

ABSTRACT

Jackson, Sharon Lynn Jennette. Influence of pre-feeding a semi-solid hydrated supplement, OASIS™, on development and performance of turkey poults.

The purpose of this thesis was to examine the effects of prefeeding a hydrated nutrient compound (OASIS™, Novus, St. Louis, MO) on (1) resistance to naturally occurring Salmonella infection and monitor three week performance of control and PEMS-infected poults, (2) pancreatic and mucosal digestive enzymes in control and PEMS-infected poults, and (3) post-hatch organ weight. The results of the first investigation indicated that provision of OASIS™ as a hydrated pre-feeding supplement before placement induced gut development in poults, and reduced 24h post-hatch loss before placement. Although the provision of OASIS™ as a pre-feeding supplement induced gastrointestinal development and reduced post-hatch body weight loss, the results of this investigation did not show consistently improved body weight gain at 7d of age. Neither body weight gain nor feed conversion improved as the result of OASIS™ pre-feeding, but 7d livability of pre-fed turkey poults was improved significantly. Histology from this investigation suggested that OASIS™ pre-fed PEMS-infected gut sections frequently looked as if nothing had changed their morphology, especially at 21d of age. The second objective of this investigation was to determine if the pre-feeding of OASIS™, before the poults were PEMS-challenged, affected performance of the poults through the first 3 weeks post-hatch. We found that impaired digestion in PEMS-infected poults was due to decreased digestive enzyme activity and that pre-feeding OASIS™ immediately after hatch had some ameliorating properties that might aid in the recovery from PEMS. OASIS™ in this investigation was found to stimulate intestinal

development in poult in both control and PEMS infected poult. The third objective of this thesis was to investigate the effects of pre-feeding OASIS™ on visceral organ growth and development of the small intestine of poult. This investigation produced data that suggest concurrent OAS and normal feed and water had little influence on growth of the turkey poult through 16d post-hatch. When one compares fasting versus feeding and then examines body weight data over the first 24h, it is clear that fed poult gain substantial weight while fasted poult lose hatching weight. Even when poult are fasted, there is a redistribution of body mass in the poult with some organs and the small intestine changing relative size. Part of the change in relative size of visceral organs and small intestine are due to loss of moisture, but part of the change in visceral organ and small intestine relative size can be attributed to the utilization of yolk sac nutrients for the purpose of growth and metabolism which has been long established. The strong advantage achieved when OASIS™ is provided before provision of a normal feed and water regimen can not be gained with concurrent feeding of OASIS™ and normal feed and water. Whatever advantage is gained with concurrent feeding is transitory being lost within 7d of initiation of concurrent feeding. In this study, the concurrent feeding of OASIS™ with normal feed and water influenced development of neither the small intestine, heart, lungs, pancreas, nor the bursa of Fabricius. These observations do not suggest any negative influence of OASIS™ concurrent with normal feeding, only that there is no need to provide OASIS™ when feed and water are already present. The results provided in this thesis suggested that it is very beneficial to pre-feed OASIS™ when there is a delay in placement and when the poult will be denied access to normal

feed and water consumption. It is beneficial to pre-fed OASIS™ in order for poult to utilize the nutrients in complex poultry diets.

**INFLUENCE OF PRE-FEEDING A SEMI-SOLID HYDRATED
SUPPLEMENT, OASIS™, ON DEVELOPMENT AND
PERFORMANCE OF TURKEY POULTS**

By

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DEDICATION

For Daddy and Shannon

BIOGRAPHY

Sharon Lynn Jennette Jackson was born on January 27, 1977 in Clinton, North Carolina. She is the middle child and only daughter to B.J. and Linda Jennette. Growing up on the family tobacco and turkey farm, she was exposed to the poultry industry at an early age. She continued her agriculture experience through high school by becoming an active member in the FFA. In 1995, Sharon graduated from Southern Wayne High School in Dudley, NC. From there, she received a B.S. in Poultry Science from North Carolina State University in 1999. After receiving her B.S., she decided to pursue a Master of Science degree in Poultry Science under the direction of Dr. Frank Edens.

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LIST OF ABBREVIATIONS

AcP	Acid phosphatase
AE	Attaching/effacing
AEC	Affacing effector cells
ALP	Alkaline phosphatase
ATP	Adenosine triphosphate
BW	Body weight
CD	Cluster of differentiation
ConA	Concanavalin
DNA	Deoxyribonucleic acid
DPI	Days post inoculation
<i>E. Coli</i>	<i>Escherichia coli</i>
<i>E. maxima</i>	<i>Escherichia maxima</i>
EPEC	Enteropathogenic e coli, R98/5
EPM	Early poult mortality
FACS	Fluorescence-activated cell sorter
FCR	Feed conversion ratio
GI	Gastrointestinal tract
Hgb	Hemoglobin
IL-1	Interleukin 1

IL-6	Interleukin 6
LPDV	Lymphoproliferative disease virus
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular concentrations
MCV	Mean corpuscular volume
OAS	OASIS™
PBL	peripheral blood lymphocytes
PCV	Packed cell volume
PEMS	Poult enteritis and mortality syndrome
RBC	Red blood cell
RBCL	Randombred control line
RNA	Ribonucleic acid
<i>S. arizona</i>	<i>Salmonella arizona</i>
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SPF	Specific pathogen-free
SRV	Small round virus
Tast-OSU	Turkey astrovirus – Ohio State University
TCV	Turkey coronavirus
TNF- α	Tumor necrosis factor – alpha
TRBC	Total red blood cell
TWBC	Total leukocytes

CHAPTER 1

LITERATURE REVIEW

HISTORY OF POULT ENTERITIS AND MORTALITY SYNDROME

Poult enteritis and mortality syndrome (PEMS) was first observed in 1991 in western North Carolina (Barnes and Guy, 1995). Since that time, it developed into one of the worst diseases to ever affect commercial turkeys (Edens and Doerfler, 1998). PEMS is classified as a disease with a multifactoral etiology owing to the fact that several other organisms were found to be associated with the disease process (Heggen-Peay et al., 2002ab). Viral agents such as coronavirus (Lin, et al., 2002; Yu et al., 2000ab), adenovirus (Yu et al., 2000ab), astrovirus (Yu et al., 2000ab; Qureshi et al., 2000a; Koci et al., 2000) and reovirus (Schat et al., 1998) were implicated as well as bacterial agents like; salmonella, campylobacter, clostridia and *E. coli* types I and II (Edens, et al., 1997ab).

Characteristics of the disease include diarrhea, dehydration, loss of weight, anorexia, immunodysfunction, 100% morbidity, and 2% mortality or more between 7 and 28 days of age. The mortality usually developed within 4 days after exposure to conditions where the etiologic agents were harbored (Edens, 1994; Edens and Doerfler, 1998; Brown, 1995; Barnes and Guy, 1995; Barnes et al., 1996). Older birds, including those of market age, can show signs that resemble PEMS (Edens and Doerfler, 1998; Edens et al., 1997ab; Doerfler et al.,

1998; Edens et al., 1998). Poults with PEMS show signs of agitation characterized by high-pitched vocalization and constant movement. Poults refused to eat normal amounts of feed and behaved as if feed and water intake irritated the gastrointestinal tract (Edens and Doerfler, 1998). The disease occurred throughout North Carolina and other mid-Atlantic states (Barnes, 1997) and caused great economic losses.

There have been two “forms” of PEMS-associated enteritis identified. The first type of enteritis is a severe form known as “spiking mortality in turkeys”. The mortality rate for this form of PEMS is about 9% at 7 to 28 days of age. The mild form of PEMS is known as “excess mortality of turkeys”. Other related disorders include stunting syndrome/feed refusal, poult growth depression, poult enteritis complex and “flushing”. Flushing is the acute diarrhea of older turkeys (Barnes, 1997; Edens and Doerfler, 1998).

EARLY POULT MORTALITY

Early poult mortality is a difficult and costly problem in the turkey industry. Many field characteristics have been associated with early poult mortality. Contributors to early poult mortality include disease inputs, managerial inputs, and the poult itself (Edens, 1994).

DIGESTION IN POULTRY

Newly-hatched turkey poult must make a rapid transition from dependence on absorbed egg contents as a source of nourishment to reliance on a relatively complex diet. During this transition, physical and functional development of the gastrointestinal tract is completed and is characterized by increased tissue mass and composition and synthesis and secretion of digestive enzymes (Sell, 1996).

Digestive anatomy and function in birds are similar to that in mammals with some exceptions (Hill, 1983; Duke, 1986ab). The esophagus in poultry includes a crop, which serves for temporary food storage and minor enzymatic microbial digestive activity. The gastric area includes the proventriculus (glandular stomach) and the ventriculus (muscular stomach or gizzard). The proventriculus secretes mucus, pepsin, and hydrochloric acid (HCl). Pepsin and HCl are responsible for the initial stages of dietary protein breakdown within the gizzard. The secreted mucus forms a protective coating on the proventriculus to prevent damage from pepsin and HCl induced protein hydrolysis. The primary function of the gizzard is maceration of feed. Ingested feed will empty from the gizzard into the duodenum when the particle size has been reduced to approximately 1 mm. Reflux of digesta from the duodenum back through the ventriculus and proventriculus and back to the duodenum occurs about four times per hour (Duke, 1986ab).

The small intestine consists of the duodenum and the ileum (Duke, 1986a). There is no histological differentiation of the lower small intestine (ileum) into the jejunum and ileum, but many avian physiologists use Meckel's diverticulum as an arbitrary anatomical site to divide the small intestine into ileum and jejunum (Duke, 1986a). The primary function of the duodenum is to complete the chemical digestion, which was initiated in the proventriculus (Duke, 1986b). To complete digestion, the duodenum secretes amylase, lipases for lipid breakdown, dipeptidases for protein breakdown, and disaccharidases for breakdown of simple carbohydrates. It receives pancreatic and biliary secretions, which participate in chemical digestion. The pancreas secretes amylase, lipase, trypsin, and chymotrypsin (protein breakdown) into the duodenal lumen. Bile is delivered from the liver via two ducts- one from the gall bladder (cystic) and one directly from the liver (hepatic) and provides acids to help emulsify fats in the gut lumen aiding in their further digestion and absorption. The ileum serves to absorb the sugars, amino acids, fatty acids, and cholesterol as they are released from the ingesta moving distally in the duodenum. Almost all digestion and absorption of nutrients is complete by the time the digesta reaches the end of the small intestines or ileocecorectal junction (Duke, 1986ab).

Birds have paired ceca that contain microorganisms capable of some fiber digestion, which releases carbohydrates (Duke, 1986b). The released carbohydrates are used locally by microbes, but birds can also utilize the

carbohydrates from the ceca. The cecal microbes also convert some uric acid from urine into amino acids, which can be used by the bird. The ceca also are involved in water reabsorption. Avian kidneys account for 85% of water reabsorption, the ceca account for 10-12%, and the colon accounts for 3-5%. The terminal organ of the gastrointestinal tract in birds is the cloaca, which serves as the common opening of the digestive, urinary, and reproductive tracts.

Gastric motility of the components of the avian stomach is different from that of mammals. The gizzard macerates food substances using two pairs of muscles, which are called the thin and thick pairs. The thin pair of ventriculus muscles mix while the thick pair of ventriculus muscles grind the feed in the gizzard. The motility of the duodenum is coordinated with the gizzard motility. The motility of the avian gastrointestinal tract allows for re-mixing through a reflux mechanism back through the gizzard and proventriculus and then back to the duodenum (Duke, 1986ab). This reflux allows for remixing of digesta with HCl, pepsin, and mucus to improve the early digestive process (Duke, 1986b).

There is another type of reflux (backward flow) that occurs in birds (Duke, 1986ab). The second reflux occurs when duodenal and upper ileal contents move back into the gizzard. This process has three functions: (1) slow overall passage through the gut, (2) re-mix intestinal digesta with gastric secretions, and (3) to increase the digestibility of feeds. The third reflux occurs in the cloacal-large intestine region. It involves a nearly continuous, low amplitude antiperistalsis

that moves urine out of the cloaca and into the ceca where water is absorbed (Duke, 1994).

INTESTINAL MORPHOLOGY

The morphology of the broiler chicken small intestine undergoes dynamic changes in villus height and volume, particularly in the jejunum and ileum, from 4 days to 10 days after hatching. Enterocytes per villus increase with age and have the greatest density in the jejunum and the ileum (Uni, 1995).

In chicks and poults, the rate of development of the gastrointestinal tract exceeds the rate of the body weight gain on both a physical (relative weight) and morphological (villus height, diameter, and volume) basis (Uni, 1995). This rapid development can be found in the duodenum, jejunum, pancreas, liver, cecum, and large intestine of poults (Phelps et al., 1987a) and chickens (Uni, 1995).

The utilization of feed is influenced significantly by the anatomy of the avian gastrointestinal tract. The avian intestine is structurally similar to that of monogastric animals with the exception of lacteals, which are not found in birds. Intestinal epithelium damage may decrease nutrient absorption, whereas epithelial replacement results in improved nutrient utilization (Turk, 1982).

There is variation in the time at which maximum specific activities of digestive enzymes in the pancreas and intestinal brush border occur, but it most often occurs at or shortly after hatch (Uni et al., 1998; Noy et al., 2001; van

Leeuwen et al., 2004). These activities frequently decrease with age. The total digestive enzyme activity tends to increase during the early post-hatch period due to the rapid increase in the intestinal and pancreas weight and concomitantly, their secretory activity also increases. This is predicated upon the fact that feed is present in the intestinal tract. However, delay in feeding or feed refusal might cause a delay in intestinal development and body growth (Butzner and Gall, 1990). Under these conditions, the normal increase in total enzyme activity may be delayed and contribute to relatively poor utilization of some dietary constituents. Some lipids, carbohydrates, and proteins are utilized less efficiently during the first week or two after hatch (Jin, 1998; Bedford, 1996).

DIGESTIVE ENZYMES

The role of digestive enzymes in the etiology and progression of PEMS has not been determined. Nonetheless, the multiple and diverse signs of PEMS suggests that malabsorption and maldigestion is a critical part and possible fatal characteristic of the disease. Typical characteristics of PEMS such as severe diarrhea, high morbidity and mortality, stunting, wasting of musculature, and loss of nearly all adipose tissue strongly suggest a dysfunction in the digestion and absorption of nutrients. Even though PEMS-infected poult is eating some feed, the nutrient intake is not sufficient to meet body requirements for maintenance and growth, especially if not absorbed completely.

One of the viral agents implicated in PEMS is the reovirus designated as ARVCU98 (Heggen-Peay et al., 2002ab). Avian reovirus is classified as an important economic disease of poultry with a worldwide distribution. Viral arthritis and leg weakness-related problems are traditionally-associated with reovirus infection (Goodwin, et al., 1993; Robertson and Wilcox, 1986; Rosenberger and Olson, 1991), but numerous other disease conditions such as myocarditis, pericarditis (Sternier et al., 1989), hepatitis (Mandelli et al., 1978), splenitis (Hieronymus et al., 1983), bursal atrophy (Kibenge and Dhillon, 1987), enteric problems and malabsorption syndrome (Kibenge and Wilcox, 1983), respiratory disease (Fahey and Crawley, 1954), and immunosuppression (Sharma et al., 1994) have been reported in avian reovirus-infected chickens.

Normal functions of digestion and absorption have been characterized in broiler chickens up to 21 d of age (Nitsan et al., 1991ab; Nir et al., 1993; Noy and Sklan, 1995). The body weight and feed intake increased more rapidly after 10 days of age. Also, the time of food passage through the intestines decreased by approximately 33%. The duodenal secretion of amylase, trypsin, and lipase was low at 4 days of age and increased 100-, 50-, and 20- fold respectively by 21 days of age. Enzyme activity was less in the distal end of the small intestine. Fatty acid absorption in the ileum decreased after 7 days of age. Nitrogen digestion in the small intestine increased from 78% at 4 days of age to 92% at 21 days of age. However, the fatty acid and starch digestion ranged from 82% to 89% in this time

period (Noy and Sklan, 1995; Nitsan et al., 1991ab). Vitelline residue decreased from 4.6 grams at hatching to negligible values at 4 days of age (Nitsan et al., 1991a; Nir et al., 1993).

Sell et al. (1991) made observations on development of turkey poult pancreatic digestive enzymes. The specific activities of pancreatic amylase, lipase, and trypsin were low until hatch. Then specific activity of amylase increased nearly three-fold by day 6 post-hatch. The specific activity of lipase remained constant between day 1 and 8 post-hatch. The trypsin specific activity increased only slightly. The post-hatch increase in enzyme activity was associated with increased pancreas weight (Uni, 1995). The small bowel maltase specific activity was high at hatching, but decreased significantly after day 4. Decreased specific activity of maltase resulted in a reduction in the total maltase activity despite the increase in small bowel relative weight (Sell et al., 1991).

Zimber et al. (1985) reported that pancreatic acid and alkaline phosphatase activities were not affected significantly by challenge with the lymphoproliferative disease virus. Shapiro et al. (1998) reported that stunting syndrome, which has an etiology similar to PEMS, results in depression of intestinal maltase and saccharase activities in affected poult, but disaccharidases increased activities. In turkey poult given the stunting syndrome inoculation, both amylase and trypsin activities were increased at 2 and 3 weeks after the inoculation. Ali and Reynolds (1998) challenged poult with a stunting syndrome

agent and observed decreased maltase activity along with decreased D-xylose uptake. D-xylose is a pentose sugar that is absorbed from the upper small intestinal tract and is an indicator of malabsorptive conditions (Godwin et al., 1984ab). It is poorly metabolized and is readily excreted in urine. Changes in plasma D-xylose concentrations over a 3-hour period are indicative of its absorption in the gastrointestinal tract of poult (Doerfler et al., 2000).

EMBRYONIC GROWTH AND SURVIVAL RELATED TO POST-HATCH DEVELOPMENT

There is a negative correlation between selection for increased growth and livability (Nester and Noble, 1995). Furthermore, embryonic survival is negatively correlated with increased post-hatch growth rate in turkey breeders. Embryonic growth is influenced by two factors: (1) egg weight and (2) length of the incubation period (Ricklefs, 1987). When incubation periods are either shortened or lengthened, poult quality at hatching is affected because the development period is determined by the average time of incubation and not adjusted for only a few eggs. Poults that hatch early tend to be dehydrated and typically do not thrive after placement. Late hatching poults have a slower post-hatch maturation period, which results in poor performance (Christensen et al., 2000).

Depressed embryonic survival may also be associated with tissue glycogen concentrations or other products of carbohydrate metabolism (Christensen et al., 1993 and 1999ab). Due to the hypoxia from inadequate egg shell conductance, carbohydrates are essential to embryonic viability (Freeman, 1965). Christensen et al. (1999b) observed that breeder hens producing heavier poults with elevated blood glucose concentrations had lower hatchability than those whose progeny hatched with lower weights and lower glucose concentrations. Therefore, within limits of genetic diversity, embryonic growth may not be conserved in all individuals following selection for commercial traits.

Christensen et al. (1993) reported that growth selected lines of turkey embryos had lower metabolism at pipping than randombred and egg production lines of turkeys. Hatchability of growth selected embryos was improved even though oxygen consumption was reduced at external pipping and at hatch compared with randombred controls, but during incubation, oxygen consumption was elevated in growth selected embryos. During embryogenesis, hepatic and cardiac glycogen was elevated in the growth selected embryos, but plasma glucose was less than in randombred controls. At hatch, hepatic and cardiac glycogen in growth selected poults was less than that found in randombred controls. Christensen et al. (1993) concluded that there is a relationship between carbohydrate storage and utilization during pipping and embryonic survival during pipping and at hatch. Christensen et al. (2000) later reported that there was

a negative correlation between selection for rapid growth and embryonic survival. Hatchling body weight was highly correlated with blood glucose at hatch, demonstrating that the negative relationship between growth and embryonic survival was related to energy metabolism of the rapidly growing embryo (Christensen et al., 2000).

A similar study was also conducted to examine the influence of supplementation of iodide to maternal diet on growth and energy metabolism (as influenced by whole body glycogen content) of different strains of turkeys selected for increased 16 week body weight (F line) or increased 180 day egg production (E line) and compared to a randombred control line (RBC1) (Christensen et al., 1999b). Maternal iodide supplementation affected egg and embryo weights of each line differently. Increased egg and poult weight were observed in the RBC1, but there was no effect in the selected lines given iodide supplementation. These results suggested that iodide supplementation, which can influence thyroid function, affects egg weight and embryo development also.

Thus, genetic selection of turkeys for growth may affect thyroid function with impact on embryonic growth (Christensen et al., 1999b). The embryos selected for growth (F line) actually grew at a slower rate than RBC1 line embryos, but embryos selected for egg production (E line) grew faster than the RBC1. Possibly, there is a physiological mechanism for maternal and growth-related influences on embryonic survival. Genetic line and maternal iodide

supplementation can influence embryonic growth independent of egg weight (Christensen et al., 1999b) and also influence embryonic survival (Christensen, 1999a).

POST-HATCH GROWTH OF TURKEY POULTS

There are several factors which influence post-hatch growth rate of turkey poults. These factors include egg size (Wiley, 1950; Kosin et al., 1952; Godfrey et al., 1953), the timeliness of hatch (Kushner and Veilman, 1950), environmental temperature (Barrot and Pringle, 1947; Tretyakov, 1950; Clark and Das, 1974; Huston, 1965), nutrition (Daghir and Balloum, 1962; Daghir et al., 1966), and genetic strain (Hoffman et al., 1953). Phelps et al. (1987a) examined the development of different organ systems of the poult. At the time of hatching, the pancreas relative weight was greater than that reported for hatching chicks (Latimer, 1925) and increased parallel to body weight gain. Female pancreas weights reached maximum sooner than the males (Phelps et al., 1987a).

Poult liver relative weights reported by Phelps et al. (1987a) were greater at hatch than those reported in chicks (Latimer, 1925; Al-Dabagh and Abdulla, 1963). A transitory decrease in liver relative weight was observed at day 3 post-hatch and between days 6 and 8 and was more evident in females than in males. A peak liver relative weight was found at 4 days post-hatch in females but it was not until 6 days post-hatch that peak liver relative weight was observed in males.

After the peak relative weights were found, liver relative weight began to decline through 21 days post-hatch (Phelps et al., 1987a).

At the time of hatch, poult heart relative weight has been reported to be greater than the chicks' heart relative weight (Matsuzana, 1981; Dagher and Pellet, 1967). Phelps et al. (1987a) reported a significant variation in the increasing heart relative weights of the male poult on post-hatch days 3 and 9.

The spleen weights for newly hatched poult by Phelps et al. (1987a) were less than those reported in chicks (Latimer, 1925; Al-Dabagh and Abdulla, 1963). Since the spleen is a late maturing organ (Hafez, 1955), no peak was reported by Phelps et al. (1987a).

The bursa of Fabricius matures reaching maximum relative weight around 5 to 6 weeks of age in chickens (Glick, 1956) and then regresses. The turkey poult bursa relative weights were still increasing at the end of the 10 day trials reported by Phelps et al. (1987a).

Phelps et al. (1987a) concluded that in the turkey poult the pancreas is the fastest growing organ during the first 10 days post-hatch, followed by the spleen and the bursa of Fabricius. The liver grew somewhat faster than the total body early post-hatch, but the heart grew at the slowest rate. The early post-hatch development favors the development of organs over whole body gain, but this trend reverses later (Phelps et al., 1987a).

Phelps et al. (1987b) hypothesized that early poult mortality (EPM) may occur from the refusal to eat or drink during the first week post-hatch. The refusal to eat properly had profound influences on the hematological developed of the turkey poult during the first ten days post-hatch. There were six components to the post-hatch turkey poult hematological profile. The first was the total red blood cells. The values reported by Phelps et al. (1987b) day of hatch agree closely with those reported for hatchling poult (Christensen et al., 1982). Total red blood cell numbers (TRBC) increased significantly for developing turkey poult of both sexes through 10 days post-hatch.

Packed cell volume (PCV), which estimates the TRBC numbers as well as cell size, was the second component analyzed by Phelps et al. (1987b). The PCV decreased immediately after hatch, and then increased to sub-adult levels. A surge in PCV values was seen from day 3 to 4, but there were no increases in total RBCs during this period, suggesting that cell size/volume had increased.

The mean corpuscular volume can be used to support the hypothesis of Phelps et al. (1987b) that the increase in PCV was due to increased numbers of erythrocytes concomitant to reduced cell size. The MCV at day 1 post-hatch reported by Phelps et al. (1987b) were slightly higher than those values reported for hatchling poult (Christensen, 1982). However, the high post-hatch MCV decreased over the next several days (Phelps et al. (1987b).

Hemoglobin (Hgb) levels were also measured by Phelps et al. (1987b). It

has been reported that poult blood Hgb levels were low and then gradually increased with age (Wolterink et al., 1947). In the study by Phelps et al. (1987b), Hgb values were initially high at hatch and then decreased significantly 2 to 3 days following hatch. This decrease was due to a combination of decreased TRBC numbers and possibly replacement of a molecular species of embryonic Hgb with an adult species (Hall, 1934).

Mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentrations (MCHC) have been reported to be decreased after hatching (Phelps et al., 1987b). These observations suggest that there might have been an increase in the tissue demand for oxygen necessary for growth and development. This may have been a required physiological signal for the hematopoietic system to increase the number of erythrocytes to provide more Hgb for oxygen transport.

In general, total leukocytes (TWBC) increase with age in turkey poults (Venkataratnam and Clarkson, 1962; Phelps et al., 1987b). However, the number of leukocytes varies greatly from day to day (Phelps et al., 1987a). Male poults develop a physiological anemia more quickly than female poults and recover in a more slowly. Phelps et al. (1987c) have shown that nutrient solutions and antibiotic injections could counteract the latency in production of erythrocytes and leukocytes.

When birds hatch, they are in a lipemic condition and in a state of ketosis (Entenman et al., 1940), but the ketosis state is quickly eliminated when the hatchlings consume carbohydrates (Best, 1966). This transition is associated with altered levels of many blood borne metabolites (Best, 1966), and in turkey poults these changes occur in association with yolk depletion, increasing feed consumption, and elevation of glucose concentrations (Phelps et al., 1987c). Significant changes in blood glucose, including an increase from hatch to day 5 to 6, then a decrease to day 8, followed by yet another increase through day 10 might be a reflection of feed refusal. However, it appeared that there was a sexual dimorphism associated with the biphasic regulation of blood glucose with males being less capable than females to regulate blood glucose. The biphasic glucose levels after hatch appear to result of rapid utilization of yolk material and initiation of feeding. The decline in blood glucose between days 5 and 8 post-hatch coincides with yolk depletion, and this increase in blood glucose was coincident with heavy feeding of the poults through day 10 post-hatch (Phelps et al., 1987b). Phelps et al. (1987b) also observed a transitory decrease in blood osmolarity. The data presented by Phelps et al. (1987b) suggest that development of a transitory anemia, leukopenia, and malabsorption of nutrients limit the physiological responsiveness of the poult at hatch.

