0 to 5 weeks of age as affected by dietary amylase and xylanase supplementation. Means within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

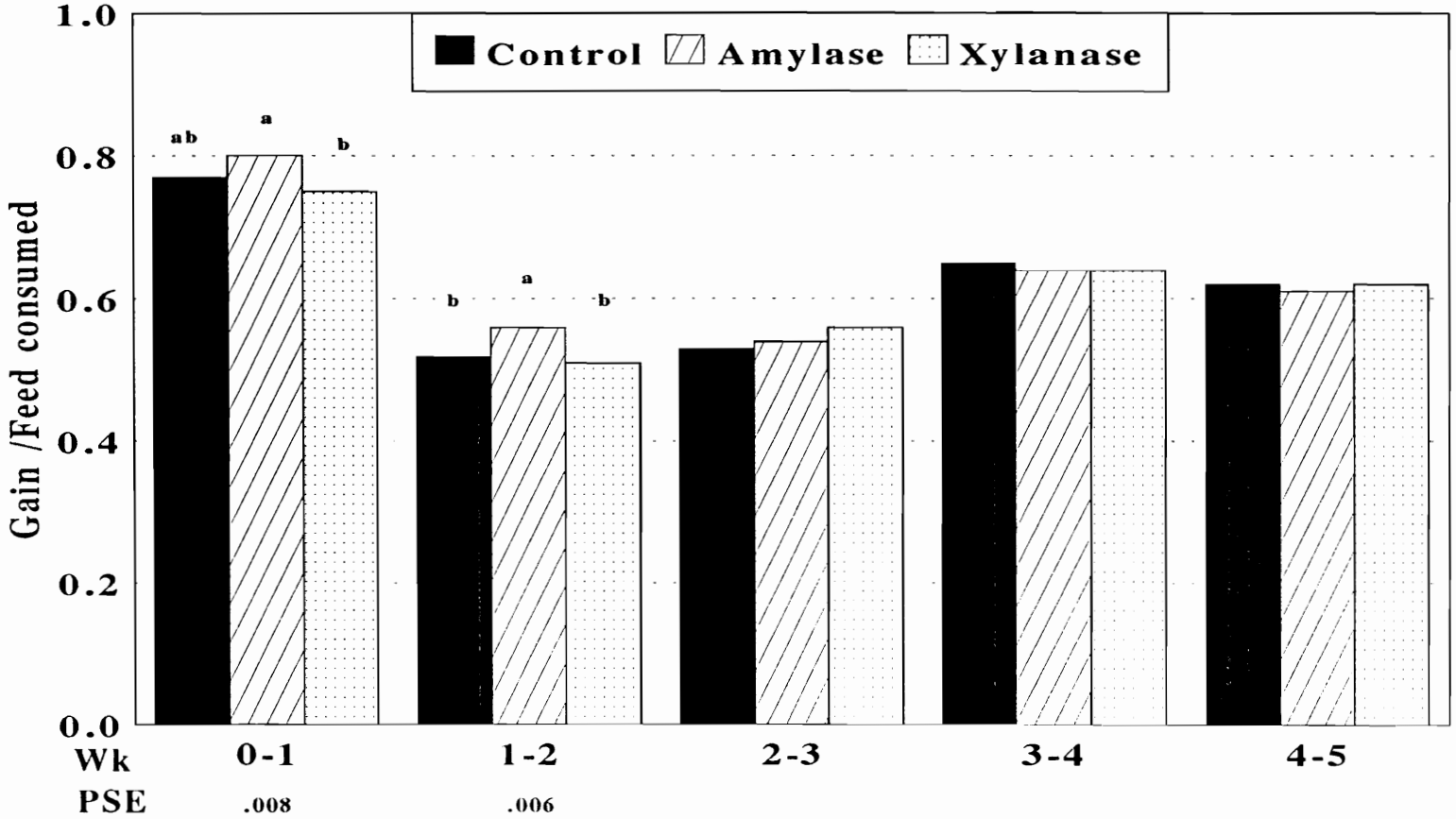

Figure 4. Mean feed efficiency of male turkeys 0 to 5 weeks of age as affected by dietary amylase and xylanase supplementation. Means within an age with different superscripts are significantly different ($P < .05$). PSE, pooled standard error of the mean.

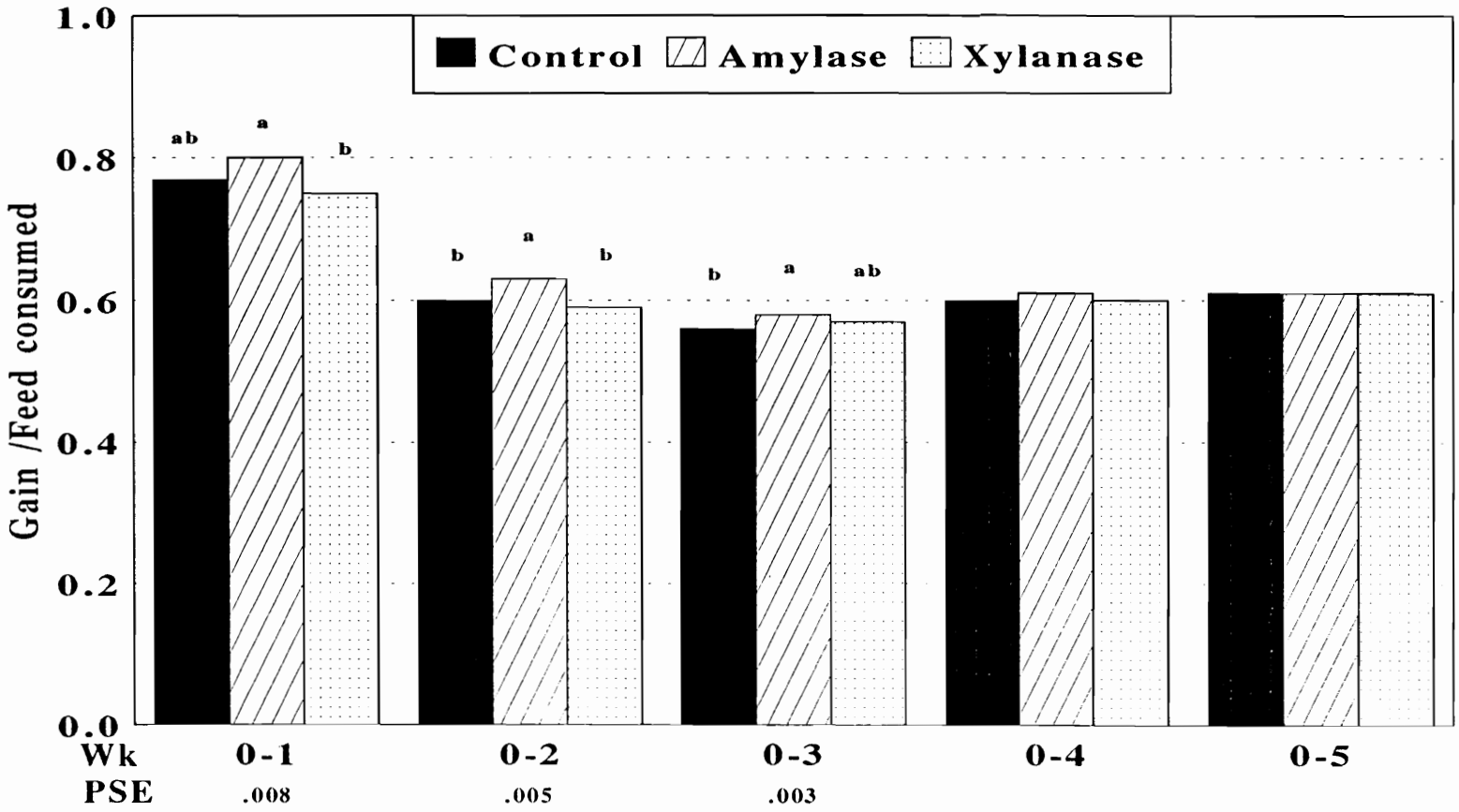

Figure 5. Cumulative feed efficiency of male turkeys 0 to 5 weeks of age as affected by dietary amylase and xylanase supplementation. Means within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

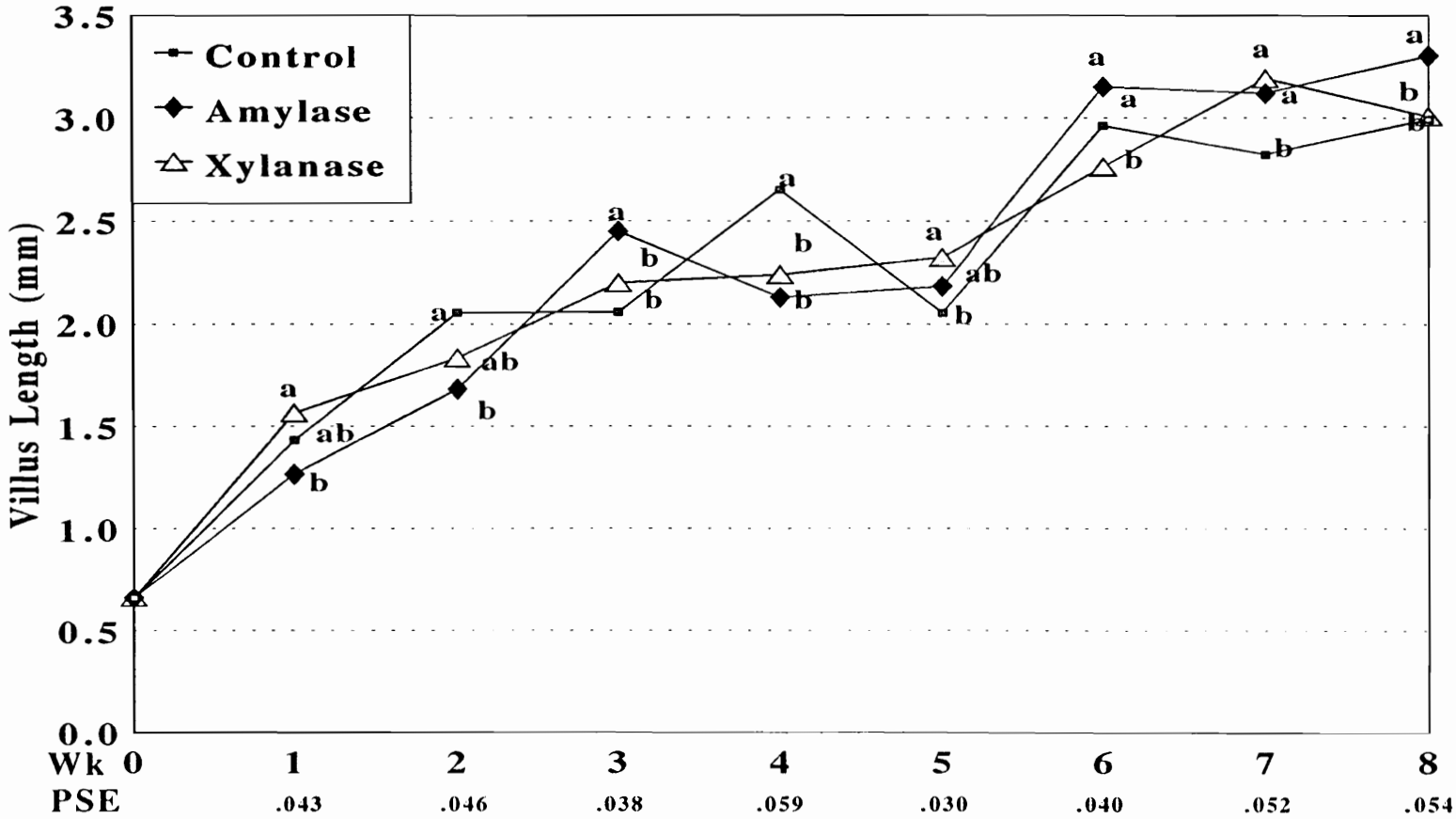

Figure 6. Mean duodenal villus length for male turkeys 0 to 8 weeks of age. Means (n = 12) within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

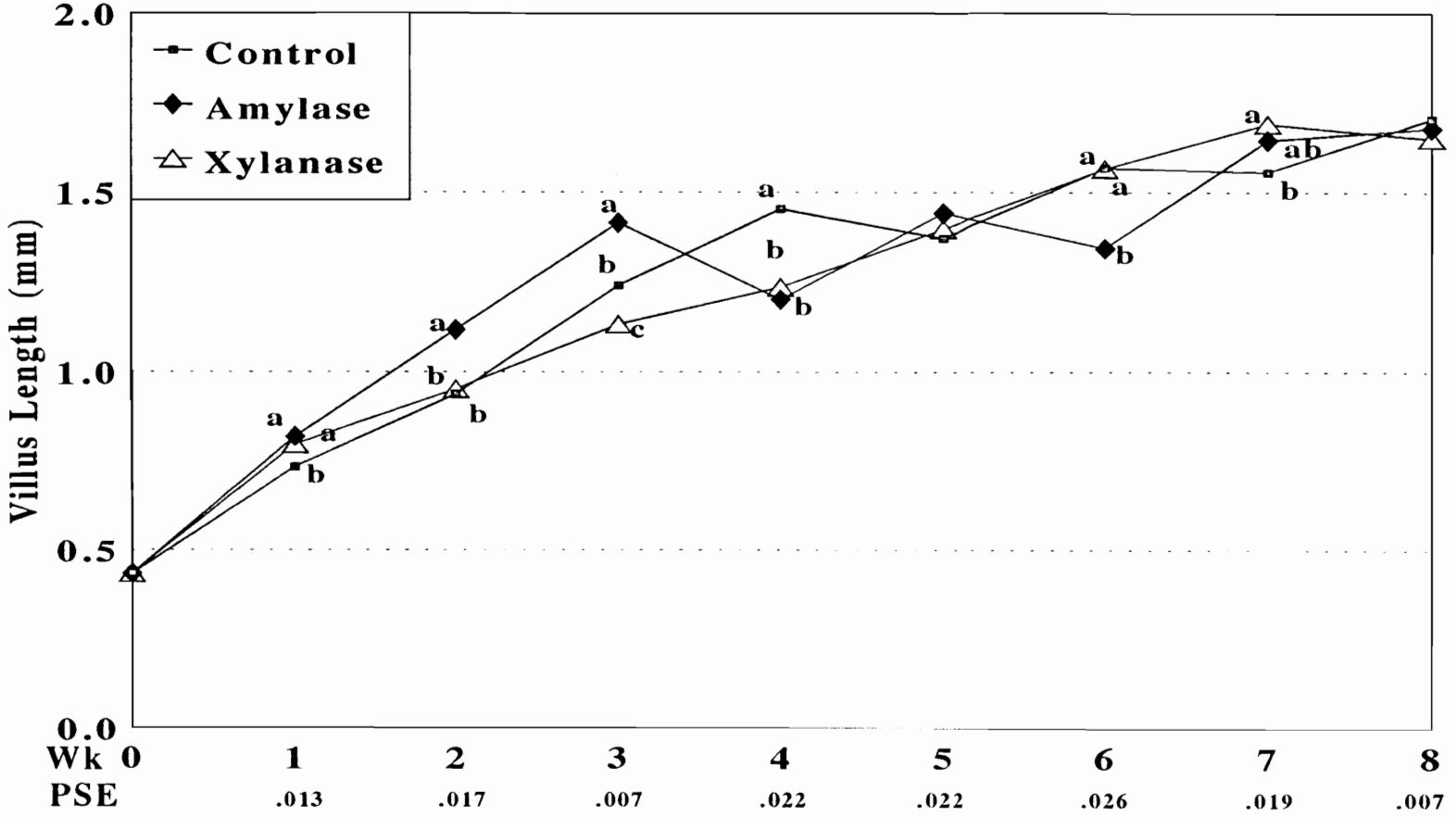

Figure 7. Mean jejunal villus length for male turkeys 0 to 8 weeks of age. Means (n = 12) within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

