NUTRITIONAL PARAMETERS ASSOCIATED WITH ENTERIC *ESCHERICHIA COLI* AND ROTAVIRUS IN POULTS

by

Gregory P. Schmidt

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APPROVED:

L. M. Potter,
Major Advisor

D. R. Bevan

J. A. Cherry

C. H. Domermuth

C. T. Larsen

J. H. Wolford

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Blacksburg, Virginia

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INTRODUCTION

The effects of enteric pathogens on the nutrition of the host have recently become more important, not only in poultry but also in other animals, including man. While subclinical enteric diseases have always existed in poultry, the economic losses due to reduced nutritional performance were negligible compared to the losses from diseases such as Marek's and Salmonella pullorum. However, advanced management practices and improved production efficiency have made the losses due to malabsorption of nutrients more apparent. Similar explanations exist for other food producing animals and for man.

The poultry industry has recognized a condition in recent years, perhaps best described as infectious stunting syndrome (Wyeth, 1982). The syndrome is characterized by large variation within birds in a flock. Severely affected birds have a stunted and unthrifty appearance with noticeable diarrhea in some cases. Losses occur as a result of morbidity and mortality and also from substantial condemnations during processing. The fact that feed is consumed but not converted to body tissue greatly increases the economic losses, estimated as high as several million dollars annually. Both chickens and turkeys have been reported to be affected.

Eradication or control of infectious stunting syndrome can be achieved through an understanding of the course of infection and of the nutritional response of the host. Therefore, the research presented in this dissertation was designed with the general objective of identifying the nutritional response of turkeys to infectious stunting syndrome from

various origins. The more specific objectives were:

1. To characterize the malabsorption of nutrients in poults selected from commercial turkey flocks exhibiting infectious stunting syndrome, and
2. to identify the effects of Escherichia coli and rotavirus on poult performance. The parameters of poult performance considered in addition to body weight gain, feed consumption, and feed efficiency, were digestible dry matter and nitrogen, and metabolizable energy of the diet. Finally, the effects of the pathogens on intestinal mucosal enzyme activities were also investigated.

REVIEW OF LITERATURE

Malabsorption or Pale Bird Syndrome

Recently, considerable attention has been given to field conditions of malabsorption in poultry. Although "malabsorption syndrome" may be a misnomer, various other titles have been used to describe the condition such as helicopter disease, pale bird syndrome, runting and field rickets. Perhaps the most accurate name has been coined by Wyeth (1982) in England as "infectious stunting syndrome". Found in turkeys and chicks, the stunting syndrome was transmitted experimentally from intestinal isolates of unknown pathogens. On commercial farms, both lateral and vertical transmission occurs (Wyeth, 1982).

Rosenberger (1982) has identified reoviruses as the most probable cause of the infectious stunting or pale bird syndrome in broilers. Certain isolates of reovirus were capable of reproducing the mortality and stunting. The multiple clinical signs of reovirus infection are demonstrated by the fact that reovirus isolated from the gut can produce tenosynovitis and bone lesions (Hieronymus et al., 1983). Regardless of the origin of the virus or the inoculation route, Hieronymus et al. (1983) observed severe mortality from experimental reovirus infection. In turkeys, enteritis, morbidity and loss of weight are among the symptoms of naturally occurring reovirus (Gershowitz and Wooley, 1973).

The term "pale bird syndrome" arose from the decreased pigmentation observed in stunted broilers. Lilburn et al. (1982)

reported decreased lipid absorption and metabolizable energy for broilers exhibiting pale bird syndrome. Control birds selected from the same flock also had lower lipid absorption than normal indicating that a subclinical level of infection probably existed. Excreta nitrogen has also been shown to increase with pale bird syndrome (Nelson et al., 1982). Thus, overall nutrient utilization appears to be depressed. The loss of pigmentation has been attributed to poor carctenoid absorption (Colnago et al., 1983). These authors have also reported some benefit of excess vitamin E and selenium in preventing mortality and depressed weight gain.

While reovirus has been implicated in some instances, other causes of pale bird syndrome have been identified. Stale feed containing molds has been implicated as the cause of the condition (Winstead et al., 1982). Mycotoxins have been shown to reduce serum levels of carotenoids independent of any effect on lipid absorption (Osborne et al., 1982). Various mycotoxins affect absorption parameters at different rates, however, decreased utilization of some nutrients is generally apparent. Aflatoxin, even at low levels, reduces absorption of fats, while ochratoxin did not produce fat malabsorption (Osborne et al., 1982). In contrast, digestible dry matter and amino acids were significantly reduced by ochratoxin, with no observable difference due to aflatoxin (Nelson et al., 1982). Thus, multiple symptoms and etiologies appear to exist for "infectious stunting syndrome".

Rotavirus

Rotaviral infections have been identified as the primary cause of

infantile gastroenteritis in humans (Banatvala et al., 1978; Middleton, 1978), resulting in severe diarrhea and sometimes death. The condition is very similar to transmissible gastroenteritis in swine. The ubiquitous nature of rotavirus, independent of clinical outbreaks, makes control of viral infections difficult. Viruses with common antigenic background have been identified for a wide spectrum of animals as summarized by Bartz (1981). The first isolation of rotavirus in turkeys was by Bergland et al. (1977), and later McNulty (1978) and Jones et al. (1979) identified rotavirus from turkeys and laying hens, respectively, exhibiting severe diarrhea. Subsequently, McNulty et al. (1980) classified various turkey and chicken rotavirus strains from eight field isolates. The eight distinct serotypes were found to share common group antigens, in addition to being related to calf rotavirus.

Experimentally, reproduction of rotaviral disease has been difficult for several reasons. Isolation and propagation of the virus in cell culture was slow until the requirement for proteolytic enzymes in the media was identified (McNulty et al., 1980). Also, the viral surface is coated by a glycoprotein which in vivo binds to lactase in the brush border membrane prior to uncoating the virion (Holmes et al., 1976). Holmes et al. (1976) have demonstrated hydrolysis of the glycoprotein coat in vitro with B-galactosidase of E. coli. Establishment of suitable conditions in vitro to mimic the high lactase activity in the intestinal mucosa is difficult. The requirement for high lactase activity may partially explain the pathogenicity of rotavirus primarily in infants, where lactase activity is elevated. Finally, the common antigenic

background of the many rotavirus strains produces antibody responses across species without clinical signs of infection (Woode and Crouch, 1978). As a result, antigen of human or mouse origin can stimulate antibody production in poultry or other domestic animals. Unfortunately, the presence of circulating serum antibodies does not preclude viral outbreaks under natural conditions.

Until recently, the severity of rotaviral infection was assumed related to the size of the dose of virus (Malherbe, 1978). However, Schwers et al. (1983) found low doses of rotavirus to be more effective than high doses when administered to newborn calves. These authors conclude that the high dose may elicit an interferon response in the calf, thereby decreasing the susceptibility to virus. This situation would clearly differ from environmental accumulation of virus particles, which has been shown to increase the severity of infection in piglets (Leece et al., 1978).

Nutritionally, the effects of rotavirus alone are not fully understood. Severe mucosal epithelial damage resulting in malabsorption has been reported for rotavirus in combination with pathogenic E. coli (Leece et al., 1983; Tzipori et al., 1983). Halpin and Caple (1976) observed a reduction in the length of the villi and decreased lactase activity in calves infected with a virus-like agent and E. coli. Rotavirus alone was shown to decrease lactase activity in calves, independent of any morphological effects on the mucosal epithelium (Tzipori et al., 1983). When these same authors combined rotavirus and E. coli as stated above, severe mucosal damage and impaired lactase

activity resulted. In humans, rotavirus infection substantially impairs sodium transport, and in the acute phase of infection a net flux of sodium into the intestinal lumen occurs (Middleton, 1978). Carbohydrate digestion and absorption decrease in infants with rotavirus diarrhea (Sack et al., 1982), resulting in large amounts of undigested carbohydrate in the feces. The presence of undigested carbohydrates in the intestinal lumen may induce an osmotic shift toward the lumen resulting in diarrhea. One may speculate that other nutrients (i.e., electrolytes) are carried with the fluid into the lumen. The decreased absorption of nutrients probably results from the villar atrophy resulting in a predominance of cuboidal crypt cells rather than columnar villous cells (Middleton, 1978; Tzipori et al., 1983).

Enteric Escherichia Coli Infection

While E. coli is known to cause pericarditis and air sacculitis in poultry, the effect of intestinal E. coli infection has not been reported. Although coliforms comprise part of the normal intestinal microflora in most animals, increases in the number of coliforms are associated with diarrhea and unthriftiness. Problems resulting from enteropathogenic E. coli infections are well documented in swine (Armstrong and Cline, 1976; Prochazka et al., 1982), especially related to weanling pigs. Recent work has elucidated some of the nutritional responses of animals to E. coli infection.

In most cases, E. coli is associated with severe diarrhea which is due to hypersecretion in the intestinal lumen in response to enterotoxin (Tzipori et al., 1983). The hypersecretion of fluids results in losses of

water and ions mainly, however, high losses of protein (albumin) have been demonstrated to accompany the efflux of fluid into the intestinal lumen (Prochazka et al., 1982). The loss of albumin into the lumen can occur independently of clinical signs of diarrhea. Damage to the epithelial lining is probably responsible for the increased permeability. Decreases in proteinase and amylase activity have been observed in the intestinal contents of pigs infected with E. coli (Liven, 1978). Conversely, lipase activity was shown to increase in the same study. Mucosal lactase activity was examined in E. coli infected calves (Tzipori et al., 1983) and found to be unchanged.

Dietary manipulation has been unsuccessful in alleviating colibacillary diarrhea in swine (Armstrong and Cline, 1976). Changes in dietary protein or energy level did not prevent the accumulation of fluid in ligated intestinal segments inoculated with E. coli. Leece et al. (1983) proposed that the nutrient surge of a high calorie-high protein diet may actually increase the severity of diarrhea and shedding of E. coli in the feces. At weaning, the introduction of a high density feed in combination with environmental stresses makes the piglet highly susceptible to diarrhea.

Substantial protection from mortality due to E. coli infection has been achieved from Lactobacillus inoculation in poultry (Watkins et al., 1982), however, little effect on body weight gain has been observed. Variable results are reported concerning the ability of Lactobacillus to control the numbers of E. coli in the birds intestine. While Watkins et al. (1982) were able to control the numbers of E. coli by competition

with Lactobacillus, Adler and DaMassa (1980) were unable to depress viable E. coli concentrations with Lactobacillus. Such conflicting results are more than likely due to the different strains of both organisms used. The interactions between various enteric bacteria and bacteria and viruses have only recently been investigated. For example, E. coli has been shown to play a significant role in rotavirus infections (Tzipori et al., 1983; Woode and Crouch, 1978) in mammals, while limited complications may result from either pathogen alone.

Malabsorptions from Other Infectious Agents

Malabsorption syndromes have been identified with bluecomb disease in turkeys (Duke et al., 1969b; Duke et al., 1970a, b), and with coccidial infections in chickens (Turk and Stephens, 1969; Turk, 1972). The effects of the infectious agent on the nutrition of the host appear to be comparable for both species. A very marked difference between coccidial infections and bluecomb infection is that during the infectious phase and the recovery phase of coccidiosis, absorption of most nutrients actually increases. Following the recovery period, absorption generally returns to the level observed in uninfected birds.

In bluecomb infected turkeys, absorption of water, Na^+ and Cl^- were significantly lower than in normal birds (Duke et al., 1969b). Gastrointestinal transit times were decreased in infected birds (Duke et al., 1970a) to a greater extent than in fasted birds as early as 28 hours after infection. Decreased dry matter digestibility was observed in infected turkeys as early as one day after inoculation in ad libitum fed turkeys (Duke et al., 1970b). Unfortunately, these

experiments were not continued long enough to observe whether the birds recovered from the condition.

The effect of coccidial infections on the absorption of various nutrients has been researched extensively and reviewed thoroughly by Turk (1978). Coccidial infections of the upper intestinal tract were found to decrease both zinc and oleic acid absorption from 5 to 10 days after inoculation (Turk and Stephens, 1967). The relative absorption rates vary for different nutrients depending on the infectious agent and the time post infection.

Protein digestion and absorption were shown to decrease during the acute phase of coccidial infections of the jejunum (Turk, 1972). Infections of other areas of the intestinal tract had little effect on protein absorption. The level of crude protein in the diet has been shown to influence the severity of weight loss during coccidiosis (Sharma et al., 1973). Increasing the protein level protected chicks against weight reduction. Mortality and oocyst production were also greatest with the highest level of protein. The amount of protein retained from the diet, however, was significantly reduced during the chronic period of infection (Sharma and Fernando, 1975). This same pattern was also observed for the percent fat and metabolizable energy of the diet.

The absorption of free amino acids in the presence of coccidial infections varies with the area of the intestine affected. Ruff (1978) observed methionine absorption to increase in the jejunum and decrease in the duodenum of isolated intestinal segments infected with various

species of coccidia. Turk (1978) observed decreased methionine uptake from the duodenum of intact chicks. The absorptions of other amino acids were found to be unaffected by coccidial infections. The absorption of free amino acids followed the patterns similar to other nutrients in that absorption was enhanced during the infective and recovery phases of the disease.

The increase in absorption of all nutrients observed during the recovery phase from coccidial infection appears to cause a period of compensatory growth (Sharma et al., 1973; Turk, 1978). Weight gain of infected birds during this period can result in average weights similar to that of uninfected controls. The period of compensatory growth due to hyperabsorption may result from the increase in villus height during the recovery phase of the intestinal lining (Turk, 1978). The malabsorption syndrome present in the turkey industry today does not appear to exhibit this compensatory growth found after coccidial infections. The decreased absorption of nutrients may result from permanent damage to the digestive tract lining or from a continual reinfection by the infectious agent.

Since the final site of protein digestion occurs at the mucosal membrane of the small intestine, any damage to the mucosal lining will severely affect protein digestion and absorption. The activities of intestinal dipeptidases have been examined in swine, rats and humans (Lindberg, 1966). As a result of impaired protein absorption from mucosal destruction, enzyme synthesis will be inhibited which prevents the animal from recovering from the condition of protein depletion.

Decreases in intestinal dipeptidase activities have been observed in primary protein malnutrition (Hazuria et al., 1974) even when morphological changes in the mucosa were not apparent. This effect on enzyme activity may extend to other important enzymes of the mucosal surface.

In pigs parasitized by the nematode, Strongyloides ransomi, the activities of both intestinal dipeptidases and disaccharidases were decreased (Enigk et al., 1976). Disaccharidase activity was reduced in a mild infection while dipeptidase activity remained normal. The mild infection damages the brush border where the disaccharidases are mainly located. Dipeptidase activity occurs predominantly in the cytosol. Evidently, the activity of the enzymes located in the intestinal epithelium alters with gross changes in the mucosal surface. Intestinal diseases associated with flattening of the mucosa particularly affect the level of enzyme activities (Sadikali, 1971).