It has been hypothesized that pre-feeding poults would decrease mortality and promote growth (Kienholz and Ackerman, 1970; Moreng et al., 1970; Enos et

al., 1971); Waldroup et al., 1974; Twinning et al., 1978). Phelps et al. (1987c) determined that antibiotic and nutrient solution pre-feeding altered physiological parameters associated with EPM but did not affect mortality. However, pre-feeding did cause a significantly increased feed consumption, body weights, TRBC, MCV, PCV, and increased locomotor behavior when compared to controls.

Phelps et al. (1987c) speculated that the pre-feeding regime had stimulated the gastrointestinal tract and enhanced feeding behavior. A nutritionally stimulated, vigorous poult would have a more active hemopoietic system and would permit the poult to be more capable to cope with or prevent anemia, have improved growth, and overall have improved welfare.

POULT ENTERITIS

Poult enteritis is a term that describes infectious intestinal disease that affects young turkeys. Some of these diseases, such as coronaviral enteritis and stunting syndrome, have been well characterized. Others, such as transmissible viral enteritis, poult growth depression and poult enteritis and mortality syndrome (PEMS), are ill defined. All forms of poult enteritis are multifactoral, transmissible, and infectious. Stunting and poor feed utilization are usually a result of enteritis. In the more severe forms, runting, immune dysfunction, mortality, and morbidity have been characterized. The gross lesions associated

with poult enteritis are diverse and tend to be nonspecific (Barnes et al., 2000). Edens (1994) has made observations for more than 25 years characterizing some of the major events associated with EPM and now has collected data associated with PEMS infections for comparison (Edens and Doerfler, 1998).

PATHOLOGY OF PEMS

The pathology of PEMS includes alterations of the intestinal mucosa caused by one or more viruses infecting enterocytes, inflammation, and proliferation of secondary agents, usually bacteria. Diarrhea associated with enteritis/PEMS might be and be related to maldigestion and malabsorption, but the diarrhea might also be secretory. The transmission of enteritis/PEMS is mechanical and is facilitated via the fecal-oral route. To prevent enteritis/PEMS, poultry managers have focused on eliminating infectious agents on farms that are at risk and prevention of its passage to future flocks using effective cleaning and disinfection. To facilitate an effective biosecurity program, managers practice all-in/all-out production using separate brooding and finishing units. To date, there are no vaccines available to aid in the prevention and/or control of enteritis (Barnes et al., 2000).

Epithelial cells in the gastrointestinal tract appear to be a target of the reovirus and astrovirus agents (Yu et al., 2000a; Qureshi et al., 2000a and 2001) that cause PEMS. Perry et al (1991a) examined the histopathology of poult

enteritis that was defined as malabsorption of nutrients. Day old poult were given natural exposure to enteritis-causing agents by placing the birds on litter on which poult had previously developed diarrhea, increased mortality, and stunting. The small intestine, pancreas, and liver were examined histologically. D-xylose and lipid absorption tests were used to evaluate malabsorption (Eberts et al., 1979). D-xylose is a pentose sugar that is absorbed from the upper small intestinal tract and is an indicator of malabsorptive conditions (Godwin et al., 1984ab). It is poorly metabolized and is readily excreted in urine. Changes in plasma D-xylose concentrations over a 3-hour period are indicative of its absorption in the gastrointestinal tract of poult (Doerfler et al., 2000). When compared to control poult, the gastrointestinal tract of PEMS-afflicted poult were distended grossly, fluid-filled, and had thin, flaccid walls at days 5 and 8 post-infection. The ceca were distended with brown watery fluid and gas on days 5, 8, and 12. Villous atrophy and crypt hypertrophy were evident in the small intestine on days 5, 8, 12, 16, and 21. Villous length was decreased significantly and the crypt depth was increased. D-Xylose absorption and lipid absorption were decreased significantly on days 8 and 11 (Doerfler et al., 2000). The intestinal epithelial damage caused by infectious agents and subsequent villous atrophy were credited with the development of malabsorptive diarrhea (Perry, 1991a).

Similar results were demonstrated by Doerfler et al. (2000) who used an experimentally induced PEMS model. D-Xylose absorption peaked 30 to 60

minutes after oral treatment in the healthy, non-infected poult. However, PEMS-infected poult did not show a peak in absorption and had delayed D-Xylose absorption at 4, 7, and 11 days after the PEMS challenge. The microvilli and the mitochondria within the enterocytes were severely damaged in PEMS-infected poult (Edens et al., 1997b; Doerfler et al., 2000).

Poult hatch with a relatively immature intestinal tract (Moran, 1985; Sell et al., 1991; Uni et al., 1998). During the early post-hatch period, poult have a limited absorptive capacity for carbohydrates, proteins, and lipids (Phelps et al., 1987a). Edens and Doerfler (1997ab) have suggested that in PEMS-infected poult, the malabsorption of nutrients from the gastrointestinal tract and the inability to utilize nutrients was related to marked mitochondrial hypertrophy and degeneration in enterocytes and hepatocytes. The lack of Na^+ -dependent active transport of nutrients such as glucose and amino acids would result in many signs of PEMS including diarrhea, wasting of musculature, lack of growth, stunting, and high mortality.

The absorption of glucose, xylose, and some amino acids across brush borders of enterocytes is dependent upon the interaction between the monosaccharides and Na^+ - K^+ -ATPase-dependent co-transporters (Alvarado, 1966; Alvarado and Monreal, 1967; Stevens et al., 1984). If a nonmetabolizable sugar such as D-xylose is the monosaccharide, the transport of D-xylose and sodium can be greatly depressed if the cell is not provided energy in the form of

glucose. It has been reported that PEMS infection impairs glucose metabolism, and affected birds tend to be hypoglycemic (Edens and Doerfler, 1997b; Doerfler et al., 1998). Therefore, a decrease in absorption of D-xylose would be expected and could be related to decreased co-transport of sodium into the enterocytes of the intestinal tract. It also could be related to reduced movement of fluids through the cell (Doerfler et al., 2000).

Perry et al. (1991b) determined that enteritis also affected the integrity of the skeletal system of poult by altering plasma calcium, phosphorus, and 25-hydroxyvitamin D₃ concentrations over a 3 week period. Body weights and shank lengths were decreased significantly as the result of enteritis. Plasma 25-hydroxyvitamin D₃, which plays a significant role in calcium metabolism, was decreased significantly causing a decrease in plasma calcium concentration and a concomitant increase in plasma phosphorus on day 8, but plasma phosphorus concentrations were decreased significantly on days 15, 18, and 22 which might be a reflection of malabsorption of nutrients. The growth plate in the long bone epiphysial region in the birds was thinned on days 8 and 11, but they were expanded on days 15, 18, and 22. Bone mineralization was also decreased in poult experiencing enteritis. Perry et al. (1991b) concluded that the skeletal lesions associated with poult malabsorption syndrome evolved from an early osteoporosis lesion associated with hypocalcemia with depleted vitamin D and hypophosphatemia (Perry, 1991a).

During the early years of the PEMS investigations, there was no definitive etiology (Brown, 1995; Barnes and Guy, 1995; Barnes et al., 1996; Barnes, 1997; Edens et al., 1997ab; Qureshi et al., 1997). Numerous potential viruses were investigated, including adenovirus, coronavirus, enterovirus, astrovirus, birnavirus (Serotype 2), rotavirus (Type D), reovirus, bursa epithelial virus, and others. Alone, those viruses did not induce fulminating cases of PEMS in either laboratory or field environments (Brown, 1995; Barnes and Guy, 1995; Barnes et al., 1996). However, Brown (1995) reported that a coronavirus-like particle and Serotype 2 birnavirus could both reduce growth in 3 weeks old poult, and dual challenge with these agents significantly depressed growth and feed conversion and increased mortality to 60%.

Cryptosporidiosis was reported to complicate the PEMS problem, but it did not cause PEMS (Brown, 1995; Barnes and Guy, 1995; Barnes et al., 1996). Barnes et al. (1996) suggested that poult with an unidentified virus infection were more susceptible to opportunistic enteric bacteria (*Salmonella*, *Escherichia coli*, or *Clostridium*) that further complicated the PEMS problem. Two atypical *Escherichia coli* colony types, identified by colony morphology, were isolated consistently from PEMS-infected poult (Edens et al., 1997ab). Type 1 was smooth, raised, mucoid, and slow-growing. Type 2 was rough, flat, Congo red-positive, and fast-growing. These colonies were designated as atypical forms based upon their BBL biochemical profiles (Edens et al., 1997ab).

Edens et al. (1997ab and 1998) suggest that specific virulent bacterial organisms are involved in PEMS and reported that the presence of the atypical *E. coli* colony types 1 and 2 in the moribund PEMS-afflicted turkey poult caused severe diarrhea, depressed body weight gain, bursa cores, and high rates of mortality in both infected and infected/cyclophosphomide-immunodepressed poult similar to PEMS.

Among the signs of PEMS is inhibited or reduced growth accompanied by wasting of the muscle mass (Brown 1995; Barnes and Guy, 1995; Barnes et al., 1996). Bacterial infections can result in whole body nitrogen loss proportional the duration and severity of the disease (Beisel, 1984). Edens et al. (1997ab) noted that the atypical *E. coli* strains did not always cause wasting of muscle tissue, but in many of the survivor poult, there was very little muscle mass remaining at 21 days of age. This was similar to the condition in field cases of PEMS.

Virulent *E. coli* strains can cause diarrhea, wasting, and mortality (Leitner and Heller, 1992). Leitner and Heller (1992) noted that stressors, such as inanition after virulent *E. coli* infection, exacerbated the disease and subsequent mortality. This suggested that the atypical *E. coli* strains, which have binding and penetrating ability for the avian epithelial cells, have the opportunity to translocate from the intestine to the viscera causing septicemia during the time when PEMS-afflicted poult exhibit reduced feed intake but increased

consumption of litter, which can be heavy laden with atypical *E. coli* colony Types 1 and 2 (Edens et al., 1997ab)

The disruption of the cellular integrity of the intestinal epithelium in response to infections by atypical *E. coli* colony Types 1 and 2 suggested that there might be a malabsorption problem associated with PEMS. The breakdown of the epithelial cell tight junction complex integrity would also aid in the translocation of the atypical *E. coli* (Edens et al., 1997ab)

The two *E. coli* colony types were characterized through colony morphology, biochemical, cultural, and structural characteristics, antibiotic resistance and serotyping. Edens et al. (1997ab) found that the two colony types did not appear to be greatly different when compared to the general biochemical profile of *E. coli* genus (bioMerieux Vitek, 1993). Colony type 1 has ornithine decarboxylase and ferments ducitol, L-rhamnose, sucrose, and melibiose, but colony type 2 does not have these properties. However, it is negative for sucrose and melibiose fermentation. Colony type 1 is a potent colicin producer whereas type 2 is not. Colony type 1 is nonserotypable and non-motile, whereas colony type 2 is serotyped as O136 and is motile. Both colony types are resistant to gentamicin, ceftiofur, tetracycline, neomycin, streptomycin, lincomycin, aparmycin, erythromycin, nalidixic acid, furazolidine, and sulfisoxazole. Both strains rapidly developed resistance to both sarafloxacin and enrofloxacin after an initial exposure (Edens et al., 1997ab).

VIRUSES AND PEMS

Many viruses have been associated with development of PEMS. Using immunoelectromicroscopy and double-stranded RNA virus genome electropherotyping, intestinal samples from PEMS-infected birds were examined for viruses. Four viruses were isolated: turkey coronavirus (TCV), avian rotavirus, a small reovirus, and an undefined small round virus (SRV) later identified as an astrovirus. Challenge with SRV, TCV, or both resulted in mortality and clinical responses similar to those associated with PEMS (Yu et al., 2000a). In PEMS-infected poultts there is significant atrophy of the bursa (75%), thymus (99%), and spleen (75%). Atrophy of the lymphoid tissues is highly correlated with lower anti-SRBC antibody titers in PEMS-infected poultts (Qureshi, 1997).

SMALL ROUND VIRUS (ASTROVIRUS)

The SRV was detected by electron microscopy and was observed to be 30-32nm in diameter without distant surface features. Enteroviruses, astroviruses, caliciviruses, and other unidentified viruses are among the many SRVs associated with diarrheal diseases (Caul, 1996). These viruses were originally differentiated on their surface features by electron microscopy, but this method of identification may not always be accurate. Additionally, it is very difficult to differentiate among the SRVs on the basis of their physicochemical properties. The viruses

have similar buoyancy densities in cesium chloride, and they are nonenveloped, stable at pH 3.0, and are relatively heat resistant. Yu et al. (2000a) set out to characterize the PEMS SRV by analysis of its physicochemical properties, its capsid protein profiles, and its genomic size and sequence information.

The SRVs in the experiment were titrated in specific-pathogen-freed (SPF) turkey embryos (Reed and Muench, 1938). Yu et al. (2000ab) found the PEMS SRV to be resistant to chloroform treatments, stable at pH 3, and partially resistant to heat. The buoyant density in CsCl was estimated to be between 1.34 and 1.36 g/cm³.

It is often difficult to grow enteric virus in cell culture. The Caco-2 cells, BGM cells, and addition of trypsin have been used in the attempt to cultivate viruses. Yu et al. (2000a) were unable to cultivate SRV in any media, and this indicated that the PEMS SRV is a fastidious virus. Using SDS-PAGE analysis, the PEMS SRV was found to have three capsid proteins with molecular weights similar to those of the three capsid proteins of the astrovirus (Belliot et al., 1997; Willcocks et al., 1994) and the three larger capsid proteins of enteroviruses (Johnston and Martin, 1971; Kuan, 1997; Rueckert, 1990). It was concluded that the molecular weights of the capsid proteins were more like the astrovirus than the enterovirus and that the SRV was not a DNA virus based on genomic analysis.

A parvovirus also was suspected, but parvovirus is much smaller in size and the capsid profile is different from the SRV (Young, 1996).

Electropherotyping revealed that the SRV was a single-stranded RNA virus. No similarities in the genomic sequences between SRV and any enterovirus were found. With these characterizations, Yu et al., 2000ab) concluded that the SRV is a member of the astrovirus family.

The interaction between a PEMS-turkey astrovirus (Tast-OSU; Yu et al., 2000ab) and macrophage viability, bacterial uptake, killing and clearance, and the production of cytokines and metabolites were examined by Qureshi et al. (2001). The poult challenged with Tast-OSU recruited almost 50% fewer Sephadex-elicited inflammatory cells when compared to the control. Birds given an oral challenge with Tast-OSU had reduced macrophage viability relative to controls and decreased phagocytosis and intracytoplasmic killing of *E. coli* after a 42-48 hour exposure. The challenged poult had a greater number of viable *E. coli* in their spleens after an intravenous *E. coli* challenge as compared to the control poult. Tast-OSU challenge resulted in a reduction of both interleukin (IL)-1 and IL-6 activity, but the nitrite level in culture supernatant fraction from Tast-OSU-challenged macrophages was elevated significantly. Tast-OSU has been shown to reduce weight gain and induce PEMS-like morbidity and mortality in turkey poult (Qureshi 2000a). An astrovirus challenge to poult can result in significant immune alterations, such as atrophy of lymphoid organs, reduced lymphoproliferative response against mitogens, and altered CD4/CD8 lymphocyte subpopulations (Qureshi et al., 2000b; Schultz-Cherry, 2000). Qureshi et al.

(2001) observed that Tast-OSU exposure was low to moderately cytotoxic to macrophages. Tast-OSU proteins could not be detected in macrophages co-incubated with Tast-OSU. Although no evidence of Tast-OSU replication was noted, serious macrophage functional defects were observed. Phagocytic and bactericidal functions were reduced significantly after Tast-OSU exposure. This was accompanied by reduced IL-1 and IL-6 production by macrophages. IL-1 and IL-6 play a key role in the induction of inflammatory responses.

Thymus atrophy and reduced responsiveness of T lymphocytes to mitogens is a consistent immunologic defect seen in PEMS (Qureshi et al. 1997 and 2000b; Schultz-Cherry, 2000). Reduced cytokine production by activated macrophages appeared to be coupled with reduced responsiveness to inflammatory signals. This is evident from the Sephadex-elicited AEC (accessory effector cells) numbers, which were decreased by almost 50% in Tast-OSU challenged poult. These researchers concluded that Tast-OSU binding at the macrophage cell surface might trigger intracytoplasmic events leading to macrophage defects such as reduced phagocytosis and cytokine production partially explaining the increased incidence of secondary opportunistic pathogens, including *E. coli* (Edens et al., 1997ab). The secondary agents are considered a crucial part of the PEMS multifactoral etiology that is responsible for stimulating IL-1 and IL-6 production by macrophages. Qureshi et al. (2001) found that Tast-OSU exposure resulted in prolonged survival of inoculated *E. coli* in the spleen.

Through these observations, it has been demonstrated that the turkey astrovirus had the potential to compromise the functional characteristics of the mononuclear phagocytic system. Macrophages play a key role in both nonspecific and adaptive immune responses (Qureshi et al., 2000b). Therefore, any compromise or defect in macrophage functions has the potential to result in the generalized immune dysfunctions (Qureshi et al., 2001).

IMMUNE RESPONSE

The purported immunodysfunction in PEMS (Tast-OSU)-infected poult was supported by research published by Heggen et al. (2000). She quantified IL-1, IL-6, and tumor necrosis factor-alpha (TNF- α) bioactivities and nitrate levels in abdominal macrophage cultures in 6 trials. In one trial, PEMS-infected poult had a greater IL-6-mediated index of stimulation index compared to uninfected control poult. In three trials, the IL-1 activity was significantly higher in PEMS-infected poult than in controls. TNF- α production decreased in PEMS-infected poult. The nitrate levels in PEMS-infected poult were significantly higher in two out of three trials. These results suggest that enhanced production of pro-inflammatory cytokines/metabolites by activated macrophages in PEMS-infected poult might be responsible for intestinal inflammation, and gut motility that characterize PEMS (Heggen et al., 2000).

In vivo and *in vitro* investigations have defined mononuclear phagocytic system functions and expression of lymphocyte subset cell surface markers in the thymus and bursa of Fabricius, and peripheral blood lymphocyte subset dynamics during PEMS infection (Heggen et al., 1998). Control poulters cleared blood-borne *E. coli* from their circulation within 60 minutes, but in PEMS-infected poulters, viable *E. coli* cells were still present in the circulation at 60 minutes after inoculation. The inflammatory response was assessed by Sephadex-elicited abdominal exudate cell recruitment, which was reduced in PEMS-infected poulters, but the adherence potential of the abdominal exudate cells was not significantly different between the control and the PEMS-infected poulters. The ability of the macrophages from PEMS-infected poulters to phagocytize sheep red blood cells and the average number of sheep red blood cells per phagocytic macrophage were lower when compared with the control poulters (Heggen et al., 1998).

When *E. coli* was injected intravenously into uninfected and coronavirus-positive PEMS-infected poulters, the bacteria remained in the bloodstream of coronavirus-positive PEMS-infected poulters longer than in the uninfected poulters. This is important in the field when PEMS-infected poulters develop a lower threshold of tolerance to bacterial challenge. Both control and coronavirus-positive PEMS poulters elicited a similar number of inflammatory cells in the abdominal cavity in response to Sephadex injection, but the overall phagocytic function of the macrophages was less than in coronavirus-positive PEMS-infected

poults. Heggen et al. (1998) concluded that coronavirus-positive PEMS-infected poults do not suffer from a numerical reduction in mononuclear phagocytic cells, but they have a reduced functional capacity to perform bacterial/antigen uptake and processing.

It has been noted that depopulation of PEMS-infected flocks followed by clean-out and thorough disinfection of the contaminated houses failed to prevent infection of astrovirus in subsequent flocks. The unique astrovirus from the thymus and intestines of PEMS-infected poults has been examined to determine whether it could be heat inactivated or killed with disinfectants. The PEMS-associated astrovirus was resistant to inactivation by heat, acidification, detergent treatment, and treatment with phenolic, quaternary ammonium, or benzalkonium chloride-based products (Shultz-Cherry, 2001). Formaldehyde, beta-propiolactone, or the peroxymonosulfate-based product Virkon S were the only treatments that could inactivate PEMS-associated astrovirus. Therefore, Shultz-Cherry (2001) concluded that it was very difficult to decontaminate a poultry barn that harbors astrovirus.

Poults challenged with Tast-OSU and/or turkey coronavirus (TCV) show signs of altered immune responsiveness. Peripheral blood lymphocytes from Tast-OSU-inoculated poults and from healthy poults were examined for lymphoproliferative potential against concanavalin A (Con A) using flow cytometry. Tast-OSU challenge induced diarrhea, growth suppression, and

atrophy of thymus and bursa of Fabricius resembling those from PEMS-infected poult. Tast-OSU was detected in intestinal tissues 2 and 4 days post-inoculation (DPI). The lymphoid tissues such as the thymus, bursa, and spleen were positive for Tast-OSU at 4 and 8 DPI. The responsiveness of peripheral blood lymphocytes (PBL) to Con A was reduced significantly in the virus-challenged groups as compared to uninfected groups at 2 DPI. However, the suppressed lymphoproliferation was no longer evident at 7 DPI. When Tast-OSU was incubated with normal thymocytes and splenocytes, there was a significantly reduced lymphoproliferative response to Con A. Flow cytometry (FACS) analysis of PBL from Tast-OSU-infected poult at 2 DPI showed a decrease in the numbers of CD4-CD8⁺ lymphocytes. At 2 and 4 DPI, the Tast-OSU-challenged poult had a higher percentage of CD4⁺CD8⁻ lymphocytes than controls, but at 8 DPI, the Tast-OSU-challenged poult had greater CD4-CD8⁺ lymphocyte numbers (Qureshi et al., 2000a). The Tast-OSU infection may compromise the lymphocyte-mediated immune defenses through reduction of lymphoproliferation and CD4-CD8⁺, CD4⁺CD8⁻, and CD4⁺CD8⁺ lymphocyte numbers during the acute stage of SRV infection (Qureshi et al., 2000).

TURKEY CORONAVIRUS (TCV)

The involvement of TCV was often associated with PEMS, but a direct association between TCV and PEMS was never demonstrated. Nevertheless, it was assumed that areas having a high prevalence of TCV infection also experienced an increased incidence of PEMS. A survey of 54 commercial flocks in areas with and without a history of TCV infection were monitored for mortality and antibodies for TCV using indirect fluorescent antibody assay (Carver et al., 2001). Using the clinical definition of PEMS, mortality greater than 2% during any 3-week period from 2 weeks of age through the end of brooding due to unknown causes was used. From the study, four main health groups were determined: (1) healthy, (2) PEMS positive, (3) TCV positive, and (4) PEMS positive/TCV positive. There were 24 healthy flocks, 23 PEMS flocks, and 7 TCV positive, PEMS negative flocks. There were 10 flocks that were experienced PEMS positive, TCV positive, 13 flocks were PEMS positive, TCV negative. TCV was associated with PEMS in 43% of the field cases, but 57% of the PEMS field cases were TCV-negative. Furthermore, 41% of the TCV cases did not experience excess mortality (PEMS). TCV can be associated with PEMS but not necessary or sufficient to cause PEMS (Carver et al., 2001).

ENTEROPATHOGENIC *E. COLI* AND TCV

Guy et al. (2000) studied the interaction of *E. coli* and TCV as related to development of signs similar to PEMS infection. Six days old poults were inoculated with TCV and the enteropathogenic *E. coli* (EPEC; R98/5), isolated from PEMS-infected poults. No clinical development of disease was seen in turkeys inoculated with only R98/5. Only mild disease and moderate growth depression were observed when inoculated with only TCV. Poults inoculated with both TCV and R98/5 developed severe enteritis with high mortality (79%) and marked growth depression. The R98/5 EPEC expressed an attaching/effacing (AE) gene and caused intestinal lesions, which were characteristic of most EPEC infections. Such lesions include the adherence of bacterial micro-colonies to the intestinal epithelium with degeneration and necrosis of epithelium at sites of bacterial attachment. AE lesions were more extensive and were detected for a prolonged period in poults inoculated with TCV and R98/5 than those inoculated with only R98/5. When poults were inoculated with both TCV and R98/5, increased mortality, growth depression, and exacerbated AE lesion development indicated a synergistic effect. This suggested that TCV promotes intestinal colonization by R98/5 but, R98/5 does not alter TCV infection (Guy et al., 2000).

ENVIRONMENT

Other factors such as the environment also come into play with PEMS. Edens et al. (1998) observed that litter moisture and brooding temperatures affected the development of PEMS as indicated by body weights, relative weights of lymph organs, and mortality. The moisture levels tested were at 40% (high moisture) and 20% (low moisture). The brooding temperatures tested were 38C (high) and 34C (normal). It was determined that there was a significant interaction between litter moisture and brooding temperatures, and this significantly influenced body weight. The brooding temperature had little effect on body weight, but body weight was affected significantly by litter moisture. However, body weight of poult brooded at a higher temperature and lower humidity had significantly greater body weight than those brooded at normal brooding temperatures and higher humidity. It was concluded that litter moisture influences productivity and mortality associated with PEMS, but brooding temperature had the greatest influence on PEMS associated mortality. Therefore, poult at risk for PEMS exposure should be brooded in an environment that has higher than traditional brooding temperatures (Edens et al., 1998).

PEMS AFFECTS ON POULT PHYSIOLOGY AND BIOCHEMISTRY

Doerfler et al. (1998) exposed control poult to PEMS-infected poult for 16 hours and test poult began to show signs of PEMS and huddle. When poult

were separated from the groups, their body temperature was depressed significantly. Body temperatures of PEMS-infected poult decreased progressively for 8 days after exposure and returned to normal level at 18 days after exposure. Similar patterns were seen in serum glucose, inorganic phosphorus, triiodothyronine, and thyroxine levels. The mortality for the PEMS poult began at day 6 after exposure and peaked at day 9 after exposure. The mortality then decreased. The decreases seen in the serum glucose, inorganic phosphorus, triiodothyronine, thyroxine, body temperature, and mortality did not coincide with decreased feed intake associated with PEMS. Doerfler (1998) concluded that the agents causing PEMS might have a direct effect on energy metabolism.