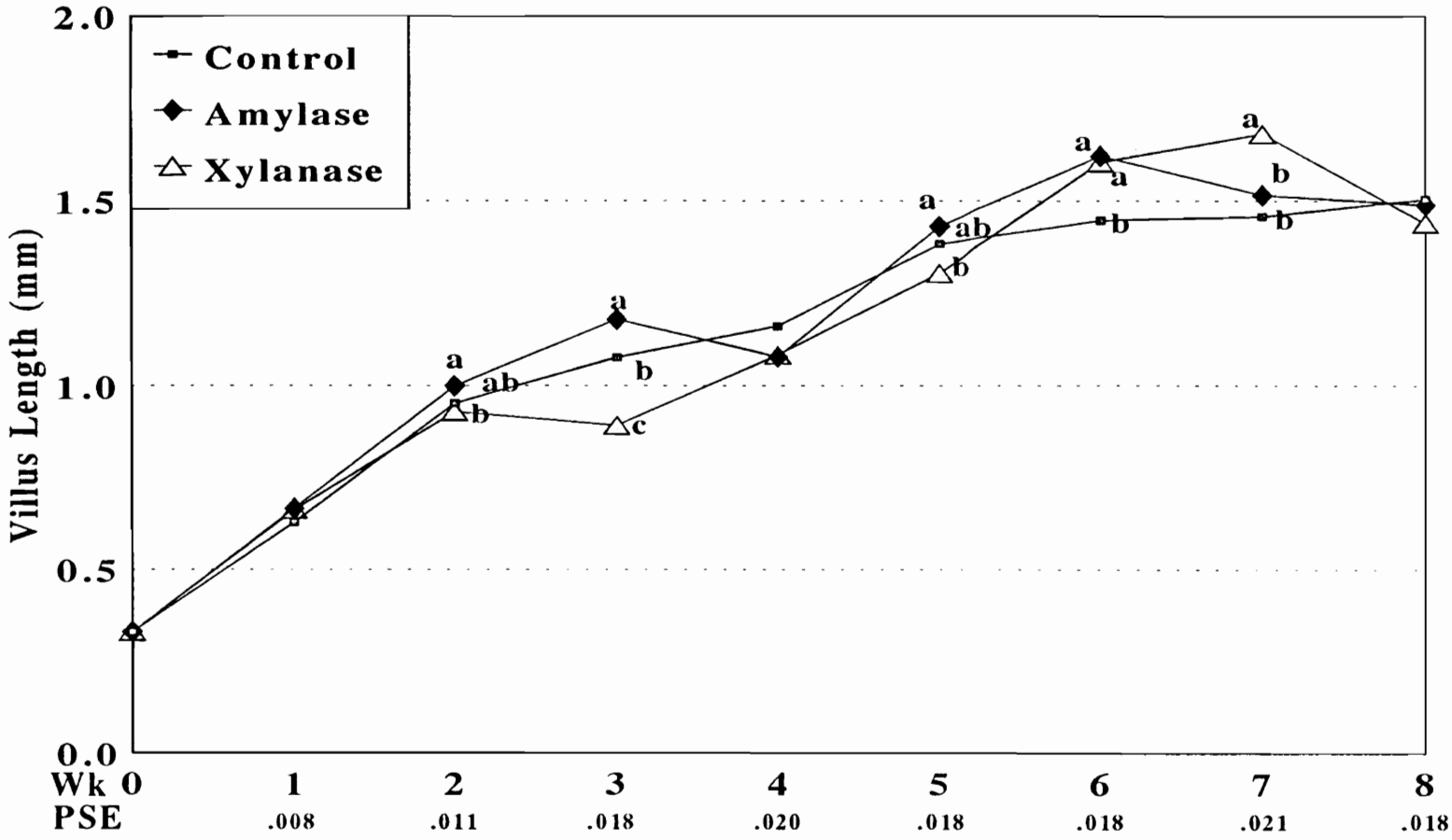

Figure 8. Mean ileal villus length for male turkeys 0 to 8 weeks of age. Means (n = 12) within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

CHAPTER 3

EFFECTS OF PROTEIN LEVEL AND ENZYME SUPPLEMENTATION UPON RATE OF DIGESTA PASSAGE AND GROWTH OF MALE TURKEYS

Effects of Protein Level and Enzyme Supplementation Upon Rate of Digesta Passage and Growth of Male Turkeys

ABSTRACT An experiment was conducted to determine the effect of feeding enzyme supplements (Avizyme® and protease) at two levels of dietary crude protein (24 and 28%) to 900 Nicholas male turkey poults from 0 to 5 weeks of age. The Avizyme®/protease enzyme mixture was fed at five levels; 0 enzyme, Avizyme® alone at 1.1 g/kg diet, 1.1 g Avizyme® + 10 units protease, 1.1 g Avizyme® + 30 units protease, and 1.1 g Avizyme® + 50 units protease. The Avizyme® mixture was an experimental product which supplied 1,000, 500 and 75 activity units/g supplement of α-amylase, xylanase and pectinase, respectively. The enzyme supplement and crude dietary protein treatments were arranged in a 5 X 2 factorial with 9 replicate pens (10 males per pen) per dietary treatment.

Birds fed the 28% protein diet had improved body weight, feed intake, and feed efficiency of 11.5, 6.5, and 4.4% respectively, when compared to birds fed the 24% protein diet. Enzyme addition to the 24% protein corn-soybean meal diet increased feed efficiency 1.2% compared to the control. When the enzyme mixture was added to the 28% protein diet, performance was not altered. Although enzyme supplementation improved poult utilization of the 24% protein diet, growth and feed utilization were not equal to the performance of the poults fed the 28% protein diet. Rate of digesta passage was

not statistically different between the levels of dietary protein or among levels of enzyme supplementation.

INTRODUCTION

Recent interest in feeding of low cost, high fiber feed ingredients supplemented with enzymes has led to renewed efforts to determine the effect that enzymes have upon the nutritional utilization of feed components and growth. Reduced digesta viscosity within feedstuffs (Inborr, 1990; Classen *et al.*, 1991), and increased growth and feed efficiency in monogastrics (Inborr, 1990; Graham, 1991) are among the benefits of enzyme supplementation currently documented.

Numerous researchers have observed the action of enzymes derived from fermentation products supplemented within poultry feeds, particularly within those diets that contain grains with a lower content of readily digestible nutrients, such as barley (Clickner and Follwell, 1926; Classen *et al.*, 1988; Rotter *et al.*, 1989), oats (Edney *et al.*, 1989) and rye (Pettersson and Aman, 1989). Most enzyme products are fermentation extracts prepared from the growth of *Aspergillus niger, Bacillus subtilis, and Trichoderma longibrachiatum,* and contain primarily amylases, pectinases, cellulases, and proteases.

The protein utilization and metabolizable energy (ME) of feeds fed to chickens have been found to improve with enzyme supplementation. Leong *et al.* (1962) showed that the metabolizable energy (ME) value of barley produced in the

western states was increased by the addition of enzyme preparations derived from fungal fermentation. Potter *et al.* (1965) showed that the ME content of barley was increased 18 to 20% by supplementation of the diet with fungal enzymes. The increase in ME was attributed to increased digestibility of the protein, fat, and carbohydrate fractions. Herstad and McNab (1975) and Mannion (1981) have also reported increases in ME values of barley after the addition of enzyme preparations.

Pettersson *et al.* (1990) found that supplementing broiler diets with enzymes allowed for a reduction in crude protein levels without affecting growth rate. Growth in turkey poults fed dietary protein levels of 24 and 28% was found to be affected by enzyme supplements (Potter *et al.*, 1991). They found that growth associated with the 24% protein diet was enhanced, whereas growth associated with the 28% protein diet was not affected. However, the unsupplemented 28% protein diets produced significantly greater growth than the 24% protein supplemented diets.

Much of the initial experimentation has dealt with improving high fiber diets. Studies with turkeys investigating enzyme supplementation of traditional corn-soybean diets of high nutritional value and low fiber have shown inconsistent results (Miner and Denton, 1957; Parkany-Gyarfas, 1975; Harper *et al.*, 1982). Therefore, the

objective of this experiment was to evaluate the growth and feed utilization responses of male turkey poults fed diets containing low and adequate protein levels and supplemented with enzyme mixtures. Dietary treatment effects on rate of passage of the digesta through the gastrointestinal tract was also evaluated to determine possible enzyme or protein effects.

MATERIALS AND METHODS

This experiment was designed as a 2 X 5 factorial with 2 levels of protein (24% and 28%) and 5 levels of enzyme supplementation (Table 1). The enzyme supplements were a multi-enzyme cocktail (Avizyme-TK®) and protease supplement, as provided by FinnFeeds International LTD. The Avizyme® cocktail was an experimental product which supplied 1,000, 500 and 75 activity units/g supplement of α-amylase, xylanase and pectinase, respectively. The supplements were added to a conventional corn-soybean meal turkey starter diet (Table 2) at an inclusion rate of 1.1 g supplement/kg feed. The treatments included a control with no enzyme supplement, three levels of the enzyme mixture with protease, and one level of enzyme mixture without protease. Each treatment was fed to 90 Nicholas male poults (9 pens, 10 poults per pen) obtained from a commercial hatchery and randomly allocated into Petersime brooder batteries within a windowless, environmentally-controlled facility. All birds had free access to mash feed and water throughout the entire experiment. Body weight, feed consumption and feed efficiency were determined weekly for each pen of turkeys.

Rate of digesta passage for each of the diets was determined to evaluate viscosity of the digesta, as manifested by digesta motility variations within the gastrointestinal tract that may have occurred among the treatments. Rate of

digesta passage was determined each week for the first 4 weeks, utilizing gelatin-encapsulated ferric oxide as an nondigestible marker (Dansky and Hill, 1952; Blake, 1986). The capsules were dipped in vegetable oil and placed into the esophagus of the birds, then gently massaged into the crop to prevent regurgitation. Capsules were placed into 4 randomly selected birds from each of the 10 diets once a week for four weeks. Drop pans were checked every fifteen minutes to determine passage of the ferric oxide. Time of capsule placement within the bird and corresponding time of ferric oxide emergence within the fecal material were recorded for each bird.

Analysis of variance and Duncan's multiple range test procedures were used for data analysis and mean comparisons (SAS, 1985). Significance was determined at the $P<.05$ level.

RESULTS AND DISCUSSION

Growth Values

Dietary protein level significantly influenced body weight gain, feed consumption and feed efficiency after the first week (Table 3). From 0 to 5 weeks of age, body weight gain was increased 11.5%, feed consumption 6.5% and feed efficiency 4.4% with the 28% protein diets, compared to the 24% protein diets. Enzyme supplementation with the multi-enzyme cocktail and protease series did not affect growth of the poults (Table 4) when protein level was not included as a factor. This observation indicates that the addition of the enzyme supplement formulated for corn-soybean meal diets containing the levels of protein used in this study did not benefit or improve the growth of poults. Lack of response to the protease supplement may have been a factor of inclusion rate, since Rexen (1981) found a significant improvement in gain and feed conversion when protease was fed to chickens at higher levels than those used in this study.

The unsupplemented 28% protein control diet produced significantly higher growth values than either supplemented or unsupplemented 24% protein diets (Table 3). This observation is similar to the growth results of Fry *et al.* (1958) wherein supplemented barley diets, though significantly superior over unsupplemented barley diets, were still of lower value when compared to unsupplemented corn diets. These results also

correlate to those of Potter *et al.* (1991) wherein enzyme supplemented 24% protein diets produced lower growth values than unsupplemented 28% protein diets.

Rate of Digesta Passage

Protein level and enzyme supplementation did not significantly influence the rate of digesta passage within the poults at any time during the four weeks of observation (Table 5). The incidence of sticky droppings, an indicator of increased viscosity of the digesta and decreased rate of passage, were not noticeably different between the two protein levels or among the enzyme levels. Enzyme supplementation has been shown to have a direct effect upon the viscosity of digesta by reducing the amount of non-starch polysaccharides (NSP) within barley, rye and wheat diets. Graham *et al.* (1993) demonstrated that NSP are characteristic of feed components typically associated with the endosperm cell walls and can increase the incidence of sticky droppings. Corn-soybean diets are low in NSP and, therefore, may not exhibit the same response typically associated with other feedstuffs. The results from this study are in contrast to that observed by Pettersson *et al.* (1990) where feeding of high protein (22.7%) diets to broilers increased the incidence of sticky droppings to approximately twice that of birds fed low protein (19.2%) diets. When the high protein diet was supplemented

with glucanase, the frequency of sticky dropping decreased to a level associated with the low protein diet. However, the diets within their study were formulated with barley, wheat, and rye which have been shown to inherently increase the incidence of sticky droppings as a result of high levels of NSP within the endosperm cell wall structure.

The results from this experiment further demonstrate that diets associated with high protein values and low fiber (3-4%) are superior in promoting growth of turkey poults when compared with turkeys fed enzyme supplemented diets containing lower crude protein and higher fiber. Additionally, enzyme supplementation as applied in this study, appeared to not affect rate of digesta passage associated with either the high or low protein diet.

ACKNOWLEDGEMENTS

Enzyme supplements were formulated by Cultor LTD. Technology Center, 02460 Kantvik, Finland, and provided by FinnFeeds International LTD., Market House, Ailesbury Court, High Street, Marlborough, Wiltshire, SN8 1AA, U.K.