SERIES 1. CHARACTERIZATION OF MALABSORPTION SYNDROME IN STUNTED TURKEYS

In this study, two ten-day digestibility experiments were conducted to determine if stunted turkeys from commercial flocks resulted from malabsorption of nutrients per se. The ability to consume feed, but not absorb nutrients, is a more serious problem than a lack of feed consumption, since the expense of feed will be invested on turkeys that do not convert feed to body tissue. The differences in protein, amino acids and dry matter digestibilities and metabolizable energy between normal and stunted turkeys were investigated. Scanning electronmicrographs also were obtained from the intestinal mucosal lining to examine differences in mucosal morphology of both groups of turkeys in the second experiment.

EXPERIMENTAL PROCEDURES

Two digestibility trials were conducted with Large White turkeys selected from commercial flocks based on body size. In Experiment 1, 13 normal females and 20 stunted females were obtained at 8 weeks of age and divided into three pens per treatment (normal vs. stunted). Prior to the start of the experiment, the birds had received no vaccinations. For Experiment 2, 5-week old female turkeys were obtained in the same manner as described above, such that 18 normal females and 21 stunted females were each divided into three pens.

Each experiment consisted of a 10-day digestibility trial in which feed consumption per pen was recorded daily on days 1 through 9, with total excreta collection conducted on days 2 through 10. The excreta collected on any given day was assumed derived from the previous days feed in all digestibility calculations. In all cases digestibility refers to the difference between the quantity of feed consumed and the total excreta collected. Excreta contained both fecal and urinary products. In addition, individual body weights were recorded daily.

The excreta were dried at 60 C in a forced-draft oven, and weighed and stirred every 24 hr until dry. In most cases, 48 hr was sufficient time for complete drying. After obtaining total dry excreta weights for each pen per day, the dry excreta for each pen for all nine days were mixed and a representative sample taken. The six resulting samples were ground to pass through a 60-mesh screen in preparation for chemical analyses. Samples of the experimental diets were also ground in a similar manner. For Experiment 1, a commercial turkey

grower diet was obtained from the house at the time the birds were selected. The diet used in Experiment 2 is presented in Table 1.

Gross energy analyses of feed and excreta were conducted with an adiabatic bomb calorimeter. Metabolizable energy (AME) was calculated using the equation of Hill and Anderson (1958). Nitrogen contents of feed and excreta were determined using the Kjeldahl procedure (AOAC, 1980). Amino acid analyses of feed and excreta were conducted on a Beckman Model 121 Amino Acid Analyzer following a 24-hr hydrolysis.

At the end of Experiment 2, two birds from each treatment were sacrificed and sections of the intestine prepared for Scanning Electron Microscopy (SEM). Sections were removed from midway of the ascending duodenum, mid-jejunum, and mid-ileum, opened to remove the intestinal contents and rinsed in physiological saline. After rinsing, the sections were fixed immediately in 5% glutaraldehye in sodium cacodylate fixative.

Average dry matter digestibilities for each day, and average nitrogen and amino acid digestibilities and AME_n for each group were compared using the Students t-test.

RESULTS

Experiment 1.

Average daily dry matter digestibilities for normal and stunted turkeys are presented in Table 2. Over the ten-day period, the average digestibility value of 64.8% for the stunted turkeys was significantly less than that of 69.2% for the normal turkeys. On all days, the stunted turkeys had a lower digestibility value than the controls. The standard errors were also much greater for the stunted birds indicating the large variation in that group.

When expressed on an as-fed basis, the feed consumption of the stunted group was significantly less than that of the controls (Table 3). However, when feed consumption is expressed as a percent of body weight, the stunted birds consumed significantly more feed than the normal turkeys.

The nitrogen content of the excreta collected over the 10-day period was larger for the stunted turkeys resulting in a significantly higher percent nitrogen digestiblity for the normal controls (Table 4). Also presented in Table 4 are the AME and AME_n of the diet for both treatment groups. Although differences were not significant, the data for excreta energy and metabolizable energy, whether uncorrected or corrected to nitrogen equilibrium, paralleled that of the nitrogen digestibility data. Individual amino acid digestibilities for both groups are presented in Table 5. Only proline digestibility was significantly different between the controls and stunted turkeys. The average amino acid digestibilities were 83.6% and 81.6% for the normal and stunted

birds, respectively.

Experiment 2.

When compared to Experiment 1, the differences in dry matter digestibility obtained in this experiment were small and non-significant (Table 6). Over the ten-day period, an average of 60.9% was obtained for the stunted turkeys and 62.9% for the controls ($.20 \leq P \leq .10$).

The data for feed consumption expressed in grams or as a percent of body weight are presented in Table 7. On the average, the stunted birds consumed about 2.0% more feed than the controls when expressed as a percent of body weight (10.3 vs. 8.3%, respectively). The results of this experiment were more variable than Experiment 1. However, this difference in feed consumption was significant.

A significant difference in excreta nitrogen and nitrogen digestibility between the stunted and normal turkeys was observed in this experiment (Table 8). As in Experiment 1, a small but non-significant difference was noted for AME and AME_n.

Average amino acid digestibilies were 84.8% for the controls and 83.4% for the stunted birds (Table 9). No significant differences for any individual amino acids were observed.

Scanning Electron Micrographs of duodenal mucosa from normal and stunted turkeys are presented in Figure 1. Note in the section taken from the stunted turkey that the epithelial cells are less defined, lacking the distinct proliferation of those from the normal section. This flattening of the columnar epithelial cells is characteristic of decreased mucosal surface area.

DISCUSSION

The data obtained in Experiments 1 and 2 indicate that a malabsorption *per se* occurred in the turkeys obtained from commercial flocks. In Experiment 1, a significant difference ($P \leq .05$) in dry matter digestibility was observed between normal and stunted turkeys while in Experiment 2 the difference was not significant. The diet used in Experiment 1 was a pelleted commercial formulation. In Experiment 2, the feed was supplied as mash and contained only 2% added fat, which may account for the lower dry matter digestibility for the controls in Experiment 2 compared to those in Experiment 1. Since the birds in Experiment 2 were younger than those in Experiment 1 (5 weeks *vs*. 8 weeks), one would expect higher digestibility values for the former. However, a higher soybean meal content of the diet could explain the lower digestibility values.

The difference between experiments may be due to the fact that the turkeys for Experiment 2 were removed from the flock about one week before the start of the digestibility trial, whereas those in Experiment 1 were placed on the digestibility trial the day after removal from the flock. The trend towards improved dry matter digestibility over the experimental period in Experiment 1 supports this claim. Presented graphically in Figure 2, this linear improvement in digestibility for the stunted turkeys compared to the controls was significant ($P \leq .01$), while no trend was observed in Experiment 2.

Duke *et al*. (1970a) observed a marked decrease in digestibility for about 14 days following inoculation with bluecomb in 7-week old

turkeys. By 15 to 17 days post inoculation, the bluecomb inoculated turkeys had dry matter digestibilities similar to that of the controls. The present condition found in commercial turkeys flocks appears to occur at about two weeks of age, however, the stunted turkeys do not seem to recover. A similar stunting has been reported in Missouri (Trampel et al., 1983), apparently resulting from a parvo-like viral infection. The appearance of affected turkeys is similar to the "pale bird" syndrome described for broilers (Rosenwald, 1982; Wyeth, 1982), characterized by stunted birds with ruffled feathers.

The difference in feed consumption when expressed as a percentage of body weight between stunted and normal turkeys support the dry matter digestibility data. Stunted turkeys consumed adequate feed for their body size as did the control turkeys, however, the stunted turkeys were less able to absorb or metabolize nutrients from the feed. The stunted turkeys may have experienced a period of anorexia which predisposed the malabsorption condition. Duke et al. (1970b) observed a decrease in dry matter intake post inoculation. Force-feeding to restore intake two days post inoculation did not improve digestibility to the level of controls. Since the turkeys at a common age obtained for the present study differed so greatly in body size between stunted turkeys and controls (788 vs. 1,982 g, respectively), as-is feed consumption would be a misrepresentation.

The percent nitrogen in the excreta of the stunted turkeys was greater than for the controls in both experiments. The protein component of the diet may be the nutrient most affected by the

malabsorption. During chronic protozoa infections, protein digestion and absorption decreases in the jejunum (Turk, 1972) as does protein retention (Sharma and Fernando, 1975). In Experiment 1, a 12% difference in nitrogen digestibility was found between normal and stunted turkeys, while a 5% difference occurred in Experiment 2. The larger difference for Experiment 1 reflects the significant difference in dry matter digestibility observed in that experiment. The 5% decrease in nitrogen digestibility in Experiment 2 occurred independent of a difference in dry matter digestibility.

Since the total nitrogen content of the excreta was determined via the Kjeldahl procedure (AOAC, 1980), the resulting value includes nitrogen of uric acid origin. The key point of interest is whether the increase in excreta nitrogen for the stunted turkeys results from undigested protein or from an increase in uric acid. In both experiments only small differences in amino acid digestibilities were found between normal and stunted turkeys. By calculating the nitrogen contribution to the excreta from amino acid origin, the difference in excreta nitrogen cannot be fully accounted for by undigested protein (amino acid nitrogen in excreta). In Experiment 1, 1.85% nitrogen was obtained from the amino acids in the excreta for both groups. In Experiment 2, 1.06% and 1.02% nitrogen was derived from amino acids in the excreta for the stunted and normal turkeys, respectively. Therefore, the difference in excreta nitrogen may be due to increased uric acid excretion in the stunted birds.

Rosenberger (1982) has suggested that the stunting syndrome may

be the result of impaired liver function caused by reovirus infection in broilers. The increase in uric acid in the excreta of stunted birds would result from the breakdown of protein in the liver and an inability to assimilate protein as body tissue. Lilburn et al. (1982) conducted an experiment similar to the present one using broilers exhibiting pale bird syndrome. In their experiment, lipid absorption and AME_n were impaired in the infected group compared to the controls. However, the control birds selected from the commercial flock also exhibited lower lipid absorption and AME_n than University of Georgia controls, indicating subclinical infection in the controls. Differences in lipid content of the excreta were also observed between the three groups of broilers. The decrease in lipid absorption did not account for the total difference in AME_n suggesting some other nutrient is also involved.

Scanning electron micrographs of the duodenal mucosa indicate some change has occurred in the absorptive cells of the stunted birds in Experiment 2. The condition of the mucosa reflects a lack of maturity and proliferation of the columnar epithelial cells. This observation differs from that of Michael and Hodges (1975), who observed protruding cells infected with Eimeria acervulina in the duodenum. Various coccidia species were found to have different affects on the intestinal surface (Witlock and Ruff, 1977). The mucosa examined from the stunted turkey may represent immature cells, replacing those sloughed from the serosa by an infectious agent.

Based on the present study, the stunting condition appears to result from an overall unthriftiness of the affected turkeys. The

differences in digestibility and AME_n observed in this study do not seem large enough to account for the great difference in the body weights of the turkeys. The condition appears to represent a true malabsorption in as much as feed utilization is decreased in the affected turkeys. Impairment of biosynthetic processes following absorption may also play a major role in the stunting syndrome.

SUMMARY

Two experiments were conducted with stunted poults selected from commercial turkey flocks to evaluate the nutritional performance of stunted poults in contrast to control birds from the same flocks. In Experiment 1, dry matter digestibility was 64.8% for the stunted birds, while the control groups had a value of 69.2%. As expected, feed consumption was reduced in the stunted birds. However, when feed consumption was expressed as a percent of body weight, stunted poults consumed an average of 2.6% more feed. In Experiment 2, the difference in dry matter digestibility was small and nonsignificant (60.9 vs. 62.9% for stunted and controls, respectively). As a percentage of body weight, the stunted birds consumed 2.0% more feed than the control groups in Experiment 2.

Nitrogen digestibility decreased for the stunted poults in both experiments as a result of a marked increase in excreta nitrogen content. In a similar manner, gross energy of the excreta was increased while metabolizable energy decreased for the stunted groups. These increases in excreta nitrogen and energy were indicative of a malabsorption of nutrients in the stunted turkeys. Average amino acid digestibilities were not substantially changed for the stunted birds in either experiment. Differences between the two test groups were 2.0% in Experiment 1 and 1.4% in Experiment 2.

Alterations in the intestinal mucosa of stunted poults was observed using scanning electron microscopy in Experiment 2. A general lack of cell proliferation of the epithelium from stunted poults compared to

controls supports the hypothesis that an infectious agent or toxin was involved.

Table 1. Experimental diet in Experiment 2

Ingredient	g/kg
Ground yellow corn	426.339
Stabilized fat	20.
Dehulled soybean meal	445.
Meat and bone meal	25.
Menhaden fish meal	50.
Defluorinated phosphate	19.
Ground limestone	5.
Sodium chloride	4.
DL-Methionine	3.
Trace mineral mix[1]	0.385
Vitamin and feed additives[2]	2.276

[1]Supplied the following amounts of trace minerals in mg/kg complete diet: 150 manganese, 100 zinc, 70 iron, 10 copper, 2.6 iodine and .8 cobalt from manganese oxide, zinc oxide, ferrous sulfate, copper oxide, calcium iodate and cobalt carbonate, respectively.

[2]Supplied the following quantities of vitamins and feed additives in mg/kg complete diet unless otherwise stated: 13,228 IU vitamin A, 3,312 ICU vitamin D_3, 11 IU vitamin E, 7.05 menadione sodium bisulfite complex, 1.1 thiamine HCl, 5.5 riboflavin, 16.5 calcium pantothenate (D), 66 niacin, 1,000 choline chloride, 3.3 mcg vitamin B_{12}, 1.1 folic acid, 110 mcg biotin, 2.2 pyridoxine HCl, 125 ethoxyquin and .2 selenium.

Table 2. Average dry matter digestibilities of stunted and normal turkeys (Experiment 1)

Day	Stunted	Normal	Difference
1	57.6±2.3[1]	67.0±1.9	9.4*
2	60.5±3.7	68.5±1.1	8.0
3	63.0±2.8	68.0±0.3	5.0*
4	65.3±1.7	69.4±0.4	4.1
5	66.5±1.9	67.9±0.5	1.4
6	66.4±1.3	69.4±0.5	3.0
7	67.2±0.7	70.4±1.0	3.2
8	67.1±0.9	68.7±1.2	1.6
9	69.6±0.9	73.1±1.2	3.5
Average	64.8±1.3	69.2±0.6	4.4*

*$P \leq .05$

[1]Mean ± SEM. N = 3 observations per treatment.