THESIS OBJECTIVES

It appears that a definitive etiology of PEMS remains equivocal, but it is clear that whatever causes the disease also alters the biochemistry and physiology of the poult. Therefore the objectives of this thesis were to examine the effects of prefeeding a hydrated nutrient compound (OASISTM, Novus, St. Louis, MO) on (1) resistance to naturally occurring Salmonella infection and monitor three week performance of control and PEMS-infected poult, (2) pancreatic and mucosal digestive enzymes in control and PEMS-infected poult, and (3) post-hatch organ

weight. The first objective was important because Salmonella has been implicated in PEMS infections.

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CHAPTER 2

OASIS™ PRE-FEEDING IMPROVES EARLY PERFORMANCE IN TURKEYS

ABSTRACT The failure of newly hatched turkey poults to thrive has been attributed to delayed placement accompanied by dehydration and inability to find feed and water after placement. Poults, suffering from failure to thrive, appear to be more susceptible to diseases, especially enteric diseases. Use of a hydrated nutritional supplement, OASIS™ (OAS; Novus International, Inc.) for hatchlings can overcome some of the problems associated with failure to thrive. The influence of OAS on 7d performance of poults was investigated in three trials in which 480 (Trials 1 and 2) and 520 (Trial 3) Hybrid tom poults were weighed and placed in groups of 10 into each of the four quadrants of shipping boxes. OAS (50g) was placed in 2 quadrants in each box. The boxed poults were then placed into isolation rooms where the ambient temperature was held at 35°C. After 24h, the poults in each quadrant of the boxes were re-weighed and placed onto pinewood shavings-covered floors in the isolation rooms and were fed a common starter diet. At the time of placement, random samples of OAS-fed and Control poults were killed for histological examination of the GI tract. At 7d of age, 10% of the Control and 10% of the OAS-fed poults were contact-exposed to poults known to be carriers of the agent(s) that cause poult enteritis and mortality

syndrome (PEMS). BW and FCR were determined at 7d intervals through 21 d of age. Mortality was determined daily. During the 24h preplacement period, Control poult lost 6.21% of their hatch weight as compared to a 4.18% loss in OAS-fed poult, giving OAS-fed poult about 1 g BW advantage ($p \leq 0.05$) at placement. BW at 1 week after placement showed no differences (139 g for Control and 140 g for OAS-fed). The one week mortality rates for Control were 8.33% vs. 5.11 % ($p \leq 0.05$) for OAS-fed (data included mortality due to *Salmonella arizona* infection in Trial 2). The 8d-21d mortality rates including PEMS exposure were as follows: Control- 1.22%, OAS-fed- 1.06%, PEMS- 49.10%, and PEMS + OAS-fed- 34.17%. FCR was as follows: Control- 1.98, OAS-fed- 1.92, PEMS- 18.32, and PEMS + OAS-fed- 5.35. OAS feeding stimulated villus growth and development in the GI tract. The data suggested that OAS pre-feeding had beneficial effects in turkey poult.

Key Words: OASIS™, turkey, PEMS, performance, intestine, development

INTRODUCTION

Poor turkey poult quality is viewed by growers with increasing concern and has been linked to poor hatching quality (Meir and Ar, 1987; Phelps et al., 1987ab; Christensen et al., 1999 and 2001; Huff et al., 2001), early poult mortality (Phelps et al., 1987ab; Edens, 1994; Christensen et al., 1999), lack of uniformity in the poults (Meir and Ar, 1987; Breeding et al., 1994; Edens, 1994; Christensen et al., 1999), and low resistance to bacterial and viral pathogens (Edens, 1994; Dibner et al., 1998a; Carver et al., 2002). In addition to the concerns about poult quality, there is the specter of the potential for development of poult enteritis and mortality syndrome in some of the major turkey growing regions, especially in the southeastern United States (Barnes et al., 1996).

Poult enteritis and mortality syndrome (PEMS) has received much attention since 1995 when it was discovered as a multietiology-induced disease of poults (Barnes et al., 1996). One of the most prominent characteristics of PEMS infection is growth depression (Edens et al., 1998) and reduced ability to digest and assimilate nutrients from the intestinal tract (Doerfler et al., 1998 and 2000ab; Edens and Doerfler, 1997ab and 1998). The GI tract in PEMS-afflicted poults appears to be targeted by at least one and possibly more of the viral and bacterial etiologic agents known to be associated with the disease (Edens et al., 1997ab; Guy et al., 2000; Heggen-Peay et al., 2002ab; Qureshi et al., 2000ab and 2001).

Thus, the intestinal tract can become morphologically compromised by invading bacteria and viruses alike, preventing normal nutrient digestion and uptake.

Even though commercial hatcheries make every attempt to deliver poults as rapidly as possible, occasionally delayed placement can not be avoided. In these cases, dehydration is a serious problem especially in warm seasons (Moran, 1990; Edens, 1994; Carver et al., 2000 and 2002). Poults hatch with an immature gastrointestinal tract, which requires 2 to 19 d post-hatch to develop full functional activities (Noy et al., 2001; Sklan and Noy, 2003). Furthermore, delayed placement of poults has been shown to lead to delayed feeding, which is associated with delayed intestinal and immune system development (Dibner et al., 1998ab).

Total dependence of the poult on residual yolk for all of its metabolic needs during the first days after hatching carries costs that are very expensive (Phelps et al., 1987ab; Edens, 1994; Dibner et al., 1998ab). Much of the protein derived from the residual yolk is maternal antibody used for resistance to various pathogens (Dibner et al., 1998ab). Lipid normally used for membrane growth will be lost (Phelps et al., 1987ab). Therefore, delayed feeding of poults can have serious consequences such as significant reductions in 7d and 14d BW and increased early mortality (Pinchasov and Noy, 1993; Edens, 1994; Noy and Sklan, 1999; Carver et al., 2000 and 2002).

OASIS™ (OAS) is a hydrated feed supplement that was developed by NOVUS International, Inc. for use in the poultry industry. It originally contained 7% crude protein, 0.5% crude fat, 1.5% fiber, and 60% moisture (Dibner et al., 1996), but the current version of OAS contains a minimum of 25% moisture, 20% protein, 0.5% fat, and a maximum of 3% fiber (Dibner and Knight, 1999; Dibner et al., 1998b). The remaining ingredients included grain products, soybean meal, cornstarch, egg products, propionic acid, citric acid, phosphoric acid and water (Dibner et al., 1996). Originally used at a rate of 454g to 570g/100 birds in shipping boxes and currently used at a rate of 2.0-2.5g/bird/day or roughly 230g/100 birds/day, it provides nutrients and moisture for hatchlings before placement and stimulates development of the gut and other systems (Dibner et al., 1996; Dibner and Knight, 1999; Dibner et al., 1998b). Furthermore, the low pH of OAS may assist in reducing risk of exposure to potential bacterial pathogens.

The objectives of this investigation were to (1) determine the influence of preplacement OAS supplementation on growth, gut development and livability of turkey poults through the first 7 d after placement and (2) determine the influence of preplacement OAS supplementation on the ability of turkey poults to resist development of PEMS after an experimental challenge.

METHODS AND MATERIALS

Animal Welfare. This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

Animals and Husbandry. In Trials 1 and 2 (480 poult/trial) and Trial 3 (520 Poults), 1d old Hybrid tom poults from a commercial hatchery were obtained and transferred in poultry shipping boxes to the North Carolina Agriculture Research Service poultry isolation facility maintained within the Department of Poultry Science. A total of 10 poults were in each quadrant of each shipping box. On arrival at the isolation facility, body weight was determined for each poult. Before placement onto a pinewood shavings-covered floor in brooding rings, poults were held in the shipping boxes for 24h. Ambient temperature of the room holding the poults in the shipping boxes was 35°C, the starting temperature in the brooding rings. After placement into the brooding rings, ambient temperature was decreased 3°C at 7d and 14d. Continuous lighting for the poults was provided by incandescent lamps in the ceiling of each room. North Carolina Agriculture Research Service turkey starter feed (2915 kcal /kg ME, 28.13% CP) and water were given for *ad libitum* access.

Treatments: In each trial, poultS were wing-banded, weighed, and returned to their respective shipping box quadrant after arrival. Quadrants of the boxes were assigned randomly to one of four different treatments consisting of (1) Control, (2) Control + OAS, (3) PEMS, and (4) PEMS + OAS. A total of 50g of OAS was placed onto the excelsior pads in the bottom of two quadrants per box. The boxes were then taken to isolation rooms where the poultS were allowed to peck and eat the OAS over the following 24h. They were then reweighed and placed on 4 inches of pinewood shavings in brooder rings. Any remaining OAS in the quadrants of the boxes was dumped onto the pinewood shavings covering the floor.

PEMS Challenge: At 7 d of age, 10% of poultS (12 birds/PEMS group in Trials 1 and 2 and 13 birds/PEMS group in Trial 3) in the PEMS exposure groups were contact-exposed overnight to known PEMS-infected poultS in another isolation room (Qureshi et al., 1997). Contact-exposed poultS were then returned to their respective treatment groups to expose the remaining birds in the PEMS treatment groups.

Measurements. Body weights were determined before OAS treatment, at 24h after OAS treatment, and at 7d, 14d, and 21d of age. Feed conversion (FCR) was determined at 21d of age. Daily records of mortality were made. Histopathology

information on the intestinal tract was collected at 24h post treatment and at 21d of age for poult in the four treatment groups. Two centimeter samples of the duodenum midway in the loop, of the mid-jejunum, of the ileum 2 cm distal to Meckel's diverticulum, of the cecum midway to the blind end, and of the large intestine (21d only) midway between the ileocecal junction and the cloaca were dissected from five randomly selected poult from each treatment. From each treatment, separated tissues were placed into a cassette and fixed in 7% neutral buffered formalin. The tissues were dehydrated, imbedded in wax, and sectioned at 5 μ . Glass-mounted sections were stained with hematoxylin and eosin or the Luna's stain using a standard procedure. Luna's staining can be used to differentiate among heterophils and eosinophils in poultry species (Andreasen and Latimer, 1990) and mast cells (Simoes and Schoning, 1994).

Experimental Design. A completely randomized, factorially arranged experimental design was used. Data were analyzed by analysis of covariance using the GLM procedure of SAS (SAS Institute, 1996). If a significant F statistic for main effects or their interaction was found, means were separated by least significant difference (SAS Institute, 1996). Statements of statistical significance are based on $P \leq 0.05$ or less as indicated in the text.

RESULTS

OAS supplementation significantly reduced the 24h post-hatch weight loss of turkey poults in Experiment 1 (Table 2.1). This resulted in OAS supplemented poults weighing about 1g more than controls at the time of placement. There was a significant OAS-related increase in 7d BW in Trial 1 but not in Trial 2. Pooled 7 d BW data from the 2 trials showed no differences between control and OAS pre-feeding. However, 7 d livability was improved significantly by the OAS supplementation before placement (Table 2.3).

OAS supplementation influenced the development of experimentally induced PEMS and the course of the disease was equivocal. Feed conversions were improved significantly as a result of preplacement OAS supplementation (Figure 2.1). This indicated that the gut of the preplacement OAS supplemented poults was in better condition than in the non-supplemented poults during and after PEMS infection. The 21d BW of non-infected control poults given the preplacement OAS supplementation was less than those that did not get the supplement (Table 2.2). On the other hand, preplacement OAS supplementation did not improve significantly the body weight of PEMS-infected poults. However, over the course of 3 trials, livability was improved significantly in the preplacement OAS supplemented PEMS-infected poults and in Control poults as well (Table 2.3). There was a 56.4% mortality rate in PEMS infected poults, but in the preplacement OAS supplemented PEMS-infected poults, mortality was

50%. There was considerable variability in the mortality rates due to PEMS infection, but pre-fed PEMS-infected poult had better livability in all three trials including Trial 2 when there was a natural *S. Arizona* infection also in the PEMS-infected poult (Figure 2.2). When the mortality due to *S. arizona* was removed from the data set, a mortality rate of 34% was found in OAS-pre-fed PEMS-infected poult compared with a mortality rate of 54% in normal-fed PEMS-infected poult (Figure 2.3). Additionally, in non-infected poult, pre-feeding of OAS also reduced 21d mortality rates (Table 2.3; Figures 2.2 and 2.3).

Developments of the intestinal tract in 2d old preplacement OAS supplemented and in control poult and in 21d old control and PEMS-infected poult from OAS and non-pre-fed treatments are shown in Figures 2.4-2.7. In the duodenum and cecum of 2d old OAS-pre-fed poult, the mucosa and villi are increased in height and volume, and there are thicker appearing gut walls which included the submucosa, muscularis externa and the serosa (Figures 2.4 and 2.5). Goblet cell numbers on the duodenal villi in OAS-fed poult did not change in number compared with non-fed controls, but in the cecum, there was a small increase in goblet cell numbers per villus (Figures 2.4 and 2.5). The 24h OAS pre-feeding regimen did not alter the morphological appearance or size of the villi in either the jejunum or the ileum regions of the small intestine, and goblet cells per villus was not altered by the pre-feeding regimen either (Figures 2.4 and 2.5).

Representative photomicrographs from 14d and 21d old Control and PEMS-infected gastrointestinal segments are presented in Figures 2.6-2.15. Several distinct observations were made. First, as the poult ages, the length and volume of the ileum villus enlarges and goblet cells per villus also increased. Second, pre-fed poult had even greater time-dependent increases in villus length. Third, PEMS infection caused non-fed villus morphological changes characterized by decreased villus length and decreased villus volume. Additionally, villi in PEMS-infected non-fed poult showed characteristic alterations often times called blunting and fusing in earlier PEMS reports. However, in the pre-fed PEMS-infected poult, villi were longer than in the non-fed poult's intestinal segments, but the villi were not as long as those found in pre-fed controls. Even though there were longer villi in the pre-fed PEMS-infected gastrointestinal segment sections, there were also signs of mild blunting and thickening of those same villi in many instances.

At 14d of age the villi in the duodenum were significantly longer than those in 2d old poult. In control poult, the duodenum villi were elongated and tended to peak at the tips (Figure 2.6). Pre-feeding of OAS caused the duodenum villi to be thicker and have rounded tips in control poult. On the other hand, PEMS infection did little to alter villus length or thickness, but the crypt regions of the segments were deeper than in controls (Figure 2.6). Pre-feeding of OAS

tended to increase villus length in PEMS-infected poult, but crypt depth remained similar to that seen in PEMS-infected but not pre-fed.

Representative morphology of the jejunum in 14d old poult is presented in Figure 2.7. There are distinct differences between control and PEMS-infected jejunum villus morphology. In PEMS-infected jejunum, the villi are elongated with very narrow tips. Crypt depth is increased compared with control. Pre-feeding of OAS did not appear to affect morphology of the 14d old poult's jejunum villi, but in pre-fed PEMS-infected poult, villi were not as elongated and narrow as in PEMS control without OAS.

The villi of the ileum region of the small intestine of OAS-pre-fed 14d old poult responded by increasing length in both control and PEMS-infected poult (Figure 2.8). Ileum villi in control poult were shorter than expected, but OAS pre-feeding caused an increase in villus length (Figure 2.8). In PEMS-infected poult, the ileum villi were severely damaged as can be seen in Figure 2.8C, but in OAS-pre-fed PEMS-infected poult, the ileum villi were elongated showing few signs of an infection (Figure 2.8D).

The distal cecum of a control poult is represented in Figure 2.9A and is characterized by low profile plicae covered with short villi. In OAS-pre-fed controls represent by a section through the mid-cecum, villi are elongated and numerous on each of the plicae (Figure 2.9B). In PEMS-infected and OAS-pre-fed PEMS-infected poult, the mid-cecum showed signs of degeneration in which

the plicae were shortened and villi also were shortened (Figures 2.9C and 2.9D, respectively). Villus morphology of the large intestine also responded to OAS pre-feeding by showing increased villus lengths (Figure 2.10C and 2.10D, respectively). However, in PEMS-infected poult, the large intestine villi were shortened in comparison with controls (Figure 2.10A and 2.10C, respectively).

At 21d of age, the duodenum villi in controls (Figures 2.11A and B) had increased in length as compared with 14d old poult (Figures 2.6A and B, respectively). Even in PEMS-infected poult (Figure 2.11C and 2.11D), duodenum villi were elongated in comparison with younger poult. Villi of OAS-pre-fed poult also appeared to have responded with increased length even in the PEMS-infected birds (Figures 2.11B and 2.11D, respectively).

Jejunum villi in 21d old poult can be examined in Figure 2.12. Generally, the villi were elongated as previously observed in controls, and the pre-feeding of OAS appeared to cause a small increase in villus size of controls (Figure 2.12A and 2.12B). PEMS infection had a highly significant negative influence on villus morphology (Figure 2.12C). The villi were shortened, blunted and the tips were showing signs of degeneration with cells sloughing-off into the lumen of this intestinal segment. At this age, the poult had been infected with the agents causing PEMS for a period of 14d, and it was obvious that the PEMS group had not recovered at this time (Figure 2.12C). However, in the OAS-pre-fed group, there were signs of intestinal recovery as indicated by numerous elongated villi

that were equal in length to the controls. Nevertheless, there were still some short, blunt, fused villi in the OAS-fed PEMS-infected group (Figure 2.12D).

OAS pre-feeding did not cause persistent noticeable change in ileum villus morphology in 21d old poult (Figure 2.13A and 2.13B). Villi in the controls, with and without OAS pre-feeding, were equivalent in size, and crypt depth was relatively shallow. In the PEMS-infected group, there was obvious degeneration of the villi and crypt depth was increased (Figure 2.13 C), and in the OAS-pre-fed PEMS-infected group, there was an apparent beginning of recovery based on the generalized elongation of ileum villi and a decrease in crypt depth (Figure 2.13D).

Morphology of the cecal villi can be seen in Figure 2.14. In the 21d old control group, proximal cecal plicae are elongated with numerous folds covered with villi (Figure 2.14A), which result in large surface areas for the cecum. OAS pre-feeding generally caused the mid-cecal plicae in controls to increase in height and increase villus volume (Figure 2.14B). In PEMS-infected poult, there was apparent shortening of the mid-cecal plicae, and villi on the arborized plicae were shortened and smaller in size compared with control (Figure 2.14A and 2.14C). In OAS-pre-fed PEMS-infected poult (Figure 2.14D), the mid-cecal plicae were increased in length such as those found in OAS-pre-fed controls (Figure 2.14B), and villi were increased in thickness as compared with PEMS-infected without OAS pre-feeding. Crypt depth in control ceca was relatively shallow but crypt

depth was increased somewhat in the PEMS-infected poult. OAS pre-feeding did not alter crypt depth or morphology for PEMS-infected poult.

The morphology of the large intestinal villi is very different from the villi in the small intestine and the ceca. At 21d of age, the large intestine villus can be described as being composed of ridges of various heights covered with villi of various lengths, generally elongated (Figure 2.15A). In OAS pre-fed poult, that pre-feeding regime had no impact on the villus morphology. However, in PEMS-infected poult (Figure 2.15C), the villus ridge was frankly swollen, the villi were shortened and the villi were in a degenerate state representing a condition of severe dysfunction in those poult. In OAS-pre-fed PEMS-infected poult, the villus morphology (Figure 2.15D) was similar to that observed in control poult (Figure 2.15A and 2.15B) suggesting an early recovery from the PEMS infection.

DISCUSSION

The pre-feeding of OAS has been reported to increase body weight in young chicks and poult (Dibner et al., 1996; Knight and Dibner, 1998; Noy and Sklan, 1999), and provision of OAS as a pre-feeding supplement promoted development of the gastrointestinal tract of hatchlings (Dibner et al., 1996 and 1998ab; Dibner and Knight, 1999; Yi et al., 2005). Development of the gastrointestinal tract is dramatic and intense during the first week post-hatch and is critical for the continued healthy development and growth of poultry species

(Uni et al., 1998). Knight and Dibner (1998) reported that poult fed OAS for 72 h post-hatch had improved growth rates of small intestine and visceral organs associated with the digestive tract. Early induction of feeding in poults through provision of OAS has been reported to improve 140d market weights of turkeys (Noy and Sklan, 1999).

The possibility of disruption of early growth due to enteric disease in turkey poults and chicks is an ever-present problem. Growth depression is one of the characteristics of PEMS, and PEMS is a disease that appears to initiate its negative growth influences in hatchlings via the actions of multiple etiologic agents, which include bacteria (Edens et al., 1997ab and 1998; Guy et al., 2000) and viruses (Guy et al., 2000; Heggen-Peay et al., 2002ab; Qureshi et al., 2000ab and 2001). Characteristics of PEMS include feed refusal in many cases, inhibition of intestinal development, and severe lesions in the intestinal mucosa (Barnes et al., 1996; Edens and Doerfler, 1998; Doerfler et al., 2000a), which cause decreased nutrient absorption and utilization in infected poults (Doerfler et al., 1998 and 2000ab).

The results of this investigation clearly indicated that provision of OAS as a hydrated pre-feeding supplement before placement induced gut development in poults, and reduced 24h post-hatch weight loss before placement. Part of the reduction in post-hatch weight loss can be attributed to intake of the OAS and its provision of nutrients and water. Additionally, provision of feed and water

induces intestinal development in chicks (Maiorka et al., 2003) and in poult as seen in this study.

Although the provision of OAS as a pre-feeding supplement induced gastrointestinal development and reduced post-hatch body weight loss, the results of this investigation did not show consistently improved body weight gain at 7d of age. This observation was supportive of observations in chickens and turkeys made by Noy and Sklan (1999), and by Yi et al. (2005), who provided the OAS pre-feeding supplement along with water to chickens, and with the observations made by Batal and Parsons (2002), who provided OAS for 24h to 48h after hatch. At 21d of age, OAS-pre-fed poult body weight was slightly less than controls, and there was no difference in body weight between control and OAS-pre-fed PEMS-infected poult. Knight and Dibner (1998) found improved body weights in poult fed OAS immediately after placement compared with 72h fasted poult.

Neither body weight gain nor feed conversion improved as the result of OAS pre-feeding, but 7d livability of pre-fed turkey poult was improved significantly in this investigation. Yi et al. (2005) did not see a difference between control-fed and OAS-pre-fed chick 7d percent livability. When data for livability was assessed after PEMS infection, it was found that even with the debilitating PEMS infection, OAS-pre-fed poult had improved livability compared with non-OAS-fed PEMS controls. With PEMS infection, either with OAS pre-feeding or no OAS pre-feeding, livability was less than in controls either with OAS pre-

feeding or no OAS pre-feeding. However, it was unexpected to see improved livability in OAS-pre-fed poult in the face of PEMS and also *S. arizona* infection in this study.

Observation of the intestinal mucosal morphology in control and PEMS-infected poult given either no OAS pre-feeding or with OAS pre-feeding revealed that OAS pre-feeding was probably involved in recovery of the gastrointestinal tract after PEMS infection. Yi et al. (2005) suggested that OAS pre-feeding followed by vaccination against *E. maxima* had stimulated the gut associated immune system as indicated by elevated IFN- γ and IL-2. In PEMS-infected poult, it has been reported that there is an immunodysfunction (Qureshi et al., 1997), but curiously not all immunological endpoints are suppressed and that some recovery pathways, specifically those mediated by nitric oxide synthase may be initiated (Qureshi et al., 2001). Because much of the mortality due to PEMS can be attributed to secondary bacterial infection (Edens et al., 1997ab), it is possible that one or more immunologically active mechanisms in the gut associated lymphoid tissues might be activated by the simple process of feeding. Zekarias et al. (2002) have shown that as the intestine ages, there are dramatic increases in the numbers of T cells and macrophages in the lamina propria of the intestinal villi. The histological results from this investigation show that there was a biological aging, possibly even a chronological aging of the intestinal villi in OAS-pre-fed poult. Therefore, if the observations by Zekarias et al. (2002) are

true, then the ability of the OAS-pre-fed poult to resist both viral and bacterial infection might be enhanced. Histological information suggested that OAS-pre-fed PEMS-infected gut sections frequently looked as if nothing had changed their morphology, especially at 21d of age. Thus, one must conclude that gut repair was enhanced in the OAS-pre-fed poult and that there was a shortened period in which PEMS had its most severe influence in the infected poult. This concept of enhanced recovery in the OAS-pre-fed PEMS-infected poult is supported by the significantly improved feed conversion ratio in 21d old OAS-pre-fed PEMS-infected poult and in the improved livability of the OAS-pre-fed PEMS-infected poult. Thus, it is likely that the improved performance of OAS-pre-fed poult subjected to enteric disease from PEMS infection is due to improved physiology of gut function resulting from improved villus morphology and function and to early and enhanced repair of damaged tissues in the gut. Under these conditions, OAS appears to be a useful management tool for poult at risk for enteric infections, especially PEMS.

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Table 2.1 Influence of OASIS™¹ feed supplement on body weight loss of turkey poults between hatch and placement and body weight at 7 days of age.

Treatment	Trial	Hatching Weight, g	24 Hour % Weight Loss	Placement Weight, g	7 Day Weight, g
Control	1	56.1^a	5.85^a	53^b	138^b
Pre-Feed	1	56.1^a	3.95^b	54^a	143^a
Control	2	55.9^a	6.90^a	51^b	140^a
Pre-Feed	2	55.3^a	5.50^b	52^a	129^b

^{a,b} Within a column and within a trial, means with unlike superscripts differ significantly ($p < 0.01$).

¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

Table 2.2 Body weight responses of female turkey poult s given the OASIS^{TM1} feed supplement before placement and subjected to PEMS² challenge at 7 days of age.

Treatments	BW at 7 Days grams	BW at 14 Days grams	BW at 21 Days grams
Control (non-fed)	140.2 ± 1.4^a	326.6 ± 3.3^a	459.7 ± 6.2^a
Control (pre-fed)	135.3 ± 1.3^b	257.8 ± 3.1^b	401.6 ± 5.5^b
PEMS (non-fed)	138.0 ± 1.3^a	159.4 ± 3.4^c	290.3 ± 9.3^c
PEMS (pre-fed)	136.6 ± 1.3^{ab}	173.1 ± 3.1^c	291.1 ± 7.5^c

^{a, b, c, d} In a column, means with unlike superscripts differ significantly (p < 0.01).