REFERENCES

Blake, J. W., 1986. Methodology for evaluating the digestibility and metabolizable energy of poultry feedstuffs. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0332.

Classen. H., G. Campbell, and J. Grootwassink, 1988. Improved feeding value of Saskatchewan-grown barley for broiler chickens with dietary enzyme supplementation. Can. J. Anim. Sci. 68:1253-1259.

Classen, H. L., H. Graham, J. Inborr, and M. R. Bedford, 1991. Growing interest in feed enzymes to lead to new products. Feedstuffs 63 (4):1-5.

Clickner, F. H., and E. H. Follwell, 1926. Application of "protozyme" (*Aspergillus orizae*) to poultry feeding. Poultry Sci. 5:241-247.

Dansky, L. M., and F. W. Hill, 1952. Application of the chromic oxide indicator method to balance studies with growing chickens. J. Nutr. 47:449-459.

Edney, M. J., G. L. Campbell, and H. L. Classen, 1989. The effect of beta-glucanase supplementation on nutrient digestibility and growth in broilers given diets containing barley, oat groats and wheat. Anim. Feed Sci. Technol. 25:193-200.

Fry, R. E., J. B. Allred, L. S. Jensen, and J. McGinnis, 1958. Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. Poultry Sci. 37:372-375.

Graham, H., 1991. Enzymes in monogastric feeds. International Milling Flour and Feed July, 1991 pages 33-36.

Graham, H., M. Bedford, and M. Choct, 1993. High gut viscosity can reduce poultry performance. Feedstuffs February 1, 1993 pages 14-15.

Harper, J. A., G. H. Arscott, W. B. Roush, and M. P. Goeger, 1982. The comparative feeding value of corn and wheat with and without an enzyme supplement in turkey breeder rations. Poultry Sci. 61:1183-1187.

Herstad, O., and J. M. McNab, 1975. The effect of heat treatment and enzyme supplementation on the nutritive value of barley for broiler chicks. Br. Poult. Sci. 16:1-8.

Inborr, J., 1990. Practical application of feed enzymes. Feed Compounder 10:41-49.

Leong, K. C., L. S. Jensen, and J. McGinnis, 1962. Effect of water treatment and enzyme supplementation on the metabolizable energy of barley. Poultry Sci. 41:36-39.

Mannion, P. F., 1981. Enzyme supplementation of barley based diets for broiler chickens. Aust. J. Exp. Agric. Anim. Husb. 21:296-302.

Minor, J. J., and C. A. Denton, 1957. Supplementation of a practical diet with enzymes for turkey poults. Poultry Sci. 36:1321-1323.

Parkany-Gyarfas, A., 1975. Utilization of alpha-amylase-supplemented diet in turkey feeding tests. Acta Alimentaria 4:23-35.

Pettersson, D., and P. Aman, 1989. Enzyme supplementation of a poultry diet containing rye and wheat. Brit. J. Nutr. 62:139-149.

Pettersson, D., H. Graham, and P. Aman, 1990. Enzyme supplementation of low or high crude protein concentration diets for broiler chickens. Anim. Prod. 51:399-404.

Potter, L. M., M. W. Stutz, and L. D. Matterson, 1965. Metabolizable energy and digestibility coefficients of barley for chicks as influenced by water treatment or by presence of fungal enzyme. Poultry Sci. 44:565-573.

Potter, L. M., R. M. Hulet, and C. W. Ritz, 1991. Effects of added enzyme supplements to diets of turkeys. Poultry Sci. 70 (Suppl 1):176.(Abstr)

Rexen, B., 1981. Use of enzymes for improvement of feed. Animal Feed Science Technology 6:105-114.

Rotter, B., M. Neskar, R. Marquardt, and W. Guenter, 1989. Effects of different enzyme preparations on the nutritional value of barley in chicken diets. Nutr. Rep. Int. 39:107-120.

SAS Institute, 1985. SAS® User's Guide: Statistics. Version 5 Edition. SAS Institute Inc., Cary, NC.

Table 1. Enzyme activity of dietary supplements

Diet	Avizyme-TK®[1]	Protease
	Activity units/g supplement	
(24% protein)		
1	-	-
2	100	-
3	100	10
4	100	30
5	100	50
(28% protein)		
6	-	-
7	100	-
8	100	10
9	100	30
10	100	50

[1]Commercial enzyme supplement.
Supplement inclusion rate = 1.1g/kg diet.
Activity unit = micromole substrate hydrolyzed/min/g.

Table 2. Basal diet composition

Ingredient	g/kg diet
Ground yellow corn	561.2
Stabilized fat[1]	20.0
Dehulled soybean meal	334.2
Menhaden fish meal	25.0
Meat and bone meal	25.0
Defluorinated phosphate	22.5
Ground limestone	2.5
Iodized salt	4.0
DL-Methionine	3.0
Trace mineral mix[2]	1.0
Vitamin and additive mix[3]	1.7
Total	1000.0
Crude protein (%)	24*
Metabolizable energy (kcal/kg diet)	3148

[1]Commercial animal-vegetable blend.

[2]The trace mineral mix provided (per g mix): 0.45 mg cobalt, 5 mg copper, 2.5 mg iodine, 120 mg manganese, 120 mg zinc, and 40 mg iron, with calcium carbonate as a diluent.

[3]The vitamin and additive mix provided (per g mix): 1764 IU vitamin A, 661.5 ICU vitamin D_3, 1.1 IU vitamin E, 0.88 mg riboflavin, 1.76 mg d-calcium pantothenate, 8.82 mg niacin, 74.97 mg choline chloride, 0.002 mg vitamin B_{12}, 0.22 mg thiamine mononitrate, 0.220 mg pyridoxine-HCl, 0.02 mg d-biotin, 0.04 mg selenium, 198 mg methionine, 0.22 mg folic acid, 1.06 mg menadione sodium bisulfite complex, and 125 mg ethoxyquin (as a preservative).

*The 28% protein diet was formulated by adding 10% dehulled soybean meal in place of equal amount of ground yellow corn within the 24% diet.

Table 3. Effects of 24 and 28% protein diets upon male turkeys 0 to 5 weeks of age.

	Week	24% Protein	28% Protein	Pooled SEM
Body weight gain (g)	0-1	48	51	1.6
	1-2	119[b]	147[a]	2.3
	2-3	189[b]	208[a]	4.3
	3-4	264[b]	298[a]	5.6
	4-5	389[b]	420[a]	5.5
	0-5	1011[b]	1127[a]	12.7
Feed consumption (g)	0-1	70	72	1.5
	1-2	156[b]	174[a]	2.4
	2-3	318[b]	348[a]	4.9
	3-4	472[b]	494[a]	7.9
	4-5	698[b]	736[a]	8.7
	0-5	1716[b]	1827[a]	21.4
Feed efficiency	0-1	0.692	0.709	.0128
	1-2	0.760[b]	0.845[a]	.0083
	2-3	0.600	0.599	.0144
	3-4	0.562[b]	0.603[a]	.0103
	4-5	0.559	0.571	.0065
	0-5	0.590[b]	0.616[a]	.0036

Means within an age with different superscripts are significantly different ($P<.05$).

450 birds per protein level (45 pens, 10 birds per pen).

Table 4. Effects of Avizyme® enzyme supplement and protease upon male turkeys 0 to 5 weeks of age.

	Week	Control	TK[1]	TK-10[2]	TK-30[3]	TK-50[4]	Pooled SEM
Body weight gain (g)	0-1	51	50	49	49	52	2.5
	1-2	129	137	136	135	131	3.7
	2-3	193	210	201	196	196	6.8
	3-4	278	278	285	283	284	8.9
	4-5	400	404	420	394	407	8.7
	0-5	1051	1079	1091	1057	1070	20.1
Feed consumption (g)	0-1	70	71	72	70	72	2.4
	1-2	162	167	167	168	165	3.8
	2-3	332	341	333	332	329	7.7
	3-4	492	486	479	486	475	12.6
	4-5	703	717	721	729	718	13.7
	0-5	1758	1783	1771	1785	1758	33.8
Feed efficiency	0-1	0.716	0.691	0.683	0.696	0.716	.0203
	1-2	0.800	0.820	0.820	0.800	0.790	.0131
	2-3	0.586	0.622	0.602	0.590	0.597	.0227
	3-4	0.574	0.569	0.592	0.582	0.596	.0163
	4-5	0.570^{ab}	0.562^{ab}	0.582^{a}	0.543^{b}	0.568^{ab}	.0103
	0-5	0.599^{ab}	0.604^{ab}	0.614^{a}	0.592^{b}	0.608^{ab}	.0056

Means within an age with different superscripts are significantly different ($P<.05$).

180 birds per treatment (18 pens, 10 birds per pen).

[1]Avizyme®, a commercially prepared enzyme supplement manufactured by Cultor LTD.
[2]Avizyme® + 10 units protease.
[3]Avizyme® + 30 units protease.
[4]Avizyme® + 50 units protease.

Table 5. Mean rate of digesta passage (min) for diets supplemented with enzymes fed to male turkeys 0 to 4 weeks of age.

	24% protein (Diets 1-5)					28% protein (Diets 6-10)					
Week	Diet 1 control	Diet 2 TK	Diet 3 TK10	Diet 4 TK30	Diet 5 TK50	Diet 6 control	Diet 7 TK	Diet 8 TK10	Diet 9 TK30	Diet 10 TK50	Pooled SEM
1	117	129	128	143	98	105	143	120	116	139	14.5
2	114	123	150	105	139	124	120	139	146	135	15.2
3	141	138	139	139	143	143	143	143	154	143	8.5
4	129	135	161	146	154	158	150	150	139	150	9.9

10 treatments, 4 observations per treatment.

TK = Avizyme, a commercially prepared enzyme supplement manufactured by Cultor LTD.

TK series designates the units of protease added to the commercial supplement.

APPENDIX I

RESPONSE OF MALE TURKEYS TO VARYING LEVELS OF AMYLASE AND XYLANASE SUPPLEMENTATION

Response of Male Turkeys to Varying Levels of Amylase and Xylanase Supplementation

ABSTRACT Two experiments were conducted with 900 and 960 Nicholas male turkey poults from 0 to 5 weeks of age, to determine the effects on body weight gain, feed consumption, and feed efficiency by adding enzyme supplements to their diets. In the first experiment, the enzyme mixtures were experimental products which supplied progressively increasing levels of amylase and xylanase. Ten diets were formulated, five containing amylase and four containing xylanase, and a control with no added enzyme. Each diet was fed to 9 pens of 10 poults per pen. The second experiment involved the addition of supplements containing predominantly amylase, protease, pectinase, and α-galactosidase. Each of 12 diets was fed to 8 pens with 10 poults per pen. Endogenous levels of amylase within the crop, pancreas, and small intestine were also quantified for birds in the first experiment fed the control diet as a measure of amylase activity of turkeys fed a conventional corn-soybean meal diet.

Amylase supplementation in Experiment 1 significantly increased feed efficiency when compared to the control and numerically improved the performance of the poults when compared to the xylanase diets. Xylanase supplementation did not improve bird performance over the control. Endogenous amylase was found to be increasing 34 days post hatch in

contrast to earlier data reporting a stabilization of amylase activity after 21 days post hatch.

The complexity of the serial enzyme applications within Experiment 2 disallowed any real determinations with regard to a 'best' supplement mixture. However, amylase was included in those supplement combinations that produced the best overall results, indicating that amylase is beneficial for inclusion in turkey starter diets.

INTRODUCTION

Until recently, research on the use of enzymes in feeds has not been very systematic in approach, and crude enzyme preparations have been used with little knowledge of their array of enzyme activity. Results collected from enzyme trials are difficult to assess and compare because of the diversity and variation of enzyme supplements used. Information regarding supplement enzyme activity and possible optimal or detrimental inclusion rates of the supplements is lacking.

Rotter *et al.* (1989a, 1989b) found that enzyme form, and not concentration, improved growth and feed efficiency when selected commercially prepared crude enzyme supplements were fed to broilers, and presumably the lowest concentration of enzyme inclusion was sufficient to produce the maximal response. Reese *et al.* (1983) found similar responses to graded levels of multi-enzyme supplements fed to broilers.

In order to evaluate the effectiveness of amylase and xylanase supplementation, two experiments were conducted with male turkeys to observe 1) response to incremental increases of amylase and xylanase supplementation, 2) corresponding amylase activity levels in the pancreas, small intestine, and crop of turkeys fed a reference corn-soybean meal diet, and 3) response of turkeys to serial application of different multi-enzyme supplements to turkey starter diets.

MATERIALS AND METHODS

Experiment 1

Experiment 1 was designed to evaluate enzyme supplements containing incremental increases of amylase and xylanase activity (Table 1). The enzyme supplements were multi-enzyme cocktails (Avizyme-TK®), as provided by FinnFeeds International LTD. The supplements were added to a conventional corn-soybean meal turkey starter diet (Table 2) at an inclusion rate of 1.1 g supplement/kg feed. In the first experiment, 10 diets were formulated; five containing amylase with activities of 50, 100, 200, 400, and 800 units per gram supplement, four containing xylanase with activity units of 40, 80, 160, and 320 per gram supplement, and a control with no enzyme added. Each treatment was fed to 90 Nicholas male poults (9 pens, 10 poults per pen) obtained from a commercial hatchery and randomly allocated into four 6-tier Petersime brooder batteries within a windowless, environmentally-controlled facility. All birds had free access to feed and water throughout the entire experiment, and all diets were fed in mash form. Body weight, feed consumption and feed efficiency were determined weekly for each pen of turkeys by group pen weight, from which mean bird values were calculated.

Additionally, endogenous amylase activity corresponding to the unsupplemented diet was evaluated from hatch to 34 days

of age following the amylase assay procedures outlined in Chapter 1.

Experiment 2

Experiment 2 was conducted to evaluate the serial application of different enzyme supplements predominantly containing amylase, protease, pectinase, and α-galactosidase. Enzyme preparations used were in the form of a dry powder or liquid concentrate. The enzyme supplements were provided by FinnFeeds International LTD (Table 3), with application occurring at the time of feed formulation. The supplements were added to a conventional corn-soybean meal turkey starter diet (Table 2) either as a dry ingredient or sprayed on the feed by hand at the time of mixing, with inclusion rates as outlined in Table 3. The treatments included three controls based on enzyme delivery method, individual supplements predominantly containing either amylase, protease, pectinase or α-galactosidase, and combinations of the four supplements. Each treatment was fed to 80 Nicholas male poults (8 pens, 10 poults per pen) obtained from a commercial hatchery and reared according to the procedures outlined in Experiment 1.

Analysis of variance and Duncan's multiple range test procedures were used for data analysis and mean comparisons (SAS, 1985). Significance was determined at the $P<.05$ level.