Table 3. Average feed consumption expressed in grams and as a percent of body weight for stunted and normal turkeys (Experiment 1)

Day	Feed consumption		Feed consumption/Body weight		
	Stunted	Normal	Stunted	Normal	Difference
1	73.9±2.3[1]	133.6±8.7	8.3±0.4	5.9±0.4	2.4*
2	83.4±2.1	136.5±3.9	9.0±0.1	5.9±0.2	3.1*
3	85.9±3.0	138.3±6.4	8.9±0.3	5.8±0.2	3.1*
4	86.7±2.4	144.8±7.0	8.7±0.2	5.9±0.2	2.8*
5	87.1±1.6	149.4±4.1	8.4±0.2	5.9±0.1	2.5*
6	95.4±4.0	153.9±6.9	8.7±0.1	6.0±0.2	2.7*
7	96.4±5.9	158.7±5.7	8.4±0.1	6.0±0.1	2.4*
8	95.5±6.7	156.3±1.5	7.9±0.3	5.7±0.1	2.2*
9	111.3±5.3	168.8±7.4	8.3±0.1	5.9±0.2	2.4*
Average	90.6±2.0	148.9±5.1	8.5±0.1	5.9±0.2	2.6*

[1] Mean ± SEM. N = 3 observations per treatment.

*$P \leq .01$

Table 4. Excreta nitrogen, nitrogen digestibility, excreta energy, AME and AME_n on a dry matter basis for stunted and normal turkeys (Experiment 1)

Treatment	Excreta nitrogen (%)	Nitrogen digestibility (%)	Excreta energy (kcal/g)	AME (kcal/g)	AME_n (kcal/g)
Stunted	7.39±.38[1]	43.53±7.5*	3.959±.021	3.440±.116	3.283±.090
Normal	6.64±.08	55.10±7.5	3.838±.043	3.619±.021	3.420±.018

[1] Mean ± SEM. N = 3 observations per treatment.

*$P \leq .001$

Table 5. Apparent amino acid digestibilities for stunted and normal turkeys, % (Experiment 1)

Amino acid	Stunted	Normal	Difference
LYS	86.0±1.8[1]	89.6±1.2	3.6
HIS	91.9±0.8	92.6±0.9	.5
ARG	93.2±0.4	93.3±1.3	.1
ASP	83.3±1.1	85.9±0.2	2.6
THR	78.5±0.9	81.1±0.5	2.6
SER	80.0±1.1	81.8±0.2	1.8
GLU	85.9±0.9	87.7±0.2	1.8
PRO	81.5±0.7	85.0±0.3	3.5*
GLY	67.5±1.9	74.0±1.5	6.5
ALA	47.9±2.7	50.9±0.4	3.0
VAL	82.9±0.8	83.3±0.2	.4
ILE	85.9±1.2	87.5±0.8	1.6
LEU	87.3±0.6	88.2±0.5	.5
TYR	84.4±1.0	86.5±0.1	1.1
PHE	86.7±1.3	88.4±0.3	1.7
Average	81.6±1.5	83.6±0.3	2.0

[1]Mean ± SEM. N = 3 observations per treatment.

*$P \leq .05$

Table 6. Average dry matter digestibilities of stunted and normal turkeys (Experiment 2)

Day	Stunted	Normal	Difference
1	63.7	65.0	1.3
2	57.7±3.9[1]	65.3±1.9	7.6
3	64.3±2.4	63.0±1.1	-1.3
4	58.3±1.0	60.8±1.3	2.5
5	59.5±0.9	60.7±1.0	1.2
6	60.1±1.3	61.2±0.7	1.1
7	62.6±1.0	63.7±1.4	1.1
8	64.6±0.9	64.5±0.4	-0.1
9	59.7±2.6	62.9±0.9	3.3
Average	60.9±1.1	62.9±0.3	2.0

[1]Mean ± SEM. N = 3 observations per treatment in all cases but day one, when N = 1.

Table 7. Average feed consumption expressed in grams or as a percent of body weight for stunted and normal turkeys (Experiment 2)

Day	Feed consumption		Feed consumption/Body weight		
	Stunted	Normal	Stunted	Normal	Difference
1	74.1± 5.0[1]	148.2± 5.0	10.8±0.5	8.6±0.1	2.2*
2	70.4± 9.5	158.7±13.4	10.0±1.4	8.7±0.6	1.3
3	77.6± 2.6	156.9± 6.6	10.5±0.5	8.2±0.3	2.3*
4	82.5± 1.1	173.9± 4.5	10.6±0.1	8.6±0.1	2.0*
5	80.6± 3.5	161.6± 4.4	9.9±0.4	7.8±0.3	2.1*
6	86.7± 7.9	176.3± 3.4	10.1±0.8	8.1±0.0	2.0*
7	93.3± 6.7	188.4± 4.9	10.5±0.5	8.3±0.1	2.2*
8	102.6± 8.5	192.5± 6.3	10.4±0.4	8.1±0.2	2.3*
9	100.0±14.6	192.8± 7.9	9.8±0.9	7.8±0.2	2.0*
Average	85.3± 5.5	172.1± 5.3	10.3±0.5	8.3±0.1	2.0*

[1] Mean ± SEM. N = 3 observations per treatment.

*$P \leq .01$

Table 8. Excreta nitrogen, nitrogen digestibility, excreta energy, AME and AME_n for stunted and normal turkeys (Experiment 2)

Treatment	Excreta nitrogen (%)	Nitrogen digestibility (%)	Excreta energy (kcal/g)	AME (kcal/g)	AME_n (kcal/g)
Stunted	7.80±.06*	45.85±.75*	3.769±.019	3.173±.007	2.975±.005
Normal	7.29±.09[1]	50.59±.49	3.749±.032	3.212±.023	2.995±.023

[1] Mean ± SEM. N = 3 observations per treatment.

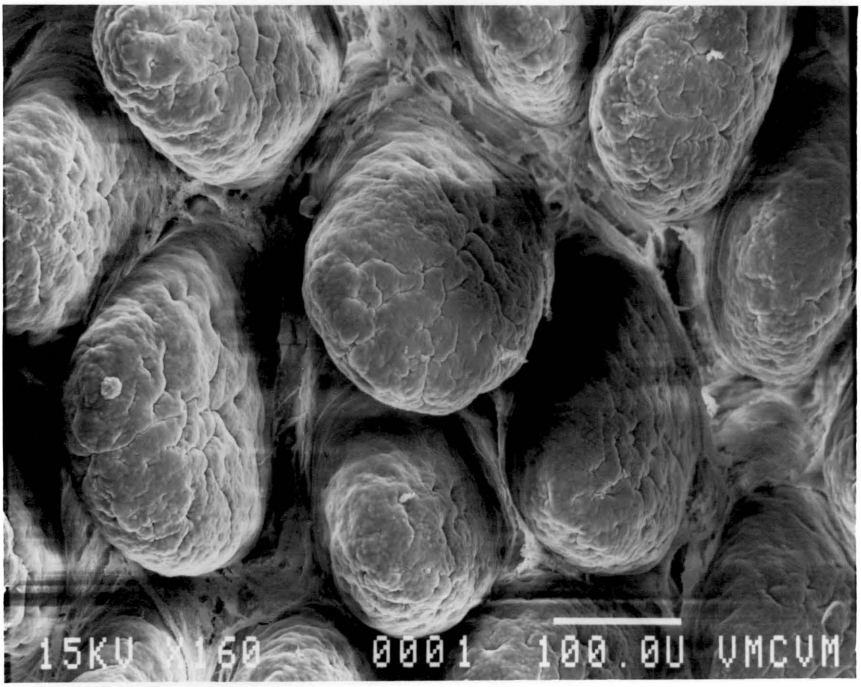
*$P \leq .05$

Table 9. Apparent amino acid digestibilities for stunted and normal turkeys (%) (Experiment 2)

Amino acid	Stunted	Normal	Difference
LYS	87.6±0.9[1]	89.6±5.4	2.0
HIS	92.5±0.7	91.6±1.9	-.9
ARG	93.2±0.5	93.7±0.4	.5
ASP	84.3±0.9	84.7±0.2	.4
THR	78.4±1.8	78.7±0.2	.3
SER	79.3±2.1	81.4±0.4	2.1
GLU	87.4±0.9	88.1±0.3	.5
PRO	84.8±1.3	85.4±0.3	.6
GLY	67.4±5.0	66.6±1.8	-.8
ALA	81.0±1.4	82.3±0.1	1.3
VAL	80.2±1.4	81.7±0.5	1.5
ILE	86.3±1.3	88.0±0.8	1.7
LEU	87.0±0.9	87.4±0.4	.4
TYR	83.7±1.3	87.3±1.1	3.6
PHE	86.7±1.1	87.8±0.4	1.1
Average	83.4±1.3	84.8±0.2	1.4

[1]Mean ± SEM. N = 3 observations per treatment.

Figure 1. Scanning electron micrographs of the duodenal mucosa of stunted and normal turkeys. Plate 1: Stunted (a) and normal (b) turkeys X160. Plate 2: Stunted (a) and normal (b) turkeys X1500.



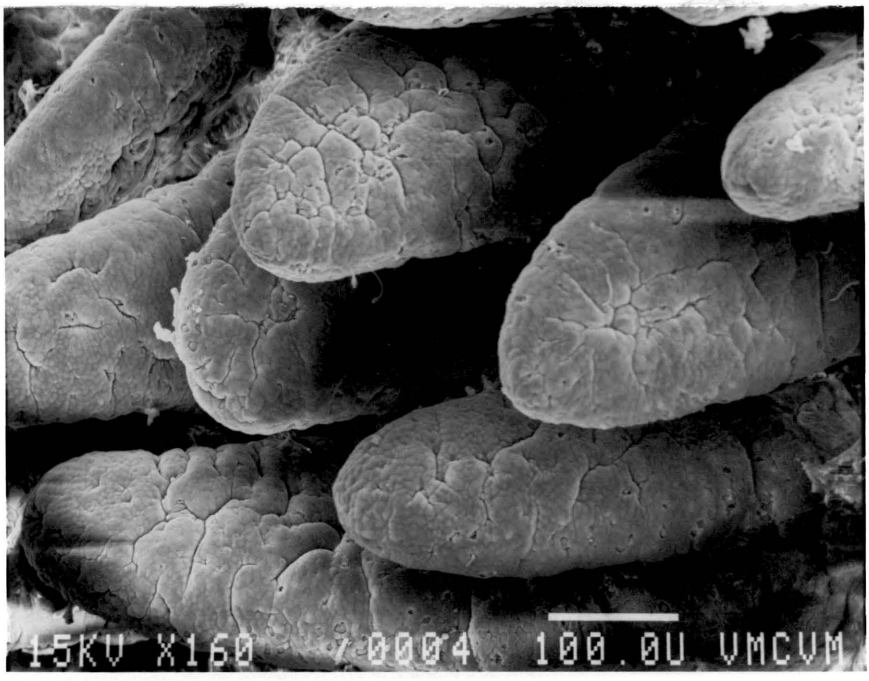
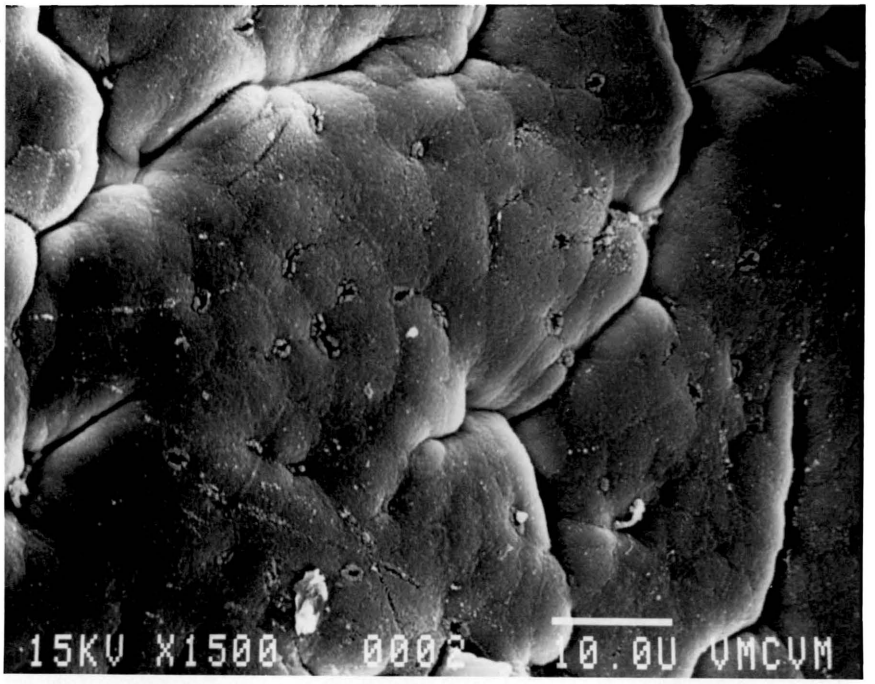