¹ OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

² PEMS – Poult Enteritis and Mortality Syndrome

Table 2.3 Influence of OASIS^{TM1} feed supplement on livability (percent of total placement) of turkey poult before and after PEMS² challenge.

TREATMENT	PERCENT LIVABILITY						
	TRIAL 1		TRIAL 2		TRIAL 3		
	1d-7d	8d-21d	1d-7d	8d-21d	1d-7d	8d-21d	Pooled
Non-fed	98.0 ^A		91.7 ^B		86.3 ^B		92.0 ^B
Pre-fed	97.4 ^A		95.0 ^A		92.0 ^A		94.8 ^A
Control Non-fed		45.3 ^b		98.0 ^b		100.0 ^c	81.1 ^b
Control Pre-fed		98.7 ^c		98.7 ^b		99.3 ^c	98.9 ^a
PEMS Non-fed		21.3 ^a		43.3 ^a		66.0 ^a	43.6 ^d
PEMS Pre-fed		12.0 ^a		48.0 ^a		90.0 ^b	50.0 ^c

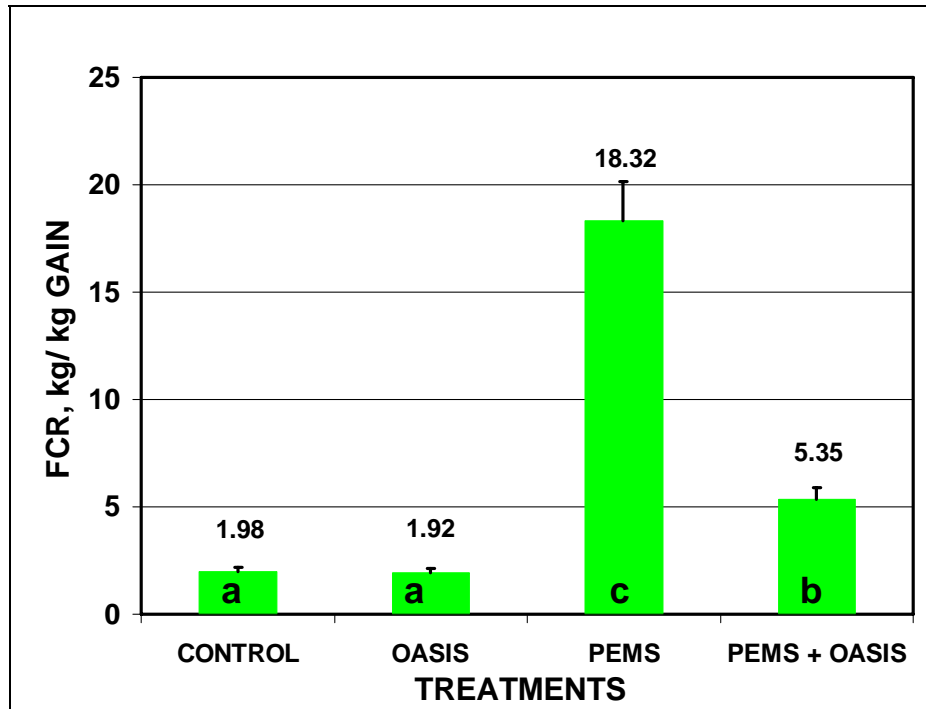
^{A,B} In a column, means with unlike superscripts differ significantly ($p < 0.01$).

^{a,b,c} In a column, means with unlike superscripts differ significantly ($p < 0.01$).

¹ OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

² PEMS – Poult Enteritis and Mortality Syndrome

FIGURE 2.1 Influence of OASIS^{TM1} pre-feeding (mean \pm SEM, n = 1480) from hatch through one day post-hatch on 21-day feed conversion ratios (FCR) of turkey poultlets given a PEMS² challenge at seven days of age.

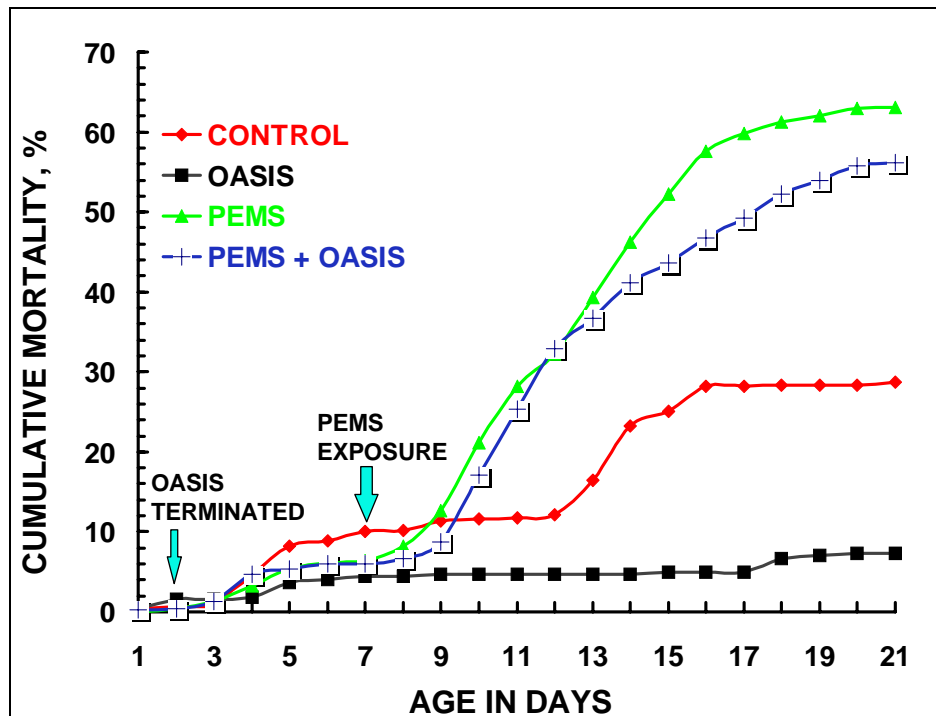


^{a,b,c} Different lower case letters in the histogram bars indicates a significant difference among treatment means ($P < 0.05$)

¹ OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

² PEMS – Poult Enteritis and Mortality Syndrome

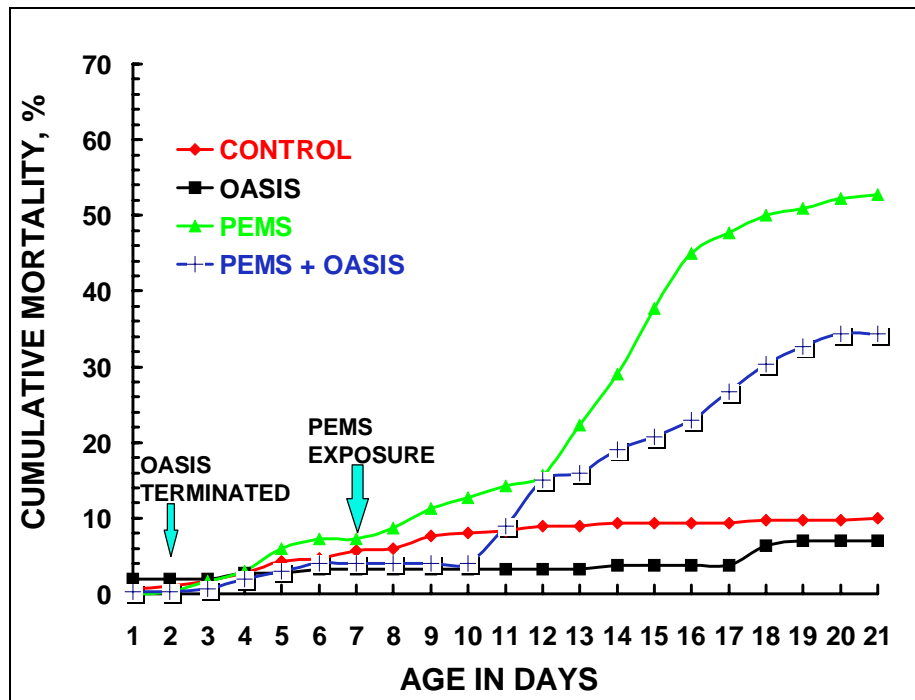
FIGURE 2.2 Mortality profile (including *Salmonella arizona* infection) of turkey poult s given the OASIS^{TM1} pre-feeding regime from hatch through one day post-hatch and subjected to PEMS² challenge at seven days of age.



¹OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poul t Enteritis and Mortality Syndrome

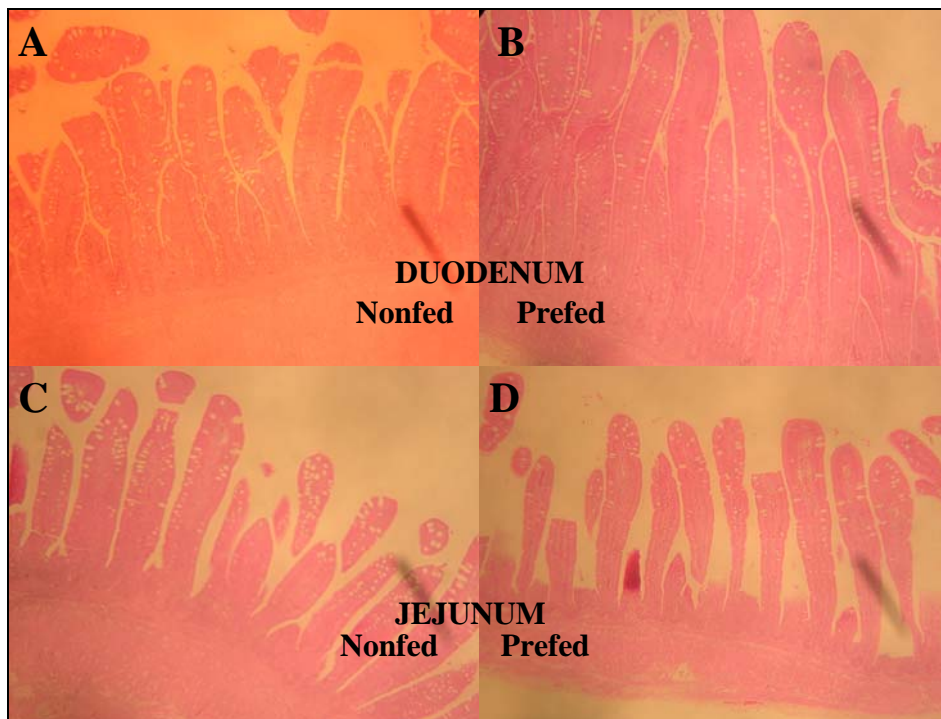
FIGURE 2.3 Mortality of poult given the OASIS^{TM1} pre-feeding regime from hatch through one day post-hatch and subjected to PEMS² challenge at seven days of age.



¹OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poult Enteritis and Mortality Syndrome

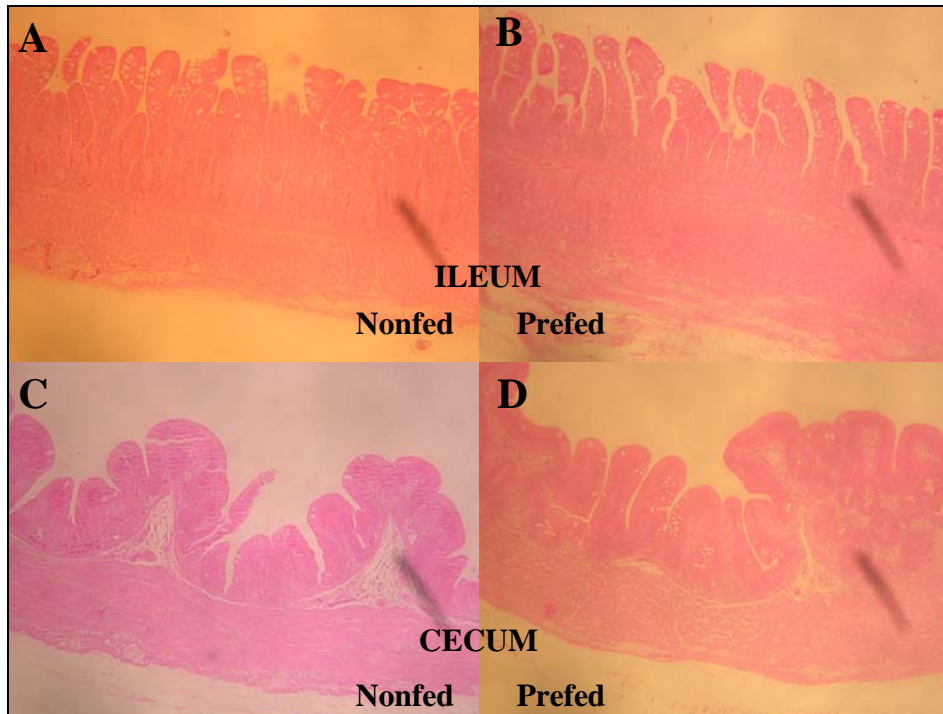
FIGURE 2.4 Induction of villus growth and development in two days old turkey poults subjected to a 24 hour OASIS™¹ pre-feeding or 24 hour holding period without feed or water in shipping boxes.



¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

A: duodenum in non-fed Control; **B:** duodenum in pre-fed Control; **C:** jejunum in non-fed Control; **D:** jejunum in pre-fed Control. (Hematoxylin & Eosin stain; 12.5X magnification)

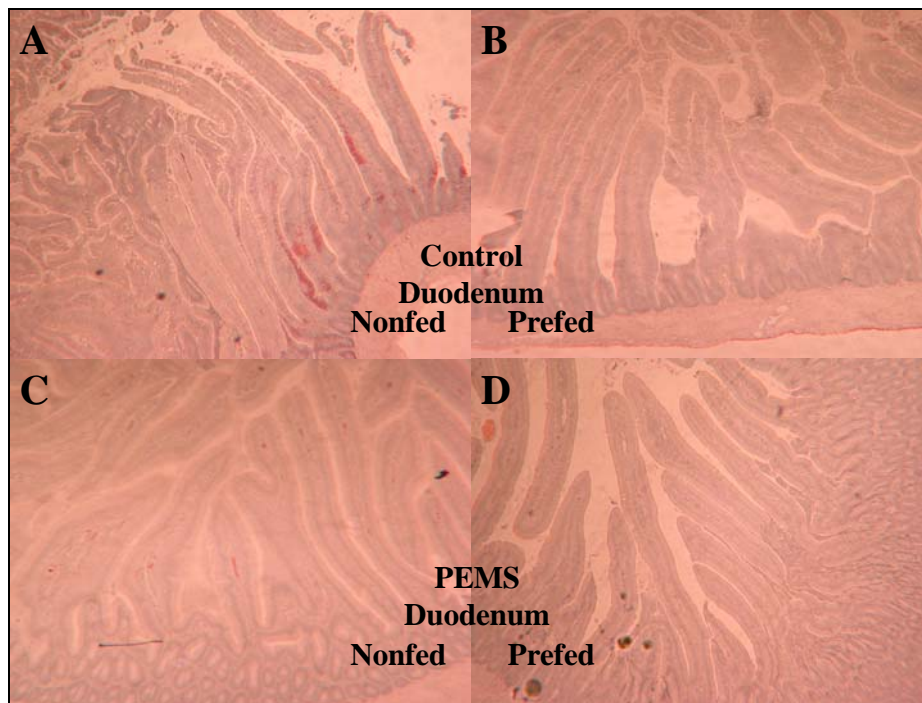
FIGURE 2.5 Induction of villus growth and development in two days old turkey poults subjected to a 24 hour OASIS™¹ pre-feeding or 24 hour holding period without feed or water in shipping boxes.



¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

A: ileum in non-fed Control; **B:** ileum in pre-fed Control; **C:** cecum in non-fed Control; **D:** distal cecum in pre-fed Control. (Hematoxylin & Eosin stain; 12.5X magnification)

FIGURE 2.6 Influence of OASIS™¹ pre-feeding on duodenum villus morphology in fourteen days old turkey poult subjected to experimental challenge to PEMS² etiological agents at seven days of age.

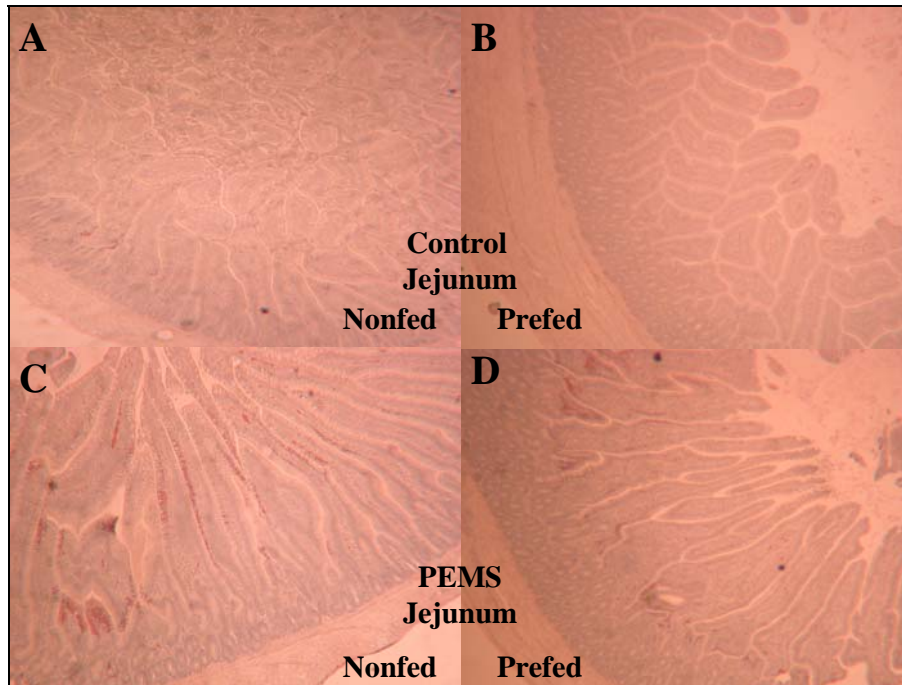


¹ OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

² PEMS – Poult Enteritis and Mortality Syndrome

A: non-fed Control; B: pre-fed Control; C: non-fed PEMS²-infected; D: pre-fed PEMS²-infected. (Luna's stain; 3.1X magnification)

FIGURE 2.7 Influence of OASIS™¹ pre-feeding on jejunum villus morphology in fourteen days old turkey poult subjected to experimental challenge to PEMS² etiological agents at seven days of age.

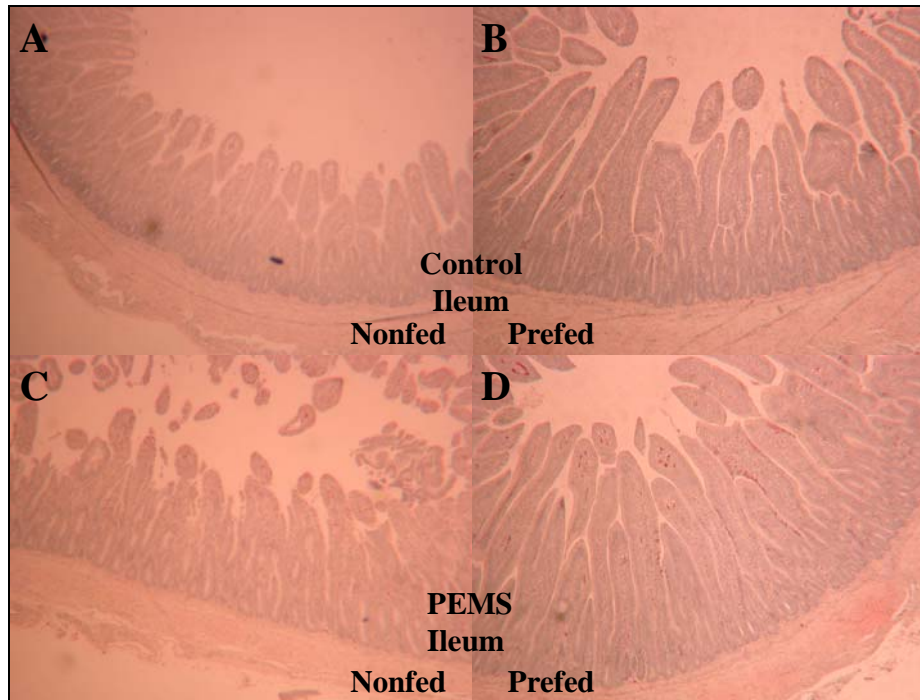


¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poultry Enteritis and Mortality Syndrome

A: non-fed Control; **B:** pre-fed Control; **C:** non-fed PEMS²-infected; **D:** pre-fed PEMS²-infected. (Luna's stain; 3.1X magnification)

FIGURE 2.8 Influence of OASIS™¹ pre-feeding on ileum villus morphology in fourteen days old turkey poult subjected to experimental challenge to PEMS² etiological agents at seven days of age.

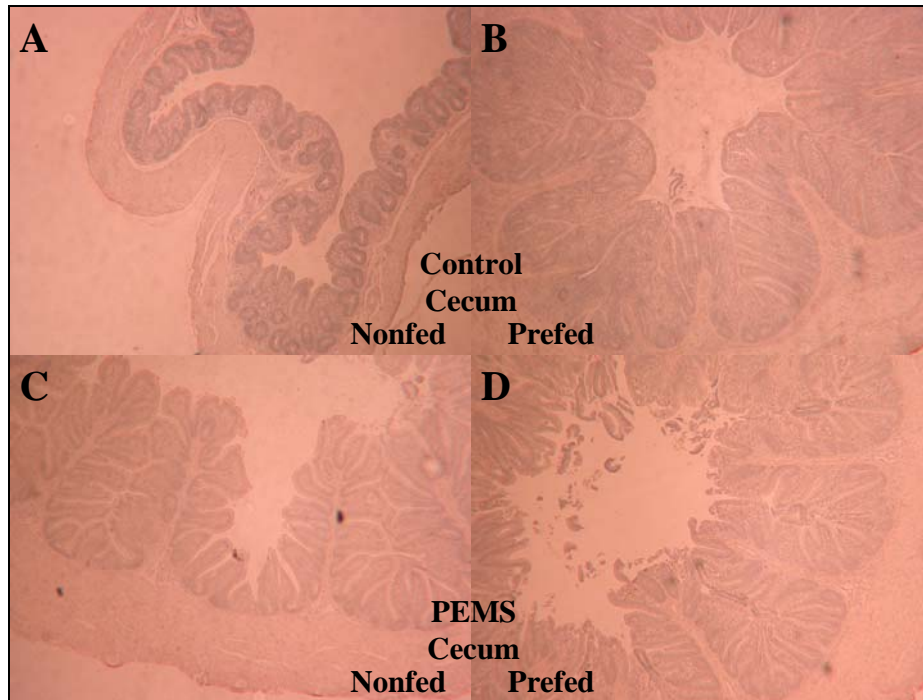


¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poulter Enteritis and Mortality Syndrome

A: non-fed Control; **B:** pre-fed Control; **C:** non-fed PEMS²-infected; **D:** pre-fed PEMS²-infected. (Luna's stain; 3.1X magnification)

FIGURE 2.9 Influence of OASIS™¹ pre-feeding on cecum villus morphology in fourteen days old turkey poult subjected to experimental challenge to PEMS² etiological agents at seven days of age.

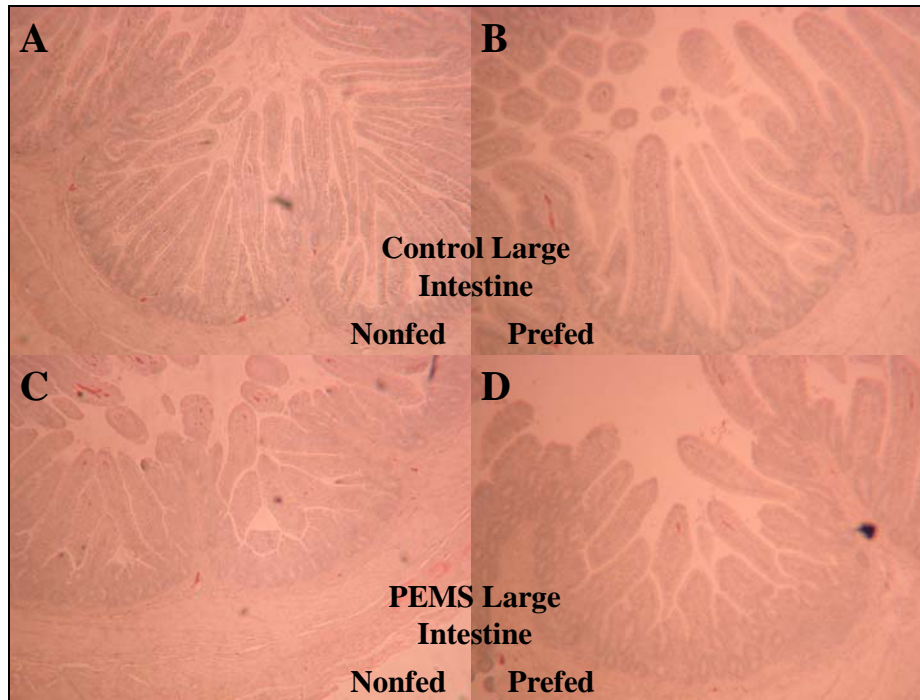


¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poult Enteritis and Mortality Syndrome

A: non-fed Control (distal cecum); **B:** pre-fed Control (mid-cecum); **C:** non-fed PEMS²-infected (mid-cecum); **D:** pre-fed PEMS²-infected (mid-cecum). (Luna's stain; 3.1X magnification)

FIGURE 2.10 Influence of OASIS™¹ pre-feeding on large intestine villus morphology in fourteen days old turkey poult subjected to experimental challenge to PEMS² etiological agents at seven days of age.