RESULTS AND DISCUSSION

Experiment 1

Response to amylase supplementation at all levels of inclusion were similar in value when compared to the control (Table 4). Diets supplemented with xylanase did not improve bird growth over the control-fed birds (Table 5). Similar observations with xylanase were found by Bedford *et al.* (1992) with swine. Variability in feed efficiency indicates a lack of response to xylanase supplementation.

When enzyme supplements are compared solely on enzyme activity (Table 6), amylase significantly improved body weight gain and feed efficiency over the control during the 0 to 1 and 2 to 3 week periods. Growth responses to dietary amylase supplementation were not significant over the responses to xylanase supplementation, however, amylase supplementation did numerically improved performance throughout the experiment.

Krogdahl and Sell (1989) reported that endogenous amylase activity reached a plateau at 21 days of age within poults. The assay performed in the present experiment also demonstrated a plateau at 21 days. However, activity was observed to be increasing again at 34 days (Figures 1, 2). This increase at 34 days indicates that amylase activity may continue to increase with age. Additional experimentation is needed to verify increasing enzyme activity with age.

Experiment 2

Because of the composite nature of the multi-enzyme applications, assessment of the results as to the best overall enzyme within the supplements is difficult to evaluate (Table 7). The results from this experiment are similar to the that found with crude enzyme supplements (Willingham *et al.*, 1960), wherein crude supplements yielded better performance than did purified enzyme supplements.

The supplements in this experiment that produced the highest poult performance values contained amylase and would indicate that amylase is beneficial for inclusion in turkey starter diets. The diets that produced the lowest growth performance contained higher levels of protease.

Hesselman (1983) and Potter *et al.* (1991) determined that feed and water applications of enzymes were equally effective as modes of enzyme delivery and produced similar performance results. Collier and Hardy (1986) concluded that spray application of enzymes after pelleting resulted in the highest activity recovery. The dry and spray methods of enzyme application in this experiment did not appear to be a factor since similar growth performance was observed with diets associated with each mode of enzyme delivery.

The results from these two experiments further illustrate the responses of feeding multi-enzyme supplements containing amylase, by increased growth performance of poults in the

early weeks of life. Amylase appeared to be associated with this increased growth performance when supplemented in the feed. Additionally, endogenous amylase did not appear to reach maximal activity at 21 days of age as originally reported, and may continue to increase with age, as observed in Figures 1, 2, and 3.

ACKNOWLEDGEMENTS

Enzyme supplements were formulated by Cultor LTD. Technology Center, 02460 Kantvik, Finland, and provided by FinnFeeds International LTD., Market House, Ailesbury Court, High Street, Marlborough, Wiltshire, SN8 1AA, U.K.

REFERENCES

Bedford, M. R., J. F. Patience, H. L. Classen, and J. Inborr, 1992. The effect of dietary enzyme supplementation of rye- and barley-based diets on digestion and subsequent performance in weanling pigs. Can. J. Anim. Sci. 72:97-105.

Collier, B., and B. Hardy, 1986. The use of enzymes in pig and poultry feeds. The Feed Compounder 6:28-30.

Hesselman, K., 1983. Effects of beta-glucanase supplementation to barley based diets for broiler chickens. Report 112. Sveriges Lantbruksuniversitet, Uppsala.

Krogdahl, A., and J. L. Sell, 1989. Influence of age on lipase, amylase, and protease activities in pancreatic tissue and intestinal contents of young turkeys. Poultry Sci. 68:1561-1568.

Potter, L. M., R. M. Hulet, and C. W. Ritz, 1991. Effects of added enzyme supplements to diets of turkeys. Poultry Sci. 70 (Suppl 1):176.(Abstr)

Reese, G. L., R. S. Karg, B. I. Fancher, and P. W. Waldroup, 1983. The use of supplemental enzymes in the diet of broiler chickens. Nutr. Rep. Int. 28:919-929.

Rotter, B. A., R. R. Marquardt, and W. Guenter, 1989a. Optimizing responses from enzyme in poultry and pig diets: new methods for ensuring response. Proceedings of Alltech's Annual Symposium. Nicholasville, Kentucky.

Rotter, B. A., M. Neskar, R. R. Marquardt, and W. Guenter, 1989b. Effects of different enzyme preparations on the nutritional value of barley in chicken diets. Nutr. Rep. Int. 39:107-119.

SAS Institute, 1985. SAS® User's Guide: Statistics. Version 5 Edition. SAS Institute Inc., Cary, NC.

Willingham, H. E., K. C. Leong, L. S. Jensen, and J. McGinnis, 1960. Influence of geographical area of production on response of different barley samples to enzyme supplements or water treatment. Poultry Sci. 39:103-108.

Table 1. Enzyme activity of dietary supplements - Exp. 1

Diet	Amylase	Protease	Pectinase	Xylanase
	Activity units/g supplement			
1 (control)	-	-	-	-
3	50	100	750	80
4	100	100	750	80
5	200	100	750	80
6	400	100	750	80
7	800	100	750	80
8	0	100	750	40
2	0	100	750	80
9	0	100	750	160
10	0	100	750	320

Supplement inclusion rate = 1.1g/kg diet.
Activity unit = micromole substrate hydrolyzed/min/g.

Table 2. Basal diet composition (Exp. 1, 2)

Ingredient	g/kg diet
Ground yellow corn	459.7
Stabilized fat[1]	20.0
Dehulled soybean meal	435.0
Menhaden fish meal	25.0
Meat and bone meal	25.0
Defluorinated phosphate	22.5
Ground limestone	2.5
Iodized salt	4.0
DL-Methionine	2.8
Trace mineral mix[2]	1.0
Vitamin and additive mix[3]	2.5
Total	1000.0
Crude protein (%)	28
Metabolizable energy (kcal/kg diet)	2960

[1]Commercial animal-vegetable blend.

[2]The trace mineral mix provided (per g mix): 0.45 mg cobalt, 5 mg copper, 2.5 mg iodine, 120 mg manganese, 120 mg zinc, and 40 mg iron, with calcium carbonate as a diluent.

[3]The vitamin and additive mix provided (per g mix): 1764 IU vitamin A, 661.5 ICU vitamin D_3, 1.1 IU vitamin E, 0.88 mg riboflavin, 1.76 mg d-calcium pantothenate, 8.82 mg niacin, 74.97 mg choline chloride, 0.002 mg vitamin B_{12}, 0.22 mg thiamine mononitrate, 0.220 mg pyridoxine-HCl, 0.02 mg d-biotin, 0.04 mg selenium, 198 mg methionine, 0.22 mg folic acid, 1.06 mg menadione sodium bisulfite complex, and 25 mg ethoxyquin (as a preservative).

Table 3. Enzyme activity of dietary supplements - Exp. 2

Diet		Amylase[1]	Protease[2]	Pectinase[3]	α-Galacto-sidase[4]
		Activity units/g supplement			
1	(dry appl. control)[5]	-	-	-	-
2	(water appl. control)[6]	-	-	-	-
3	(dry/water control)[7]	-	-	-	-
4	(dry appl.)	200	-	-	-
5	(dry appl.)	-	324	-	-
6	(water appl.)	-	90	9	165
7	(dry appl.)	-	5	40	-
8	(dry/water)	-	419	49	165
9	(dry appl.)	200	324	-	-
10	(dry/water)	200	90	9	165
11	(dry appl.)	200	5	40	-
12	(dry/water)	200	419	49	165

[1]Amylase inclusion rate = 1.1g/kg diet.
[2]Protease inclusion rate = 0.617g/kg diet.
[3]Pectinase inclusion rate = 1g/kg diet.
[4]α-Galactosidase inclusion rate = 0.606g/kg diet.
[5]Control diet for dry enzyme application diets.
[6]Control diet for spray enzyme application diets.
[7]Control diet for combination dry/spray application diets.

Activity unit = micromole substrate hydrolyzed/min/g.

Table 4. Effect of amylase supplementation upon male turkeys 0 to 5 weeks of age (Exp. 1).

		Activity units					
	Week	Control	50	100	200	400	800
Body weight gain (g)	0-1	65	66	65	66	67	68
	1-2	136	138	135	140	136	138
	2-3	165	171	174	181	180	176
	3-4	312	322	310	330	318	323
	4-5	442	446	450	456	442	453
	0-5	1120	1143	1134	1173	1143	1159
Feed consumption (g)	0-1	102	102	98	97	97	100
	1-2	218	230	220	225	218	224
	2-3	344	350	338	352	336	344
	3-4	521	540	528	543	533	536
	4-5	770	782	782	793	780	781
	0-5	1955	2005	1966	2010	1964	1984
Feed efficiency	0-1	0.633^{b}	0.645^{ab}	0.666^{ab}	0.682^{a}	0.687^{a}	0.686^{a}
	1-2	0.624	0.601	0.615	0.623	0.624	0.615
	2-3	0.481^{b}	0.492^{ab}	0.507^{ab}	0.515^{ab}	0.535^{a}	0.512^{ab}
	3-4	0.598	0.590	0.586	0.608	0.596	0.603
	4-5	0.574	0.570	0.575	0.574	0.567	0.581
	0-5	0.572	0.570	0.576	0.583	0.581	0.584

Means within an age with different superscripts are significantly different ($P<.05$).

90 birds per treatment (9 pens, 10 birds per pen).

Table 5. Effect of xylanase supplementation upon male turkeys 0 to 5 weeks of age (Exp. 1).

		Activity units				
	Week	control	40	80	160	320
Body weight gain (g)	0-1	65	64	67	65	65
	1-2	136	141	134	137	134
	2-3	165	177	176	171	176
	3-4	312	310	318	320	312
	4-5	442	450	452	461	444
	0-5	1120	1146	1147	1154	1131
Feed consumption (g)	0-1	102	95	104	102	98
	1-2	218	214	231	215	230
	2-3	344	349	350	350	346
	3-4	521	540	539	533	530
	4-5	770	791	801	790	775
	0-5	1955	1988	2025	1990	1979
Feed efficiency	0-1	0.633	0.673	0.640	0.644	0.663
	1-2	0.624^{ab}	0.675^{a}	0.580^{b}	0.637^{ab}	0.587^{b}
	2-3	0.481	0.509	0.504	0.492	0.510
	3-4	0.598	0.573	0.591	0.601	0.587
	4-5	0.574	0.574	0.563	0.583	0.573
	0-5	0.572	0.576	0.566	0.580	0.571

Means within an age with different superscripts are significantly different ($P<.05$).

90 birds per treatment (9 pens, 10 birds per pen).

Table 6. Comparison of no enzyme, amylase and xylanase supplementation upon male turkeys 0 to 5 weeks of age (Exp. 1).

	Week	No enzyme (N=1)	Amylase (N=5)	Xylanase (N=4)
Body weight gain (g)	0-1	65	66	65
	1-2	136	138	136
	2-3	165[b]	176[a]	175[ab]
	3-4	312	321	315
	4-5	442	450	453
	0-5	1120	1151	1145
Feed consumption (g)	0-1	102	99	100
	1-2	218	223	222
	2-3	344	344	349
	3-4	521	536	535
	4-5	769	784	789
	0-5	1955	1986	1995
Feed efficiency	0-1	0.633[b]	0.674[a]	0.656[ab]
	1-2	0.624	0.616	0.620
	2-3	0.482[b]	0.513[a]	0.505[ab]
	3-4	0.599	0.598	0.588
	4-5	0.574	0.574	0.574
	0-5	0.573	0.579	0.573

Means within an age with different superscripts are significantly different ($P<.05$).

N = number of treatments (1 control, 5 amylase, 4 xylanase) with 90 birds per treatment (9 pens, 10 birds per pen).

Table 7. Effects of enzyme supplements upon male turkeys 0 to 5 weeks of age (Exp. 2).

Amylase (AU*)		-	-	-	200	-	-	-	-	200	200	200	200	
Protease (AU)		-	-	-	-	324	90	5	419	324	90	5	419	
Pectinase (AU)		-	-	-	-	-	9	40	49	-	9	40	49	
alpha-Galactosidase (AU)		-	-	-	-	-	165	-	165	-	165	-	165	
	Week	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12	Pooled SEM
Body weight gain (g)	0-1	75	77	75	79	75	78	80	75	74	78	75	79	.7
	1-2	149	151	146	153	144	157	146	144	150	148	145	149	1.7
	2-3	217 ab	213 ab	216 ab	217 ab	197 b	204 ab	220 ab	215 ab	226 a	215 ab	216 ab	219 ab	2.0
	3-4	382	390	373	386	366	377	379	376	373	369	393	377	3.7
	4-5	505	482	500	513	506	499	514	512	518	493	514	499	4.4
	0-5	1327	1316	1313	1350	1310	1327	1313	1326	1346	1303	1342	1325	7.7
Feed consumption (g)	0-1	100	102	102	106	103	103	104	101	100	102	102	103	.7
	1-2	240 ab	244 ab	235 ab	246 a	236 ab	245 a	239 ab	230 b	241 ab	243 ab	240 ab	237 ab	1.9
	2-3	376	392	381	381	375	378	389	376	383	381	386	363	2.4
	3-4	586 ab	598 ab	583 ab	597 ab	569 b	586 ab	598 ab	572 b	615 a	584 ab	596 ab	576 ab	4.8
	4-5	850	848	859	826	849	847	851	847	842	915	818	842	7.5
	0-5	2152	2184	2160	2156	2132	2160	2181	2126	2181	2225	2141	2140	12.5
Feed efficiency	0-1	0.749	0.750	0.740	0.751	0.729	0.750	0.768	0.743	0.742	0.760	0.737	0.766	.0054
	1-2	0.620	0.617	0.618	0.623	0.610	0.640	0.610	0.629	0.623	0.606	0.607	0.630	.0061
	2-3	0.576 ab	0.554 b	0.573 ab	0.575 ab	0.585 ab	0.572 ab	0.577 ab	0.579 ab	0.603 a	0.565 ab	0.555 b	0.582 ab	.0018
	3-4	0.652 a	0.652 a	0.640 ab	0.649 a	0.643 ab	0.643 ab	0.634 ab	0.660 a	0.608 b	0.632 ab	0.660 a	0.654 a	.0018
	4-5	0.594 abc	0.568 bc	0.583 abc	0.623 a	0.596 ab	0.589 abc	0.605 ab	0.605 ab	0.620 ab	0.543 c	0.633 a	0.592 abc	.0066
	0-5	0.617 a	0.603 ab	0.608 ab	0.627 a	0.615 a	0.614 a	0.616 a	0.624 a	0.618 a	0.586 b	0.627 a	0.619 a	.0029

Means within an age with different letters are significantly different ($P < .05$).

80 birds per treatment (8 pens, 10 birds per pen).

