Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
ABSTRACT

Severe wasting of body tissues, diarrhea, high morbidity and mortality, and stunting are all characteristics of poult enteritis and mortality syndrome (PEMS). The wasting of musculature and loss of nearly all adipose tissue suggested that even though the PEMS-infected poults were eating some feed, nutrient intake was not sufficient to meet body requirements for maintenance and growth. Because epithelial cells in the gastrointestinal tract appeared to be a target of the undefined etiological agent (or agents) that causes PEMS, a study was conducted in which PEMS-infected poults were evaluated for malabsorption through 3 wk of age. D-Xylose, a poorly metabolized pentose, was given per os as a bolus, and blood samples were obtained from the ulnar vein in the wing of control and PEMS-infected poults over a 3-h period to estimate intestinal absorption. D-Xylose absorption in control poults peaked 30 to 60 min after the oral treatment, similar to results reported earlier. The PEMS-infected poults did not show a peak in absorption. The PEMS-infected poults showed significant delays in D-xylose absorption at 4, 7, and 11 d after PEMS challenge. The severe malabsorption and metabolic deficiency problem associated with PEMS was postulated to be a direct effect of the undefined infectious agent or agents that cause the disease.

(Key words: poult, enteritis, mortality, D-xylose, malabsorption)

INTRODUCTION

Poult enteritis and mortality syndrome (PEMS) is an acute transmissible, infectious disease with an unknown etiology that affects turkey poults between 7 and 28 d of age (Barnes et al., 1996, 1997). Poults with PEMS exhibit high-pitched vocalization, marked anorexia, and a strong behavioral tendency to huddle into very tight and, often times, piled-up groups (Edens et al., 1997; Doerfler et al., 1998; Edens et al., 1998). Edens and Doerfler (1997a,b) reported that glucose metabolism in PEMS-infected poults appeared to be impaired because hepatic glycogen was depleted, and hepatic glucose-6-phosphatase activity was increased without concomitant increases in serum glucose. Even with addition of sucrose to the drinking water of infected poults, these conditions were not corrected (Edens and Doerfler, 1997a).

The D-xylose absorption test, first introduced by Eberts et al. (1979) and later modified by Goodwin et al. (1984a,b), has been used as an indicator of malabsorptive conditions in the small intestine of normal turkeys and turkeys with enteric diseases. D-Xylose is a pentose sugar that is absorbed from the upper small intestinal tract, similar to the sodium-dependent active transport of glucose and amino acids (Goodwin et al., 1984a,b). In the chicken, xylose is actively absorbed and shares a common mobile-transport carrier with glucose and amino acids, similar to the condition found in mammals (Alvarado, 1966, 1967; Alvarado and Monreal, 1967). This substance has proven quite useful as an indicator of intestinal absorptive function, as D-xylose is poorly metabolized by the body and is readily excreted in the urine. Because urine collection can be difficult in avian species, changes in plasma D-xylose concentrations over a 3-h period are indicative of its absorption from the intestinal tract. The objective of the present study was to evaluate the absorptive function of the small intestine in PEMS-infected poults compared with healthy, control poults.

MATERIALS AND METHODS

Animal Welfare

This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee, which has adopted Animal Welfare

Received for publication March 8, 1999.
Accepted for publication January 28, 2000.

Abbreviation Key: PEMS = poult enteritis and mortality syndrome; PI = postinoculation.
Care and Use Guidelines governing all animal use in experimental procedures.

**Poult and Husbandry**

British United Turkey male poult from a commercial hatchery were obtained and transported to the Dearstyne Avian Research Center at North Carolina State University, where they were wing-banded and weighed and were placed into pens in heated metal battery brooders with raised wire floors. The poult were not subjected to hatchery services such as beak or nail trimming, antibiotic administrations, or vaccinations. The control poult and the poult designated for PEMS exposure were assigned to separate but identical controlled-environment isolation rooms and were placed in heated batteries. In each of two trials, six replicate pens of 12 control poult and eight replicate pens of 12 PEMS-challenged poult were utilized. The two additional replicate pens of PEMS-challenged poult were necessary to ensure adequate numbers of PEMS-challenged poult at the termination of the experiment at 21 d of age. Continuous lighting was provided by incandescent lamps in the ceiling of each room and on each deck of the brooding battery.

Ambient temperature for brooding was maintained by room air conditioning with a thermostatically controlled hot water and cold water heat exchange system mediated by a forced draft. Initial room brooding temperature for both the control and PEMS rooms was set at 34 ± 1°C, and this temperature was decreased 3°C in each room at 7 and 14 d of brooding. Humidity in the experimental rooms was not controlled and varied from 47 to 63% relative humidity. Continuous light was provided from deck brooder lights and from incandescent lamps in the ceiling. The North Carolina Agricultural Research Service Corn- and soybean-based turkey starter feed (2,915 kcal/kg ME and 28.13% crude protein) and water were provided ad libitum in stainless-steel feeders.