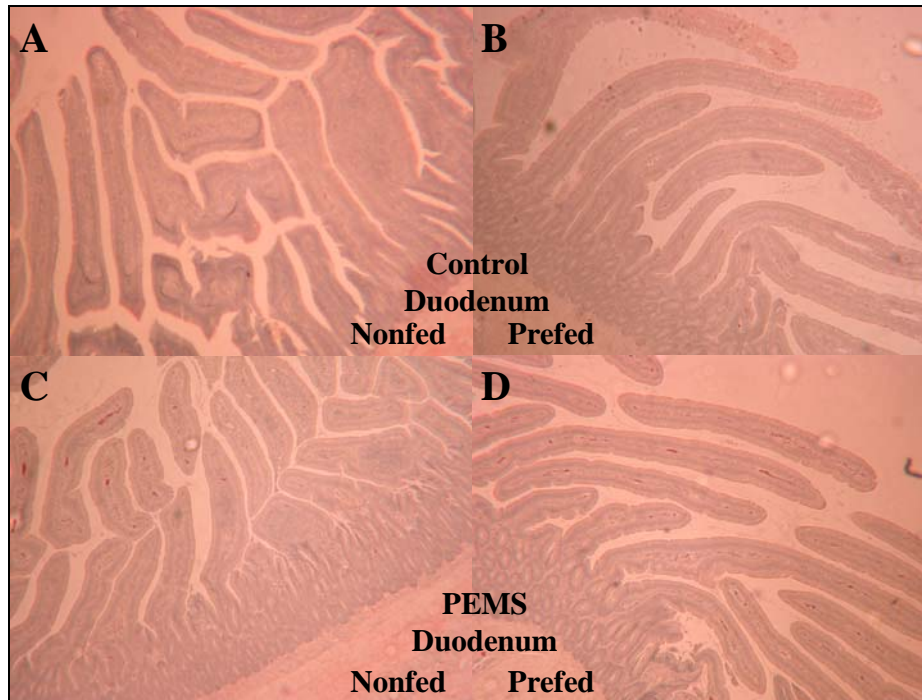


¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poult Enteritis and Mortality Syndrome

A: non-fed Control; **B:** pre-fed Control; **C:** non-fed PEMS²-infected; **D:** pre-fed PEMS²-infected. (Luna's stain; 3.1X magnification)

FIGURE 2.11 Influence of OASIS™¹ pre-feeding on duodenum villus morphology in twenty-one days old turkey poult subjected to experimental challenge to PEMS² etiological agents at seven days of age.

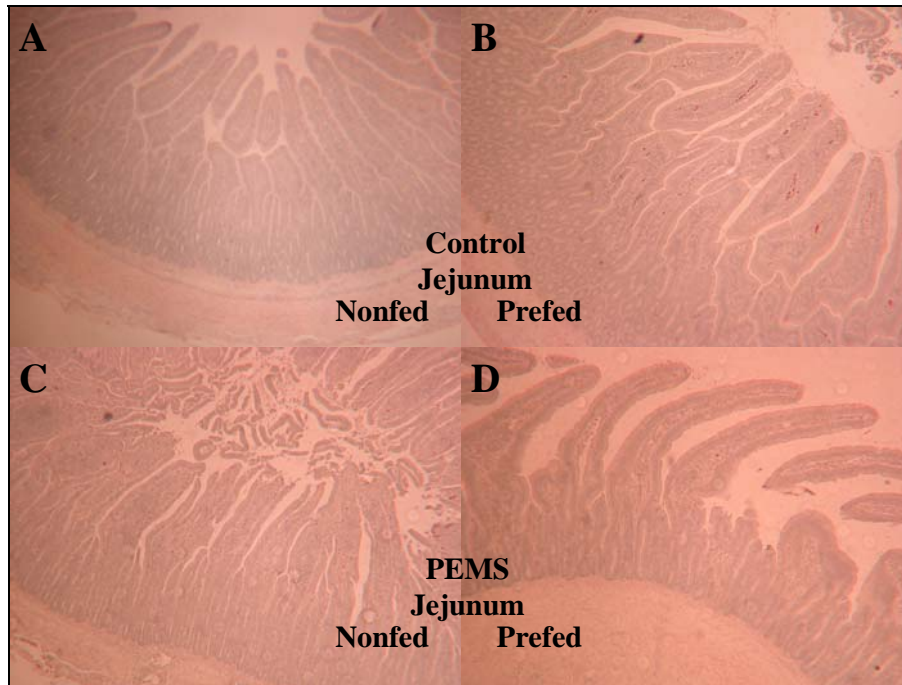


¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poult Enteritis and Mortality Syndrome

A: non-fed Control; **B:** pre-fed Control; **C:** non-fed PEMS²-infected; **D:** pre-fed PEMS²-infected. (Luna's stain; 3.1X magnification)

FIGURE 2.12 Influence of OASIS™¹ pre-feeding on jejunum villus morphology in twenty-one days old turkey poult subjected to experimental challenge to PEMS² etiological agents at seven days of age.

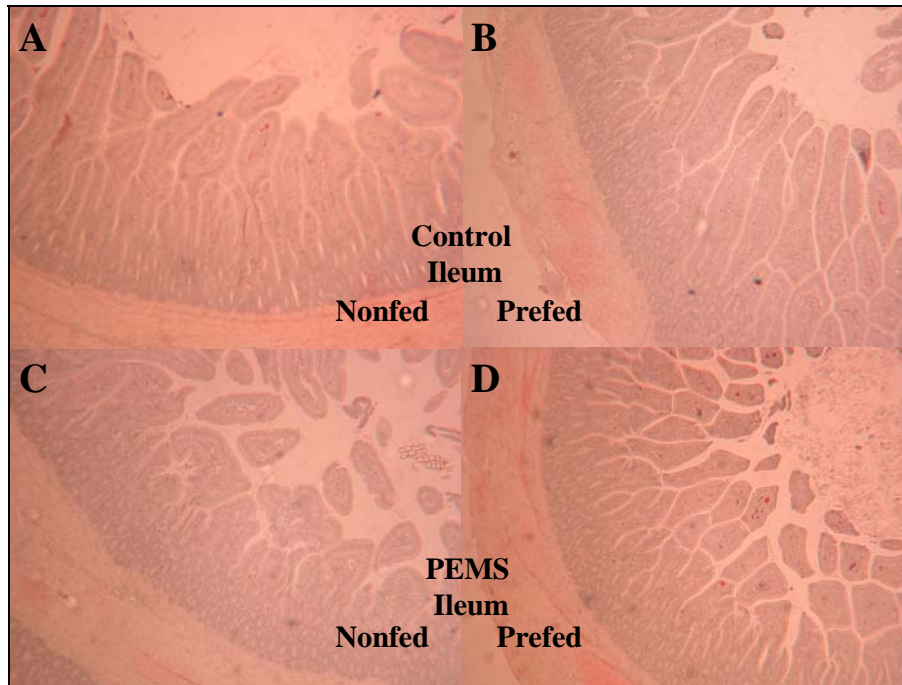


¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poult Enteritis and Mortality Syndrome

A: non-fed Control; **B:** pre-fed Control; **C:** non-fed PEMS²-infected; **D:** pre-fed PEMS²-infected. (Luna's stain; 3.1X magnification)

FIGURE 2.13 Influence of OASIS™¹ pre-feeding on ileum villus morphology in twenty-one days old turkey poultts subjected to experimental challenge to PEMS² etiological agents at seven days of age.

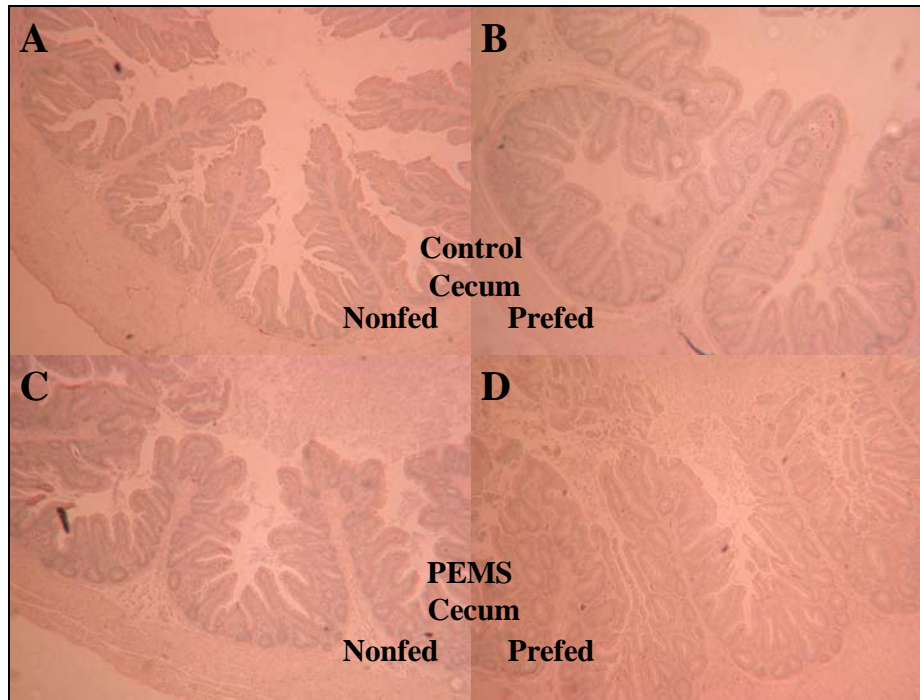


¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poult Enteritis and Mortality Syndrome

A: non-fed Control; **B:** pre-fed Control; **C:** non-fed PEMS²-infected; **D:** pre-fed PEMS²-infected. (Luna's stain; 3.1X magnification)

FIGURE 2.14 Influence of OASIS™¹ pre-feeding on cecum villus morphology in twenty-one days old turkey poult subjected to experimental challenge to PEMS² etiological agents at seven days of age.

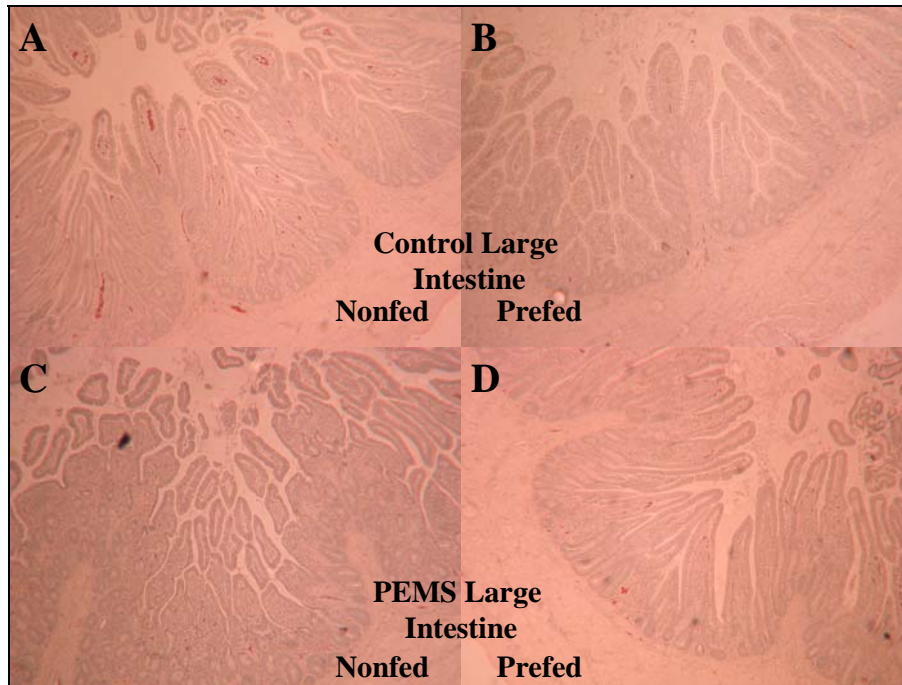


¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poult Enteritis and Mortality Syndrome

A: non-fed Control (proximal cecum); **B:** pre-fed Control (mid-cecum); **C:** non-fed PEMS²-infected (mid-cecum); **D:** pre-fed PEMS²-infected (mid-cecum. (Luna's stain; 3.1X magnification)

FIGURE 2.15 Influence of OASIS™¹ pre-feeding on large intestine villus morphology in twenty-one days old turkey poult subjected to experimental challenge to PEMS² etiological agents at seven days of age.



¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²PEMS – Poult Enteritis and Mortality Syndrome

A: non-fed Control; **B:** pre-fed Control; **C:** non-fed PEMS²-infected; **D:** pre-fed PEMS²-infected. (Luna's stain; 3.1X magnification)

CHAPTER 3

INFLUENCE OF PRE-FEEDING OASIS™ ON DIGESTIVE ENZYMES IN TURKEY POULTS CHALLENGED WITH FECES-BORNE AGENTS THAT CAUSE POULT ENTERITIS AND MORTALITY SYNDROME (PEMS)

ABSTRACT Poult enteritis and mortality syndrome (PEMS) targets the gastrointestinal (GI) tract causing an array of lesions, maldigestion, and malabsorption. OASIS™ (OAS; Novus International, St. Louis, MO), a hydrated pre-feeding nutrient supplement, enhances GI tract development and improves growth and feed conversion of PEMS infected poult. This investigation was conducted to describe the effects of OAS pre-feeding on turkey poult small intestinal digestive enzyme activity using a 2 X 2 factorial arrangement of treatments as follows: (1) Negative Control, no pre-feeding; (2) Positive Control, OAS pre-feeding; (3) PEMS, no pre-feeding; (4) PEMS, OAS pre-feeding. Commercial, non-serviced poult were held in boxes divided into quadrants. Poults in each box quadrant were wing-banded and returned to their respective quadrant before OAS was added to two quadrants per poult box. After 24 hours, poults were placed, 10 per pen, into brooding batteries and given a turkey starter diet. Control (120) and designated PEMS-challenge (280) poults were placed in separate isolation rooms. At 7 days of age, PEMS challenge was accomplished by oral gavage with 0.1 mL of a 10% suspension of turkey coronavirus-negative

PEMS-positive fecal material. Duodenum, jejunum, and ileum were collected every 2 days over two 21 day study periods and assayed for alkaline phosphatase, acid phosphatase, lipase, maltase, and sucrase. Enzyme activity was increased ($P \leq 0.05$) in certain small intestinal segments in OAS-pre-fed Control and PEMS-infected poult compared to non-pre-fed poult. The improved performance reported for OAS-pre-fed, PEMS-challenged poult was associated with improved digestion as indicated by increased activity of acid and alkaline phosphatases, disaccharidases, and lipase.

Key Words: PEMS, pre-feeding, digestive enzymes, OASIS™

INTRODUCTION

At the time of hatch, poult s have an immature gastrointestinal tract and require from 2 to 19 days post-hatch to develop a fully functional digestive system (Noy et al., 2001; Sklan and Noy, 2003). Sklan and Noy (2003) noted that digestive enzymes begin to be secreted with the first feeding of the poult, and their ability to digest nutrients improves through 19 days of age. However, if there is an early interruption in feed intake, glucose and amino acid uptake can be inhibited. It has been noted that increased mortality can be observed in poult s given a normal feeding regime (Edens, 1994). This can be exacerbated if the poult s are forced to be without feed for extended periods of time or if the ambient temperature is too high (Edens, 1994).

Poult enteritis and mortality syndrome (PEMS) is a highly contagious and transmissible disease of young turkeys which causes high rates of morbidity, mortality, and stunting in survivor poult s. Pathological alterations in the poult, including well characterized biochemical and physiological changes associated with the disease have been thoroughly described (Barnes et al., 1996; Doerfler et al., 1998 and 2000a; Edens and Doerfler, 1997ab and 1998; Edens et al., 1997ab and 1998b). The GI tract in PEMS-afflicted poult s appears to be targeted by at least one and possibly more of the viral and bacterial etiologic agents known to be associated with the disease (Edens et al., 1997ab; Guy et al., 2000; Heggen-Peay et al., 2002ab; Qureshi et al., 2000ab, 2001). Thus, the intestinal tract can become

morphologically compromised by invading bacteria and viruses alike, preventing normal nutrient digestion and uptake. Therefore, it was hypothesized that during PEMS, when there is feed refusal, digestive functions might have become inhibited by the agents causing PEMS. If this were to be proven true, then the severe growth depression associated with the disease could be explained and possibly corrected by pre-feeding a nutrient-rich compound before exposure to PEMS-causing agents.

OASIS™ (OAS; Novus International, St. Louis, MO), a nutrient-rich dietary supplement used to pre-feed poultry that will be shipped or held for short times before placement, has been reported to increase body weight in young chicks and poults (Dibner et al., 1996; Knight and Dibner, 1998; Noy and Sklan, 1999). Poults pre-fed OAS showed increased growth of the liver, GI tract, and pancreas compared with fasted poults, and increased pancreas weight persisted through 3 weeks of age. It was concluded that early improvement in growth and digestive functions contributed to later growth and performance of turkey poults that had been pre-fed OAS (Noy and Sklan, 1999). Pre-feeding for the purpose of enhancing the development of the GI tract and its barriers against bacterial and viral pathogens is important to the turkey industry especially in cases where PEMS is a risk factor. Thus, the objective of this investigation was to determine if the pre-feeding of OAS, before the poults were PEMS-challenged, affected performance of the poults through the first 3 weeks after hatch.

METHODS AND MATERIALS

Animal Welfare. This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

Animals and Husbandry. Two 21 d long trials were conducted with Nicholas male poult obtained within three hours after hatching from a commercial hatchery and transported to North Carolina State University, where they were wing-banded, and segregated into treatment groups of either control or pre-fed for a 24 hour period before placement into pens in heated metal battery brooders with raised wire floors. The poult were not subjected to hatchery services such as beak or claw trimming, antibiotic administrations, or vaccinations. The control poult and the poult designated for PEMS-exposure were assigned to separate but identically controlled-environment isolation rooms.

Ambient temperature for brooding was maintained by room air conditioning using a thermostatically controlled hot water/cold water heat exchange system mediated by a forced draft. Initial room brooding temperature for both the Control and PEMS rooms was set at $34 \pm 1^{\circ}\text{C}$, and this temperature was decreased 3°C in each room at 7 and 14 d of brooding. Humidity in the experimental rooms was not controlled and varied from 45 to 70% relative

humidity. Continuous light was provided from brooder deck lights and from incandescent lamps in the ceiling.

Pre-feeding and Feeding. Pre-feeding of OAS was done immediately after body weight measurements and wing-banding of the newly hatched poult had been completed. The weighed and wing-banded-poults were returned to their respective quadrants of their original shipping boxes. The negative control poult groups were given no OAS, poult in the positive control groups were given 100g OAS per box quadrant, PEMS-Control groups were given no OAS, and PEMS-pre-fed groups were given 100g OAS per box quadrant. At the end of the 24 hour holding period with or without OAS, the treatment groups were placed in heated brooder battery pens and were provided the North Carolina Agricultural Research Service corn and soybean based turkey starter feed (2,915 kcal/kg metabolizable energy and 28.13% crude protein) and water in stainless steel feeders and waterers for *ad libitum* consumption.

PEMS Challenge. At 7 d post-hatch, each poult in the PEMS-designated groups was given an oral PEMS inoculation. The PEMS inoculation consisted of a 0.1 mL oral gavage of a 10% suspension in sterile 0.9 % saline of fresh, raw feces derived from coronavirus-negative PEMS-infected poults maintained at the North Carolina State University College of Veterinary Medicine.

Treatments. A 2 x 2 factorial arrangement of treatments was used with the treatments assigned as follows: (1) control- no pre-feeding (60 poult divided into six replicates of 10 poult per replicate pen); (2) control-OAS pre-feeding (60 poult divided into six replicates of 10 poult per replicate pen); (3) PEMS-no pre-feeding (140 poult divided into ten replicates of 14 poult per replicate pen); (4) PEMS-OAS pre-feeding (140 poult divided into ten replicates of 14 poult per replicate pen). Negative Control and OAS positive Control groups were placed into a room separate but with an identically controlled environment compared with the isolation room containing the PEMS-challenged poult.

Tissue Collection. Tissue samples of the duodenum, jejunum, and ileum were collected at 1 d and every other day for a total of 11 samplings. These tissue samples were collected for the determination of maltase, sucrase, acid phosphatase, alkaline phosphatase, and lipase.

The 10 cm segments of the duodenum, jejunum, and ileum were excised rapidly from the intestinal tract, flushed with 20 mL of sterile 0.85% saline, weighed and placed into pre-weighed polypropylene tubes, flash-frozen in liquid nitrogen, and stored at -70°C until analyzed for digestive enzymes. Before digestive enzyme analysis, the intestinal segments were thawed and homogenized in 50 mL polypropylene tubes using 20 mL of ice-cold sterile 0.85% saline.

Tissues were homogenized on ice with an ultra-turax homogenizer (Thomas Scientific, Swedesboro, N.J. 08085) at 20,000 rpm. The samples were then centrifuged at 400 X g for 30 min at 4°C to remove large debris from the mixture. After the centrifugation, 1.5 mL of the sample supernatant was transferred to a 2.0 mL microcentrifuge tube and frozen at -70°C until analyzed. Before analysis, total protein for each sample was determined by the method described by Bradford (1976).

Digestive Enzyme Analyses. Sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) activities were determined using a colorimetric procedure developed by Dahlqvist (1968) and modified for microplate assay by Black and Moog (1978), commercial kits (Sigma Chemical Co, St. Louis. MO 63178-9916) were obtained for determination of activities of acid phosphatase (EC 3.1.3.2) (Sigma procedure # 435), alkaline phosphatase (EC 3.1.3.1) (Sigma procedure # 245), and lipase (EC 3.1.1.3) (Sigma procedure # 805). Enzyme and reagents were prepared following the vendor's directions. Brief descriptions of the assay conditions for each enzyme are presented below.

Acid Phosphatase Activity (AcP, U/mg protein with one unit (U) representing 1 μ M α -naphthyl phosphate/min) (Sigma procedure # 435) : Room temperature AcP reagent (250 μ l) was added to each of each sample of 25 μ l volume of known

protein concentration in flat-bottomed microplate wells. The solution was mixed and incubated for five minutes at 30°C. After incubation, the absorbance was read at 405 nm versus water as a reference providing the initial absorbance reading. Incubation was continued at room temperature with two 5 min absorbency readings which terminated after 10 min providing the final absorbance reading. Activity of the of the AcP was calculated following procedures provided by the vendor of the kit.

Alkaline Phosphatase Activity (ALP, U/mg protein with one unit (U) representing 1 μ M para-nitrophenol produced/min) (Sigma procedure # 245): The ALP reagent was prepared according to the instructions provided with the kit. Blank and test flat-bottomed microplate wells were setup in triplicate. To the test wells, 250 μ l of the ALP reagent was added and incubated at 30°C. The 5 μ l of each sample of known protein concentration was added and mixed in each microplate well. The plate was then incubated at 30°C for 30 sec and read at 405 nm versus water as a reference to obtain the initial reading. The incubation was continued at 30°C and the absorbency was again recorded at 1 and 2 min intervals to observe linearity of the reactions. The absorbance reading after 2 min was the final reading. Activity of the ALP was calculated following procedures provided by the vendor of the kit.

Lipase Activity (U/min; U is 1 mM quinone diimine/min at 37°C) (Sigma procedure # 805): The microplate was set up for blanks, standards, and samples. To each well, 210 μ L of substrate solution was added. Then, 4 μ L of deionized water, standard solution, and samples were added into corresponding wells. The solutions were mixed gently and incubated for 3-5 min at 37°C and read at 550 nm for the initial reading. Incubation continued at 37°C and final absorbency was recorded after 2 min. The total lipase activity (U/mg protein) was calculated following procedures provided by the vendor of the kit.

Maltase and Sucrase Activity (μ M glucose hydrolyzed from either maltose or sucrose/hour/mg protein): The microplate was set up for triplicate wells for blank, glucose standard, and sample blank using the following solutions: 40 μ L of 0.85% saline solution was added to each blank well, and 40 μ L of glucose standard was added to glucose standard wells. The microplate was placed on ice and 20 μ L of each homogenate was added to each well. In two of the three wells, 20 μ L of maltose or sucrose substrate was added. The third well in the triplicate served as a sample blank. The solutions in the microplate wells were mixed and after a 60 min incubation at 37°C, the microplate was removed and placed directly on ice. A volume of 250 μ L of Tris-glucose oxidase reagent was added to each well, including the plate blank and standards, and this was followed by addition of 20 μ L of maltose or sucrose substrate to the third well for each sample. Microplate

well contents were mixed and the plate was incubated at 37°C for an additional 60 minutes for color development. The microplate was removed from the incubator to stop color development and was read at 415 nm in the plate reader.

Statistical Analyses. The experiment was conducted as a completely randomized design with a factorial arrangement of treatments (Control *vs.* PEMS *vs.* Control-OAS *vs.* PEMS-OAS). The data were subjected to analysis of covariance of main effects of Disease (Control *vs.* PEMS), Pre-feeding (OAS *vs.* no OAS), Time (days) and their interactions, using the SAS General Linear Models procedures for analysis of variance and regression analysis (SAS Institute, 1996). Because there was no trial effect, the data from both trials were pooled and analyzed using disease, pre-feeding and day main effects, and when significant interactions of main effects were found, means were separated by least significant difference (SAS Institute, 1996). Statements of significance were based on $P \leq 0.05$ or less.

RESULTS

Acid phosphatase activity in the duodenum was characterized generally by daily increases in activity before birds were challenged at 7 d post-hatch with feces-contained unidentified agents that cause PEMS (Figure 3.1). From 9d through 15d, duodenal AcP activity was generally decreased in PEMS-infected poults regardless of pre-feeding program. After 13d, there was an apparent

physiological and biochemical alteration in AcP activity in the PEMS-infected poult as evidenced by a general slow increase in AcP activity (Figure 3.1). There were instances in which individual treatment groups showed either increased or decreased AcP activity in comparison with Control, but those events appeared to be random with no discernible pattern.

In the jejunum, AcP activity generally showed an increasing profile regardless of treatment from 1d to 7d post-hatch (Figure 3.2). On d7, before PEMS challenge, the AcP activity in those birds designated to receive the PEMS challenge, AcP activity was increased significantly over their respective controls. However, by d11, AcP activity in PEMS-challenged poult was decreased relative to their controls and remained decreased through d15. From d17 through d21, there was a general recovery of AcP activity in the jejunum in PEMS-challenged poult with those having received the OAS showing the greater increases.

The AcP activity in the ileum also was characterized by daily increases in activity from 1d to 7d post-hatch, but on d7, the AcP activity in PEMS designated poult was less than their respective controls (Figure 3.3). After PEMS-challenge on d7, AcP activity declined in challenged birds relative to their respective controls. By d13 PEMS-challenged poult still had lower AcP activity compared with Control, OAS and PEMS/OAS, but by d19, AcP in both PEMS-challenged groups showed signs of recovery as evidenced by AcP activities that were not different from their respective controls. By d21, the AcP activity in both PEMS-

challenged groups was significantly greater than Control but not OAS. OAS treatment appeared to provide some stimulatory effect and recovery ability on AcP activity in PEMS-infected poult.