*AU = enzyme activity units per gram supplement.

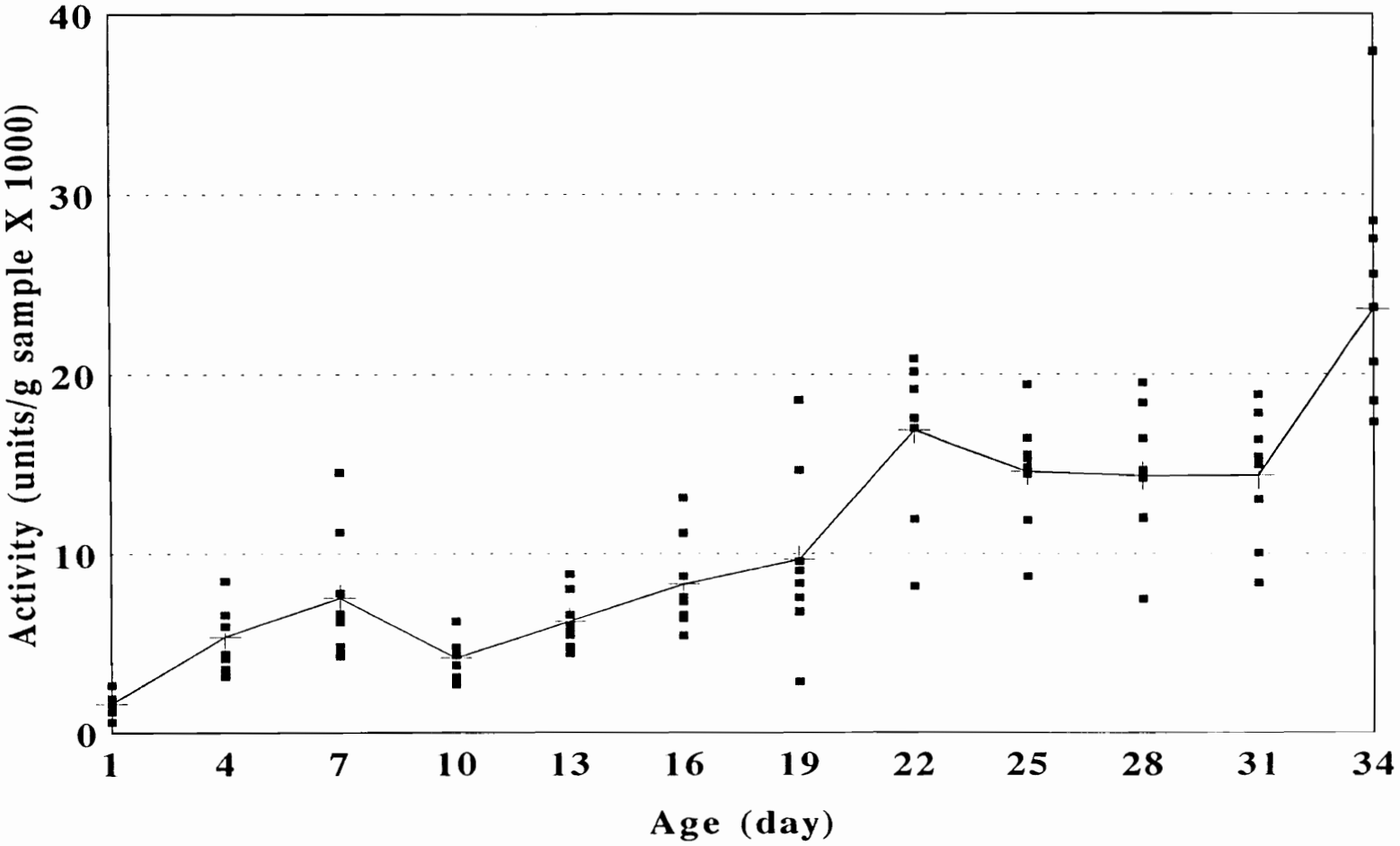

Figure 1. Pancreatic amylase activity of male turkeys 0 to 5 weeks of age. Each point represents a sample assay (n=8).

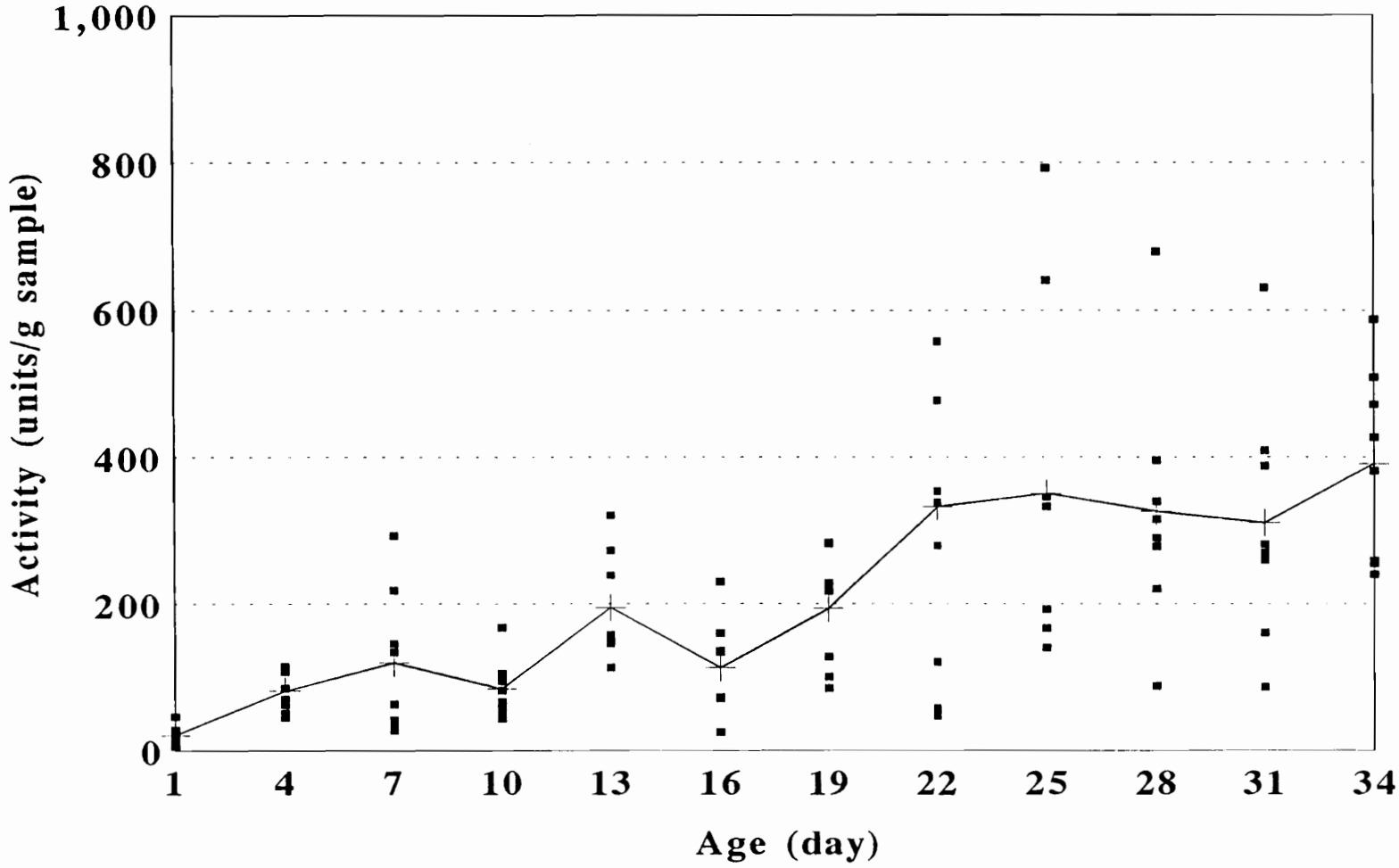

Figure 2. Intestinal amylase activity of male turkeys 0 to 5 weeks of age. Each point represents a sample assay (n=8).

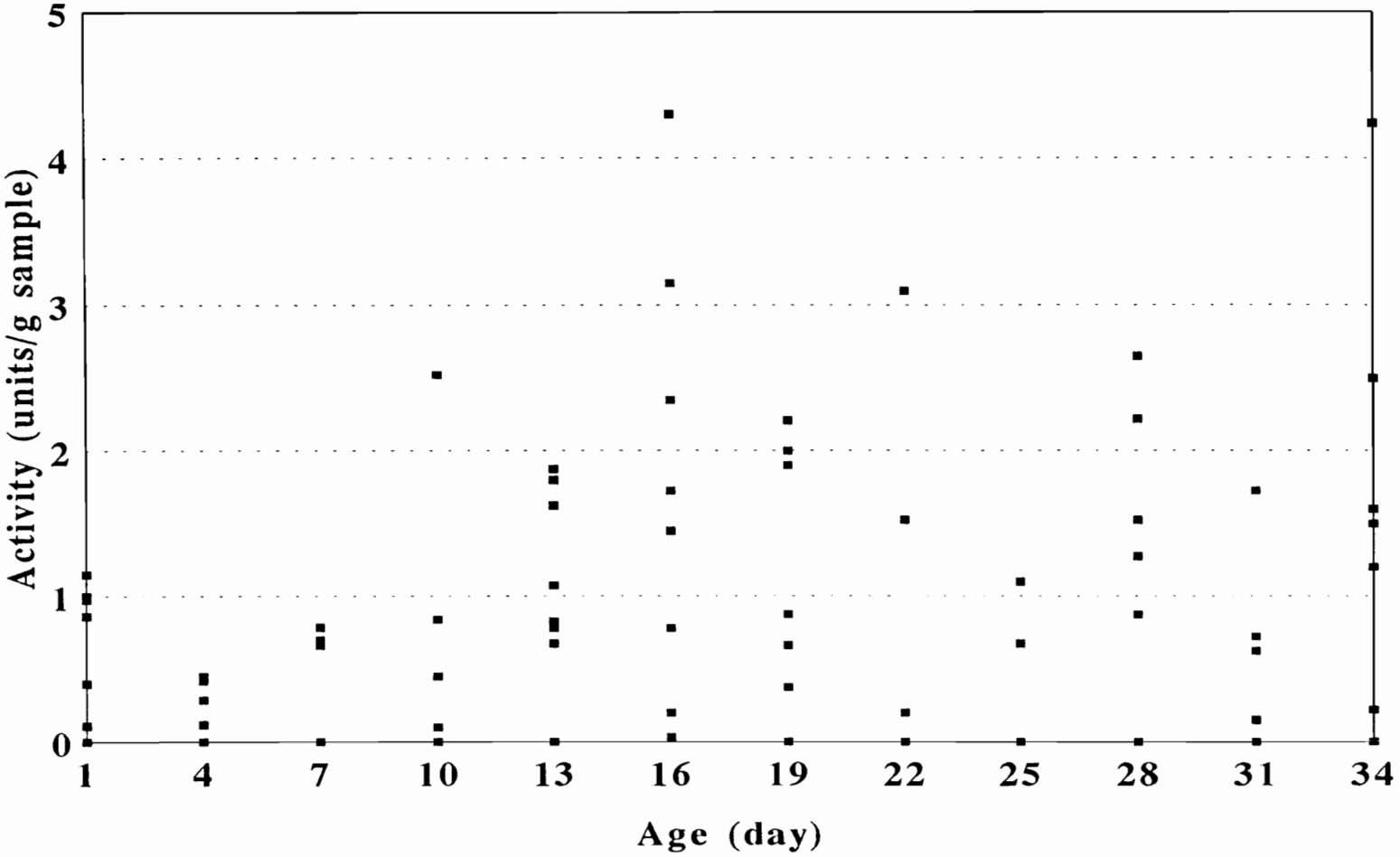

Figure 3. Crop amylase activity of male turkeys 0 to 5 weeks of age. Each point represents a sample assay (n=8). Multiple points of zero activity within an age represented by a single point.

Table 1. Analysis of variance of feed efficiency of male turkeys 1 to 2 weeks of age

Week	Source of variation	df	Mean square	F	p
1-2	Amylase	4	0.0025	0.74	0.594
	Xylanase	3	0.0114	3.42	0.013 *
	Error	80	0.0033		
	Total	87			

*P<.05.

Table 2. Analysis of variance of cumulative feed efficiency in male turkeys 0 to 2 weeks of age

Week	Source of variation	df	Mean square	F	p
0-2	Amylase	4	0.0027	1.85	0.112
	Xylanase	3	0.0046	3.15	0.019 *
	Error	80	0.0015		
	Total	87			

*P<.05.

Table 3. Analysis of variance of feed consumption of male turkeys 4 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
4-5	Amylase	1	41	0.01	0.905
	Protease	1	1250	0.44	0.507
	α-galactosidase	1	13700	4.84	0.030*
	Pectinase	1	7050	2.48	0.118
	Error	91	2840		
	Total	95			

*P<.05.

Table 4. Analysis of variance of feed efficiency of male turkeys 2 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
2-3	Amylase	1	0.0003	0.03	0.866
	Protease	1	0.0085	7.55	0.007*
	α-galactosidase	1	0.0003	0.30	0.586
	Pectinase	1	0.0008	0.67	0.415
	Error	91	0.0011		
	Total	95			
3-4	Amylase	1	0.0012	1.05	0.308
	Protease	1	0.0010	0.86	0.355
	α-galactosidase	1	0.0004	0.36	0.550
	Pectinase	1	0.0036	3.11	0.081
	Error	91	0.0012		
	Total	95			
4-5	Amylase	1	0.0024	1.09	0.300
	Protease	1	0.0027	1.20	0.276
	α-galactosidase	1	0.0174	7.80	0.006*
	Pectinase	1	0.0093	4.18	0.044*
	Error	91	0.0022		
	Total	95			

*P<.05.

Table 5. Analysis of variance of cumulative feed efficiency of male turkeys 0 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
0-3	Amylase	1	0.000005	0.01	0.923
	Protease	1	0.0023	4.58	0.035*
	α-galactosidase	1	0.0002	0.33	0.567
	Pectinase	1	0.0003	0.53	0.470
	Error	91	0.0005		
	Total	95			
0-4	Amylase	1	0.0003	0.86	0.356
	Protease	1	0.0001	0.35	0.556
	α-galactosidase	1	0.0002	0.77	0.384
	Pectinase	1	0.0003	0.94	0.336
	Error	91	0.0003		
	Total	95			
0-5	Amylase	1	0.00002	0.04	0.834
	Protease	1	0.0007	1.49	0.226
	α-galactosidase	1	0.0019	3.90	0.051
	Pectinase	1	0.0025	5.18	0.025*
	Error	91	0.0005		
	Total	95			

*P<.05.