PLATE 1



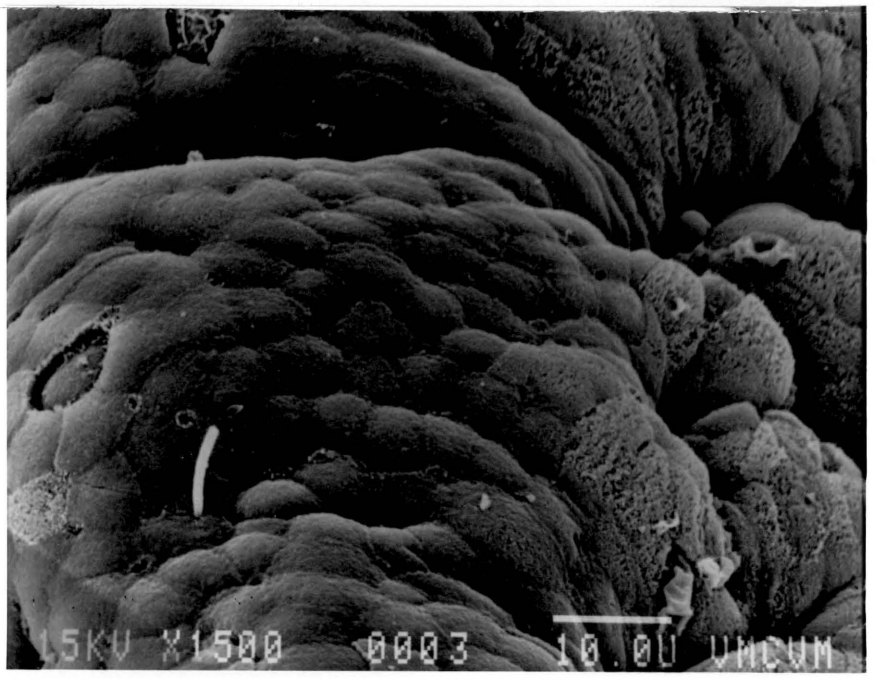


PLATE 2

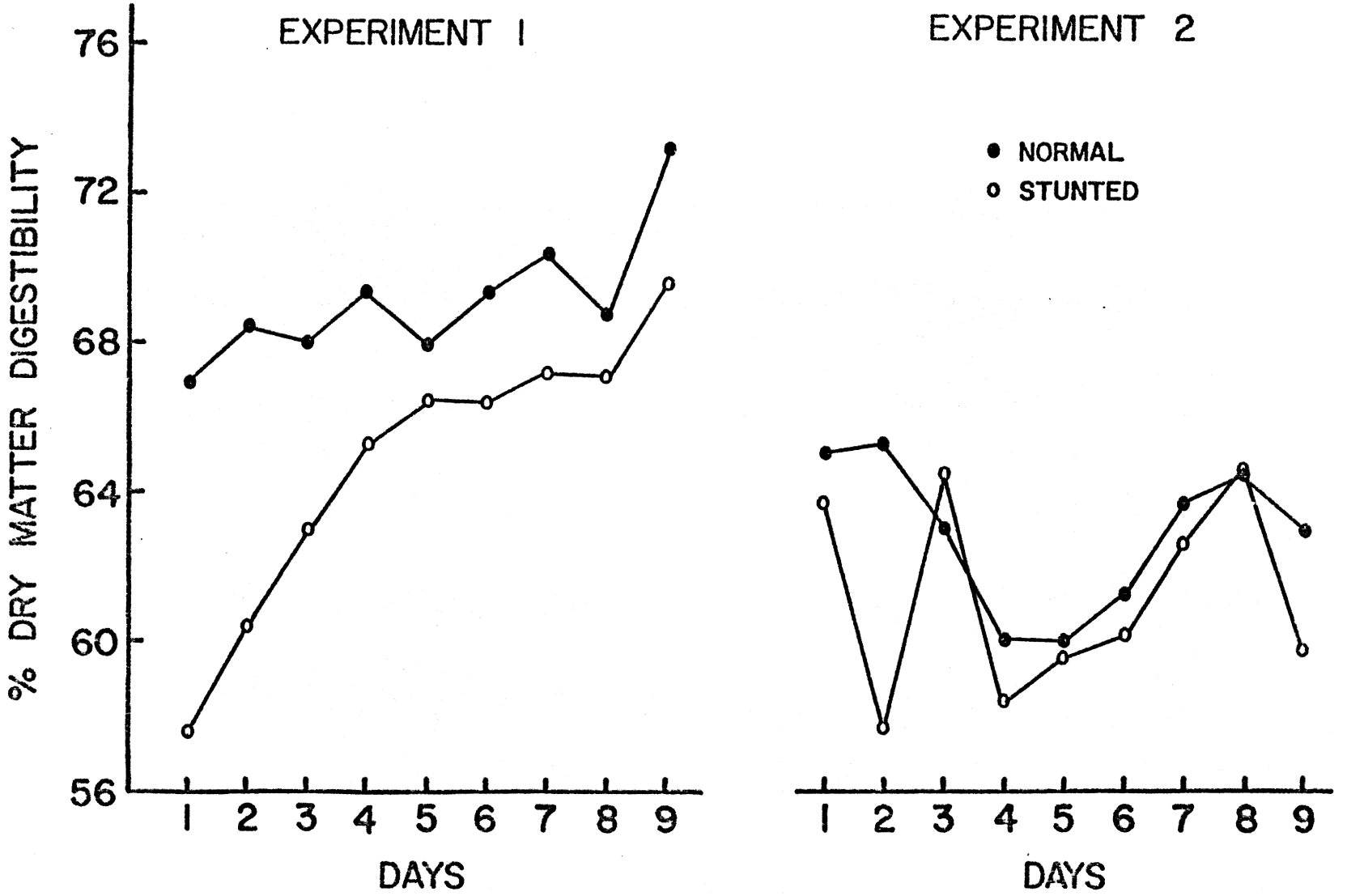


Figure 2. Average daily dry matter digestibilities for stunted and normal turkeys.

SERIES II. THE EFFECT OF ORAL INOCULATION OF *ESCHERICHIA COLI* ON POULT PERFORMANCE

The overall objective of this experiment was to colonize a known bacterial population in the intestinal microflora of young turkeys. A pathogenic strain of *E. coli* isolated from the yolk sac of poults was introduced orally into conventional day-old poults. This experiment served as a pretreatment for poults later to be inoculated with rotavirus in order to study the effect of a virus-bacterium interaction. The effects of adequate and low protein diets (28 *vs*. 22%) were examined with respect to the poult's response to an oral challenge with *E. coli*.

EXPERIMENTAL PROCEDURES

For this experiment, 360 Large White turkeys, 200 male and 160 female, were hatched at the VPI&SU Turkey Research Center and randomized into 36 pens of Petersime starter batteries. The poults were wingbanded and weighed in groups of 10 at one day of age. At five days of age, all birds were debeaked. The turkeys were group weighed by pen weekly for the three-week experiment. Feed consumption and mortality records were recorded by pen so that weekly feed consumptions and feed efficiencies could be calculated. All data were subjected to analysis of variance.

The 28% protein basal diet used in the experiment is presented in Table 10. The 22% protein diet was formed by substituting 15.3% ground yellow corn for dehulled soybean meal in the basal diet. The two diets and water were provided ad libitum for the duration of the experiment. When one day of age at the start of the experiment, poults were inoculated orally with .1 ml of either sterile Todd Hewitt broth or a 10^{-2} dilution of a 24-hr Escherichia coli culture. Four treatments resulted from the 2 x 2 factorial arrangement of the two protein levels with the two dilutions of E. coli. Each of the four treatments was assigned to five pens of male and four pens of female poults.

The E. coli culture was obtained from culturing the yolks of stunted poults in an earlier experiment. Yolks were cultured using a sterile swab and plated onto TG-7 (Difco) agar plates, selective for gram negative enteric bacilli. After 24 hr of incubation at 37 C,

individual colonies were picked and again plated onto TG-7 agar. Following incubation, a single colony was again selected and inoculated onto Kligler iron agar slants for the detection of sugar fermentation. The pure culture obtained on the Kligler iron slants was then used to inoculate 10 ml of sterile Todd Hewitt broth. After incubation for 24 hr, this culture was frozen at -70 C until needed.

The 10^{-2} dilution was chosen based on the results of an earlier experiment in which day-old poults were inoculated via the yolk sac with serial E. coli dilutions. This dilution was found to reduce 12-day body weights by 15% with low mortality. Dilutions lower than 10^{-4} resulted in body weights equal to the controls.

Daily (24-hr) feed consumptions by pen were recorded on days 7 through 9 and on days 17 through 19 for two pens of males and two pens of females per treatment. Total excreta collection was conducted on days 8 through 10 and on days 18 through 20 for these same pens so that digestible dry matter and protein, and apparent metabolizable energy (AME) could be calculated. The excreta collected on a given day was assumed derived from the previous days feed consumption.

Excreta samples were dried at 60 C in a forced draft oven and ground to pass through a 60-mesh screen. After obtaining dry excreta weights for each day, the excreta from each three-day period within a pen were pooled and the resulting samples prepared for subsequent chemical analyses. Gross energy of feed and excreta were determined using an adiabatic bomb calorimeter. Nitrogen determination was conducted by the method of Kjeldahl (AOAC, 1980). Nitrogen corrected

AME (AME_n) was calculated using the equation of Hill and Anderson (1958).

RESULTS

The average body weight gain, feed consumption and feed efficiency for the turkeys in each treatment are presented in Table 11. An oral inoculation of E. coli at one day of age resulted in increased body weight gains and feed consumptions at both levels of protein. The effect of oral inoculation of E. coli was greater at the 28% protein level, however, this protein by E. coli interaction was not significant. The percent increase in body weight gain was 6.1% for the 28% protein group and 2.7% for the 22% protein group, yielding an average increase of 4.5% as shown in Table 12. Feed consumption was increased 2.8% at the 28% protein level and 1.3% at the 22% protein level for an average increase of 2.1%. The feed efficiencies at each protein level were increased 3.2 and 1.4%, respectively, by oral E. coli inoculation. The average improvement in feed efficiency of 2.4% due to E. coli inoculation was significant for the 0 to 3-week data (Table 12).

No significant differences were observed for the digestibility parameters examined for the 7 to 10-day collection period post inoculation (Table 13). As shown in Table 14, for the 18 to 20-day post inoculation collection period, nitrogen retention increased 4.86 g at the 28% protein level due to oral E. coli inoculation. No change was observed in nitrogen retention at the 22% protein level. This protein by E. coli interaction was approaching significance ($P \leq .058$). When expressed as nitrogen digestibility, E. coli inoculation had no effect at either protein level. Differences for the 28% protein level vs. the 22% protein level were significant ($P \leq .05$) for all parameters studied except

AME and AME_n for the 7 to 10-day collection period.

DISCUSSION

The results of this experiment indicate that oral inoculation with a low level of E. coli significantly improved feed efficiency of turkeys from 0 to 3 weeks of age. This observation is very interesting in light of the research demonstrating detrimental effects from E. coli inoculation (Adler and DaMassa, 1980; Watkins et al., 1982). This difference could be due to the level of inoculation. In the present experiment, a low level inoculation was given (.1 ml of 10^{-2} dilution), which may allow colonization of E. coli in the intestinal tract without a toxic response. Watkins et al. (1982) inoculated chicks with 1 ml of a pure 24-hr E. coli culture, estimated to contain approximately 10^9 organisms. This higher dose resulted in 66.7% mortality when given at two days of age.

Although the resulting microflora was not defined, one could speculate that the E. coli inoculation given at hatching induced a beneficial change in the intestinal microflora of the turkeys in this experiment. Establishment of the inoculated E. coli strain would occur at the expense of some other organism, probably another coliform. These results support the hypothesis that an "optimum microflora" exists which, once defined, will allow for superior performance through the synergism between the bird and the intestinal microorganisms. Research work with antibiotics and Lactobacillus inoculation to poultry also have supported this hypothesis.

Although much of the research with Lactobacillus has concentrated on the prophylactic use of Lactobacillus against E. coli infection

(Watkins et al., 1982) and also on the growth promotion effects of Lactobacillus (Tortuero, 1973), recent works by Buenrostro and Kratzer (1983) and Watkins and Kratzer (1983) have demonstrated growth depression from oral Lactobacillus inoculation. Again, the difference in results obtained by various workers is probably due to the level of inoculation. One should note the effects of Lactobacillus on body weight gain are seldom significant whether as a growth promotant or a growth inhibitor. Using Bobwhite quail, Miles et al. (1981) observed a numerical decrease in body weight in one experiment and a numerical increase in a second experiment from the commercial Lactobacillus preparation Probios. This organism apparently acts by excluding the development of other enteric organisms through its attachment to the crop epithelium.

The effect of low level Lactobacillus (and in the present research E. coli) inoculation parallels that of antibiotic responses in poultry diets. Namely, the environmental conditions of the experiment and the basal diet have an effect on the response to the organism. For example, bacitracin elicits a growth response in broilers in a non-isolated environment fed a soybean protein and corn based diet (Stutz et al., 1983). The response to antibiotics was shown to be due to the exclusion of Clostridium perfringens in the intestine of the chicken. Likewise, Tortuero (1973) reported a response from Lactobacillus similar to the antibiotics, in that a more pronounced effect to inoculation was observed in an "old" environment when fed a practical-type diet. The Lactobacillus inoculation resulted in an almost complete disappearance of

Enterococci from the chick intestine.

Another important finding of the current experiment is that the same E. coli isolate elicited a different response depending on the site of inoculation. When administered via the yolk sac at one day of age, significant stunting and mortality resulted. Given orally, the same dose of E. coli improved body weight gain and significantly improved feed efficiency. This interrelationship between the bird, bacteria and the environment remains to be completely understood. A more thorough understanding of this relationship would offer insight into poultry production problems.

SUMMARY

An experiment was conducted to determine the effect of a pathogenic strain of Escherichia coli administered orally to day-old Large White poults. This strain was isolated from the yolk sacs of stunted poults, and considered pathogenic based on the mortality and reduced weight gain of poults when the isolate was reintroduced via the yolk sac. The oral route of inoculation apparently was successful for establishing the E. coli as part of the intestinal microflora in the turkey. While both body weight gain and feed consumption were numerically increased 4.5 and 2.1%, respectively, for all birds inoculated with E. coli, feed efficiency was significantly increased by 2.4% from 0 to 3 weeks due to the E. coli inoculation at day one. The beneficial effect of E. coli on poult performance was greater for birds fed a 28% protein diet compared to birds fed a 22% protein diet.

Digestibility trials were conducted from 7 to 10 and from 17 to 20 days post inoculation. No changes in digestibility parameters were observed as a result of E. coli other than an increase in nitrogen retention for the 17 to 20-day period by those poults fed the 28% protein diet and inoculated with E. coli. It is postulated that changes in the intestinal tract from E. coli inoculation resulted in a faster growing bird which consumed and retained more nitrogen from the feed.

Table 10. Basal diet (28% protein)[1]

Ingredient	g/kg
Ground yellow corn	500.6
Dehulled soybean meal	391.0
Meat and bone meal	50.0
Menhaden fish meal	35.0
Defluorinated phosphate	12.0
Ground limestone	3.0
Iodized salt	1.0
DL-Methionine	2.5
L-Lysine HCl	.5
Trace mineral mix[2]	1.0
Vitamins and feed additives[3]	3.4
Total	1000.0

[1]The 22% protein diet was formed by substituting 15.3% ground yellow corn for dehulled soybean meal.

[2]Supplied the following amounts of trace minerals in mg/kg complete diet: 150 manganese, 100 zinc, 70 iron, 10 copper, 2.6 iodine and .8 cobalt from manganese oxide, zinc oxide, ferrous sulfate, copper oxide, calcium iodate and cobalt carbonate, respectively.

[3]Supplied the following quantities of vitamins and feed additives in mg/kg complete diet unless otherwise stated: 19,695 IU vitamin A, 6,598 ICU vitamin D_3, 11 IU vitamin E, 4.2 menadione dimethylpyrimidinol bisulfite, .98 thiamine, 5.5 riboflavin, 15.2 calcium pantothenate (D), 66.08 niacin, 868 choline chloride, .015 vitamin B_{12}, 1.1 folic acid, .11 biotin, 1.81 pyridoxine HCl, 125 ethoxyquin and .2 selenium.

Table 11. Average body weight gain, feed consumption and feed efficiency of turkeys inoculated orally with E. Coli[1] and fed two levels of protein

Age (weeks)	Treatment: 28% protein	28% protein + E. Coli	22% protein	22% protein + E. Coli	Standard deviation[2]
			Body weight gain (g)		
0-1	45	49	50	53	6
1-2	101	108	97	96	9
0-2	146	156	147	149	13
2-3	172	182	159	163	12
0-3	319	338	306	314	21
			Feed consumption (g)		
0-1	76	80	85	85	6
1-2	195	204	200	199	14
0-2	271	284	285	285	18
2-3	290	293	286	289	17
0-3	561	577	571	578	30
			Feed efficiency		
0-1	.591	.611	.592	.619	.059
1-2	.520	.526	.484	.481	.023
0-2	.539	.550	.516	.522	.027
2-3	.594	.621	.555	.563	.025
0-3	.568	.586	.536	.543	.016

[1]Poults inoculated orally at one day of age with a 10^{-2} dilution of a 24 hr E. Coli culture. Controls received .1 ml of sterile Todd Hewitt broth.

[2]$\sqrt{\text{mean square error}}$. N = 9 observations per treatment.