**PEMS Inoculation**

At 6 d posthatch, each poult in the PEMS-designated groups was given an oral PEMS inoculation. The PEMS inoculation consisted of a 0.1-mL oral gavage of a 10% suspension (sterile 0.9% saline) of fresh, raw feces derived from PEMS-infected poult maintained at the North Carolina State University College of Veterinary Medicine.

**D-Xylose Administration and Measurement in Plasma**

Food and water were removed from five poult per group 12 h before each sampling. Birds were selected randomly for each sampling, and even though BW was determined for each bird, it was used only for administration of D-xylose. Birds were sampled at 1, 4, 7, 11, and 15 d postinoculation (PI). During each sampling period, control and PEMS-inoculated poult were maintained and handled separately. These control and PEMS-inoculated poult were not used again but were returned to the general population of test animals.

The feed-deprived poult were weighed and were given a dose of 5% D-Xylose solution at a concentration of 0.5 g D-xylose/kg BW via oral gavage. Blood samples from the ulnar vein in the wings were collected on a 0.5-h basis for 3 h with heparinized microhematocrit capillary tubes. These hematocrit tubes (in duplicate) were centrifuged for plasma collection. The collected plasma (20 µL) was subjected to a modified micromethod (Goodwin et al., 1984a,b) first described by Eberts et al. (1979) for determination of plasma D-xylose. To each 20-µL plasma sample, 2 mL of phloroglucinol color reagent was added and heated for 4 min at 100°C. The samples were allowed to cool to room temperature in a water bath. After cooling, the absorbance of each sample was read on a Gilford UV-Vis spectrophotometer set at 554 nm.

**Analysis of Data**

The experiment was conducted as a completely randomized design (control vs. PEMS). Data were grouped by trials, treatments, day, and replicate, and all parameters were analyzed using the SAS general linear models procedures for ANOVA and regression analysis (SAS Institute, 1996). Because there was neither a trial effect nor a treatment by replicate pen interaction, the data from both trials were pooled and analyzed with treatment and day as main effects. Statements of significance were based on P ≤ 0.05. Means were separated by least significant difference when there were significant differences due to main effects in the ANOVA.

**RESULTS**

Data for D-xylose absorption were averaged across the 150-min sampling periods for 1, 4, 7, and 11 d PI (Table 1). No difference was found between D-xylose absorptions of control and PEMS-designated poult at 1 d PI. At 4, 7, and 11 d PI, the poult in the PEMS-challenged groups absorbed significantly less D-xylose than did the control poult (Table 1). The difference between plasma xylose concentrations of control and PEMS-inoculated poult varied as a function of days PI (Table 1). The difference between control and PEMS poult ranged from 20.3 mg/dL (54% less than controls at 7 d PI) to 10.3 mg/dL (34.0% less than controls at 4 d PI) and 10.5 mg/dL (36.3% less than controls for 11 d PI) (Table 1). At 15 d PI, there was no difference in D-xylose absorption between control and PEMS-inoculated poult, even though PEMS-infected poult showed 11.0% greater D-xylose uptake than did controls.

There were significant time effects that influenced D-xylose absorption in the control and PEMS-infected

---

3Sigma Chemical Co., St. Louis, MO 63178-9916.
4Fisher Scientific, Fairlawn, NJ 07410.
5Ciba-Corning Diagnostics Corp., Norwood, MA 02062-4694.

---
TABLE 1. Overall, within-days postinoculation, mean plasma D-xylose concentration comparisons between control poults and poults inoculated with poult enteritis and mortality syndrome (PEMS) at 6 d of age

| Days Post-inoculation | Treatments | D-Xylose | Control vs. PEMS |
|-----------------------|------------|----------|-----------------|
|                       |            | Numerical| Percentage      |
|                       |            | (mg/dL)  | (mg/dL) (%)     |
| 1 Control (10)         | 36.6 ± 2.2*| 0.5      | 1.4             |
| PEMS (10)              | 36.1 ± 2.2*|          |                 |
| 4 Control (10)         | 30.4 ± 1.9*| 10.3     | 34.0            |
| PEMS (10)              | 20.1 ± 1.9*|          |                 |
| 7 Control (10)         | 37.6 ± 2.5*| 20.3     | 54.0            |
| PEMS (10)              | 17.3 ± 2.6*|          |                 |
| 11 Control (10)        | 29.0 ± 2.4*| 10.5     | 36.3            |
| PEMS (10)              | 18.5 ± 2.5*|          |                 |
| 15 Control (10)        | 41.8 ± 3.0*| −4.6     | −11.0           |
| PEMS (10)              | 46.4 ± 3.0*|          |                 |

a,bWithin Days Postinoculation, means (± SEM) with unlike superscripts differ significantly (*P* ≤ 0.05).