APPENDIX II

AMYLASE ASSAY

AMYLASE ASSAY
modified from Gertler and Nitsan (1970)

EQUIPMENT:

Water bath set at 37° C
Water bath or dry block heater set at 100° C
Vortex
Centrifuge (capable of going to 30,000 g)
Polytron homogenizer and/or stomacher
Spectrophotometer
Pipet-aid
10-100 µl pipetter
100-1000 µl pipetter
2000-5000 µl pipetter
Tubs for ice water bath
Stopwatch

SUPPLIES:

30 ml plastic centrifuge tubes with caps (capable of 30,000 g)
10 ml plastic centrifuge tubes with caps (capable of 30,000 g)
Various size glass test tubes from 10 ml to 50 ml
Pasteur pipettes
Pipet tips
16 x 125 mm glass test tubes
Labeling tape
Stomacher bags (optional)
Cuvettes

REAGENTS:

1) Phosphate buffers (stock)
   a) X = 0.2M NaH_2PO_4 (28.4 g NaH_2PO_4 in 1000 ml dH_2O)
   b) Y = 0.2M $Na_2HPO_4.7H_2O$ (53.6 g $Na_2HPO_4.7H_2O$ in 1000 ml dH_2O)

2) Phosphate buffer (working)
   a) 45 ml X + 55 ml Y + .3915 NaCl. Fill to 1000 ml with dH_2O
   b) Adjust pH to 6.9

3) Sumner Reagent
   10 g dinitrosalicylic acid ($C_7H_{14}N_2O_7.H_2O$) - Sigma Cat. #D-1510
   16 g NaOH
   300 g sodium potassium tartarate ($C_4H_4KNaO_6.4H_2O$) - Sigma Cat. #S-2377
   Mix with approximately 800 ml dH_2O. Stir and heat. Once

dissolved allow to cool and bring volume up to 1000 ml with dH_2O.

4) Starch solution (prepare fresh daily)
   1% starch solution - 1 g potato starch
   (Sigma Cat. #S-2630) per 100 ml working buffer.
   Prepare as much as needed for the day
   (3 ml/sample + 1 ml/general blank)
   a) Bring half of the required amount of buffer to a boil.
   b) Meanwhile add the starch to the remaining half.
   c) Slowly add the cold starch solution to the boiling buffer (away from the heat). Pour a small amount of solution back into the cold solution container and swish to rinse all starch out.
   d) Bring entire solution back to a boil. This solution should be opaque-cloudy when cooled and should not separate.

SAMPLE PREPARATION: (keep samples cold at all times)

Excise organs and/or contents, wrap and place immediately in liquid N_2 until frozen. Store at -20° C until ready to run assay. Throughout the sample preparation procedure keep samples on ice.

1) Turn on water baths and/or dry block heater.
   Cool centrifuge and head. Warm up spectrophotometer.

2) Homogenization of sample
   a) Pancreas - cut entire sample into small pieces. Place in appropriate sized plastic centrifuge tube (10 ml for samples < .25 g, 30 ml for all others). Add 10 vol. of cold dH_2O (example, 1 g sample:9 ml dH_2O). Homogenize on ice using a Polytron, using short times; 20-30 sec total. Clean all tissue from generator with forceps and rinse with dH_2O between samples.

   b) Crop and small intestine contents - can homogenize as above (using 5 vol dH_2O) or stomach sample 2 min. in 5 vol cold dH_2O. Pour into centrifuge tubes.

3) Centrifuge 20 min. at 30,000 G (16000 rpm).

4) Transfer supernatant using pasteur pipette to glass test tube. The supernatant may be stored at -20° C for other enzyme assays. Amylase assay must be done from start to finish in one day.

5) Dilute supernatant

Since correct dilution is more or less a shot in the dark, it is advisable to take extra samples at least once, and work out a dilution before running your test samples. Some dilution factors for organs from turkeys of various ages are listed below. These dilution factors are the product of 3 dilutions: the dilution for homogenization (10X or 5X), the dilution of the supernatant, and the dilution of the sample when put in 1 ml of buffer.

Organ	Age (day)	Dilution factor
Crop	1 - 4	25
Contents	7	40
	10 - 35	50
Small	1	100
Intestine	4	150
Contents	7	200
	10 - 17	250
	20 - 35	300
Pancreas	1	9000
	4	10000
	7	12000
	10	18000
	13 - 17	24000
	20	20000
	23 - 32	24000
	35	40000

If the dilution factor is relatively low (25-200) then the dilution of the supernatant would not be necessary. For example, for 7 day small intestine contents, dilute it 5x during homogenization, then a 40X dilution of the supernatant in 1 ml buffer (25 ul supernatant in 1 ml buffer) will give a 200 dilution factor. For pancreas, three dilution steps are necessary. For 20000 dilution factor: 10x when homogenizing, 50x of supernatant (100 ul supernatant in 4.9 ml cold dH_2O), and 40x going in to buffer (25 ul in 1 ml buffer).

PROCEDURE:

1) Label three 16 x 125 mm glass tubes per sample (2 sample replicates and 1 blank). It helps to label samples, sample blank and general blank in 3 different colors. You can do a maximum of 12 samples per set, but 8 or 10 is an easier number to work with.

2) Label 1 general blank for the set.

3) Add 1 ml working buffer to each tube.

4) Add appropriate amount of sample (according to the dilution you want) to the 2 sample replicates and the sample blank tube.

5) Add identical amount of water to general blank.

6) Place tubes in 37° C water bath

7) Add 1 ml 1% starch solution to sample tubes only (2 tubes) and vortex

8) Incubate 3 minutes

9) Add 2 ml Sumner reagent (stopping solution) to each sample tube. Vortex. The following table demonstrates the timing of addition of solution to tubes on 15 second intervals:

Tube	Starch	Sumner
1a + 1b	:00	3:00
2a + 2b	:15	3:15
3a + 3b	:30	3:30
4a + 4b	:45	3:45
5a + 5b	1:00	4:00
6a + 6b	1:15	4:15
7a + 7b	1:30	4:30
8a + 8b	1:45	4:45

These steps are accomplished much easier with 2 people: 1 to time and vortex, the other to add solutions.

10) Add 2 ml Sumner to all blanks (sample + general). Vortex.

11) Add 1 ml starch solution to all blanks. Vortex.

12) Places tubes in 100° water bath or dry block heater for 5 min.

13) Allow tubes to cool.

14) Read absorbance at 540 nm. Zero spec. with general blank. Acceptable OD values are .1-1.0. If samples are not within this range you must change your dilution factor.

We were able to do 24 samples (3 sets of eight tubes - including reruns due to dilution changes) in 5 hours with 2 people working.

CALCULATIONS

1) OD reading = (OD sample A + OD sample B)/2 - OD sample blank

2) Dilution Factor = dilution when homogenized x dilution of supernatant x dilution when sample is added to buffer.

3) Activity = OD reading x dilution factor

APPENDIX III

XYLANASE ASSAY

XYLANASE ASSAY
modified from Biely *et al*,, 1999

Equipment:

Water bath set at 37°C
Water bath set at 100°C
Vortex
Centrifuge (capable of going to 4,000 g)
Spectrophotometer
100-1000 ul pipetter
1000-5000 ul pipetter

Supplies:

5-15 ml tubes - plastic or glass (We used 15 ml plastic because they fit in big centrifuge.)
Racks for tubes
Pipet tips
Labeling tape
Cuvettes
95% ethyl alcohol (ethanol)

Reagents:

1) .1 M acetate buffer
   a) X = 5.75 ml acetic acid fill to 1 liter with dH_2O
      Y = 8.204 g anhydrous sodium acetate (or 13.61 g trihydrate sodium acetate) in 1 liter dH_2O
   b) 322 ml X + 678 ml Y = .1 M acetate buffer
   c) Adjust pH to 5

2) RBB - Xylan substrate (11.5 mg/ml) - Sigma Cat. #M5019
   Dissolve 1.15 g/100 ml .1 M acetate buffer (This takes a long time to dissolve.)

Sample Preparation:

See amylase procedure. Use supernatant from Step 4.

Procedure:

1) Label 3 tubes per sample (2 sample replicates and 1 blank). The sample replicates and all sample blanks will be in a 2 separate racks. Most advantageous to work in sets of 8-10 samples. Up to 8 sets can be run in one day if you have room in the water bath.

2) Label 1 general blank for all the sets in one water bath.

3) Add 250 ul sample supernatant to each tube (total of 3 tubes/sample).

4) Add 250 ul RBB - Xylan substrate to the sample replicate tubes. Vortex then cover with foil to prevent evaporation.

5) Incubate 4 hr. at 37°C in water bath (see Step 11 for procedure for blanks.)

6) Stop reaction by adding 1.5 ml 95% ethanol. Vortex.

7) Let stand 30 min.

8) Centrifuge at 4,000 g for 10 minutes (2100 rpm).

9) Pour or pipet supernatant into cuvettes.

10) Read absorbance at 590 nm. Acceptable OD values should be .1 - 1.0. If samples are not within this range, dilute out supernatant and rerun.

11) Near the end of the 4 hr. incubation period for the sample tubes, add 250 ul of RBB -Xylan substrate to all blanks (sample blanks plus one general blank which contains 250 ul water). Vortex. Cover tubes with foil.

12) Incubate 10 min at 100°C in water bath.

13) Follow steps 6-10 above. General blank is to zero the machine, sample blank is to account for color of supernatant in samples.

Calculate activity as per amylase activity: Activity - OD reading x dilution factor

APPENDIX IV

CHAPTER 1

Table 1. Amylase activity response to handling stress

Stressor	Date	Sampling date	Diet[1]	Relative change[2]	
				Pancreas	Intestine
Weigh	Day 8	Day 10	1	-	+
			2	+	+
			3	-	o
Weigh	Day 15	Day 16	1	+	o
			2	+	o
			3	-	o
Weigh, move	Day 22	Day 25	1	-	o
			2	+	o
			3	-	o
Weigh	Day 29	Day 31	1	+	o
			2	+	o
			3	+	o
Weigh, randomize	Day 36	Day 37	1	-	o
			2	+	o
			3	-	o
Weigh	Day 43	Day 46	1	+	o
			2	+	o
			3	-	0
Weigh, randomize	Day 50	Day 52	1	-	+
			2	-	+
			3	-	+

[1] Diet 1 = control
2 = amylase supplementation
3 = xylanase supplementation

[2]Relative change in activity determined by using 2X ANOVA coefficient of variation differential between mean activity prior to and after stress.
+ = increase in activity
- = decrease in activity
o = no change

Table 2. Analysis of variance of amylase activity within the pancreas of male turkeys fed control, amylase and xylanase diets from 1 to 55 days of age

Day	Source of variation	df	Mean square	F	p
1	Diet	2	170000	0.27	0.764
	Error	19	622000		
	Total	21			
10	Diet	2	21000000	6.81	0.005*
	Error	21	3080000		
	Total	23			
19	Diet	2	2140000	0.26	0.772
	Error	21	8180000		
	Total	23			
28	Diet	2	14400000	2.17	0.140
	Error	21	6670000		
	Total	23			
37	Diet	2	24900000	2.38	0.117
	Error	21	10500000		
	Total	23			
46	Diet	2	186000000	4.84	0.019*
	Error	21	38500000		
	Total	23			
55	Diet	2	15900000	0.45	0.643
	Error	18	35200000		
	Total	20			

*P<.05.

Table 3. Analysis of variance of amylase activity within the small intestine of male turkeys fed control, amylase and xylanase diets from 1 to 55 days of age

Day	Source of variation	df	Mean square	F	p
1	Diet	2	446	2.39	0.131
	Error	13	187		
	Total	15			
10	Diet	2	16042	1.43	0.263
	Error	21	11249		
	Total	23			
19	Diet	2	326	0.10	0.907
	Error	18	3334		
	Total	20			
28	Diet	2	8308	0.65	0.533
	Error	20	12790		
	Total	22			
37	Diet	2	8084	4.22	0.029*
	Error	21	1916		
	Total	23			
46	Diet	2	196	0.01	0.992
	Error	20	24464		
	Total	22			
55	Diet	2	70770	2.34	0.121
	Error	21	30255		
	Total	23			

*P<.05.

Table 4. Analysis of variance for amylase activity within the crop of male turkeys fed control, amylase and xylanase diets from 1 to 55 days of age

Day	Source of variation	df	Mean square	F	p
1	Diet	2	1.951	2.06	0.190
	Error	8	0.948		
	Total	10			
10	Diet	2	0.005	0.01	0.992
	Error	14	0.587		
	Total	16			
19	Diet	2	1.913	1.367	0.277
	Error	21	1.401		
	Total	23			
28	Diet	2	2.848	2.31	0.138
	Error	13	1.232		
	Total	15			
37	Diet	2	2.449	2.60	0.100
	Error	19	0.940		
	Total	21			
46	Diet	2	2.919	2.19	0.144
	Error	16	1.333		
	Total	18			
55	Diet	2	0.316	0.18	0.833
	Error	20	1.714		
	Total	22			

Table 5. Analysis of variance for amylase activity within the pancreas of male turkeys fed control, amylase and xylanase diets 0 to 5 weeks of age

Diet	Source of variation	df	Mean square	F	p
1 (control)	Age	18	155000000	10.32	0.0001*
	Error	128	15000000		
	Total	146			
2 (amylase)	Age	18	173000000	10.60	0.0001*
	Error	130	16300000		
	Total	148			
3 (xylanase)	Age	18	192000000	14.35	0.0001*
	Error	131	13400000		
	Total	149			

*P<.05.

Table 6. Analysis of variance for amylase activity within the small intestine of male turkeys fed control, amylase and xylanase diets 0 to 5 weeks of age

Diet	Source of variation	df	Mean square	F	p
1 (control)	Age	18	57700	8.08	0.0001*
	Error	127	7140		
	Total	145			
2 (amylase)	Age	18	122000	10.09	0.0001*
	Error	126	12100		
	Total	144			
3 (xylanase)	Age	18	88800	7.20	0.0001*
	Error	127	12300		
	Total	145			

*P<.05.

Table 7. Analysis of variance of amylase activity between three different sample preparation procedures

Week	Source of variation	df	Mean square	F	p
1	Procedure	2	72500000	10.14	0.0008*
	Error	21	7150000		
	Total	23			

*P<.05.