Table 12. Effect of oral E. Coli inoculation on body weight gain, feed consumption, and feed efficiency at three weeks of age

E. Coli dilution[1]	Body weight gain, 0-3 weeks (g)	Feed consumption, 0-3 weeks (g)	Feed efficiency, 0-3 weeks
0	312.7[2]	565.5	.552
10^{-2}	326.8	577.3	.565
Difference	14.1	11.8	.013*
Increase (%)	4.5	2.1	2.4

*$P \leq .05$

[1]Each bird received .1 ml of inoculum.

[2]N = 17 observations per mean.

Table 13. Excreta nitrogen, nitrogen retention, nitrogen digestibility, dry matter digestibility, excreta energy, AME and AME_n on a dry matter basis for turkeys 7 to 10 days after oral inoculation with E. Coli[1]

Variable	Treatment				Standard[2] deviation
	28% protein	28% protein + E. Coli	22% protein	22% protein + E. Coli	
Excreta nitrogen (%)	4.89	4.97	3.75	3.68	.25
Nitrogen retention (g)	13.09	14.61	11.82	11.79	1.13
Nitrogen digestibility (%)	52.24	51.81	53.77	56.14	3.56
Dry matter digestibility (%)	49.61	50.07	51.19	52.79	1.86
Excreta energy (kcal/g)	4.200	4.205	4.257	4.259	.060
AME (kcal/g)	2.379	2.396	2.359	2.426	.090
AME_n (kcal/g)	2.158	2.176	2.184	2.243	.082

[1]Poults inoculated orally at one day of age with .1 ml of a 10^{-2} dilution of a 24-hr E. Coli culture. Controls received sterile Todd Hewitt broth.

[2]$\sqrt{\text{mean square error}}$. N = 4 observations per treatment.

Table 14. Excreta nitrogen, nitrogen retention, nitrogen digestibility, dry matter digestibility, excreta energy, AME and AME_n on a dry matter basis for turkeys 17 to 20 days after oral E. Coli[1] inoculation

Variable	Treatment				Standard[2] deviation
	28% protein	28% protein + E. Coli	22% protein	22% protein + E. Coli	
Excreta nitrogen (%)	6.21	6.22	4.87	4.67	.15
Nitrogen retention (g)	28.00	32.86	25.20	24.99	2.32
Nitrogen digestibility (%)	55.13	55.23	59.26	59.18	1.85
Dry matter digestibility (%)	62.77	62.89	66.81	65.12	1.70
Excreta energy (kcal/g)	3.993	4.017	4.056	4.128	.070
AME (kcal/g)	3.009	3.005	3.091	2.997	.087
AME_n (kcal/g)	2.775	2.771	2.898	2.804	.082

[1] Poults inoculated orally at one day of age with .1 ml of a 10^{-2} dilution of a 24-hr E. Coli culture. Controls received sterile Todd Hewitt broth.

[2] $\sqrt{\text{mean square error}}$. N = 4 observations per treatment.

SERIES III. NUTRITIONAL RESPONSE OF TURKEY POULTS TO YOLK SAC INOCULATION WITH ESCHERICHIA COLI

E. coli has been isolated from the yolk sac of stunted turkeys from previous experiments, and also is known to exist commercially in both turkeys and chickens. The presence of a yolk sac infection is observed by failure of the poults to completely absorb the yolk material within the first week. Many times a caseous remnant of the yolk remains for several weeks. For this reason, two experiments were conducted to determine if the stunting syndrome could be reproduced by inoculation of day-old poults with the E. coli isolate. The poults were inoculated via the yolk sac to simulate an egg transmitted infection. In addition to E. coli inoculation, two levels of dietary protein (28 vs. 22%) were fed to investigate the effect of low protein on the susceptibility of turkeys to infectious stunting. The nutritional response of poults was evaluated by body weight gain and feed consumption, digestibility parameters, and by the specific activity of mucosal hydrolytic enzymes.

EXPERIMENTAL PROCEDURES

Two experiments were conducted with Large White turkeys hatched at the VPI&SU turkey research center. At one day of age (hatch date), poults were sexed, randomized and weighed, and wingbanded by groups of 10 turkeys each. The time from setting eggs to hatch date was 29 days for Experiment 1 and 28 days for Experiment 2. The decision to remove the birds from the hatcher earlier in the second experiment was based on the poor poult quality in Experiment 1. All birds were placed in Petersime starter batteries and provided feed and water ad libitum. Body weights and feed consumptions by pen were recorded weekly for both three-week experiments. Mortality was recorded on a daily basis.

For Experiment 1, four treatments resulted from a factorial arrangement of two protein levels (28 vs. 22%) and two levels of E. coli inoculation (0 vs. 10^{-2}). Each treatment was randomly assigned to five pens of male and four pens of female poults for a total of 36 pens. Because of the high mortality observed in the first week, replicate pens were combined at one week of age yielding a total of 19 pens (11 pens of males and 8 pens of females). Each treatment contained at least two replicate pens per sex, and the zero dose of E. coli had three and four replicate pens of males at the 28% and 22% protein levels, respectively. The body weight and feed consumption data from Experiment 1 are included only for comparison with Experiment 2.

In Experiment 2, again the two levels of protein (28 vs. 22%) were fed to turkeys inoculated with three dilutions of E. coli (0, 10^{-3},

10^{-2}). The six treatments were each assigned to 4 pens of male and 4 pens of female poults yielding a total of 48 pens with 10 poults per pen. The 28% basal diet used in both experiments is presented in Table 10. The 22% protein diet was made by substituting 15.3% ground yellow corn for dehulled soybean meal in the basal diet. These diets contained no added antibiotic.

Inoculation with E. coli was done via the yolk sac at one day of age. Isolation of the strain and preparation of the E. coli culture has been described (Series II). Dilutions were made of a 24-hr culture incubated at 37 C. The zero level control consisted of sterile Todd Hewitt broth. The inoculation procedure consisted of swabbing the area around the naval liberally with wescodyne solution, so that the down and skin were thoroughly disinfected. The appropriate culture was injected into the yolk sac using a 2 cm 26-gauge needle fitted on a 1.0 ml tuberculin syringe. The needle was inserted (lateral to the naval) approximately .5 cm at an angle perpendicular to the surface. Each poult received .1 ml of the inoculum. Needles were replaced periodically to help prevent contamination. Birds were placed into pens immediately following inoculation.

Two three-day digestibility trials were conducted using two pens of males and one pen of female turkeys in Experiment 2. Daily feed consumptions were recorded on days 7 through 9 and on days 17 through 19. Total excreta collection was conducted using galvanized steel trays on days 8 through 10 and on days 18 through 20 for the same pens. Excreta collected on a given day were assumed derived

from the previous days feed. Feed and excreta were handled as described previously (Series I). Digestible dry matter and protein, apparent metabolizable energy (AME) and nitrogen corrected AME (AME_n) were calculated for each period.

Following the termination of the experiment at three weeks of age, two male turkeys from each treatment were selected with body weights close to the mean for the treatment. The remaining turkeys were killed and autopsied to verify the inoculation accuracy and to record whether or not the yolk sac had been absorbed. The 12 selected turkeys were used to prepare muscosal enzyme homogenates.

Birds for muscosal enzyme analysis were killed by cervical dislocation. Immediately after death, intestines were removed in a cold room (4 C), placed on a metal tray and separated into duodenal, jejunal and ileal segments. The sections were opened longitudinally and rinsed in ice cold .1M NaCl. Approximately one-half of the length of each section obtained from the midpoint was then scraped with a glass slide, diluted at a 4:1 volume with .1M NaCl, and homogenized for 2 min with a Polytron homogenizer similar to the procedure of Enigk and Dey-Hazra (1976). After standing in an ice bath for 30 min, the homogenates were rapidly frozen using dry ice and acetone.

Maltase activity was measured according to the procedure of Dahlqvist (1968). Homogenates were thawed and centrifuged at 2000 g and diluted with ice cold .1M NaCl. An incubation time of 1 hr at 37 C was used for the formation of glucose. Sodium maleate buffer (.1M) at pH 6.0 was used to prepare the maltose substrate (.056M). Mucosal

dipeptidase activity was measured using the procedure of Josefsson and Lindberg (1965a). Glycyl-L-valine (.03M) and glycyl-L-leucine (.0375M) were used as substrates, while .15M Borate adjusted to pH 7.4 served as the buffer. Hydrolysis time was 5 min. Absorbance was measured at 220 nm against an ethanol:water (99:1) reagent blank. Protein content of the homogenates were determined using the Coomassie Blue dye binding procedure (Bradford, 1976).

All data were subjected to analysis of variance. Comparisons of specific treatment means to the controls were made by Dunnett's procedure (Lentner and Bishop, 1978).

RESULTS

Inoculation of E. coli into the yolk sac of day old turkeys had a profound effect on poult performance. As shown in Table 15, greater than 50% mortality resulted from all treatments in Experiment 1, whether or not E. coli was included in the inoculum. The high mortality in the control groups at both protein levels resulted from poor quality poults. Poults from the same hatch used in another experiment did not exhibit substantial mortality. The mortality data from Experiment 2 shows that the inoculation procedure was not a cause of mortality. The higher dose of E. coli (10^{-2}) resulted in 40 and 45% mortality at the 28 and 22% protein levels, respectively. Lower mortality resulted from the 10^{-3} dilution at both protein levels (24 and 34% for the 28 and 22% protein diets). Differences in one-week mortality were significant ($P \leq .05$) between the three levels of E. coli inoculation in Experiment 2. At three weeks of age, 5% of the control poults had not completely absorbed the yolk sac, while 58 and 64% of the birds inoculated with the 10^{-3} and 10^{-2} dilutions of E. coli, respectively, were found to contain large remnants of the yolk.

Body weight gain and feed consumption decreased dramatically for all poults inoculated with E. coli in both experiments. Table 16 contains the weekly data for Experiment 1. Averaged over the two levels of protein, the depression in body weight gain and feed consumption was significant ($P \leq .05$) for all periods. Changes in feed efficiency were not significant at any age. For Experiment 2 (Table 17), decreases in body weight gain due to E. coli inoculation compared

to the control (zero dose) were highly significant (P≤.001) for all periods except the 2 to 3-week period. Differences between the two levels of added E. coli were not significant. A significant E. coli by protein interaction (P≤.05) on feed consumption was observed for all periods other than the 2 to 3-week period. This interaction results from an alleviation of the depression in feed consumption from E. coli (10^{-3} dilution) at 28% protein, whereas a marked depression occurred at the 22% protein level (Table 18). The response of the 10^{-2} dilution of E. coli was similar at both protein levels. Although this interaction is apparent in the body weight gain data, the differences were not significant. Changes in feed efficiency in Experiment 2 due to E. coli inoculation were significant (P≤.01) for the 1 to 2 and 0 to 2-week periods. The stronger dose of E. coli (10^{-2}) resulted in higher feed efficiency than either the control or the 10^{-3} dilution at both protein levels. No differences were observed between the control and the 10^{-3} dilution.

E. coli inoculation was most severe at one week of age, and decreased each week thereafter. Body weight gains decreased an average of 34% during the first week in Experiment 1 and an average of 37% from the two levels of E. coli in Experiment 2. By the 2 to 3-week period, the decrease in body weight gain due to E. coli inoculation averaged 11% for Experiment 1 and 7% for Experiment 2. The average changes in body weight gain, feed consumption and feed efficiency for both experiments from 0 to 3-weeks are presented in Table 18. Note that in all cases, the response of body weight gain parallels that of

feed consumption.

The results of the digestibility calculations for the 7 to 10-day excreta collection period are presented in Table 19. Significant ($P \leq .01$) protein by E. coli interactions were observed for nitrogen digestibility, dry matter digestibility, AME and AME_n. In each case, this interaction results from the improvement in a given parameter when turkeys were fed 28% protein and inoculated with a 10^{-2} dilution of E. coli. This same dose of E. coli produced a decrease in that nutritional parameter when combined with the 22% protein diet. For those birds inoculated at the 10^{-3} dilution compared to the control dose, performance was depressed at the 28% protein level and improved at the 22% protein level, although these differences were not always significant. For excreta nitrogen content and nitrogen retention, significant ($P \leq .05$) main effects of E. coli dilution were observed as shown in Table 20. Using Dunnett's procedure for comparing treatments to a control (Lentner and Bishop, 1978), increases in excreta nitrogen of 7 and 11% for the 10^{-3} and 10^{-2} dilutions, respectively, were both significantly different from the control dose. Nitrogen retention decreased 34 and 52% for the two doses of E. coli, respectively. Although not significant ($P \leq .10$), an increase in gross energy of the excreta was observed for both levels of inoculation (1.6 and 1.4% for the 10^{-3} and 10^{-2} dilutions, respectively). As shown in Table 21 for the 17 to 20-day excreta collection period, E. coli inoculation continued to cause a significant ($P \leq .01$) decrease in nitrogen retention at both dilutions. All other parameters remained unchanged.

Specific activities of mucosal maltase for the turkeys in each treatment are presented in Table 22. Because of the low disaccharidase activities observed in the ileum by other researchers (Enigk and Dey-Hazra, 1976), maltase activities for the ileal mucosa were not determined in this study. In the duodenum, the protein by E. coli interaction approached significance ($P \leq .10$). Turkeys inoculated with either level of E. coli exhibited higher activities than the controls when fed 28% protein, while E. coli inoculation depressed specific maltase activity at 22% protein. No differences were observed in maltase activity in the jejunal mucosa. On the average, activity was greater in the jejunal than in the duodenal mucosa.

Dipeptidase activity expressed as umoles of dipeptide hydrolyzed per minute per mg protein was found to be similar for both substrates. The exception is the marked reduction in glycyl-L-valine dipeptidase in the ileal mucosa for birds fed 22% protein and inoculated with the 10^{-3} dilution of E. coli. On the average, in the duodenal mucosa no differences were observed between the controls and the 10^{-2} dilution of E. coli (Table 23). Inoculation with the lower level of E. coli (10^{-3} dilution), resulted in a reduction of approximately 46% in the specific activity toward both substrates. No differences were observed due to the level of protein in the diet. In the jejunum, an increase in dipeptidase activity was observed for all birds inoculated at the 10^{-2} dilution, while the lower dilution was similar to the controls. These differences were not significant, however. In the ileum, a significant protein by E. coli dilution interaction was observed in the specific

activity for glycyl-L-leucine dipeptidase. This interaction resulted from a significant depression in activity from both E. coli dilutions at 28% protein compared to the 28% protein control. No differences were observed at 22% protein. Although the same depression occurred in the activity for glycyl-L-valine, this interaction was not significant.

DISCUSSION

These results clearly suggest that the most profound effect of E. coli inoculation in the yolk sac on poult performance is a reduction in feed consumption. A slight malabsorption accompanies the reduction in feed consumption as evidenced by decreases in dry matter and nitrogen digestibility, AME_n and feed efficiency for turkeys inoculated with the 10^{-3} dilution of E. coli and fed a 28% protein diet.