Alkaline phosphatase (AIP) activity in the duodenum of Control and OAS-pre-fed poult generally increased from hatch with the increase ranging between 100 and 300% (Figure 3.4). Within 2d after PEMS-challenge on d7, AIP activity in the duodenum decreased significantly relative to their respective control groups (Figure 3.4). Duodenum AIP activity remained depressed relative to the Control groups through d21. In the case of duodenum, OAS pre-feeding did not prevent the PEMS-associated decrease in AIP activity.

In the jejunum, AIP activity increased with age post-hatch (Figure 3.5). OAS pre-feeding did not increase activity of AIP any more than in Control until d19 and d21 post-hatch. On the other hand, after PEMS challenge on d7, both PEMS and PEMS/OAS treatment groups were found to have a significantly decreased AIP activity as compared with their respective control groups (Figure 3.5). From d15 to d21 AIP activity was showing signs of recovery from PEMS infection, but AIP activity in PEMS only poult did not begin to recover until d19 to d21. Thus, OAS pre-feeding, even in PEMS-infected poult, showed signs of recovery in parallel with increasing AIP activity in OAS only poult.

In the ileum, AIP activity in control groups showed a transitory increase from d1 to d3 followed by a transitory decrease from d5 to d11 followed by an

increased AIP activity that plateaued from d13 to d21 (Figure 3.6). When the poultts were PEMS-challenge on d7, AIP activity decreased in those poultts through d15. By d17, AIP activity in PEMS/OAS birds was beginning to recover to control levels, and by d19 PEMS only AIP activity was recovered to control levels. By d21 AIP activity in PEMS only poultts was significantly higher than in all other groups of poultts (Figure 3.6).

Maltase activities in the duodenum, jejunum, and ileum were significantly greater on d1 than at any other time during the 21d long trials (Figures 3.7, 3.8, and 3.9, respectively). At d3 post-hatch, duodenum maltase activity had declined to a level less 23% of d1 activities and by d5 it had declined to less than 20% of the d1 activity (Figure 3.7). In the jejunum, maltase activities had fallen to a level of roughly 37% of d1 activity and continued to decrease to 19 and 14% on d5 and 7, respectively (Figure 3.8). In neither the duodenum nor the jejunum, OAS had no influence on development of maltase activity or its recovery after PEMS infection (Figures 3.7 and 3.8). In the ileum, maltase activity was also elevated on d1 and declined by roughly 70% on d3, 66% on d5, 62% on d7, and by 71% on d21. OAS pre-feeding did not affect maltase activity in the ileum of control or PEMS-infected poultts (Figure 3.9).

The sucrase activity in the duodenum increased significantly from d1 through d3 post-hatch then decreased from d5 to d13. This was then followed by an increase to a plateau from d15 to d21 in Control and OAS pre-fed poultts

(Figure 3.10). PEMS challenge on d7 was followed by a significant decrease in duodenum sucrase activity on d9 to d17, and this was followed by apparent recovery on d19 and d21. OAS pre-feeding to PEMS-challenged poult did not prevent PEMS induced depression in duodenum sucrase activity. Sucrase activity in both the jejunum was significantly greater on d1 post-hatch than on d3 post-hatch (Figure 3.11). In this segment of the small intestine, OAS pre-feeding was associated with a significant decrease in sucrase activity (Figure 3.11). From d5 through d21, there were no differences among the four treatment groups for sucrase activity (Figure 3.11). No apparent trends in sucrase activities were discerned among the treatment groups.

In the ileum, OAS pre-feeding caused a significant increase in duodenum sucrase activity on d1 post-hatch, but by d3 OAS pre-fed poult duodenum sucrase activity was down to and equivalent to the sucrase activity in control poult (Figure 3.12). By d5 post-hatch there was a further decline in ileum sucrase activity that persisted through d11 and appeared to have been exacerbated by OAS pre-feeding. From d13 through d21 there was a small recovery of ileum sucrase activity in PEMS-challenged poult both with and without OAS pre-feeding. OAS pre-feeding had no apparent significant influence on ileum sucrase activity (Figure 3.12).

The lipase activity (meq/min) in the duodenum was increased significantly by OAS pre-feeding at 1d and 3d post-hatch compared with Control poult in the

same time-frame (Figure 3.13). By 5d or 7d post-hatch, duodenum lipase activity had reached a plateau for both OAS-pre-fed and Control poult. PEMS-challenge did not have any significant influence on duodenum lipase activity (Figure 3.13).

DISCUSSION

Dibner et al. (1998) wrote that a starter diet should be more reflective of the nutritional needs of the 14 to 21 day bird than of the day old bird. They challenged to long-held concept that young chickens and poult can derive all their early, needed nutrition through the assimilation of nutrients through the yolk sac. Instead, Dibner et al. (1998) suggest that in modern poultry, the yolk contains a vital source of macromolecules such as antibodies and immunoglobulins needed to allow the hatchling to resist the hostilities of an non-sterile environment of a poultry house by developing a high level of passive immunity. Therefore, it is necessary to provide the hatchling with a nutrient source as quickly as possible in order to stimulate gut development and immunity and to allow for early assimilation of nutrients to meet early demands for energy metabolism. Dibner and colleagues (1998) point out that early fasting or delay of consumption of nutrients has an immunosuppressive influence as indicated by negative effects on integrity of the bursa of Fabricius and other immunologically active tissues. Edens (1994) demonstrated that delaying feeding from one to three days post-

hatch had significant negative impact on growth, development, and livability of turkey poults.

Newly-hatched turkey poults must make a rapid transition from dependence on absorbed egg contents as a source of nourishment to reliance on a relatively complex diet. During this transition, physical and functional development of the gastrointestinal tract is completed and is characterized by increased tissue mass and synthesis, secretion, and composition of digestive enzymes (Sell, 1996). In PEMS-infected poults, feeding is greatly reduced if any consumption occurs, and the depression in feed intake begins within a short time after poults are placed in brooder facilities (Edens, 1994; Barnes et al., 1996; Doerfler et al., 1998 and 2000a; Edens and Doerfler, 1997ab and 1998).

When poults contract an enteric infection caused directly by a virus, a bacterium, or a combination, which has been suggested as the cause for PEMS, development of the digestive tract is altered (Edens and Doerfler, 1998) and leads to a severe malabsorption condition (Edens and Doerfler, 1998; Doerfler et al., 2000a). Edens et al. (1997a) reported that the PEMS-associated malabsorption was accompanied by decreased villus surface epithelium and loss of absorptive surface area similar to observations made by Perry et al. (1991) who described a malabsorption syndrome in chickens and turkeys. The reports by Doerfler et al. (1998 and 2000a), Edens and Doerfler (1997ab and 1998), and Edens et al. (1997a) all suggest that if glucose is absorbed from the GI tract, the PEMS-

infected poult had little ability to utilize the nutrient. Part of the inability to utilize glucose can be attributed to hypophosphatemia in PEMS-infected poult, and when the poult do receive a carbohydrate diet, there is insufficient phosphorus to phosphorylate incoming glucose, which then results in a fatal phosphate block (Edens, 1994). Thus, in PEMS-infected poult, malabsorption was accompanied by biochemical and hormonal defects that prevented nutrient utilization and growth.

The underlying basis for malabsorption in PEMS-infected poult appeared to be associated with maldigestion. Odetallah et al. (2001) have reported that PEMS infection resulted in depressed digestive capabilities. Specifically, impaired fat digestibility and dietary energy utilization contribute to PEMS-associated mortality, stunted growth in survivors, and reduced recovery from the disease.

The results from the current study showed that impaired digestion in PEMS-infected poult was due to decreased digestive enzyme specific activity and that pre-feeding of a hydrated nutrient supplement (OAS) immediately after hatch had some ameliorating properties that might aid in recovery from PEMS.

Acid phosphatase (AcP) and alkaline phosphatase (AIP) activities were affected by age and by location within the small intestine in this study. AcP and AIP are involved in pH-dependent hydrolysis of phytate, which binds minerals such as phosphorus, calcium, iron, copper, and others, and can also bind amino

acids (Maenz and Classen, 1998). Generally, there was an increase in the intestinal AcP activity from hatch through 7d post-hatch. Thereafter, a transitory decrease in AcP specific activity was found in all treatment groups through d13, which was followed by an increasing profile through d21. The decrease in specific activity of AcP was greatest in those poult that had been given the PEMS challenge on d7 post-hatch. The activity of AcP was lower than AIP and probably reflects the more alkaline environment of the intestinal tract (Maenz and Classen, 1998). After PEMS-challenge on d7, the transitory decrease in AcP activity in all three intestinal segments was not affected by infection. In the jejunum, AcP recovery during the period between 19d and 21d was significantly greater in OAS-pre-fed poult, but in the duodenum and ileum, there was no significant OAS effect on AcP activity in the infected poult. The AIP activity in all three segments of the small intestine generally remained elevated in Control and OAS-pre-fed poult as compared with their infected counterparts, but OAS pre-feeding did not alter AIP activities after PEMS infection.

These observations on AcP and AIP activities were not expected. During PEMS infections, feed consumption declines significantly (Barnes and Guy, 1995), and based on these observations, one might expect that specific activity of AcP and AIP might have increased. AcP specific activity decreased significantly in the duodenum, jejunum and cecum of broiler cockerels that were deprived of feed but recovered to control levels of activity on re-feeding (Majumdar and

Panda, 1989). On the other hand, AIP specific activities were decreased in the duodenum and jejunum when feed was withheld, but recovered only partially in the duodenum on re-feeding (Majumdar and Panda, 1989). Uni et al. (1999) have shown an increase in mucosal enzyme activity that was correlated significantly with growth. However, early growth of the poult is slower than that found for the chicken (Uni et al., 1995). The early slow growth pattern of turkey poults has been associated with two peaks (d4 to d5 and d7 to d9) in early post-hatch mortality (Phelps et al., 1987). These peaks in early mortality also coincide with the decreasing profile for specific activities of AcP and AIP in the intestinal segments reported here.

In a study with turkey poults infected with a lymphoproliferative disease virus (LPDV) (Zimber et al., 1985), AcP and AIP activities in serum and in the pancreas and spleen decreased. The diseases caused by LPDV and PEMS infections are vastly different, but decreased activities of AcP and AIP reflect dysfunction in the homeostatic conditions of all the body systems.

Intestinal disaccharidases of young turkeys have been studied extensively. In this investigation, both maltase and sucrase specific activities were characterized by high activity immediately post-hatch, and this was followed by sharp declines in activities as the poults aged. These observations were consistent with those reported by Thouvenelle et al. (1995). A similar pattern in young poults was reported by Sell et al. (1991) and Angel et al. (1990). However, Sell et

al. (1989) also reported that specific activities of poult intestinal disaccharidases increased with age, but this was dependent upon the type of diet consumed by the poult, e. g., high carbohydrate diets induced disaccharidases and high lipid diets inhibited disaccharidase activity.

In 1d old control poult, OAS pre-feeding was associated with a decrease in maltase activity in all three small intestine segments. Jejunum sucrase activity in 1d old poult was also decreased in poult pre-fed OAS, but in the duodenum there were no differences due to OAS pre-feeding. In the ileum, OAS pre-feeding caused significant elevations in sucrase activity. Pre-feeding with OAS tended to elevate the activity of maltase in the duodenum and ileum of PEMS-infected poult compared with PEMS only treatment. Even in PEMS only poult there were transitory decreases in duodenum and ileum maltase activity followed by an increasing profile. These changes in maltase activity might be associated with astrovirus infection reported to cause a latent increase in duodenum and ileum maltase activity (Thouvenelle et al., 1995). Astrovirus (Koci et al., 2000; Yu et al., 2000ab; Qureshi et al., 2001) and reovirus (Heggen-Peay et al., 2002ab) have been reported recently to be etiological causes of PEMS. Additionally, OAS pre-feeding also clearly increased the rate of recovery of the duodenum sucrase activity of PEMS-infected poult. Pre-feeding of OAS influenced small intestinal disaccharidase activity, and increased disaccharidase activity probably contributed to improved performance in chickens and poult pre-fed OAS even though very

early growth might not be affected (Batal and Parsons, 2002). Yi et al. (2005) also reported that hatchling chickens pre-fed OAS had advanced gut development compared with fasted and fed chickens. In PEMS infected poult, the development of the small intestine is hindered significantly, which resulted in a physical manifestation of malabsorption (Edens and Doerfler, 1998). Edens et al. (1998ab) pre-fed OAS to turkey poult and found increased villus length and width, and in PEMS infected OAS-pre-fed poult, there was a highly significant improvement in feed conversion ratios.

In this study, duodenum lipase specific activity was stimulated significantly by OAS pre-feeding. Even in PEMS-infected poult, OAS pre-feeding caused an elevation in lipase activity. This observation is significant when placed in the greater view of nutritional observations concerning PEMS. Odetallah et al. (2001) reported impaired fat digestibility and dietary energy utilization in PEMS-infected poult. The OAS-induction of lipase activity conceivably contributed to improved early performance of Control and PEMS-infected turkey poult. Krogdahl and Sell (1989) reported that poult intestinal lipase activity was low at hatching and was slow to be elevated requiring as long as eight weeks post-hatch to reach maximum activity. In this study, there was a time-dependent increase in lipase activity, OAS-stimulated increase in lipase activity, and also a PEMS-related increase in lipase activity with the PEMS/OAS treatment causing the greatest increase in activity. These observations placed into

perspective with other OAS and PEMS results clearly shows that the early post-hatch poult is not fully prepared to digest complex ingredients in their feed. Stimulation of digestive enzymes such as lipase is important for the poult to utilize lipids derived from absorbed yolk materials. If poults can receive a stimulus from the pre-feeding of a hydrated nutrient supplement such as that provided in OAS, they would be better able to grow even in the face of stressors (Noy and Sklan, 1999) and diseases as well (Yi et al., 2005).

After PEMS challenge, many biochemical and physiological manifestations of the disease can be found. One very important manifestation of PEMS infection is the depression in plasma tri-iodothyronine levels in circulation (Edens and Doerfler, 1998; Doerfler et al., 2000b). The decrease in plasma tri-iodothyronine in turkey poults can be attributed to decreased feed intake or fasting, which results in elevated plasma levels of corticosterone, the adrenal stress hormone (Edens et al., 1991). Noy and Sklan (2001) have reported that decreased plasma tri-iodothyronine level, resulting from feed deprivation for 48 to 96 hours post hatch, is associated with decreased glucose and amino acid uptake, but immediate feeding of chicks post hatch caused increased yolk secretion into the small intestine and stimulated uptake mechanisms. In chickens, intestinal development is dependent upon tri-iodothyronine (Prager et al., 1990; Suvarna et al., 1993), and in embryonic turkey poults (Christensen et al., 1999) and chickens (Moog, 1962), intestinal development is dependent upon the presence of tri-

iodothyronine. Not only the morphological development of the avian small intestine is increased by the presence of tri-iodothyronine, but enzyme activity (ALP) is also stimulated by the presence of tri-iodothyronine (Moog, 1962; Suvarna et al., 1993).

Pre-feeding of a hydrated nutrient (OAS), therefore, should stimulate intestinal development in turkeys and chickens as shown above by the cited scientific literature. Along with the increased rate of morphological development associated with OAS pre-feeding, digestive enzyme activities reported here also showed increased activity in certain segments in the small intestine of both control and PEMS-infected turkey poults. These observations underscore the importance of providing a source of nutrition as early as possible after poults hatch. The early nutrition facilitates development of the intestinal tract and, perhaps, positively influences the poult's ability to resist and recover from PEMS.

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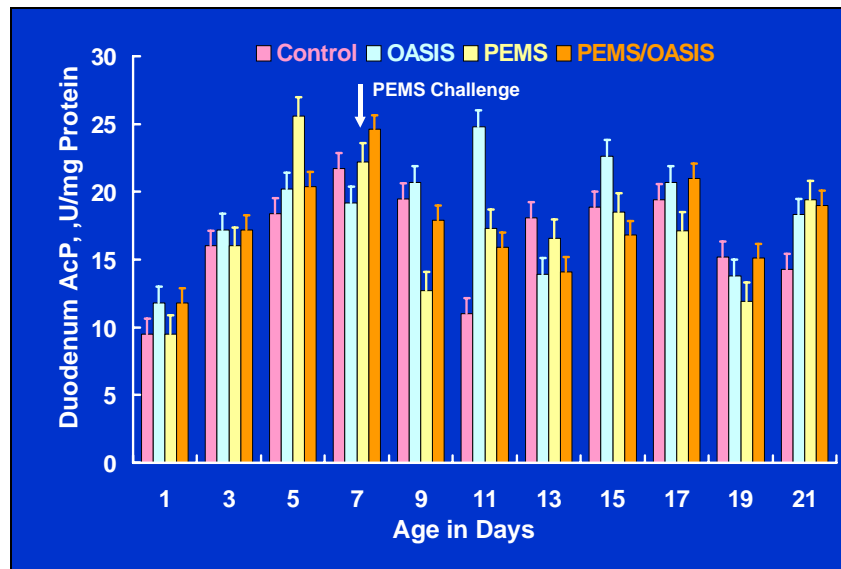
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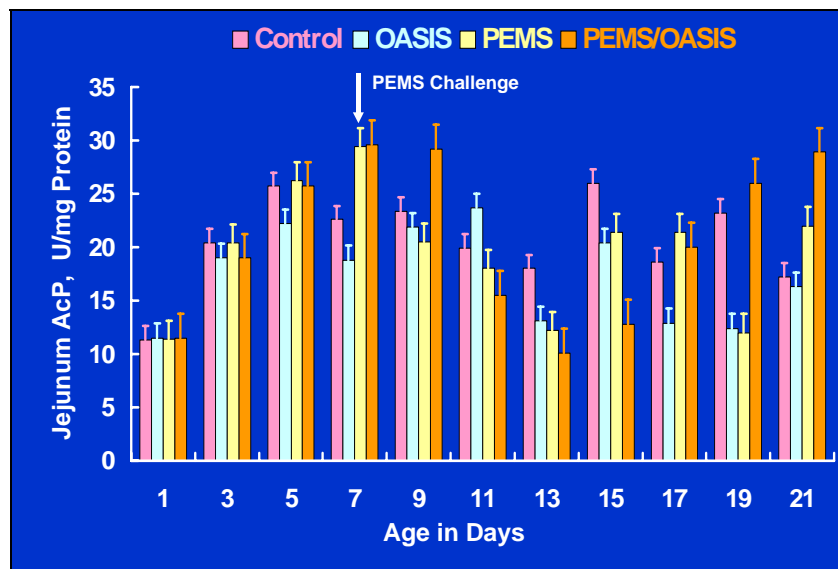
Figure 3.1 Duodenum acid phosphatase activity¹ in turkey poult given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as U/mg protein with one unit (U) representing 1 μ M para-nitrophenol produced/min).



¹Data is expressed as mean \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

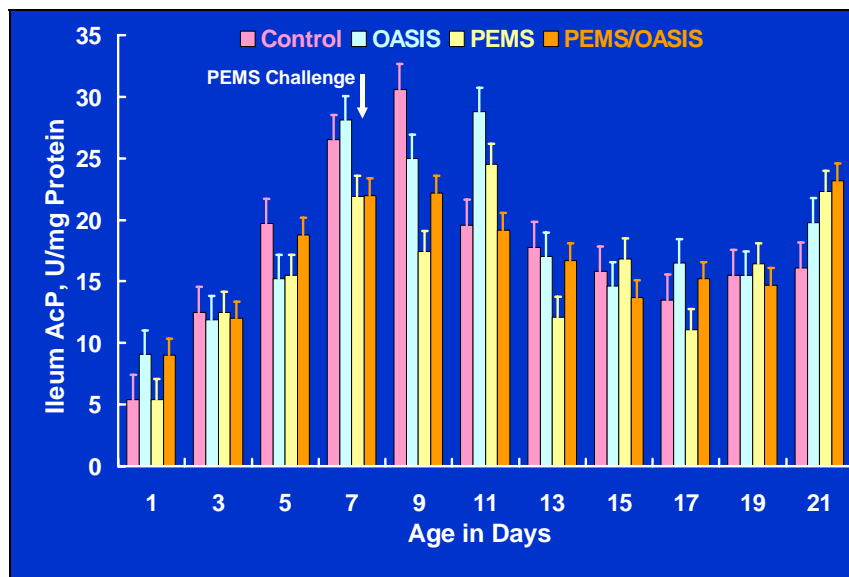
Figure 3.2 Jejunum acid phosphatase activity¹ in turkey poult given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as U/mg protein with one unit (U) representing 1 μ M para-nitrophenol produced/min).



¹Data is expressed as mean \pm SEM, n = 10 per group

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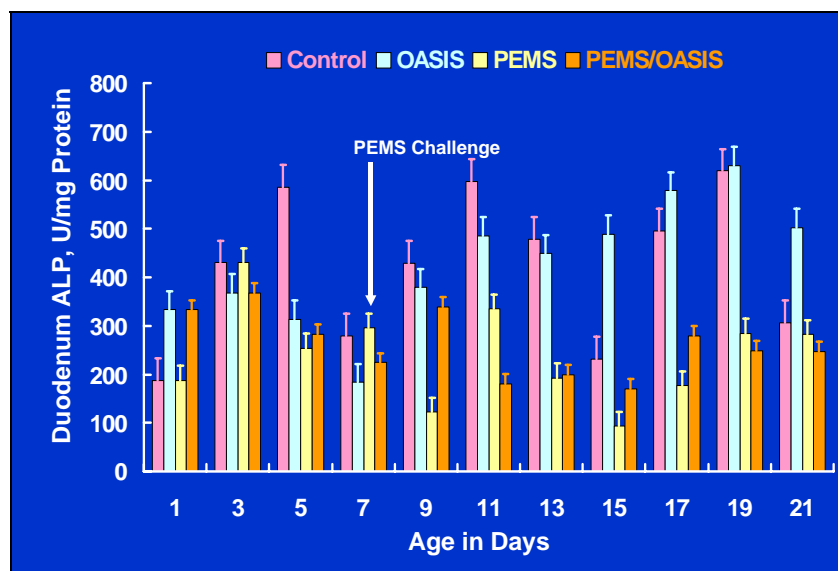
Figure 3.3 Ileum acid phosphatase activity¹ in turkey poultts given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as U/mg protein with one unit (U) representing 1 μ M para-nitrophenol produced/min).



¹Data is expressed as mean \pm SEM, n = 10 per group

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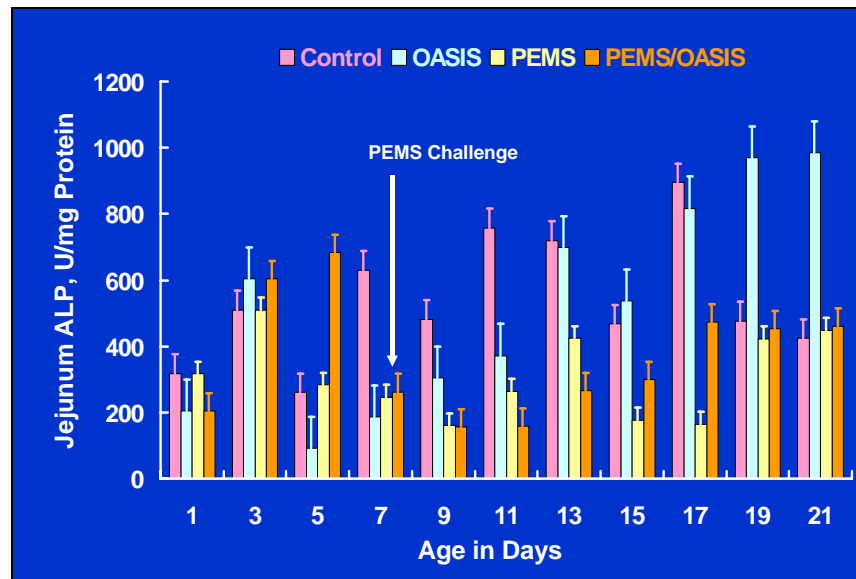
Figure 3.4 Duodenum alkaline phosphatase activity¹ in turkey poult given OASISTM¹ and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as U/mg protein with one unit (U) representing 1 μ M para-nitrophenol produced/min).



¹Data is expressed as mean \pm SEM, n = 10 per group

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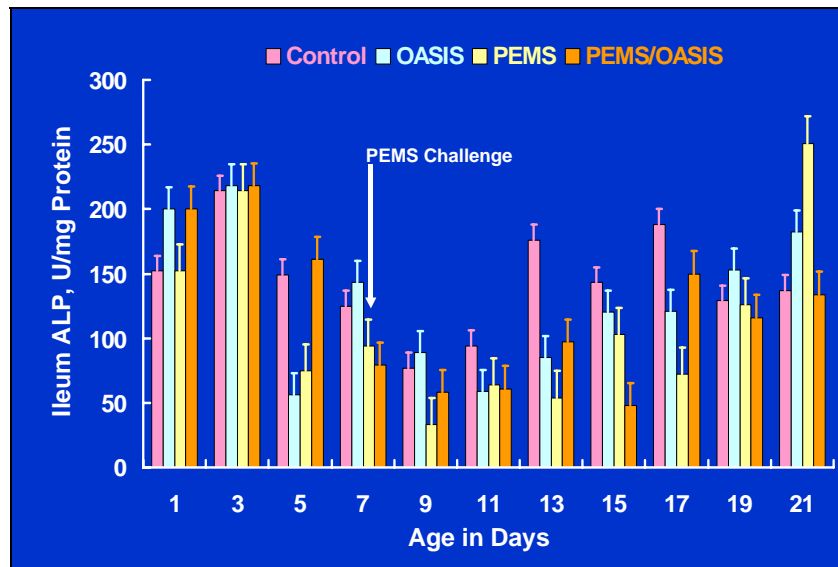
Figure 3.5 Jejunal alkaline phosphatase activity¹ in turkey poults given OASISTM² and challenged with agents in fecal material that induce poulter enteritis and mortality syndrome (PEMS). (Data is expressed as U/mg protein with one unit (U) representing 1 μ M para-nitrophenol produced/min).