1(10) Indicates the total number of poults per treatment involved in each sampling date.

poults (Figures 1, 2, and 3). However, the amount of D-xylose absorbed within the first 90 min after the oral administrations of D-xylose was always significantly less in the PEMS-infected poults. By 90 min after the administration of D-xylose, plasma concentrations in the control and PEMS-infected poults had begun to decrease significantly at 4, 7, and 11 d PI. From 120 through 150 min after D-xylose administration, control plasma D-xylose concentrations had decreased to the level measured in PEMS-infected poults (except at 7 d PI; Figure 2).

At 4 d PI, when the poults were 10 d of age, peak plasma D-xylose concentration was observed between 30 and 60 min after oral treatment in control poults (Figure 1). There was no difference between the concentrations measured at 30 and 60 min at this age in the controls. Peak concentrations were measured at 60 min after the oral treatment in PEMS-infected poults. However, during the first 90 min after the oral D-xylose treatment, the control plasma D-xylose concentrations were significantly higher than in PEMS-infected poults, but at 120 and 150 min after D-xylose administration, there were no differences between control and PEMS-infected poults plasma D-xylose concentrations.

At 7 d PI when the poults were 13 d of age, peak plasma D-xylose concentrations in the control and PEMS-infected poults were found at 60 min after the oral D-xylose treatments (Figure 2). However, at no time did the plasma concentrations in PEMS-infected poults rise to the level measured in the controls.

At 11 d PI, when the poults were 17 d of age, control poults had significantly elevated plasma D-xylose concentrations at 30 and 60 min after oral treatments (Figure 3). Peak plasma concentrations in the controls were measured at 30 min after the oral D-xylose treatments, but the peak was not observed until 60 min in the PEMS-infected poults. From 90 through 150 min after the oral treatments, there were no differences between control and PEMS-infected poults.

**DISCUSSION**

The D-xylose absorption test has been used in poultry “to provide a simple, specific, and sensitive test for intesti-
Malabsorption in PEMS-challenged poults, as indicated by significantly decreased D-xylose uptake on Days 4, 7, and 11 PI, was documented at times when the clinical manifestation of this enteric disease, using death as an indicator, was, historically, at its zenith (Doerfler et al., 1998). In this study, mortality was not considered as a variable, but more deaths occurred in the PEMS-infected group when the birds were between 10 and 13 d of age. Histologically, the malabsorptive condition was reflected as villus atrophy and crypt hypertrophy (Perry et al., 1991). Edens and Doerfler (1997a,b) have suggested that in PEMS-infected poults, malabsorption of nutrients from the intestinal tract and lack of ability to utilize nutrients such as glucose was related to marked mitochondrial hypertrophy and degeneration in enterocytes and in hepatocytes as well. Therefore, lack of Na⁺-dependent active transport of nutrients such as glucose and amino acids would result in many of the signs of PEMS such as diarrhea, wasting of musculature, lack of growth and stunting, and high rates of mortality.

In some mammals and chickens, D-xylose can be an indicator of water and sodium absorption because D-xylose is an active transport in chickens and, most likely, turkeys generally can be, but not specifically, inferred by failure to absorb xylose (Alvarado, 1967).

In the upper small intestine of birds and mammals, absorption of sugars and amino acids is largely responsible for fluid absorption. Also, intestinal absorption of sugars and amino acids is coupled with active transport of sodium from the lumen of the intestinal tract of birds and mammals (Alvarado, 1966; Alvarado and Monreal, 1967; Stevens et al., 1984). Thus, active absorption of D-xylose would indicate active absorption of Na⁺ and water. Therefore, a decrease in active absorption in chickens and, most likely, turkeys generally can be, but not specifically, inferred by failure to absorb xylose (Alvarado, 1967).
The movement of one molecular species by volume flow, diffusion, or active transport can cause the movement of another molecular species (Stevens et al., 1984). Consequently, two compartments can be separated by an interface and be at osmotic equilibrium, but diffusion or active transport of, e.g., D-xylose or glucose across that interface would dilute one solution and make the other more concentrated. In this case, sodium would also be actively cotransported with the sugar(s), causing water to move in the same direction as a result of the creation of an osmotic pressure difference between the two compartments. In PEMS-infected poults, dehyration in association with severe diarrhea is a predominant sign of the disease. Therefore, these data on reduced D-xylose absorption would also suggest that there is not only a problem with nutrient uptake but that water is not being absorbed at a rate necessary for maintenance of life processes. These observations lead to a conclusion that part of the wasting syndrome associated with PEMS infection can be explained by malabsorption of nutrients and water from the intestinal tract as proposed earlier (Edens and Doerfler, 1997a,b; Doerfler et al., 1998).