Several important points need to be clarified prior to a thorough understanding of the action of E. coli in inhibiting poult performance. This discussion will deal mainly with the results of Experiment 2 because of the excessive mortality of the control groups in Experiment 1. The excess mortality apparently resulted from the stress placed on newly hatched poults in the hatcher. A comparison of the average body weight at hatch for Experiment 1 vs. Experiment 2 (56 vs. 65 g) indicates that severe dehydration occurred during the extra 24 hr in the hatcher for those poults in Experiment 1. The temperature and humidity in the hatcher provide an environment beneficial to bacterial growth in addition to dehydrating the birds. These weakened poults could not undergo the additional stress of the yolk sac inoculation. Poults removed from that environment soon after hatching were easily able to withstand the inoculation procedure as observed in Experiment 2.

The strain of E. coli used in this study caused substantial mortality at the 10^{-2} dilution, with a significant decrease at the higher dilution. Overall, the mortality observed in this study was less

than that reported by Watkins et al. (1982) for an oral E. coli dose. The level of inoculation in the latter case was much higher than in the present study. As previously reported in Series II, a .1 ml inoculation of the same 10^{-2} dilution used in these experiments, given orally, resulted in improved performance of turkey poults with no mortality. The 10^{-3} dilution used in Experiment 2 produced the response desired at the outset, namely growth depression without high mortality. The stronger dose provided misleading results. Mortality was extensive to the point that only the strongest poults survived the E. coli infection. These poults exhibited superior feed efficiency at both levels of protein and also had improved digestibility parameters (Table 19) when fed an adequate level of protein (28%). The reduction in feed consumption, however, resulted in body weight gains significantly lower than the controls. The higher level of protein provided a slight protection from mortality due to infection, as has been reported previously (Boyd and Edwards, 1963).

The response of the 10^{-2} dilution in Experiment 1 was similar to the 10^{-3} dilution in Experiment 2 with regard to body weight gain and feed consumption. In each case, the effect of E. coli was severe for poults fed 22% protein diets, while the 28% protein diet protected the poults from a severe depression in feed consumption. Body weight gain paralleled feed consumption with some variation. On a percentage basis, birds fed the 22% protein diet and inoculated with the 10^{-3} dilution demonstrated a greater decrease in feed consumption than in body weight gain. This difference manifested itself in an increase in

feed efficiency. However, when birds were fed 28% protein diets and inoculated with E. coli, feed consumption was not depressed as much as body weight gain (6 vs. 9%, respectively), resulting in decreased feed efficiency. High protein diets have also been shown to protect against decreased body weight gain during coccidial infections (Sharma et al., 1973), however, in the present study the effect appears to be primarily on feed consumption. Birds infected with coccidiosis do not exhibit a marked depression in feed consumption as found with enteric E. coli infection.

The presence of a malabsorption of nutrients is supported by the results of the 7 to 10-day post-inoculation digestibility trial. In all birds inoculated with E. coli, nitrogen content and gross energy of the excreta increased significantly. This same phenomenon has been identified during Pale Bird Syndrome in broilers (Nelson et al. , 1982), with coccidiosis (Sharma and Fernando, 1975), and also for stunted turkeys (Series I). Increases in the ether extract of the excreta of stunted birds have also been reported (Sharma and Fernando, 1975; Lilburn et al., 1982).

The level of feed consumption of infected poults affected digestibility parameters similar to feed efficiency. Namely, for poults inoculated with the 10^{-3} dilution and fed 28% protein, a decrease was observed in nitrogen and total dry matter digestibility, AME and AME_n. However, the extreme depression in feed consumption observed for birds fed 22% protein diets and inoculated with E. coli appeared to cause modest increases in these same parameters.

Apparently, feed consumption was depressed to the point that the bird was forced to utilize the available nutrients more efficiently. These observed increases in feed efficiency and digestibility parameters are unexpected when one considers that the infected birds exhibited noticeable morbidity. Published data concerning the effect of level of feed consumption on efficiency of nutrient utilization are lacking.

Changes in mucosal activities for dipeptidases and disaccharidases were subject to considerable variation within treatments. Several reasons may exist for this finding. First, the birds selected for mucosal sampling were killed after three weeks of age and the homogenates prepared. Body weight gain and digestibility data indicate that the severity of the infection was greatly reduced by this time. The rate of turnover of the mucosal epithelial cells and the stage of maturity of the cells present can greatly affect the activities of hydrolytic enzymes. Enigk et al. (1976) reported that changes in the hydrolytic activity of mucosal preparations were related to the number of crypt cells in the villi of pigs with helminth infections. Of the poults used for enzyme homogenates, varying stages of mucosal development could have existed. Second, specific disaccharidase activity has been demonstrated to vary in certain circumstances in the rat (Yeh, 1983), however, total activity remained unchanged as a result of changes in the length of the intestine. Unfortunately, intestine length was not recorded in the present study.

The values obtained for maltase activity correspond with those presented for chickens by Enigk and Dey-Hazra (1976) and Siddons

(1969). Specific activities for dipeptidases for poultry are not available in the literature. These values are slightly higher than those presented for rats and swine (Josefsson and Lindberg, 1965b;1966). The lack of information concerning the amount of activity required for both enzymes under normal circumstances in poultry makes interpretation of the results difficult. However, several of the observed changes in activities appear to have meaning. Average maltase activity increased for infected birds fed 28% protein and decreased for birds fed 22% protein. This relationship corresponds to the digestibility parameters discussed previously. The specific activity for glycyl-L-valine dipeptidase over the length of the intestine decreased with inoculation of the 10^{-3} dilution of E. coli similar to the decrease in nitrogen digestibility. The decrease in dipeptidase activity in the intestinal mucosa may account for the noted increase in excreta nitrogen (Peternel and Bell, 1968). An alternative hypothesis to explain the increase in excreta nitrogen is leakage of serum protein into the intestinal lumen as shown to occur in E. coli infected pigs (Prochazka et al., 1982).

Overall interpretation of the data obtained in this study indicates that the most profound effect of E. coli inoculation into the yolk sac of day-old turkeys is a suppression of feed consumption. The depression in feed consumption is greatly magnified by feeding a low protein diet (22% protein), while a 28% protein diet permitted only a small decrease in feed consumption (6%) over the three-week experiment. Ingested nutrients were utilized less efficiently as demonstrated by decreases in

digestibility (dry matter and nitrogen) and AME_n. Substantial increases in excreta nitrogen and gross energy resulted from this malabsorption.

SUMMARY

Two experiments were conducted to examine the effects of pathogenic E. coli inoculated into the yolk sac of day-old turkeys. In Experiment 1, a control dose (sterile broth) or a 10^{-2} dilution of a 24-hr E. coli culture was administered to poults fed either 28 or 22% protein diets. Although mortality was excessive in all treatments, body weight gain and feed consumption were significantly depressed at both protein levels due to E. coli inoculation. The 28% protein diet provided some protection from the depression in feed consumption. Feed efficiency was unchanged.

In Experiment 2, E. coli was inoculated at the 10^{-3} and 10^{-2} dilutions in addition to the control dose at both protein levels. Both dilutions of E. coli significantly reduced body weight gain and feed consumption. The higher concentration of E. coli (10^{-2} dilution) increased mortality above the control or 10^{-3} dilution. The surviving turkeys inoculated with the 10^{-2} dilution exhibited elevated feed efficiencies compared to the controls at both protein levels. The 28% protein diet alleviated the depression in feed consumption and body weight gain for poults inoculated at the 10^{-3} dilution, while the same level of E. coli produced a substantial depression of feed consumption for birds fed 22% protein. Changes in digestibility parameters were variable due to a protein by E. coli interaction, however, nitrogen content and gross energy of the excreta increased significantly at both dilutions of E. coli for a 7 to 10-day collection period post inoculation. The observed increase in excreta nitrogen and energy was indicative of

a malabsorption of nutrients.

Changes in mucosal maltase and dipeptidase activities varied along the length of the intestine 21 days after inoculation. The effect of E. coli inoculation into the yolk sac was most severe at one week of age and decreased thereafter. Therefore, the changes in mucosal enzyme activities observed at 21 days of were not clearly understood and may have represented various stages of cellular renewal for the infected birds.

Table 15. Average mortality of poults within one week following E. Coli inoculation via the yolk sac

Protein (%)	E. Coli[1]	Experiment 1 (%)	Experiment 2 (%)
28	0^{2}	51	1
	10^{-3}	--	24
	10^{-2}	64	40
22	0	57	2
	10^{-3}	--	34
	10^{-2}	68	45

[1]Poults were inoculated via the yolk sac at one day of age with .1 ml of the appropriate dilution of a 24-hr E. Coli culture.

[2]Controls received .1 ml of sterile Todd Hewitt broth.

Table 16. Average body weight gain, feed consumption and feed efficiency of turkeys inoculated with E. Coli[1] in the yolk sac and fed two levels of protein (Experiment 1)

Age (weeks)	28% protein	28% protein + E. Coli	22% protein	22% protein + E. Coli	Standard deviation[2]
			Body weight gain[3](g)		
0-1	47	37	50	36	8
1-2	111	96	91	74	16
0-2	155	133	143	113	15
2-3	184	173	168	144	12
0-3	340	306	311	257	24
			Feed consumption (g)		
0-1	75	61	81	55	12
1-2	201	169	210	175	17
0-2	273	230	297	236	20
2-3	306	281	293	249	22
0-3	578	510	590	486	37
			Feed efficiency		
0-1	.628	.596	.620	.658	.088
1-2	.549	.589	.436	.422	.100
0-2	.569	.587	.482	.479	.061
2-3	.604	.617	.573	.577	.021
0-3	.587	.601	.527	.529	.021

[1] Poults were inoculated in the yolk sac at one day of age with .1 ml of a 10^{-2} dilution of a 24-hr E. Coli culture. Controls received .1 ml of sterile Todd Hewitt broth.

[2] $\sqrt{\text{mean square error}}$. N = 4 observations for treatments with E. Coli and N = 5 and 6 for the 28 and 22% protein controls, respectively.

[3] Average body weight of poults on day one was 56 g.

Table 17. Average body weight gain, feed consumption and feed efficiency of turkeys inoculated with E. Coli[1] via the yolk sac and fed two levels of protein (Experiment 2)

Protein	28%			22%			Standard
E. Coli	0	10^{-3}	10^{-2}	0	10^{-3}	10^{-2}	Deviation[2]
Age	Body weight gain[3] (g)						
0-1	57	45	42	55	38	39	6
1-2	124	110	111	109	82	101	14
0-2	181	155	153	163	120	139	17
2-3	201	194	191	172	151	163	21
0-3	382	349	344	336	271	303	35
	Feed consumption (g)						
0-1	90	72	62	95	62	64	9
1-2	209	196	173	204	151	176	24
0-2	299	268	236	299	213	241	29
2-3	333	326	313	321	262	301	38
0-3	631	595	549	621	476	542	62
	Feed efficiency						
0-1	.632	.622	.680	.574	.605	.597	.073
1-2	.593	.568	.639	.531	.541	.571	.045
0-2	.605	.581	.649	.545	.558	.578	.036
2-3	.603	.601	.611	.538	.576	.546	.048
0-3	.604	.590	.626	.541	.567	.559	.030

[1]Poults were inoculated via the yolk sac at one day of age with .1 ml of the appropriate dilution of a 24-hr E. Coli culture. Controls received .1 ml of sterile Todd Hewitt broth.

[2]$\sqrt{\text{mean square error.}}$ N = 8 observations per treatment.

[3]Average body weight of poults on day one was 65 g.

Table 18. Percent change in body weight gain, feed consumption and feed efficiency from 0 to 3 weeks for turkeys inoculated with E. Coli[1] and fed two levels of protein

E. Coli dilution	Protein (%)	Body weight gain	Feed consumption	Feed efficiency
		Experiment 1		
0 vs. 10^{-2}	28	-10.9*	-13.3*	2.3
	22	-21.1*	-21.4*	.4
		Experiment 2		
0 vs. 10^{-2}	28	-11.1*	-15.1*	3.6
	22	-10.8*	-14.5*	3.4
0 vs. 10^{-3}	28	- 9.4*	- 6.1	-2.4
	22	-23.8*	-30.5*	4.8*

[1]Poults were inoculated via the yolk at one day of age with .1 ml of the proper dilution of a 24-hr E. Coli culture. Controls received .1 ml of sterile Todd Hewitt broth.

* $P < .05$

Table 19. Excreta nitrogen, nitrogen retention, nitrogen digestibility, dry matter digestibility, excreta energy, AME and AME_n on a dry matter basis for turkeys 7 to 10 days after inoculation via the yolk sac with E. Coli[1] (Experiment 2)

Protein	28%			22%			Standard
E. Coli	0	10^{-3}	10^{-2}	0	10^{-3}	10^{-2}	Deviation[2]
Excreta nitrogen (%)	4.98	5.28	5.61	3.90	3.95	4.27	.26
Nitrogen retention (g)	16.32	10.53	7.93	12.23	7.75	5.69	1.48
Nitrogen digestibility (%)	53.16	49.97	54.32	53.19	53.39	47.39	1.90
Dry matter digestibility (%)	52.06	51.57	58.49	54.79	55.49	53.59	1.92
Excreta energy (kcal/g)	4.046	4.133	4.077	4.086	4.125	4.170	.057
AME (kcal/g)	2.567	2.504	2.814	2.587	2.598	2.499	.082
AME_n (kcal/g)	2.345	2.295	2.587	2.422	2.433	2.353	.082

[1]Poults inoculated via the yolk sac at one day of age with .1 ml of the appropriate dilution of a 24-hr E. Coli culture. Controls received .1 ml of sterile Todd Hewitt broth.

[2]$\sqrt{\text{mean square error}}$. N = 3 observations per treatment.

Table 20. Main effects of E. Coli dilution on excreta nitrogen, nitrogen retention and excreta energy for the 7 to 10-day collection period (Experiment 2)

E. Coli[1] dilution	Excreta nitrogen (%)	Nitrogen retention (g)	Excreta energy (kcal/g)
0	4.44	14.28	4.066
10^{-3}	4.75	9.42	4.130
10^{-2}	4.94	6.81	4.124
Difference			
0 vs 10^{-3}	.31	-4.86	.064
%	7.0*	-34.0*	1.6
0 vs 10^{-2}	.50	-7.47	.058
%	11.3*	-52.3*	1.4

[1]Poults inoculated via the yolk sac at one day of age with .1 ml of the proper dilution of a 24-hr E. Coli culture. Controls received .1 ml of sterile Todd Hewitt broth.

*Difference is significant based on Dunnett's Procedure ($P < .05$).