¹Data is expressed as mean \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

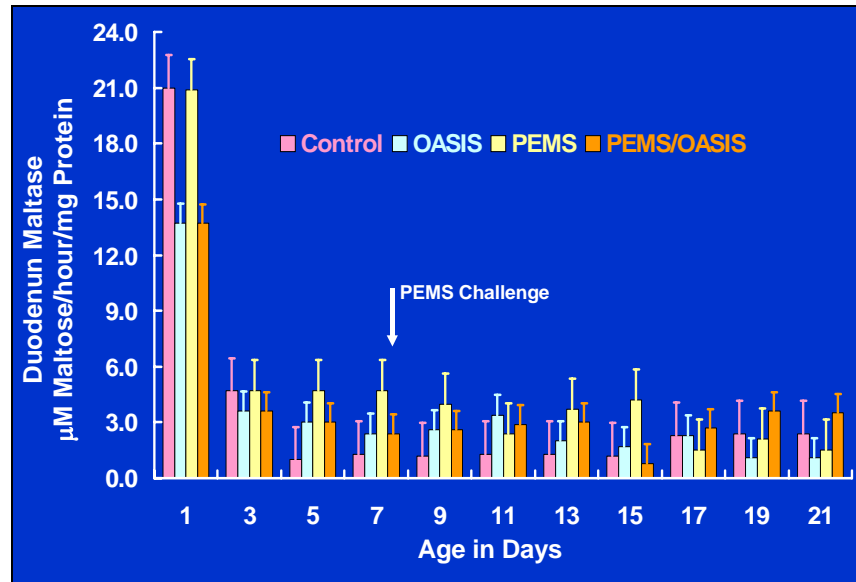
Figure 3.6 Ileum alkaline phosphatase activity¹ in turkey poult given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as U/mg protein with one unit (U) representing 1 μ M para-nitrophenol produced/min).



¹Data is expressed as mean \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

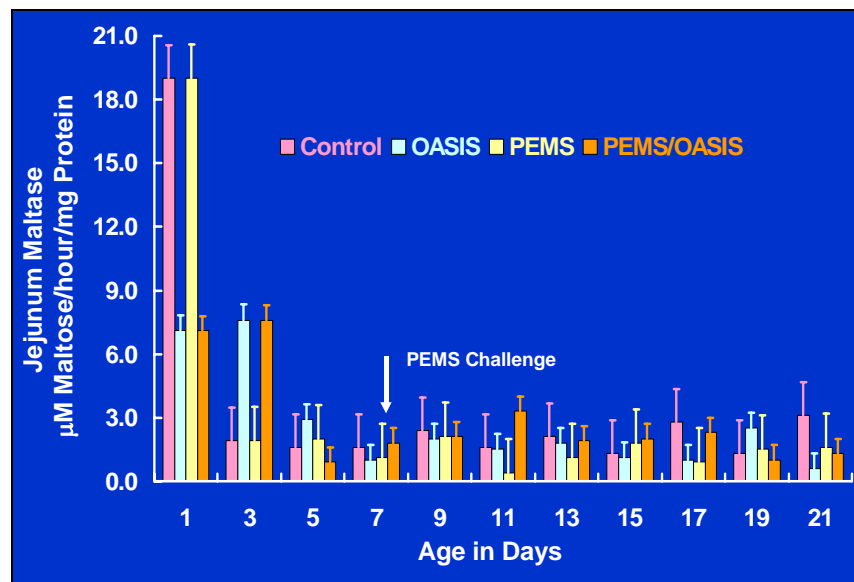
Figure 3.7 Duodenum maltase activity¹ in turkey poult given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as μM glucose hydrolyzed/hour/mg protein).



¹Data is expressed as mean \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

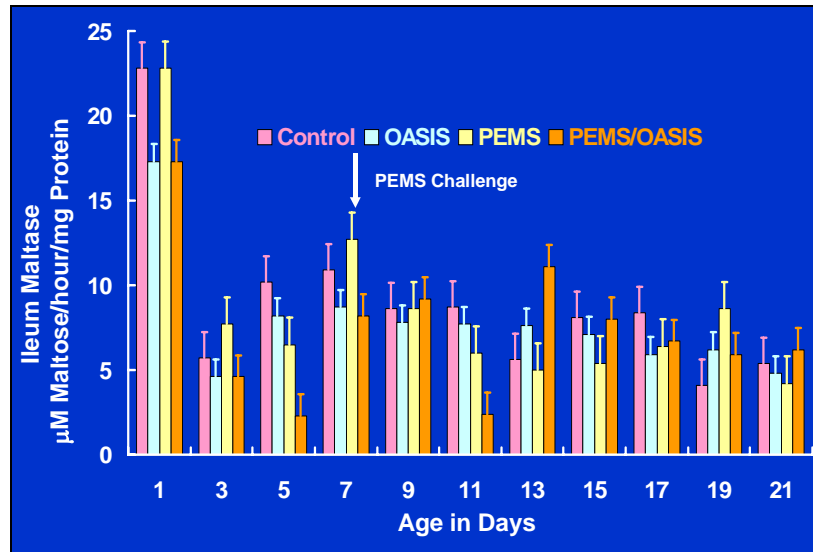
Figure 3.8 Jejunum maltase activity¹ in turkey poult given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as μM glucose hydrolyzed/hour/mg protein).



¹Data is expressed as means \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

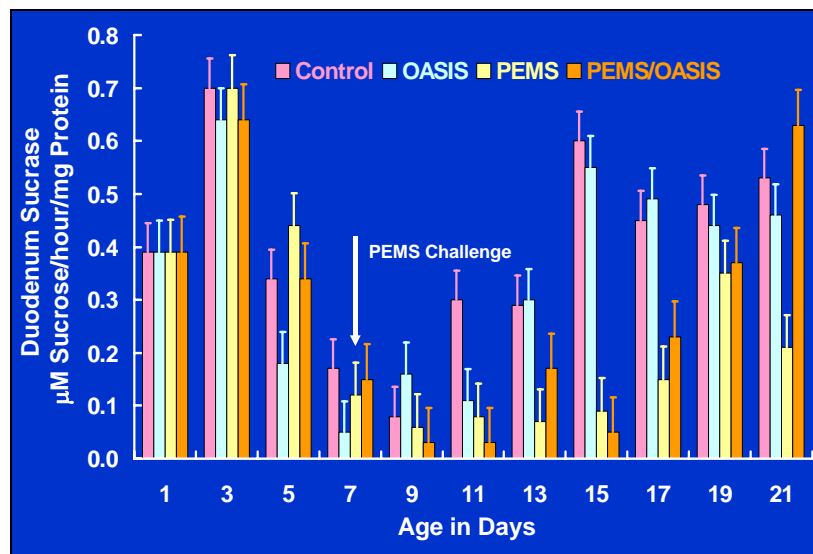
Figure 3.9 Ileum maltase activity¹ in turkey poult given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as μM glucose hydrolyzed/hour/mg protein).



¹Data is expressed as means \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

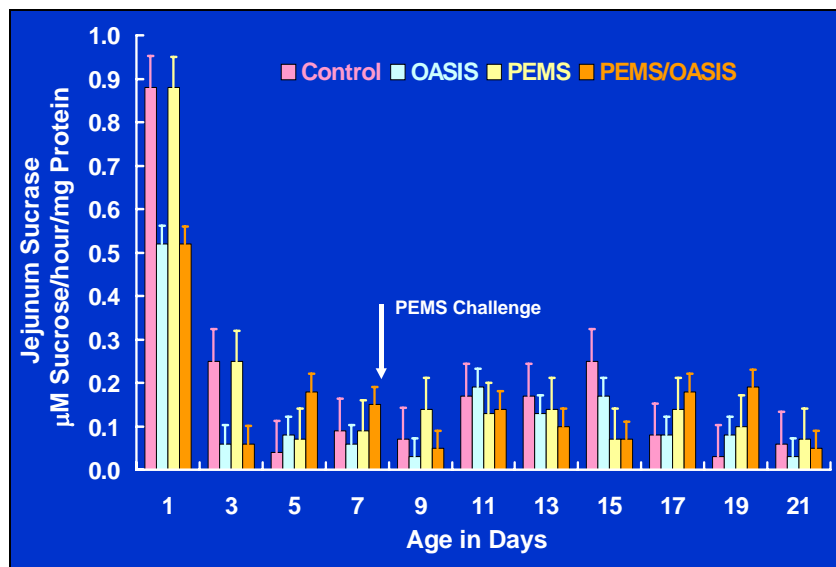
Figure 3.10 Duodenum sucrase activity¹ in turkey poult given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as μM glucose hydrolyzed/hour/mg protein).



¹Data is expressed as means \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

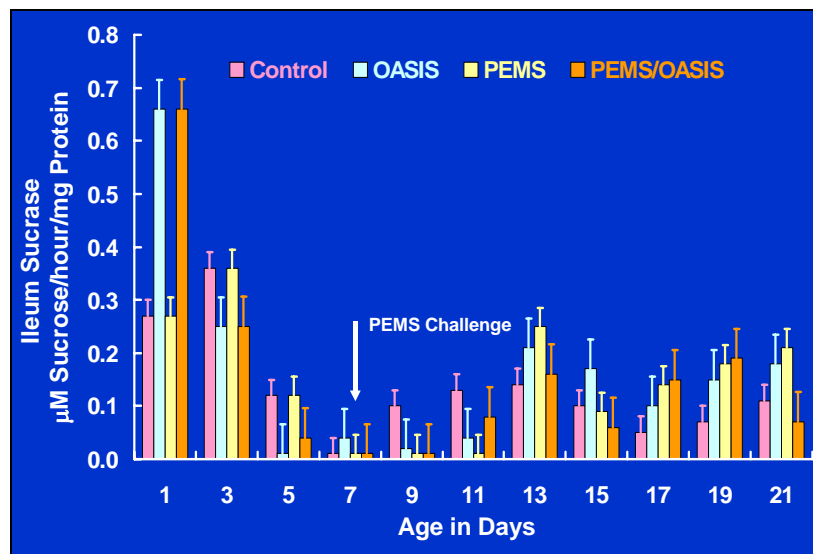
Figure 3.11 Jejunum sucrase activity¹ in turkey poult s given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as μM glucose hydrolyzed/hour/mg protein).



¹Data is expressed as means \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

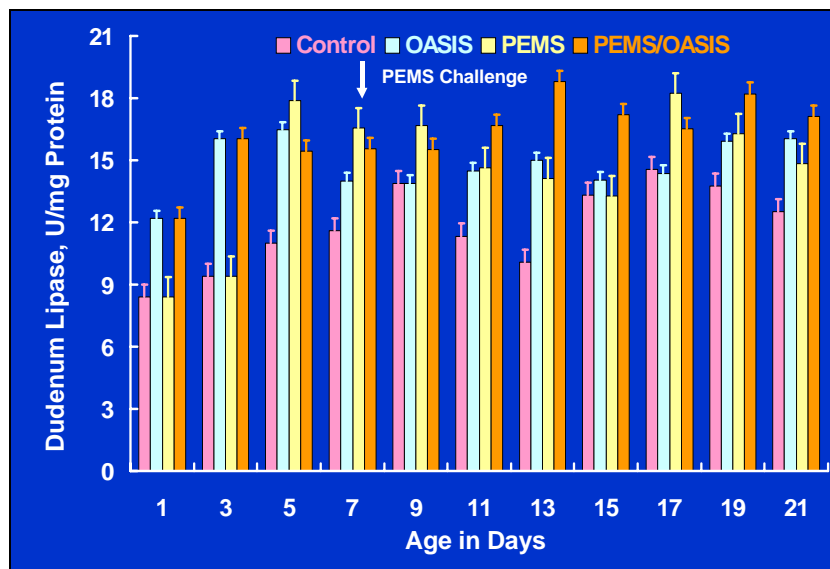
Figure 3.12 Ileum sucrase activity¹ in turkey poult given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as μM glucose hydrolyzed/hour/mg protein).



¹Data is expressed as means \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

Figure 3.13 Duodenum lipase activity¹ in turkey poult s given OASIS^{TM2} and challenged with agents in fecal material that induce poult enteritis and mortality syndrome (PEMS). (Data is expressed as U/min with U equal to 1 meq butyric acid produced/min at 37°C).



¹Data is expressed as means \pm SEM, n = 10 per group

²OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

CHAPTER 4

INFLUENCE OF OASIS™, A SEMI-SOLID HYDRATED PRE-FEEDING SUPPLEMENT, FED CONCURRENTLY WITH NORMAL FEED AND WATER ON POST-HATCH ORGAN WEIGHTS OF TURKEY POULTS

ABSTRACT After hatch, the organs of poults must develop rapidly in order to utilize the feed rather than nutrients from the yolk sac. Organ weights tend to increase rapidly as the body weight of the bird increases. OASIS™ (OAS; Novus International, St. Louis, MO), has been shown to enhance gastrointestinal development and improve growth in poults. This experiment focused on the use of OAS concurrent with provision of a normal feed and water regiment to stimulate growth and development of some visceral organs in poults. Newly-hatched poults were placed into three treatment groups: Normal-feed and water, OAS-fed with normal feed and water, and blue-green-dyed Solka-Floc®-fed with normal feed and water. Twelve were fasted for 24h before relative weights of visceral organs and small intestine were determined and compared with 24h concurrently fed poults. These data were compared with relative weight data for visceral organs and small intestinal relative weights of the newly-hatched poults. Six replicate pens of 12 poults per dietary treatment were established and fed one of the three dietary treatments described above for a period of 16d post-hatch. At 2d, 9d, and 16d post-hatch, 10 randomly caught poults from each treatment group

were killed and dissected for determination of the relative weights of bursa of Fabricius, heart, liver, lungs, pancreas, duodenum, jejunum, and ileum. Fasting for 24h caused a loss of hatching weight, but feeding of 24h caused a significant increase in body weight with OAS-fed poult gaining the most weight. There were time dependent increases in relative weights of visceral organs and intestinal segments, but there was no consistent effect of concurrent feeding of OAS and normal feed and water. The results suggest that no advantage is gained when OAS is fed concurrently with normal feed and water.

Key Words: poult, OASIS™, visceral organs, small intestine

INTRODUCTION

The post-hatch development of the turkey poult is slower than the development of the domestic chicken, and Phelps et al. (1987abc) have suggested that the slow development is due to radical physiological adjustments that occurs post-hatch. Organogenesis and hematology change dramatically and often times are correlated with peaks in early mortality. Many environmental and managerial factors interact with dynamic post-hatch developmental processes in poult and often times have negative influences on performance of the poult as it grows to market age (Phelps et al., 1987abcd; Moran, 1990; Pinchosov, and Noy, 1993).

One of the problems, believed to be associated with slow post-hatch growth and performance of poults, is prolonged dependency on residual yolk as the sole source of nutrients. However, Dibner et al. (1998) challenged the long-held concept that young chickens and poults can derive all their early, needed nutrition through the assimilation of nutrients from the yolk sac. Instead, Dibner et al. (1998) suggest that in modern poultry, the yolk contains a vital source of macromolecules such as antibodies and immunoglobulins needed to allow the hatchling to resist the hostilities of a non-sterile environment of a poultry house by developing a high level of passive immunity. Therefore, it is necessary to provide the hatchling with a nutrient source as quickly as possible in order to stimulate gut development and energy metabolism and facilitate early assimilation of immunoglobulins from the yolk sac to meet early demands for passive

immunity. Dibner and colleagues (1998) emphasized that early fasting or delay of consumption of nutrients has an immunosuppressive influence as indicated by negative effects on integrity of the bursa of Fabricius and other immunologically active tissues. Edens (1994) demonstrated that delaying feeding from one to three days post-hatch had significant negative impact on growth, development, and livability of turkey poults, and also provided evidence that delayed feeding facilitated a phosphate trap that was associated with early mortality peaks following feeding by the fasted poults.

Thus, the objective of this report was to determine if concurrent feeding of OASIS™ (OAS) with normal feed affected post-hatch development and growth of some visceral organs and the small intestine of turkey poults.

METHODS AND MATERIALS

Animal Welfare. This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

Animals and Husbandry. Within four hours of hatch, 240 unserviced Nicholas tom poults were obtained from a local turkey hatchery and were transported to North Carolina State University's Agricultural Research Service poultry disease

isolation facility. Upon arrival at the research facility, 12 poults were euthanized and necropsied. Dissected tissues were removed and processed as described below. The remaining poults were leg banded and groups of 12 poults were placed into pens with floors covered with pine wood shavings. Ambient temperature was maintained in the facility by room air conditioning, at a temperature of $34^{\circ} \pm 1^{\circ}\text{C}$, which was maintained for the duration of the experiment. Continuous light from incandescent lamps in the ceiling of the room was provided for all pens.

Treatments. This experiment consisted of three dietary treatments provided for ad libitum consumption: (1) Normal-fed- feed and water, (2) OASIS^{TMa} (OAS, a hydrated nutrient supplement)-fed- normal feed and water with top-dressed OAS, providing 2g OAS per poult (24g per pen), and (3) Solka-Floc^{®b} (Solka, a non-nutritive cellulose fiber)- fed- normal feed and water top-dressed blue-green-dyed Solka, providing 2g per poult. Twelve poults were placed in each pen, and there were a total of six pens for each dietary treatment. A 19th pen contained one group of 12 poults, which were fasted for 24 h and served as the negative control group. At the end of the 24 h fasting period, the 12 poults were killed by CO₂ asphyxiation and tissue samples were collected and weighed to determine the

^a Novus International Inc., St. Louis, MO, USA.

^b International Fiber Corporation, Tonawanda, NY, USA.

weight loss, expressed as relative weight (g/100g BW) due to 24 h fasting and compared with 24 h feeding as described below. All 18 groups of birds received the North Carolina Agriculture Research Service turkey starter diet from 1-16 days of age.

Tissue Collection. Body weights and visceral tissues were collected 1d, 2d, 9d, and 16d post-hatch. A total of 10 poultts were removed randomly from each treatment and were euthanized by CO₂ asphyxiation. Body organs and intestinal segments collected at each necropsy are listed as follows: bursa of Fabricius, heart, liver, lungs, pancreas, duodenum, jejunum, and ileum. The organs were weighed individually and relative weights (g/100g BW) were calculated. Each intestinal segment was flushed with 0.85% saline to washout its contents and residual saline was expressed by gentle application of pressure along the length of the intestinal segment before segments weighed.

Statistical Analysis. The experiment was conducted as a completely randomized design. The data were subjected to analysis of variance of main effects of dietary supplement (no supplement, OAS, or Solka) and time (days post hatch) and their interaction, using the SAS General Linear Models procedures (SAS Institute, 1996). When either a significant main effect or interaction of main effects was

found, means were separated by least significant difference (SAS Institute, 1996). Statements of significance were based on $P \leq 0.05$ or less.

RESULTS

Body weights of 24 h fasted poult (60.1g) were 11.1, 14.9 or 8.5g lighter, respectively, than poult that had been given either normal (71.2g), OAS-supplemented (75.0g), or Solka-supplemented (68.6g) feeds. The 24 h BW of fed poult was significantly greater than the BW of fasted poult, and OAS-fed poult were heavier than poult in the other treatment groups at 2d post-hatch. There were no significant differences among BW of fed poult at 9d, but at 16d post-hatch, both OAS- and Solka-fed groups were heavier than normal-fed poult (Figure 4.1).

Relative weights of visceral organs and small intestine segments from poult at hatch, fasted for 24h, or fed for 24h with either normal, OAS, or Solka supplemented feeds are presented in Table 4.1. Relative weights of several visceral organs and small intestine segments changed during the 24h fasting period when comparisons were made with those tissues from newly-hatched poult. Relative weight increases were found for duodenum, jejunum, ileum, pancreas, and liver in the fasted poult, but a decrease in the relative weights of the heart and lungs was noted in the fasted poult compared with newly-hatched

poults. There was no change in relative weight of the bursa of Fabricius due to 24h fasting.

Comparison of relative organ and small intestine relative weights from 24h fasted and relative weights of those organs and small intestine from 24h fed poults revealed significant increases in those relative weights in the 24h fed poults (Table 4.1). An exception to these observations was found in the relative weights of the heart and lungs from OAS-fed poults, which showed no differences from the relative weights of those organs in the 24h fasted group.

The influence of the 24h concurrent feeding of either OAS or Solka with normal feed on relative weights of the visceral organs and small intestine segments are presented in Figures 4.2-4.9. Heart relative weight of OAS-fed poults was smaller than the relative weights of the heart from normal-fed and Solka-fed poults through the entire 16d post-hatch period (Figure 4.2) and resulted in a significant treatment effect. As the poults aged, relative weights of the heart increased from the 2d relative weight resulting in a significant time effect, but there was no interaction of main effects.

There was a significant time effect associated with the relative weight of the lungs of the fed turkey poults in which relative weights decreased from 2d through 16d post-hatch (Figure 4.3). There was no significant effect of treatment on lung relative weights, and there was no interaction of main effects for lung relative weights.

There was a time effect associated with increasing relative weights of the bursa of Fabricius from 2d through 16d post-hatch (Figure 4.4). There was no treatment effect or interaction of main effects.

There was a significant time effect and treatment effect associated with relative weight changes of the duodenum of fed turkey poults (Figure 4.5). Poults concurrently fed Solka showed an increased duodenum relative weight at 9d post-hatch, but by 16d post-hatch, there were no differences between Solka-fed, normal-fed and OAS-fed poult duodenum relative weight. A similar profile for jejunum (Figure 4.6) and ileum (Figure 4.7) relative weights was observed. For both the jejunum and ileum, there were significant time effects in which relative weights increased at both 9d and 16d post-hatch in all three treatment groups, and in these segments, as the birds aged from 9d to 16d of age, the relative weights decreased to a level that was only slightly greater than relative weights found at 2d post-hatch. The time X treatment interactions for changes in small intestine segment relative weight changes were not significant.

Pancreas relative weights increased significantly as the poults in all three treatment groups aged from 2d to 16d post hatch (Figure 4.8). However, there were neither treatment effects nor interaction treatment with time.

Liver relative weight in all three treatment groups decreased significantly from 2d through 16d post-hatch (Figure 4.9). The decreasing relative weight

profile was equivalent in each of the three treatment groups, and there was no interaction of main effects.

DISCUSSION

Phelps et al. (1987d) administered by gavage antibiotics, vitamins and electrolytes to day old turkey poults and induced significant changes in the hematological profile and feed consumption during the first 7 days post-hatch. Pre-fed poults also gained more weight, drank more water and were more active than the control poults. Nir and Levanon (1993) noted that there was an inverse relationship between feed and water deprivation (24 and 48 h deprivation post-hatch) and subsequent growth to market weights in broiler chickens. Pinchasov and Noy (1993) observed delayed growth only when broilers were denied access to feed for at least 48h. However, turkey poults denied feed access for 24h or 48h post-hatch had reduced body weights at 14d post-hatch (Pinchasov and Noy, 1993). These reports clearly demonstrate the need to provide nutrients as soon as possible in order for the broiler chicken or turkey poult to grow at maximized rates from hatching to marketing.

The introduction of OASISTM (OAS), a hydrated pre-feeding nutrient supplement, to the poultry industry offered an alternative managerial tool to offset the problems associated with delayed placement and its influence on subsequent growth problems in both chickens and turkeys (Dibner et al., 1996 and 1998).

Dibner et al. (1996 and 1998) showed that pre-feeding altered the dynamics of intestinal development an observation that has been tested several times over the past eight years.

Turkey poults, held for 48h in transport boxes, and chicks, held for 36h in transport boxes, had at 4d of age improved BW, which persisted through growout, when they were provided OAS before provision of normal feed (Noy and Sklan, 1999). Indeed, even in disease states, results presented in this thesis (Chapter 3) suggested that OAS pre-feeding improved digestive enzyme activity in certain segments of the small intestine. Batal and Parsons (2002) reported that pre-feeding OAS compared to fasting had a beneficial influence on growth and energy utilization by broiler chickens. Presumably, the improvement was attributable to OAS stimulation of gut development. Maiorka et al. (2003) found that post-hatch water and feed deprivation decreased the rate of gastrointestinal tract development and affected mucosal development as indicated by increased villi per unit area with reduction in villus size in broiler chickens. Maiorka et al. (2003) concluded that provision of both feed and water were necessary to stimulate gut development and was required immediately upon hatching to maximize growth potential of the chicken similar to the observations reported by Dibner et al. (1996 and 1998), Batal and Parsons (2002), and Noy and Sklan (1999) when OAS was provided as a pre-feeding nutrient supplement.

Based upon published information, one must conclude that feed and water deprivation have significant negative effects on the potential for post-hatch growth and development of both turkey poults and chickens. Furthermore, it is apparent that provision of the semi-solid hydrated feed supplement, OAS, also ameliorates many of the problems associated with prolonged access to feed and water for chicks and poults. Thus, to carry these observations an additional step, one wonders if provision of OAS concurrently with normal feed and water will have any influence on growth and development of the turkey poult.

Results from this current study suggest that concurrent OAS and normal feed and water had little influence on growth of the turkey poult through 16d post-hatch. Certainly, when one compares fasting versus feeding and then examines body weight data over the first 24h, it is clear that fed poults gain substantial weight while fasted poults lose hatching weight. Furthermore, even when poults are fasted, there is a redistribution of body mass in the poult with some organs and the small intestine changing relative size. Part of the change in relative size of visceral organs and small intestine are due to loss of moisture, but part of the change in visceral organ and small intestine relative size can be attributed to the utilization of yolk sac nutrients for the purpose of growth and metabolism which has been long established (Dibner et al., 1998). The irrefutable data which have been published that show the necessity for early provision of nutrients and water for the poult and chick and its facilitation of early and persistent growth and

performance clearly point to the yolk sac as the source of materials of even more basic needs such as development of passive immunity (Dibner et al., 1998).

The strong advantage achieved when OAS is provided before provision of a normal feed and water regimen can not be gained with concurrent feeding of OAS and normal feed and water. Whatever advantage is gained with concurrent feeding is transitory being lost within 7d of initiation of concurrent feeding. In this study the concurrent feeding of OAS with normal feed and water influenced development of neither the small intestine, heart, lungs, pancreas, nor the bursa of Fabricius. These observations do not suggest any negative influence of OAS concurrent with normal feeding, only that there is no need to provide OAS when feed and water are already present.

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Table 4.1 Relative organ weights at hatch, after 24 hours fasting post-hatch, or 24 hours after feeding with either no supplement to feed (Normal), OASIS™¹ supplement to feed, or Solka Floc®² supplement to feed.