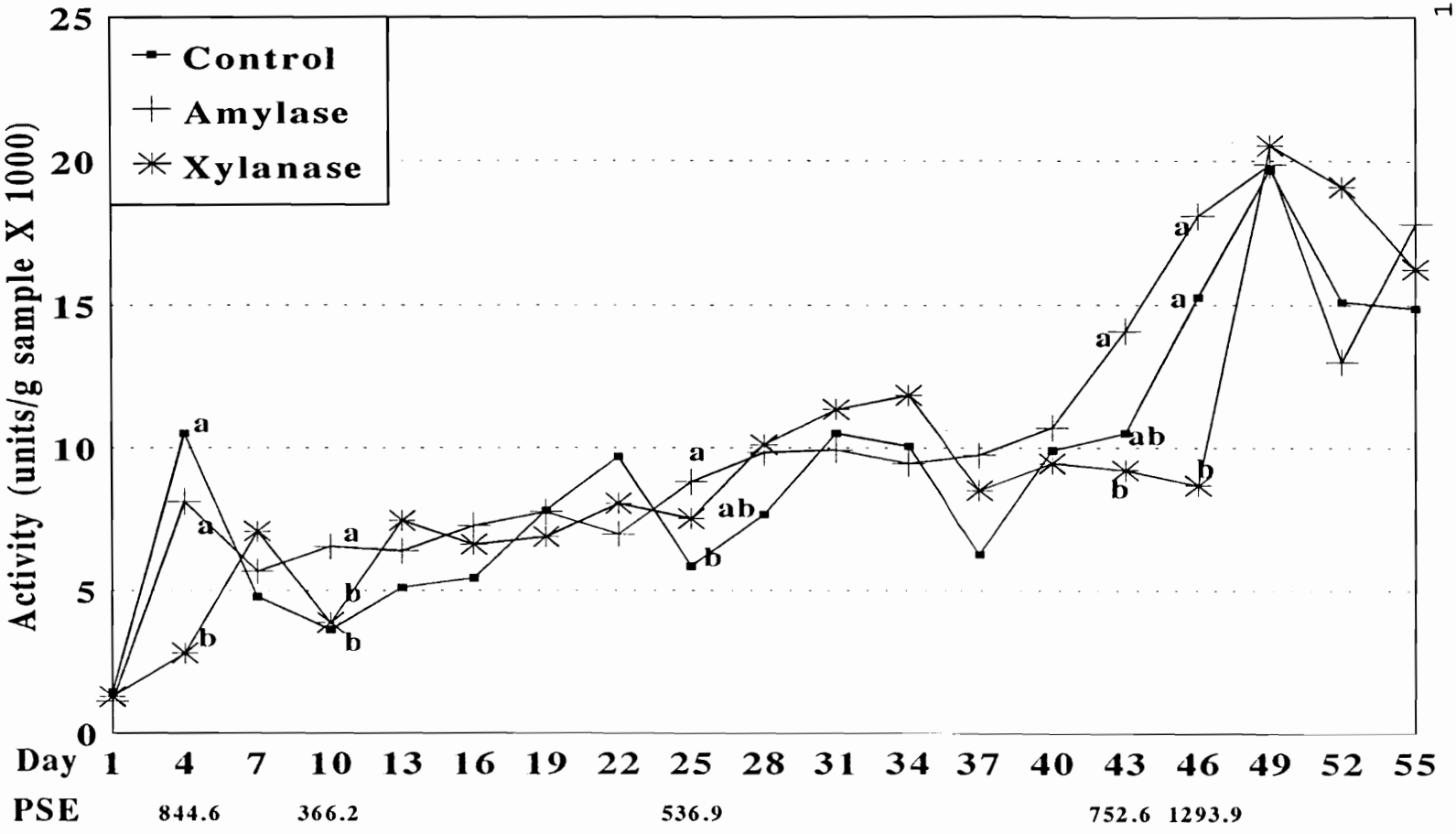

Figure 1. Pancreatic amylase activity for male turkeys 0 to 55 days of age. Means (n = 8) within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

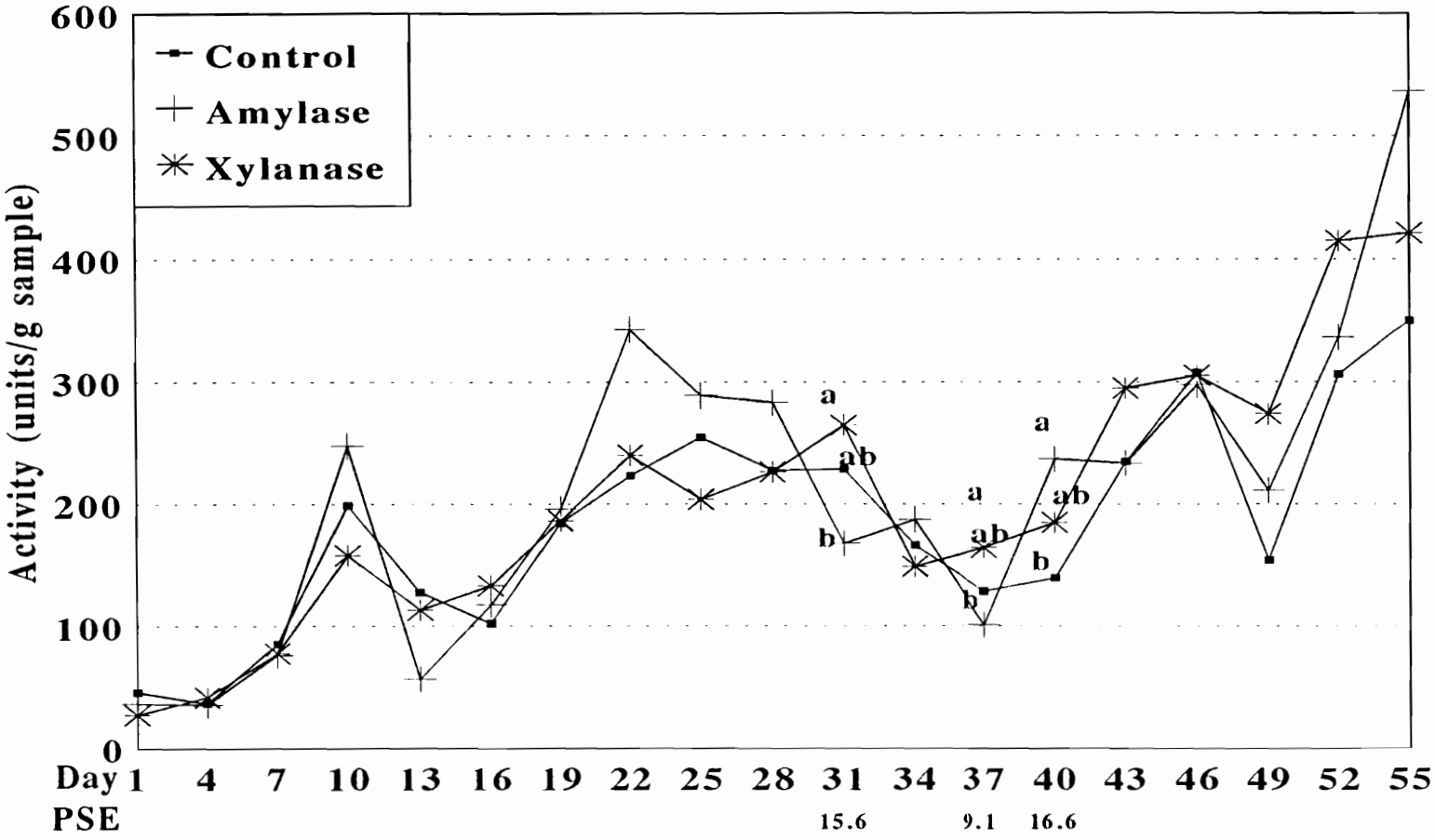

Figure 2. Intestinal amylase activity for male turkeys 0 to 55 days of age. Means (n = 8) within an age with different superscripts are significantly different (P < .05). PSE, pooled standard error of the mean.

APPENDIX V

CHAPTER 2

Table 1. Analysis of variance of body weights of male turkeys when 2 and 3 weeks of age

Week	Source of variation	df	Mean square	F	p
2	Diet	2	1350	4.64	0.019*
	Error	26	291		
	Total	28			
3	Diet	2	1949	4.48	0.021*
	Error	26	434		
	Total	28			

*P<.05.

Table 2. Analysis of variance of body weight gain of male turkeys when 1 and 2 weeks of age

Week	Source of variation	df	Mean square	F	p
0-1	Diet	2	145	3.51	0.045*
	Error	26	41		
	Total	28			
1-2	Diet	2	640	4.44	0.022*
	Error	26	144		
	Total	28			

*P<.05.

Table 3. Analysis of variance of cumulative body weight gain of male turkeys when 2 and 3 weeks of age

Week	Source of variation	df	Mean square	F	p
0-2	Diet	2	1386	4.82	0.016*
	Error	26	287		
	Total	28			
0-3	Diet	2	2013	4.67	0.018*
	Error	26	431		
	Total	28			

*P<.05.

Table 4. Analysis of variance of feed consumption of male turkeys when 2 weeks of age

Week	Source of variation	df	Mean square	F	p
1-2	Diet	2	1013	3.79	0.036*
	Error	26	267		
	Total	28			

*P<.05.

Table 5. Analysis of variance of feed efficiency in male turkeys when 1 and 2 weeks of age

Week	Source of variation	df	Mean square	F	p
0-1	Diet	2	0.0062	3.74	0.037*
	Error	26	0.0017		
	Total	28			
1-2	Diet	2	0.0052	5.55	0.010*
	Error	26	0.0009		
	Total	28			

*P<.05.

Table 6. Analysis of variance of cumulative feed efficiency of male turkeys when 2 weeks of age

Week	Source of variation	df	Mean square	F	p
0-2	Diet	2	0.0052	6.64	0.005*
	Error	26	0.0008		
	Total	28			

*P<.05.

Table 7. Analysis of variance of mean duodenal villus length of male turkeys 0 to 8 weeks of age

Week	Source of variation	df	Mean square	F	p
1	Diet	2	0.2085	4.33	0.025*
	Error	24	0.0482		
	Total	26			
2	Diet	2	0.4205	5.65	0.008*
	Error	33	0.0744		
	Total	35			
3	Diet	2	0.3856	11.76	0.0004*
	Error	21	0.0328		
	Total	23			
4	Diet	2	0.4271	3.86	0.033*
	Error	29	0.1107		
	Total	31			
5	Diet	2	0.2179	6.80	0.003*
	Error	33	0.0320		
	Total	35			
6	Diet	2	0.4538	8.10	0.001*
	Error	33	0.0560		
	Total	35			
7	Diet	2	0.2556	4.36	0.028*
	Error	19	0.0586		
	Total	21			
8	Diet	2	0.2000	3.18	0.064*
	Error	19	0.0629		
	Total	21			

*P<.05.

Table 8. Analysis of variance of mean jejunal villus length of male turkeys 0 to 8 weeks of age

Week	Source of variation	df	Mean square	F	p
1	Diet	2	0.0232	4.19	0.024*
	Error	33	0.0055		
	Total	35			
2	Diet	2	0.0962	12.04	0.0002*
	Error	26	0.0080		
	Total	28			
3	Diet	2	0.2035	155.24	0.0001*
	Error	25	0.0013		
	Total	27			
4	Diet	2	0.0975	7.04	0.004*
	Error	25	0.0139		
	Total	27			
5	Diet	2	0.0147	0.83	0.444
	Error	32	0.0176		
	Total	34			
6	Diet	2	0.1650	8.13	0.002*
	Error	27	0.0203		
	Total	29			
7	Diet	2	0.0473	4.03	0.028*
	Error	29	0.0117		
	Total	31			
8	Diet	2	0.0093	5.09	0.013*
	Error	29	0.0018		
	Total	31			

*P<.05.

Table 9. Analysis of variance of mean ileal villus length of male turkeys 0 to 8 weeks of age

Week	Source of variation	df	Mean square	F	p
1	Diet	2	0.0047	2.07	0.143
	Error	31	0.0023		
	Total	33			
2	Diet	2	0.0150	4.00	0.028*
	Error	31	0.0037		
	Total	33			
3	Diet	2	0.2431	22.45	0.0001*
	Error	32	0.0108		
	Total	34			
4	Diet	2	0.0260	1.84	0.174
	Error	33	0.0141		
	Total	35			
5	Diet	2	0.0489	4.08	0.026*
	Error	33	0.0120		
	Total	35			
6	Diet	2	0.1064	10.29	0.0004*
	Error	31	0.0103		
	Total	33			
7	Diet	2	0.1527	10.46	0.0003*
	Error	31	0.0146		
	Total	33			
8	Diet	2	0.0138	1.25	0.300
	Error	33	0.0111		
	Total	35			

*P<.05.

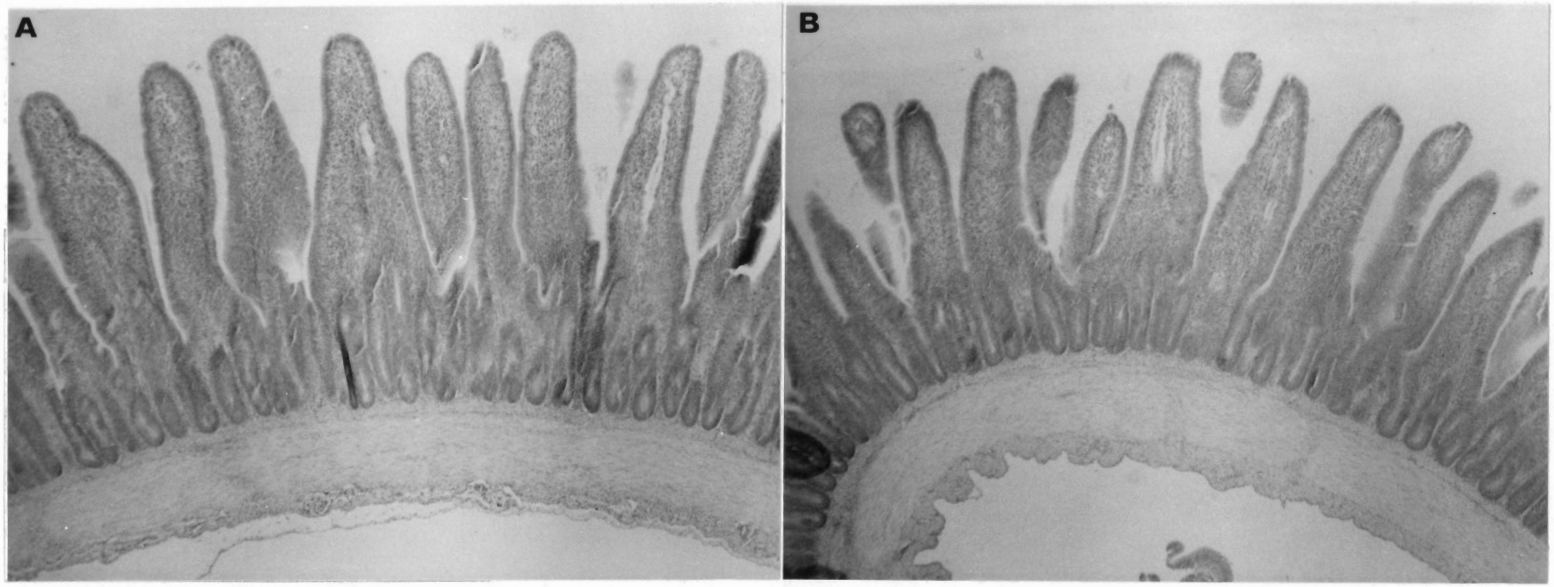

Figure 1. Cross-section view of (a) jejunal and (b) ileal mucosa at 10 X magnification at 2 weeks of age from male turkeys fed amylase supplemented diet.