Table 21. Excreta nitrogen, nitrogen retention, nitrogen digestibility, dry matter digestibility, excreta energy, AME and AME_n on a dry matter basis for turkeys 17 to 20 days after inoculation via the yolk sac with E. Coli[1] (Experiment 2)

Protein / E. Coli	28%			22%			Standard Deviation[2]
	0	10^{-3}	10^{-2}	0	10^{-3}	10^{-2}	
Excreta nitrogen (%)	6.26	5.70	6.16	4.60	4.68	4.64	.45
Nitrogen retention (g)	37.70	27.99	20.23	26.93	17.72	15.10	5.25
Nitrogen digestibility (%)	59.76	59.23	59.98	59.52	60.61	62.12	3.68
Dry matter digestibility (%)	67.08	63.64	66.87	66.86	68.33	69.07	2.64
Excreta energy (kcal/g)	3.855	3.952	3.887	4.034	4.134	4.074	.072
AME (kcal/g)	3.239	3.071	3.219	3.096	3.125	3.173	.107
AME_n (kcal/g)	2.988	2.823	2.968	2.912	2.937	2.981	.101

[1]Poults were inoculated via the yolk sac at one day of age with .1 ml of the appropriate dilution of a 24-hr E. Coli culture. Controls received .1 ml of sterile Todd Hewitt broth.

[2] $\sqrt{\text{mean square error}}$. N = 3 observations per treatment.

Table 22. Specific activity of maltase from the intestinal mucosa of turkeys inoculated with E. Coli[1] and fed two levels of protein

Protein (%)	E. Coli dilution	Specific activity[2] Duodenum	Jejunum
28	0	.257	.903
	10^{-3}	.971	.432
	10^{-2}	1.294	1.267
22	0	.391	.652
	10^{-3}	.305	.871
	10^{-2}	.142	.701
Standard deviation[3]		.288	.372

[1]Poults were inoculated via the yolk sac with .1 ml of the proper dilution of a 24-hr E. Coli culture. Controls received .1 ml of sterile Todd Hewitt broth.

[2]Specific activity expressed as μmoles of maltose hydrolyzed per minute per mg protein.

[3]$\sqrt{\text{mean square error}}$. N = 2 observations per treatment.

Table 23. Specific dipeptidase activities for glycyl-L-valine and glycyl-L-leucine from the intestinal mucosa of turkeys inoculated via the yolk sac with E. Coli[1] and fed two levels of protein

Protein (%)	E. Coli dilution	Specific activity[2] Duodenum	Jejunum	Ileum
		Glycyl-L-valine		
28	0	33.6	21.0	11.7
	10^{-3}	13.7	19.4	8.0
	10^{-2}	32.4	35.3	6.0
22	0	38.5	19.4	25.2
	10^{-3}	24.1	19.7	.5
	10^{-2}	28.5	24.6	17.4
Standard deviation		12.1	8.8	10.3
		Glycyl-L-leucine		
28	0	37.5	19.9	17.7
	10^{-3}	17.4	17.0	6.6
	10^{-2}	36.7	23.8	6.7
22	0	39.3	15.8	13.9
	10^{-3}	25.2	17.2	16.3
	10^{-2}	34.5	22.9	16.2
Standard deviation		13.9	4.3	3.0*

[1]Poults were inoculated via the yolk sac with .1 ml of the proper dilution of a 24-hr E. Coli culture. Controls received .1 ml of sterile Todd Hewitt broth.

[2]Specific activity expressed as μmoles of dipeptide hydrolyzed per minute per mg protein.

* Protein by E. Coli interaction significant ($P \leq .05$).

SERIES IV. THE ROLE OF TURKEY ROTAVIRUS IN INFECTIOUS STUNTING SYNDROME

Earlier experiments with day-old poults failed to demonstrate any effect of oral rotavirus inoculation on poult performance, however, reports in the literature suggest that rotavirus plays a role in causing gastroenteritis and stunting in poultry. The lack of response in these pilot experiments was assumed due to the presence of maternal antibodies in the yolk material. For that reason, three experiments were conducted with poults over 21 days of age to investigate the effect of rotavirus on the nutritional parameters of poult performance. Twenty-one days was chosen based on Bartz's (1981) observation that detectable antibody was gone from the serum of chicks by 21 days. Turkeys were grown to 21 days on 28 or 22% protein diets to determine whether suboptimal protein levels made poults more susceptible to the stunting syndrome caused by rotavirus.

EXPERIMENTAL PROCEDURE

Preexperimental husbandry.

For all three experiments, male poults were grown from one day of age until approximately three weeks in electrically heated battery brooders. Poults were provided feed and water ad libitum starting at one day of age. The birds were separated into two groups and fed either 28 or 22% protein diets for the preexperimental period (Table 10). By three weeks of age, the two groups of birds differed significantly in body weight as a result of the two levels of protein. All poults were debeaked at five days of age.

Prior to the start of the experiment, the birds in each protein group were individually weighed and the extreme high and low weight birds removed from each group. The remaining birds were assigned to pens of 10 birds each, such that all pens within each protein level had similar means and standard deviations.

Rotavirus inoculation.

At the start of each experiment, poults were inoculated orally with 1 ml of a 10^{-1} dilution of turkey rotavirus. The controls received 1 ml of sterile medium. The rotavirus was isolated from an intestinal filtrate obtained from a Canadian turkey flock, and grown in monkey kidney tissue culture. Live virus was stored frozen at -20 C until needed. In all experiments, controls were inoculated first, followed by those birds receiving the live virus. This same procedure was used for handling poults at all times following inoculation to prevent exposure of the control groups to viral antigen.

Experiment 1.

This preliminary 10-day experiment was conducted to determine if a response to rotavirus would be obtained from three-week old turkeys. At 23 days of age, 40 male turkeys (20 turkeys at each protein level) were placed in four pens in electrically heated batteries. Four treatments resulted from the combination of rotavirus (0 vs. 10^{-1} dilution) with protein level (28 vs. 22%). All birds were weighed individually on days 4, 7, and 10 of the experiment. Feed consumption per pen was recorded daily.

Experiments 2 and 3.

Two 10-day experiments were conducted to examine the effects of rotavirus inoculation on digestibility parameters and mucosal enzyme activities. In Experiment 2, 80 male poults were inoculated with rotavirus (or sterile medium) at 22 days of age. The design of the experiment was identical to Experiment 1, except each treatment had two replicate pens. Total excreta collection was conducted daily for the duration of the experiment on plexiglass lined trays. The daily excreta were dried and handled as previously described (Series I), and then combined for days 1-3, 4-6, and 7-10 of the trial. Birds were weighed on days 4, 7, and 10 at which times two birds per treatment with weights close to the treatment mean were removed for preparation of mucosal enzyme homogenates. Homogenates were prepared and assayed for maltase and dipeptidase activity as previously described (Series III).

For Experiment 3, two additional treatments were added to the

previous design. These treatments resulted from poults at each protein level that were orally administered E.coli at one day of age, and subsequently inoculated with rotavirus at the start of the experiment at 27 days of age. Again, birds were weighed individually on days 4, 7 and 10 of the experiment, however, excreta collection was conducted only for days 7 through 10. Feed consumption for each of the 12 pens were recorded daily. Two birds from each of the six treatments were selected at the end of the experiment for assay of intestinal maltase and dipeptidase activities.

Serum antibodies to rotavirus were determined by agar gel immunodiffusion tests with calf rotavirus antigen for both experiments. Serum was obtained via the wing vein from five birds per pen in each experiment prior to inoculation and then at two and four weeks after inoculation with rotavirus.

RESULTS AND DISCUSSION

As shown in Table 24, the results of Experiment 1 indicated that rotavirus was capable of producing infectious stunting or malabsorption syndrome. The average decrease in body weight gain of 6% over the two protein levels was significant ($P \leq .05$) for the 1 to 7 and 1 to 10-day periods. Feed consumption increased 4% over the 10 day period yielding an average decrease in feed efficiency of 10% over the two levels of protein. Unfortunately, the feed consumption and feed efficiency data were only based on one observation per treatment. Apparently those turkeys infected with rotavirus increased feed consumption in an attempt to overcome the loss of nutrients through malabsorption. This observation corresponds to findings observed in other species infected with rotavirus (Woode and Crouch, 1978). Diarrhea was not observed in the present study, as reported to accompany the increase in feed consumption in mammals.

Based on the encouraging results of Experiment 1, Experiments 2 and 3 were designed to attempt to demonstrate again the stunting effect of rotavirus and define this effect on the nutritional performance of the poult through digestibility trials and mucosal enzyme activities. In addition, it was hypothesized that the poults response to rotavirus at three weeks of age in contrast to earlier unsuccessful experiments with day-old poults was due to the loss of maternal antibody by three weeks. Bartz (1981) reported no detectable antibody to rotavirus in chicks by 21 days of age, and also that turkey poults do not acquire a significant amount of maternal antibodies. Therefore, seroconversion

studies were included in the protocol for Experiments 2 and 3.

In Experiment 2, a significant ($P \leq .05$) protein level by rotavirus interaction was observed for the 1 to 4-day body weight gain data (Table 25). For those birds inoculated with rotavirus and fed the 28% protein diet, body weight gain improved 6%. Conversely, when poults were fed the 22% protein diet, and infected with rotavirus, body weight gain decreased 11%. Although not significant, feed consumption increased at both levels of protein when rotavirus was present. The protein by rotavirus interaction was also significant for feed efficiency. Feed efficiency increased 5% due to rotavirus at the 28% protein level, and decreased 13% ($P \leq .05$) for turkeys fed the 22% protein diet and orally inoculated with rotavirus. Differences were not significant at other times in this experiment.

The removal of poults at 4 days post inoculation (pi) for mucosal enzyme studies may have been responsible for the lack of significant difference at the later periods. Rotavirus appears to cause a low level infection in which only a small percentage of those poults may contain the virus subclinically. Low level infection has also been observed for quail inoculated with turkey rotavirus (Bartz, 1981). Alternately, the lack of response after day 4 pi may result from recovery of infected poults. Pigs infected with rotavirus experimentally return to a normal rate of growth within 4 to 7 days (Woode and Crouch, 1978). The results of Experiment 2 would tend to confirm this observation in turkeys.

Differences for the three consecutive excreta collection periods

were not significant for those birds inoculated with and without rotavirus. For that reason, the results were combined for the 10 day experiment and presented in Table 26. Numerically, changes in digestibility parameters corresponded to changes in feed efficiency, however, differences were slight. Differences between the two levels of protein were significant for all parameters except gross energy of the excreta. In other experiments with E. coli infections and stunted birds from commercial flocks (Series I and III), changes in the nitrogen content of the excreta were used as an indication of malabsorption. The changes observed in Experiment 2 were slight, yet followed the trend that those birds demonstrating a decrease in feed efficiency also had an increase in the excreta nitrogen content of approximately 3% (Table 26). The change is mainly due to a 5% increase during the 1 to 4-day period post inoculation.

Differences in specific activities for maltase and dipeptidase from the intestinal mucosa were not significant for any period due to rotavirus inoculation. The average values over the length of the intestine for the three periods from Experiment 2 are presented in Table 27 for both enzymes. Similar activities were observed for glycyl-L-valine and glycyl-L-leucine dipeptidase, therefore only the values for glycyl-L-valine are presented. For both enzymes, the variation among poults within a treatment is greater than any variation due to treatment. The coefficient of variation for triplicate or duplicate assay tubes averages 3 to 5% under our assay conditions, while the coefficient of variation between poults averages about 30%. Thus, the problem

does not seem to be in the assay procedure, but more likely results from the biological variation between poults. While other researchers have reported significant changes in maltase (or other disaccharidase) activity for mice and calves as a result of enteric rotavirus (or reovirus) infections (Branski et al., 1980; Halpin and Caple, 1976; Tzipori et al., 1983), a similar conslusion cannot be made for turkey rotavirus infection.

In Experiment 3, no response was observed for rotavirus alone, or when rotavirus was given to poults pretreated with E. coli. As shown in Table 28, body weight gain and feed efficiency were slightly higher for birds inoculated with rotavirus, although these differences were not significant. The response of rotavirus observed in Experiments 1 and 2 could not be confirmed in this experiment. In addition, E. coli inoculation prior to rotavirus inoculation did not increase the poults susceptibility to rotavirus infection. The combination of rotavirus and E. coli has been shown to be more severe than either pathogen alone in other species (Tzipori et al., 1983). Leece et al. (1983) hypothesized that rotavirus damaged the gut, which favored colonization and growth of E. coli. In their work, E. coli was given 24 hr after inoculation with rotavirus. A severe clinical response is seldom observed for rotavirus alone, although the mixed infection with E. coli appears to depend on an initial disruption of the gut epithelium by rotavirus. Apparently poults were not susceptible to rotavirus in Experiment 3.

Data from the digestibility calculations for the excreta collection period 6 to 10 days pi are presented in Table 29. As expected, no

differences were observed due to rotavirus. For the poults removed after 10 days for preparation of mucosal enzyme homogenates, no significant differences were observed due to treatments. The magnitude of the specific activity values for both enzymes (Table 30) are slightly higher than those obtained in Experiment 2. This difference is due to the fact that the assays were carried out at different times with different reagents. Despite the fact that decreases in dipeptidase activity have been observed in the absence of clinical signs of malnutrition in humans (Hazuria et al., 1974), this assay system for turkeys fails to demonstrate this point as previously stated.

The seroconversion studies clarified somewhat the varied responses of poults to rotavirus in this series of experiments. Antibodies to rotavirus were noted in all poults prior to inoculation at the start of Experiments 2 and 3. This finding disagrees with Bartz (1981), who reported no detectable antibody in chicks by 21 days of age. The presence of antibodies may result from yolk transmission of maternal antibodies or from exposure of poults to rotavirus antigen during the first three weeks. Avian and mammalian rotavirus share common group antigens (McNulty et al., 1980), therefore, the latter explanation may be plausible. The ubiquitous nature of human and murine rotavirus increases the possibility of the birds exposure to antigen during that period. The presence of antibodies in the serum does not prevent a clinical response to infection. The results of Experiment 2 support this statement along with work with mammals (Woode and Crouch, 1978). Also, the response of poults in Experiment 1 probably occurred when

antibodies to rotavirus were present, since these poults were of the same background as those used in the later experiments. For this reason, use of gnotobiotic animals in studies with rotavirus is highly desired.

Overall, the response to rotavirus identified in this series of experiments corresponds to that reported in the literature. Namely, the effect of rotavirus infection is highly variable under experimental conditions as well as natural conditions. The presence of antigen is not sufficient to produce a clinical response, yet enteric disorders associated with rotavirus naturally are abundant. Therefore, one must conclude that an additional stimulus is necessary for a host to become susceptible to the pathogen. In Experiment 2, the low protein diet may have caused sufficient stress on the birds to result in a noticeable response to rotavirus. Environmental stresses such as cold temperatures have been associated with viral enteritis, especially in human infants (Melnick, 1978). The efficiency of the antigen-antibody response also may be important in prevention of rotaviral infection. A lower plane of nutrition, as observed in Experiment 2, or severe changes in nutrient composition such as occurs during weaning in mammals (Leece et al., 1983) increases the chance of a rotavirus outbreak.