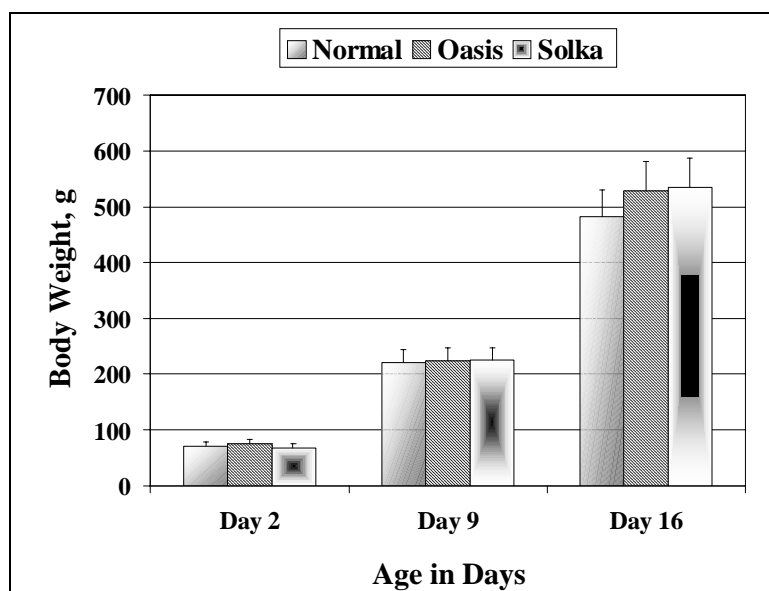
Organ Relative Weight (g/100g Body Weight)³								
Treatment Groups	Duodenum g/100g BW	Pancreas g/100g BW	Jejunum g/100g BW	Ileum g/100g BW	Liver g/100g BW	Heart g/100g BW	Lung g/100g BW	Bursa g/100g BW
Hatch	.601 ± .043	.143 ± .015	.862 ± .056	.742 ± .064	2.92 ± .105	.602 ± .024	.715 ± .049	.086 ± .008
Day 2 Fasted 24 h	.813 ± .079	.19 ± .017	1.219 ± .097	1.018 ± .083	3.105 ± .080	.562 ± .056	.654 ± .062	.088 ± .009
Normal-Fed	.826 ± .086	.186 ± .022	1.257 ± .116	1.051 ± .090	3.336 ± .072	.638 ± .032	.750 ± .048	.096 ± .015
Oasis-Fed	1.021 ± .094	.203 ± .012	1.339 ± .119	1.317 ± .056	3.496 ± .098	.584 ± .017	.672 ± .030	.099 ± .013
Solka-Fed	.965 ± .086	.195 ± .014	1.324 ± .085	1.150 ± .078	3.223 ± .133	.652 ± .022	.756 ± .035	.099 ± .009

¹OASIS™ is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²Solka-Floc® is a non-nutritive cellulose fiber. International Fiber Corp., Tonawanda, NY

³Values are expressed as mean ± SEM, n = 10.

Figure 4.1 Influence of concurrent feeding of either OASIS^{TM1}, the non-nutritive Solka Floc^{®2}, or no supplemental nutrient on male poult body weight (g) at 2, 9, and 16 days after hatch³.

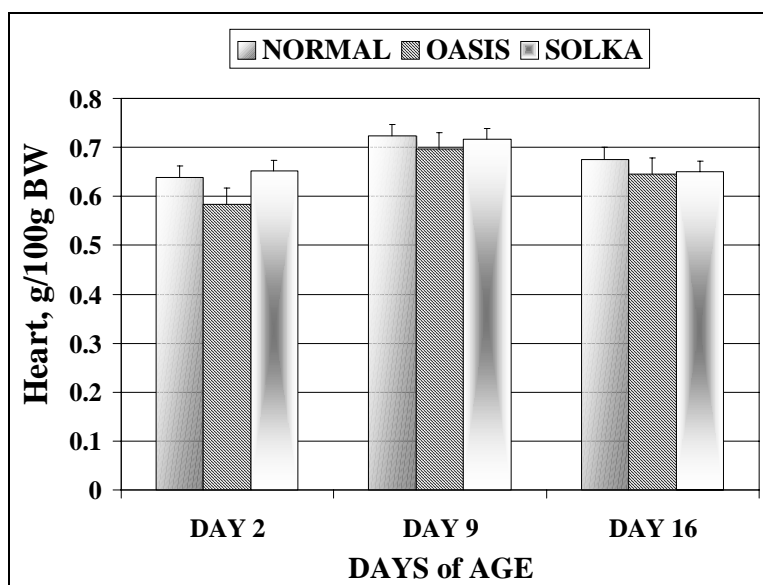


¹OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²Solka-Floc[®] is a non-nutritive cellulose fiber. International Fiber Corp., Tonawanda, NY

³Data is expressed as mean \pm SEM, n = 10 per group

Figure 4.2 Influence of concurrent feeding of either OASIS^{TM1}, the non-nutritive Solka Floc^{®2}, or no supplemental nutrient on male poult heart relative weight (g/100g body weight) at 2, 9, and 16 days after hatch³.

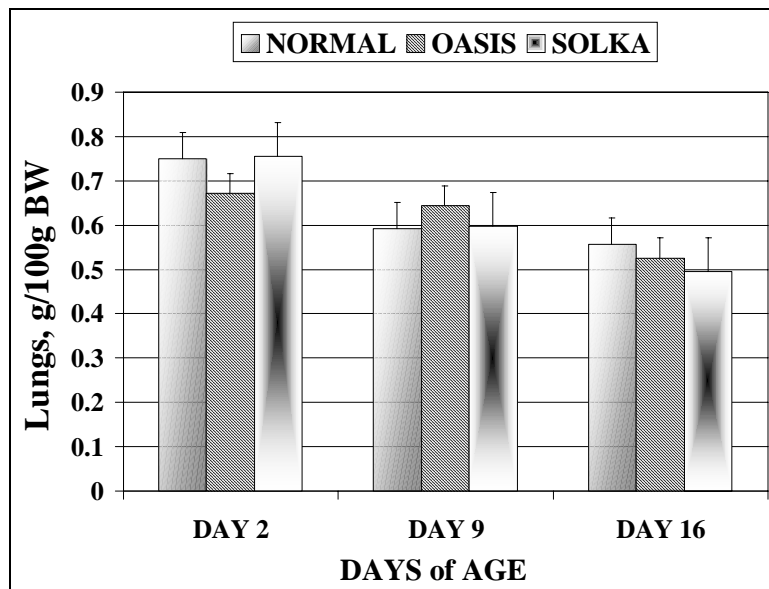


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³Data is expressed as mean \pm SEM, n = 10 per group

Figure 4.3 Influence of concurrent feeding of either OASIS^{TM1}, the non-nutritive Solka Floc^{®2}, or no supplemental nutrient on male poult paired lungs relative weight (g/100g body weight) at 2, 9, and 16 days after hatch³.

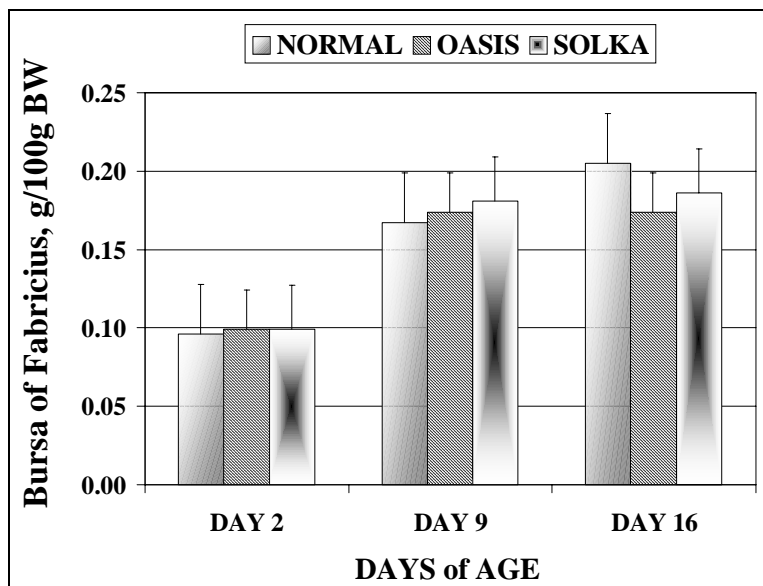


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³Data is expressed as mean \pm SEM, n = 10 per group

Figure 4.4 Influence of concurrent feeding of either OASIS^{TM1}, the non-nutritive Solka Floc^{®2}, or no supplemental nutrient on male poult bursa of Fabricius relative weight (g/100g body weight) at 2, 9, and 16 days after hatch³.

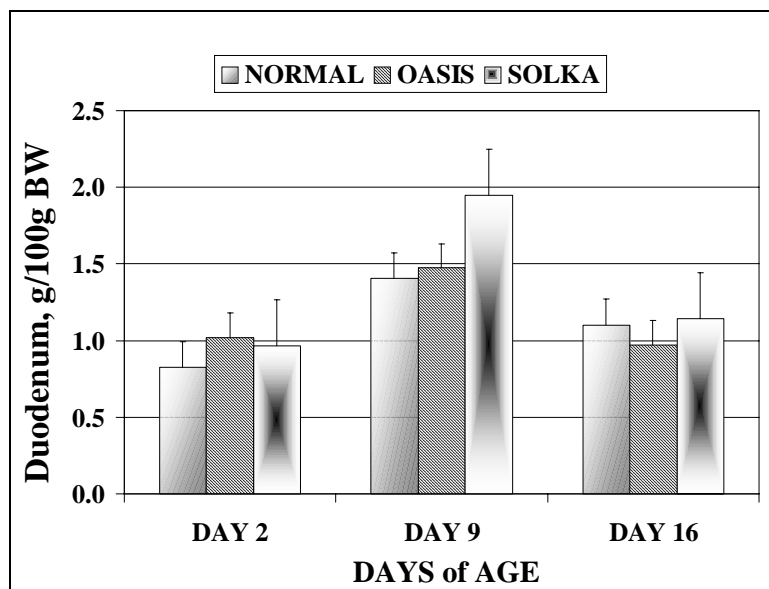


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²Solka-Floc[®] is a non-nutritive cellulose fiber. International Fiber Corp., Tonawanda, NY

³Data is expressed as mean \pm SEM, n = 10 per group

Figure 4.5 Influence of concurrent feeding of either OASIS^{TM1}, the non-nutritive Solka Floc^{®2}, or no supplemental nutrient on male poult duodenum relative weight (g/100g body weight) at 2, 9, and 16 days after hatch³.

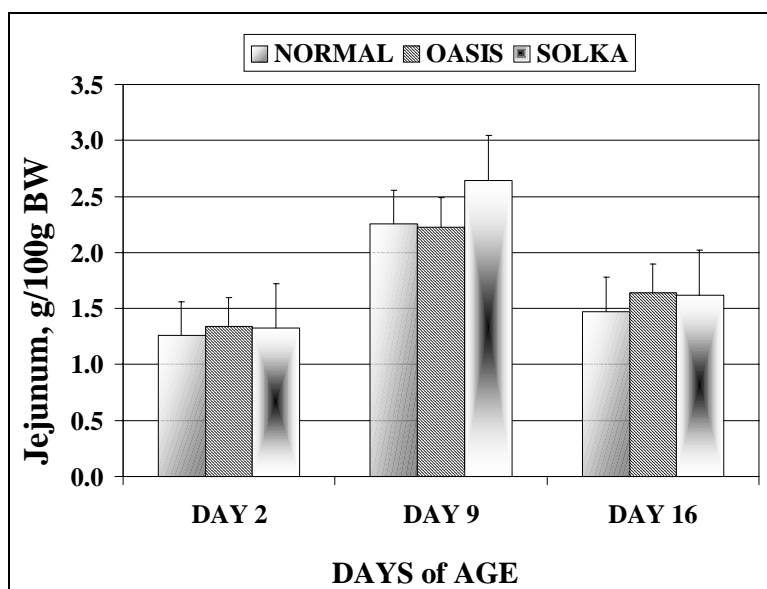


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²Solka-Floc[®] is a non-nutritive cellulose fiber. International Fiber Corp., Tonawanda, NY

³Data is expressed as mean \pm SEM, n = 10 per group

Figure 4.6 Influence of concurrent feeding of either OASIS^{TM1}, the non-nutritive Solka Floc^{®2}, or no supplemental nutrient on male poult jejunum relative weight (g/100g body weight) at 2, 9, and 16 days after hatch³.

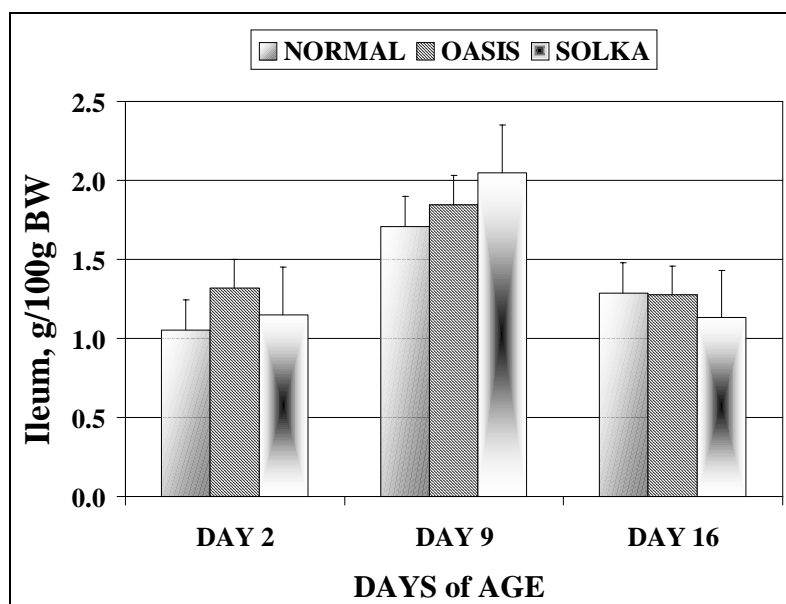


¹OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²Solka-Floc[®] is a non-nutritive cellulose fiber. International Fiber Corp., Tonawanda, NY

³Data is expressed as means \pm SEM, n = 10 per group

Figure 4.7 Influence of concurrent feeding of either OASIS^{TM1}, the non-nutritive Solka Floc^{®2}, or no supplemental nutrient on male poult ileum relative weight (g/100g body weight) at 2, 9, and 16 days after hatch³.

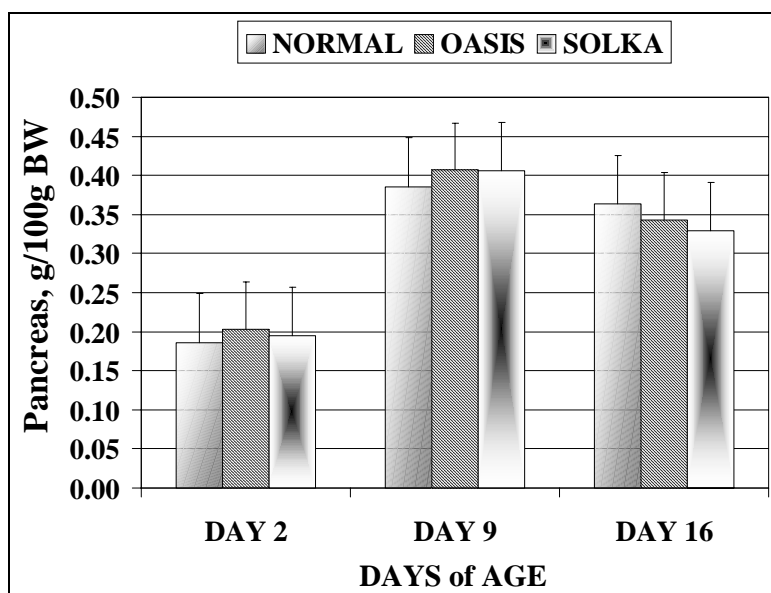


¹OASISTM is a hydrated pre-feeding nutritional supplement. Novus International, Inc., St. Louis, MO

²Solka-Floc[®] is a non-nutritive cellulose fiber. International Fiber Corp., Tonawanda, NY

³Data is expressed as means \pm SEM, n = 10 per group

Figure 4.8 Influence of concurrent feeding of either OASIS^{TM1}, the non-nutritive Solka Floc^{®2}, or no supplemental nutrient on male poult pancreas relative weight (g/100g body weight) at 2, 9, and 16 days after hatch³.

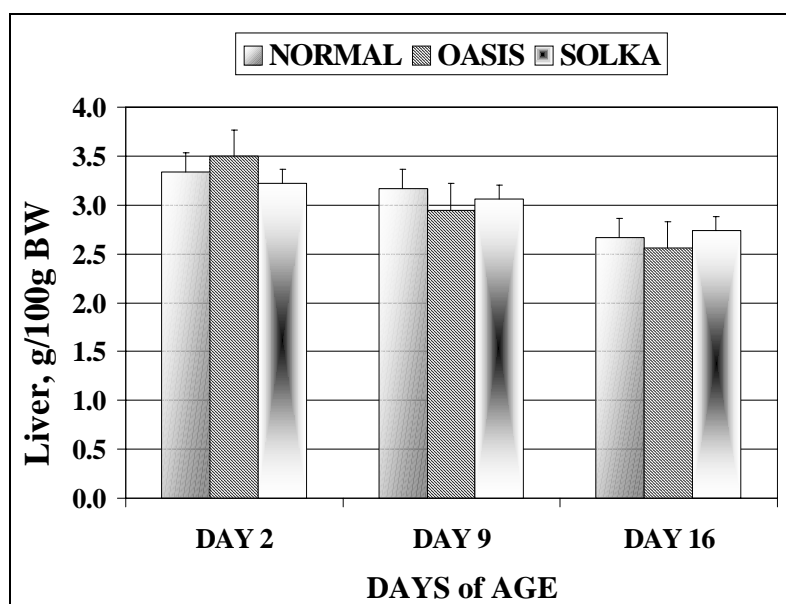


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³Data is expressed as means \pm SEM, n = 10 per group

Figure 4.9 Influence of concurrent feeding of either OASIS^{TM1}, the non-nutritive Solka Floc^{®2}, or no supplemental nutrient on male poult liver relative weight (g/100g body weight) at 2, 9, and 16 days after hatch³.



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³Data is expressed as means \pm SEM, n = 10 per group

SUMMARY AND CONCLUSIONS

The purpose of this thesis was to examine the effects of prefeeding a hydrated nutrient compound, OASIS™ (OAS; Novus International, St. Louis, MO), on (1) resistance to naturally occurring Salmonella infection and monitor three week performance of control and PEMS-infected poult, (2) pancreatic and mucosal digestive enzymes in control and PEMS-infected poult, and (3) post-hatch organ weight. The first objective was important because Salmonella has been implicated in PEMS infections. Typical characteristics of PEMS such as severe diarrhea, high morbidity and mortality, stunting, wasting of musculature, and loss of nearly all adipose tissue strongly suggest a dysfunction in the digestion and absorption of nutrients. Even though PEMS-infected poult are eating feed, the nutrient intake is not sufficient to meet body requirements for maintenance and growth, especially if not absorbed completely.

The first objective of this thesis had a two-fold purpose, to (1) determine the influence of pre-placement OAS supplementation on growth, gut development, and livability of poult through the first 7 d after placement and (2) determine the influence of pre-placement OAS supplementation on the ability of poult to resist development of PEMS after an experimental challenge. The results of this investigation indicated that provision of OAS as a hydrated pre-feeding supplement before placement induced gut development in poult, and

reduced 24h post-hatch loss before placement. Part of the reduction in post-hatch weight loss can be attributed to intake of the OAS and its provision of nutrients and water. Although the provision of OAS as a pre-feeding supplement induced gastrointestinal development and reduced post-hatch body weight loss, the results of this investigation did not show consistently improved body weight gain at 7d of age.

Neither body weight gain nor feed conversion improved as the result of OAS pre-feeding, but 7d livability of pre-fed turkey poult was improved significantly in this investigation. With PEMS infection, either with OAS pre-feeding or no OAS pre-feeding, livability was less than in controls either with OAS pre-feeding or no OAS pre-feeding. However, it was unexpected to see improved livability in OAS-pre-fed poult in the face of PEMS and also *S. arizona* infection.

The intestinal mucosal morphology in control and PEMS-infected poult given either no OAS pre-feeding or with OAS pre-feeding revealed that OAS pre-feeding was probably involved in recovery of the gastrointestinal tract after PEMS infection. Histologically, there was a biological aging, possible even a chronological aging of the intestinal villi in OAS-pre-fed poult. One can conclude that the ability of the OAS-pre-fed poult to resist both viral and bacterial infection might be enhanced. Histology from this investigation suggested that OAS-pre-fed PEMS-infected gut sections frequently looked as if nothing had

changed their morphology, especially at 21d of age. This leads one to conclude that gut repair was enhanced in the OAS-pre-fed poult and that there was a shortened period in which PEMS had its most severe influence in the infected poult. These results were supported by the significantly improved feed conversion ratio at 21d old OAS-pre-fed PEMS-infected poult and in the improved livability of the OAS-pre-fed PEMS-infected poult. This improved performance of OAS-pre-fed poult subjected to enteric disease from PEMS infection is due to improved physiology of gut function resulting from improved villus morphology and function and to early and enhanced repair of damaged tissues in the gut.

The second objective of this investigation was to determine if the pre-feeding of OAS, before the poult were PEMS-challenged, affected performance of the poult through the first 3 weeks post-hatch. We found that impaired digestion in PEMS-infected poult was due to decreased digestive enzyme activity and that pre-feeding OAS immediately after hatch had some ameliorating properties that might aid in the recovery from PEMS. There was an increase in intestinal acid phosphatase (AcP) from hatch to 7d, followed by a decrease in all treatment through d13, and then increasing through d21. The decrease in specific activity of AcP was the greatest in PEMS-challenged poult. After PEMS challenge on d7, the decrease in AcP activity in all three segments (duodenum, jejunum and ileum) was not affected by infection. Recovery in the jejunum was

greater in OAS-pre-fed poult, but this effect was not seen in the jejunum or duodenum. Alkaline phosphatase (ALP) activity remained elevated in all three segments of the intestine in Control and OAS-pre-fed poult when compared to their infected counterparts. OAS pre-feeding did not alter the ALP activities after PEMS infection.

Maltase and sucrase activities in this investigation were characterized by high activity immediately post hatch, and followed by sharp declines in activities as the poult aged. Pre-feeding OAS tended to elevate the maltase activity in PEMS-infected birds. OAS was shown to increase the rate of recovery of sucrase activity of PEMS-infected poult. Lipase activity in this investigation was significantly increased by OAS pre-feeding. The greatest increase was seen with the OAS-pre-fed PEMS-infected poult. OAS in this investigation was found to stimulate intestinal development in poult in both control and PEMS-infected poult.

Dibner et al. (1998) concluded that the yolk contains a vital source of macromolecules such as antibodies and immunoglobulins needed to allow the poult to resist hostilities of a non-sterile environment of a poultry house by developing a high level of passive immunity. Poult must make a rapid transition from dependence on absorbed egg contents as a source of nourishment to reliance on a relatively complex diet. Poult are not fully prepared to digest these complex diets. Stimulation of digestive enzymes, gut development, and energy

metabolism is important for the poult to utilize lipids derived from absorbed yolk material. Such stimulation can come from pre-feeding a hydrated nutrient supplement such as OAS.

Poults are often delayed in placement, and therefore, must depend on the absorbed yolk sac for nutrients. Dibner et al. (1998) also emphasized that delayed consumption of nutrients has an immunosuppressive influence as indicated by the negative effects on integrity of the bursa of Fabricius and other immunologically active tissues. Edens (1994) indicated that delayed feeding from one to three days had significant negative impact on growth, development, and livability of poults.

The third objective of this thesis was to investigate the effects of pre-feeding OAS on visceral organ growth and development of the small intestine of poults. Based upon published information, one must conclude that feed and water deprivation have significant negative effects on the potential for post-hatch growth and development of both turkey poults and chickens. Furthermore, it is apparent that provision of the semi-solid hydrated feed supplement, OAS, also ameliorates many of the problems associated with delayed access to feed and water for chicks and poults.

This investigation produced data that suggest concurrent OAS and normal feed and water had little influence on growth of the turkey poult through 16d post-hatch. When one compares fasting versus feeding and then examines body weight data over the first 24h, it is clear that fed poults gain substantial weight while

fasted poult lose hatching weight. Even when poult are fasted, there is a redistribution of body mass in the poult with some organs and the small intestine changing relative size. Part of the change in relative size of visceral organs and small intestine are due to loss of moisture, but part of the change in visceral organ and small intestine relative size can be attributed to the utilization of yolk sac nutrients for the purpose of growth and metabolism which has been long established.

The strong advantage achieved when OAS is provided before provision of a normal feed and water regimen can not be gained with concurrent feeding of OAS and normal feed and water. Whatever advantage is gained with concurrent feeding is transitory being lost within 7d of initiation of concurrent feeding. In this study, the concurrent feeding of OAS with normal feed and water influenced development of neither the small intestine, heart, lungs, pancreas, nor the bursa of Fabricius. These observations do not suggest any negative influence of OAS concurrent with normal feeding, only that there is no need to provide OAS when feed and water are already present.

In conclusion, pre-feeding OAS has many beneficial influences on poult that are prevented access to feed and water for prolonged periods after hatching. PEMS-infected poult do not have the proper intake of nutrients to meet body requirements, and when the poult do consume feed, the intake is not sufficient due to malabsorption of nutrients. Pre-feeding OAS, when there is delayed access

to feed and water, will stimulate the gastrointestinal tract in poults. This will allow for more complete utilization of the absorbed yolk sack and for more complete digestion and assimilation of nutrients from the complex diet the poults will consume after hatch. The second chapter of this study demonstrated an increased livability of PEMS-infected poults in the face of a *S. arizona* infection. The feed conversion was improved by OAS pre-feeding in this same trial. OAS pre-feeding facilitated the intestinal tract to become better prepared for the enteric and bacterial challenges it faces in the poultry house environment. In PEMS-infected poults, pre-feeding OAS allowed a quicker recovery from the infection.

The results provided in this thesis suggested that it is very beneficial to pre-feed OAS when there is a delay in placement and when the poults will be denied access to normal feed and water consumption. It is beneficial to pre-fed OAS in order for poults to utilize the nutrients in complex poultry diets.

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