REFERENCES

Alvarado, F., 1966. Transport of sugars and amino acids in the intestine: Evidence for a common carrier. Science 151:1010–1013.

Alvarado, F., 1967. D-xylose transport in the chicken small intestine. Comp. Biochem. Physiol. 20:461–470.

Alvarado, F., and J. Monreal, 1967. Na⁺-dependent active transport of phenylglucosides in the chicken small intestine. Comp. Biochem. Physiol. 20:471–488.

Barnes, H. J., J. S. Guy, T. P. Brown, and F. W. Edens, 1996. Pages 1–11 in: Poult Enteritis and Mortality Syndrome ("Spiking Mortality in Turkeys") and Related Disorders: An Update. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Barnes, H. J., and J. S. Guy, J. T. Weaver, and S. R. Jennings, 1997. Turkey flocks with high spiking mortality that are negative for turkey coronavirus. Proc. Amer. Vet. Med. Assoc. 134:168.

Doerfler, R. D., F. W. Edens, C. R. Parkhurst, G. B. Havenstein, and M. A. Qureshi, 1998. Hypothermia, hypoglycemia, and hypothyrosis associated with poult enteritis and mortality syndrome. Poultry Sci. 77:1103–1109.

Eberts, T. J., R.H.B. Sample, M. R. Glick, and G. H. Ellis, 1979. A simplified, colorimetric micromethod for xylose in serum or urine, with phloroglucinol. Clin. Chem. 25:1440–1443.

Edens, F. W., C. R. Parkhurst, M. A. Qureshi, and G. B. Havenstein, 1997. Atypical Escherichia coli strains and their association with poult enteritis and mortality syndrome. Poultry Sci. 76:952–960.

Edens, F. W., and R. E. Doerfler, 1997a. Glucose in metabolism in poult enteritis and mortality syndrome. Pages 106–119 in: Proceeding of the 20th Technical Turkey Conference. Pott Shrigley, Near Macclesfield, Cheshire, England.

Edens, F. W., and R. E. Doerfler, 1997b. Cellular and biochemical lesions associated with poult enteritis and mortality syndrome. Proc. Amer. Vet. Med. Assoc. 134:169.

Edens, F. W., K. A. Joyce, C. R. Parkhurst, G. B. Havenstein, and M. A. Qureshi, 1998. Effect of litter moisture and brooding temperature on body weights of turkey poults experiencing poult enteritis and mortality syndrome. Poultry Sci. 77:411–415.

Goodwin, M. A., K. S. Latimer, and O. J. Fletcher, 1984a. Quantitation of intestinal D-xylose absorption in normal turkeys. Poultry Sci. 63:1742–1747.

Goodwin, M. A., K. S. Latimer, and B. N. Nersessian, 1984b. Quantitation of intestinal D-xylose absorption in normal and reovirus-inoculated turkeys. Avian Dis. 28:959–967.

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

Perry, R. W., G. N. Rowland, and J. R. Glisson, 1991. Poult malabsorption syndrome. I. Malabsorption in poult enteritis. Avian Dis. 35:685–693.

Phelps, P. V., F. W. Edens, and R. P. Gildersleeve, 1987. The posthatch physiology of the turkey poult. III. Yolk depletion and serum metabolites. Comp. Biochem. Physiol. 87A:409–415.

Reynolds, D. L., and Y. M. Saïf, 1986. Astrovirus: A cause of an enteric disease in turkey poults. Avian Dis. 30:728–735.

SAS Institute, 1996. SAS User’s Guide. Version 6.12. SAS Institute, Inc., Cary, NC.

Sell, J. L., O. Koldovsky, and B. L. Reid, 1989. Intestinal disaccharidases of young turkeys: Temporal development and influences of diet. Poultry Sci. 68:265–277.

Shawky, S. A., Y. M. Saïf, and D. E. Swayne, 1993. Role of circulating maternal anti-rotavirus IgG in protection of intestinal mucosal surface in turkey poults. Avian Dis. 37:1041–1050.

Stevens, B. R., J. Kaunitz, and E. Wright, 1984. Intestinal transport of amino acids and sugars. Annu. Rev. Physiol. 46:417–433.

Uni, Z., Y. Noy, and D. Sklan, 1999. Posthatch development of small intestinal function in the poult. Poultry Sci. 78:215–222.

Yason, C. V., and K. A. Schat, 1986. Pathogenesis of rotavirus infection in turkey poults. Avian Path. 15:421–435.

Yason, C. V., and K. A. Schat, 1987a. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Pathology. Am. J. Vet. Res. 48:927–938.

Yason, C. V., and K. A. Schat, 1987b. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Clinical signs and virology. Am. J. Vet. Res. 48:977–983.