SUMMARY

Several experiments were conducted with poults over 21 days of age to investigate the effect of rotavirus on the nutritional parameters of poult performance. In Experiment 1, a pilot experiment, rotavirus decreased body weight gain while increasing feed consumption over a 10- day period. The result was an average decrease of 11% in feed efficiency for birds fed either 28 or 22% protein diets. The results of the experiment indicated that poults were susceptible to rotavirus infection after 21 days, whereas earlier experiments with day-old poults demonstrated no effect from rotavirus.

Subsequent experiments were less conclusive concerning the role of rotavirus in infectious stunting syndrome. In Experiment 2, a significant protein by rotavirus interaction was observed for body weight gain and feed efficiency 1 to 4 days post inoculation. An increase in both parameters resulted when birds maintained on a 28% protein diet were inoculated with rotavirus. For those poults fed at the suboptimal protein level (22%), body weight gain and feed efficiency decreased 11 and 13%, respectively. Increases in feed consumption averaged 2% at both protein levels. No significant changes were observed in digestibility parameters or intestinal mucosal enzyme activities.

In Experiment-3, no response was observed from rotavirus alone, nor when rotavirus was given to poults pretreated with E. coli at one day of age. By 10 days post inoculation, the weight gains of birds inoculated with rotavirus alone were numerically greater than the

controls or rotavirus plus E. coli groups at both protein levels. Feed efficiencies also tended to increase, although both effects were not significant. An effect of rotavirus on digestibility and mucosal enzyme activities was also not apparent in this experiment.

When one evaluates the observations made in these three experiments, it is apparent that rotavirus probably acts as an opportunist in the poult. While serum antibodies were detected in all poults prior to inoculation in Experiments 2 and 3, the low protein diet in Experiment 2 provided added sufficient stress to permit action of the virus. Because this effect was not repeated in Experiment 3, one must conclude that an additional stress, probably environmental, is required to enhance the poults susceptibility to rotavirus.

Table 24. Average body weight gain, feed consumption and feed efficiency of turkeys inoculated with rotavirus and fed two levels of protein (Experiment 1)

Protein (%)	28		22	
Rotavirus[1]	-	+	-	+
Days p.i.	Body weight gain, g			
Starting weight	474	465	373	373
1-4	126	122	98	92
4-7	146	133	112	98
1-7	272	255	210	190
7-10	233	219	178	175
1-10	505	474	388	365
	Feed consumption, g			
1-4	218	215	165	162
4-7	237	254	184	192
1-7	454	460	349	355
7-10	386	406	306	331
1-10	841	866	655	685
	Feed efficiency			
1-4	.579	.567	.591	.566
4-7	.617	.523	.610	.509
1-7	.598	.554	.601	.535
7-10	.603	.540	.582	.529
1-10	.600	.547	.592	.532

[1] Inoculation given orally at 23 days of age. Each poult received 1 ml of a 10^{-1} dilution of rotavirus.

[2] Average body weight gains based on one pen per treatment with 10 birds per pen.

Table 25. Average body weight gain, feed consumption and feed efficiency of turkeys inoculated with rotavirus and fed two levels of protein (Experiment 2)

Protein (%)	28		22		Standard
Rotavirus[1]	-	+	-	+	Deviation[2]
Days p.i.		Body weight gain (g)			
Starting weight	469	469	372	377	-
1-4	114	121	103	93	2
4-7	147	149	118	122	4
1-7	261	270	220	219	3
7-10	238	248	200	195	5
1-10	499	519	419	413	5
		Feed consumption (g)			
1-4	202	206	178	182	5
4-7	245	245	214	220	4
1-7	447	451	392	402	9
7-10	401	393	358	355	8
1-10	849	844	751	757	8
		Feed efficiency			
1-4	.564	.590	.577	.510	.015
4-7	.599	.610	.553	.557	.026
1-7	.585	.600	.561	.545	.016
7-10	.592	.631	.559	.549	.012
1-10	.588	.616	.558	.545	.012

[1]Inoculation given orally at 22 days of age. Each poult received 1 ml of a 10^{-1} dilution of rotavirus.

[2]$\sqrt{\text{mean square error}}$. N = 2 observations per treatment.

Table 26. Excreta nitrogen, nitrogen retention, nitrogen digestibility, dry matter digestibility, excreta energy, AME and AME_n on a dry matter basis for turkeys 1 to 10 days after oral inoculation with rotavirus[1] (Experiment 2)

Protein (%)	28		22		Standard[2]
Rotavirus	-	+	-	+	deviation
Excreta nitrogen (%)	6.43	6.10	4.57	4.75	.38
Nitrogen retention (g)	51.96	55.50	40.61	38.56	4.84
Nitrogen digestibility (%)	54.37	56.94	62.48	62.18	2.72
Dry matter digestibility (%)	63.45	63.58	67.35	68.18	1.28
Excreta energy (kcal/g)	3.861	3.948	3.936	3.954	.113
AME (kcal/g)	3.086	3.058	3.169	3.197	.073
AME_n (kcal/g)	2.855	2.816	2.965	2.993	.073

[1] Poults were inoculated orally at 22 days of age with 1 ml of a 10^{-1} dilution of rotavirus.

[2] $\sqrt{\text{mean square error}}$. Analysis based on the average of three consecutive collection periods. N = 2 observations per treatment.

Table 27. Average specific activities of maltase and glycyl-L-valine dipeptidase from the intestinal mucosa of turkeys inoculated with rotavirus and fed two levels of protein (Experiment 2)

Protein (%)	Rotavirus[1]	Specific activity[2]	
		Maltase	Dipeptidase
28	-	.579	17.3
	+	.488	18.1
22	-	.439	17.9
	+	.331	19.6
Standard deviation[3]		.255	5.3
n(obs/trt)		15	18

[1]Rotavirus inoculation given at 22 days of age.

[2]Specific activity expressed as μmoles of maltose or glycl-L-valine hydrolyzed per minute per mg protein. Values are the average for birds assayed 3, 6 and 10 days post inoculation.

[3]$\sqrt{\text{Mean square error}}$.

Table 28. Average body weight gain, feed consumption and feed efficiency of turkeys inoculated with rotavirus and fed two levels of protein (Experiment 3)

Protein (%)	28			22			
Rotavirus[1]	-	+	+	-	+	+	Standard
E. Coli[2]	-	-	+	-	-	+	Deviation[3]
Days p.i.	Body weight gain (g)						
Starting weight	668	663	654	574	556	545	-
1-4	160	165	163	146	158	555	7
4-7	175	175	170	142	149	145	5
1-7	336	339	334	288	307	300	6
7-10	258	276	273	239	243	230	15
1-10	593	615	606	527	550	529	18
	Feed consumption (g)						
1-4	274	270	271	250	257	244	5
4-7	284	284	290	261	272	263	9
1-7	558	554	561	511	529	507	13
7-10	446	450	460	420	417	411	15
1-10	1004	1004	1021	931	946	917	26
	Feed efficiency						
1-4	.586	.611	.602	.584	.616	.636	.036
4-7	.616	.615	.589	.544	.548	.551	.021
1-7	.601	.613	.595	.564	.581	.591	.017
7-10	.578	.613	.592	.570	.582	.560	.019
1-10	.591	.613	.594	.567	.582	.577	.014

[1] Inoculation given orally at 27 days of age. Each poult received 1 ml of a 10^{-1} dilution of rotavirus.

[2] Poults inoculated orally at one day of age with .1 ml of a 10^{-2} dilution of a 24-hr *E. Coli* culture.

[3] Mean square error. N = 2 observations per treatment.

Table 29. Excreta nitrogen, nitrogen retention, nitrogen digestibility, dry matter digestibility, excreta energy, AME and AME_n on a dry matter basis for turkeys 6 to 10 days after oral inoculation with rotavirus[1] (Experiment 3)

Protein (%)	28			22			
Rotavirus	-	+	+	-	+	+	Standard[3]
E. Coli[2]	-	-	+	-	-	+	deviation
Excreta nitrogen (%)	6.79	6.67	7.05	4.87	5.58	5.10	.27
Nitrogen retention (g)	87.35	78.36	66.14	48.65	61.26	57.30	8.26
Nitrogen digestibility (%)	57.23	58.85	56.00	62.02	58.96	63.12	1.92
Dry matter digestibility (%)	67.92	68.55	68.24	70.75	72.44	72.93	1.02
Excreta energy (kcal/g)	3.859	3.863	3.810	3.891	3.973	3.998	.056
AME (kcal/g)	3.267	3.290	3.295	3.272	3.315	3.328	.033
AME_n (kcal/g)	3.027	3.044	3.061	3.081	3.133	3.133	.031

[1]Poults were inoculated orally at 27 days of age with 1 ml of 10^{-1} dilution of rotavirus.

[2]Poults were inoculated orally at one day of age with .1 ml of a 10^{-2} dilution of a 24-hr E. Coli culture.

[3]$\sqrt{\text{mean square error}}$. N = 2 observations per treatment.

Table 30. Average specific activities of maltase and glycl-L-valine dipeptidase from the intestinal mucosa of turkeys 10 days after inoculation with rotavirus and fed two levels of protein (Experiment 3)

Protein (%)	Rotavirus	E. Coli[1]	Specific activity[2]	
			Maltase	Dipeptidase
28	-	-	.880	24.7
	+	-	.629	25.3
	+	+	.603	22.5
22	-	-	.524	23.9
	+	-	.699	25.7
	+	+	.542	28.8
Standard deviation[3]			.318	7.2
n(obs/trt)			4	6

[1] E. Coli inoculation given at one day of age while rotavirus inoculation given at 27 days of age.

[2] Specific activity expressed as μmoles of maltose or glycyl-L-valine hydrolyzed per minute per mg protein.

[3] $\sqrt{\text{mean square error}}$.

CONCLUSIONS

Experiments were conducted with pathogenic E. coli and rotavirus to compare the effects of these pathogens on the nutritional performance of poults and to produce infectious stunting syndrome. At the outset, poults from commercial turkey flocks exhibiting the stunting syndrome were studied to identify the effects of stunting on digestibility parameters of poults. The infectious stunting syndrome in commercial turkeys appeared to result from a malabsorption of nutrients per se. Decreases in digestible dry matter and nitrogen, as well as increases in excreta nitrogen and gross energy of stunted birds support this conclusion. Decreased feed consumption was not considered to cause the stunting since stunted birds consumed feed at a normal rate for the smaller body size.

As an enteric pathogen, E. coli was found to elicit multiple responses. The strain of E. coli used in these experiments was isolated from the yolk sac of stunted poults, indicative of an egg transmitted pathogen. When reintroduced into day-old poults via the yolk sac, E. coli was capable of causing mortality and stunting depending on the strength of the dose. An adequate level of protein (28% protein) was capable of preventing a drastic reduction in feed consumption and body weight gain. Suboptimal protein content of the diet (22%) resulted in a very large reduction in feed consumption. Despite the reduction in feed consumption, a slight malabsorption of nutrients was induced by E. coli infection, probably resulting from the toxic response of the intestinal epithelium. The malabsorption was greatest for birds fed 28%

protein diets, however, excreta nitrogen and gross energy increased for birds fed both levels of protein. The changes in excreta nitrogen and energy were found to be good indicators of malabsorption.

The same strain of E. coli when administered orally at hatching, apparently altered the populations of intestinal coliforms, resulting in a significant improvement in feed efficiency by three weeks of age. The improved feed efficiency resulted from a larger increase in body weight gain than feed consumption. Since the level of inoculation was comparable whether given orally or via the yolk sac, the oral route allowed colonization of the organism to the benefit of the poult, while the yolk sac inoculation elicited a severe depressing response. E. coli is a normal component of the poults flora, however, when allowed to flourish, such as in the case of yolk transmission, can become a serious threat to poult production. Yolk transmission of E. coli or any pathogen generally occurs as a result of poor management.

Experimental reproduction of rotavirus infection was found to be more difficult than E. coli infection. The ubiquitous nature of rotavirus was responsible for the detection of antibodies to rotavirus in all poults tested. However, a response to rotavirus was observed in the presence of serum antibodies when combined with low protein diets in one experiment. Rotavirus is capable of producing a severe malabsorption characterized by decreased body weight gain concurrent with an increase in feed consumption. Under normal circumstances, rotavirus can exist without problems in the intestine of the poult, however, a stressful environment may increase the poult's susceptibility

to infection. The stress may be environmental, nutritional or perhaps in combination with some other pathogen. Similar to E. coli, prevention of infectious stunting caused by rotavirus lies in sound management practices for commercial poultry production. Elimination of the pathogens may be impossible, but proper management can prevent clinical outbreaks.

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NUTRITIONAL PARAMETERS ASSOCIATED WITH ENTERIC *ESCHERICHIA COLI* AND ROTAVIRUS IN POULTS

by

G. P. Schmidt

ABSTRACT

Experiments were conducted to investigate the effects of *E. coli* and rotavirus as causative agents in producing infectious stunting syndrome of poults. The syndrome, as it occurs commercially, was characterized in two experiments to produce decreased digestible dry matter and nitrogen as well as increased excreta nitrogen and gross energy content of affected poults. The effects of *E. coli* and rotavirus on the nutritional performance of poults were compared to the commercial syndrome by digestibility trials and assay of intestinal mucosal enzyme activities.

The strain of *E. coli* used in these experiments was isolated from the yolk sac of stunted poults. Inoculation of day-old poults was done orally in one experiment and via the yolk sac in another series of experiments. Experiments were designed in a factorial arrangement of *E. coli* inoculation and dietary protein level (28 *vs.* 22%). Oral administration of the pathogenic *E. coli* at a low dose (.1 ml of a 10^{-2} dilution of a 24-hr culture) to day-old poults produced a significant increase in feed efficiency by 21 days of age for birds fed either 28 or 22% protein diets. In this case, *E. coli* apparently was established as

part of the normal intestinal microflora of the turkey without producing a toxic response. Similar concentrations of E. coli (10^{-3} and 10^{-2} dilutions) inoculated into the yolk sac of day-old poults resulted in significant mortality and morbidity. The lower dilution (10^{-3}) produced the desired response of stunted poults without substantial mortality. Body weight gain and feed consumption were severely decreased by E. coli inoculation at both levels of protein (28 or 22%). The 28% protein diet alleviated the reduction in feed consumption for birds infected with the lower concentration of E. coli. The 10^{-2} dilution caused substantial mortality and similar responses on body weight at both levels of protein. A malabsorption of nutrients accompanied E. coli infection in spite of reduced feed consumption as indicated by increased excreta nitrogen and gross energy content for all infected poults.

In three experiments, rotavirus was orally inoculated into poults over 21 days of age previously fed either 28 or 22% protein diets. The response of rotavirus was variable in the three experiments, however, the low protein diet (22%) increased the poults susceptibility to rotavirus infection. Serum antibodies to rotavirus were detected in all poults tested prior to inoculation. Ubiquitous in nature, rotavirus appears to produce clinical signs of infection in combination with stress on the poults. Effects of rotavirus on digestibility parameters were inconclusive. In experiments with either rotavirus or E. coli, activities of intestinal mucosal enzymes were found to vary more between poults within a treatment than by any effect of the various treatments.