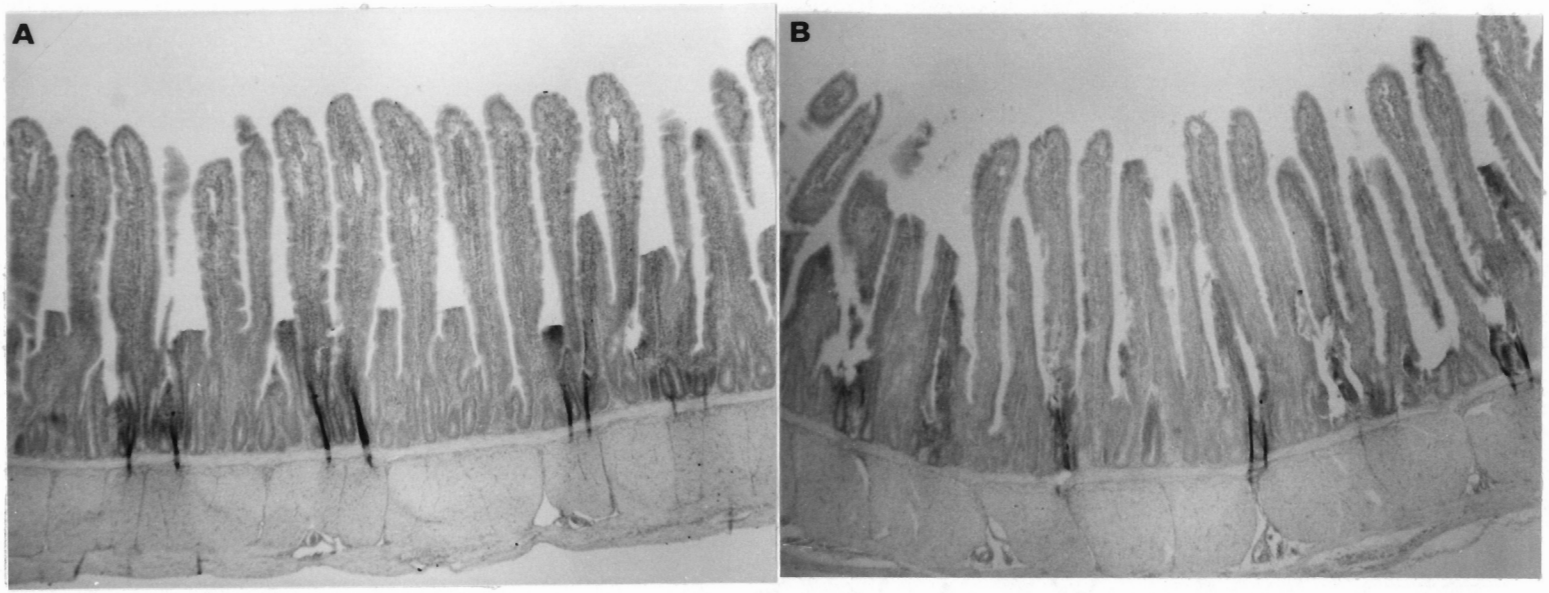

Figure 2. Cross-section view of (a) jejunal and (b) ileal mucosa at 10 X magnification at 4 weeks of age from male turkeys fed amylase supplemented diet.

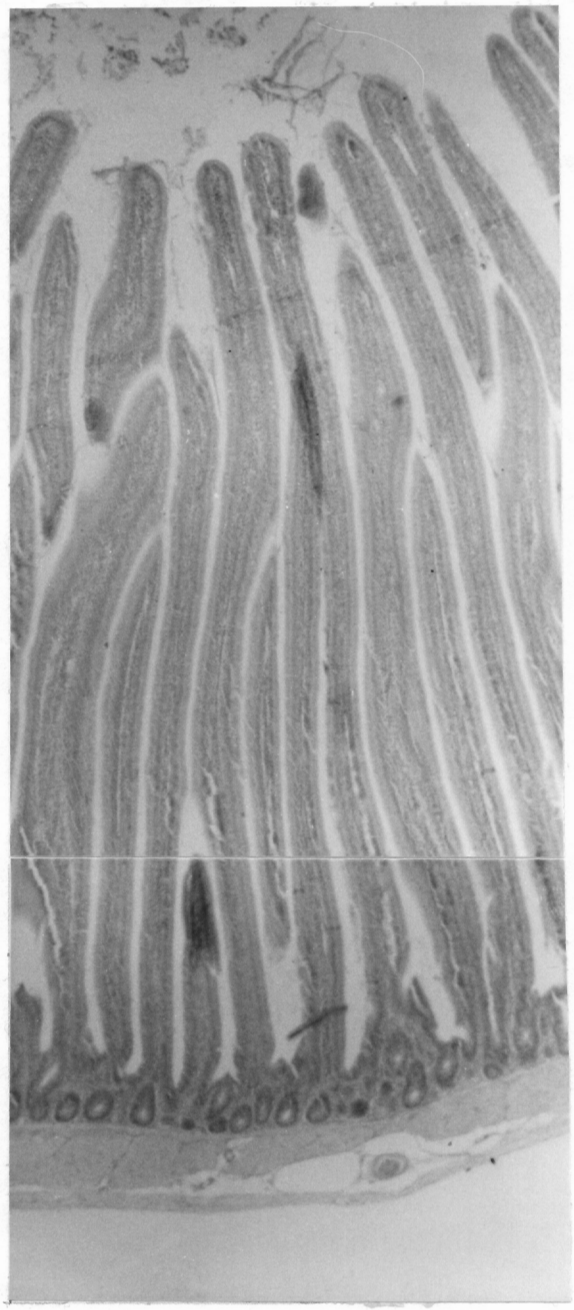

Figure 3. Cross-section view of duodenal mucosa at 10 X magnification at 4 weeks of age from male turkeys fed amylase supplemented diet.

APPENDIX VI

CHAPTER 3

Table 1. Analysis of variance of body weights of male turkeys 0 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
1	Protein	1	192	1.19	0.278
	Enzyme	4	47	0.29	0.881
	Protein*Enzyme	4	41	0.26	0.904
	Error	80	161		
	Total	89			
2	Protein	1	22656	36.39	0.0001*
	Enzyme	4	112	0.18	0.947
	Protein*Enzyme	4	133	0.21	0.930
	Error	80	622		
	Total	89			
3	Protein	1	57940	31.70	0.0001*
	Enzyme	4	1466	0.80	0.527
	Protein*Enzyme	4	121	0.07	0.992
	Error	80	1827		
	Total	89			
4	Protein	1	164277	46.34	0.0001*
	Enzyme	4	1488	0.42	0.794
	Protein*Enzyme	4	890	0.25	0.908
	Error	80	3544		
	Total	89			
5	Protein	1	301506	39.76	0.0001*
	Enzyme	4	4693	0.62	0.650
	Protein*Enzyme	4	2907	0.38	0.820
	Error	80	7584		
	Total	89			

*P<.05.

Table 2. Analysis of variance of body weight gain of male turkeys 0 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
0-1	Protein	1	137	1.18	0.280
	Enzyme	4	26	0.23	0.920
	Protein*Enzyme	4	42	0.36	0.834
	Error	80	115		
	Total	89			
1-2	Protein	1	18669	76.93	0.0001*
	Enzyme	4	200	0.83	0.511
	Protein*Enzyme	4	26	0.11	0.979
	Error	80	242		
	Total	89			
2-3	Protein	1	8133	9.70	0.0026*
	Enzyme	4	814	0.97	0.428
	Protein*Enzyme	4	392	0.47	0.759
	Error	80	838		
	Total	89			
3-4	Protein	1	27094	18.89	0.0001*
	Enzyme	4	210	0.15	0.964
	Protein*Enzyme	4	756	0.53	0.716
	Error	80	1433		
	Total	89			
4-5	Protein	1	20673	15.11	0.0002*
	Enzyme	4	1657	1.21	0.312
	Protein*Enzyme	4	664	0.49	0.746
	Error	80	1367		
	Total	89			

*P<.05.

Table 3. Analysis of variance of cumulative body weight gain of male turkeys 0 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
0-2	Protein	1	22007	41.69	0.0001*
	Enzyme	4	113	0.21	0.930
	Protein*Enzyme	4	134	0.26	0.906
	Error	80	527		
	Total	89			
0-3	Protein	1	56900	33.70	0.0001*
	Enzyme	4	1450	0.86	0.492
	Protein*Enzyme	4	150	0.09	0.985
	Error	80	1688		
	Total	89			
0-4	Protein	1	162521	49.03	0.0001*
	Enzyme	4	1490	0.45	0.772
	Protein*Enzyme	4	940	0.28	0.888
	Error	80	3314		
	Total	89			
0-5	Protein	1	299126	41.19	0.0001*
	Enzyme	4	4656	0.64	0.635
	Protein*Enzyme	4	3024	0.42	0.796
	Error	80	7261		
	Total	89			

*P<.05.

Table 4. Analysis of variance of feed consumption of male turkeys 0 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
0-1	Protein	1	81	0.76	0.386
	Enzyme	4	10	0.10	0.983
	Protein*Enzyme	4	67	0.63	0.641
	Error	80	107		
	Total	89			
1-2	Protein	1	7726	30.19	0.0001*
	Enzyme	4	113	0.44	0.777
	Protein*Enzyme	4	95	0.37	0.827
	Error	80	255		
	Total	89			
2-3	Protein	1	20525	19.27	0.0001*
	Enzyme	4	392	0.37	0.830
	Protein*Enzyme	4	513	0.48	0.749
	Error	80	1065		
	Total	89			
3-4	Protein	1	11122	3.92	0.051
	Enzyme	4	740	0.26	0.902
	Protein*Enzyme	4	2583	0.91	0.462
	Error	80	2838		
	Total	89			
4-5	Protein	1	32459	9.55	0.003*
	Enzyme	4	1524	0.45	0.773
	Protein*Enzyme	4	2602	0.77	0.551
	Error	80	3400		
	Total	89			

*P<.05.

Table 5. Analysis of variance of cumulative feed consumption of male turkeys 0 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
0-2	Protein	1	9396	15.46	0.0002*
	Enzyme	4	133	0.22	0.926
	Protein*Enzyme	4	309	0.51	0.729
	Error	80	607		
	Total	89			
0-3	Protein	1	57697	20.87	0.0001*
	Enzyme	4	701	0.25	0.907
	Protein*Enzyme	4	1302	0.47	0.757
	Error	80	2764		
	Total	89			
0-4	Protein	1	119485	13.19	0.0005*
	Enzyme	4	1462	0.16	0.957
	Protein*Enzyme	4	6025	0.67	0.618
	Error	80	9059		
	Total	89			
0-5	Protein	1	276500	13.41	0.0004*
	Enzyme	4	2937	0.14	0.966
	Protein*Enzyme	4	15336	0.74	0.565
	Error	80	20612		
	Total	89			

*P<.05.

Table 6. Analysis of variance of feed efficiency of male turkeys 0 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
0-1	Protein	1	0.0064	0.87	0.355
	Enzyme	4	0.0040	0.54	0.709
	Protein*Enzyme	4	0.0030	0.40	0.809
	Error	80	0.0074		
	Total	89			
1-2	Protein	1	0.1557	49.49	0.0001*
	Enzyme	4	0.0022	0.70	0.593
	Protein*Enzyme	4	0.0007	0.23	0.919
	Error	80	0.0031		
	Total	89			
2-3	Protein	1	0.00001	0.00	0.971
	Enzyme	4	0.0037	0.39	0.815
	Protein*Enzyme	4	0.0097	1.04	0.393
	Error	80	0.0094		
	Total	89			
3-4	Protein	1	0.0386	7.92	0.006*
	Enzyme	4	0.0024	0.49	0.747
	Protein*Enzyme	4	0.0003	0.07	0.991
	Error	80	0.0049		
	Total	89			
4-5	Protein	1	0.0033	1.66	0.201
	Enzyme	4	0.0038	1.91	0.117
	Protein*Enzyme	4	0.0002	0.11	0.977
	Error	80	0.0020		
	Total	89			

*P<.05.

Table 7. Analysis of variance of cumulative feed efficiency of male turkeys 0 to 5 weeks of age

Week	Source of variation	df	Mean square	F	p
0-2	Protein	1	0.0938	52.57	0.0001*
	Enzyme	4	0.0003	0.17	0.951
	Protein*Enzyme	4	0.0009	0.51	0.727
	Error	80	0.0018		
	Total	89			
0-3	Protein	1	0.0160	4.72	0.033*
	Enzyme	4	0.0017	0.50	0.733
	Protein*Enzyme	4	0.0040	1.19	0.321
	Error	80	0.0034		
	Total	89			
0-4	Protein	1	0.0281	37.68	0.0001*
	Enzyme	4	0.0009	1.17	0.331
	Protein*Enzyme	4	0.0008	1.13	0.349
	Error	80	0.0007		
	Total	89			
0-5	Protein	1	0.0157	27.40	0.0001*
	Enzyme	4	0.0013	2.26	0.070
	Protein*Enzyme	4	0.0004	0.69	0.601
	Error	80	0.0006		
	Total	89			

*P<.05.

VITA

Casey Warren Ritz, the son of Robert Theodore and Myrna Agnus Ritz, was born December 5, 1961 in Salem, Oregon. He competed his secondary education at Dallas High School, Dallas, Oregon, in June of 1980. He graduated with a Bachelor of Science degree from Brigham Young University in April of 1988, and continued his studies at that university under the direction of Dr. N. Paul Johnston, studying the effects of light upon turkey beeder hens and receiving a Master of Science degree in April of 1990. In August of 1990 he was admitted to Virginia Polytechnic Institute and State University as a candidate for the Doctorate of Philosophy in Poultry Management from the Department of Poultry Science. He completed his studies under the direction of Dr. R. Michael Hulet in August 1993.