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D-Xylose Absorption as a Measurement of Malabsorption in Poult Enteritis and Mortality Syndrome[1]

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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)

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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

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[1]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similar products not mentioned.

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Abbreviation Key: PEMS = poult enteritis and mortality syndrome; PI = postinoculation.

Care and Use Guidelines governing all animal use in experimental procedures.

Poults and Husbandry

British United Turkey male poults 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 poults were not subjected to hatchery services such as beak or nail trimming, antibiotic administrations, or vaccinations. The control poults and the poults designated for PEMS exposure were assigned to separate but identically controlled-environment isolation rooms and were placed in heated batteries. In each of two trials, six replicate pens of 12 control poults and eight replicate pens of 12 PEMS-challenged poults were utilized. The two additional replicate pens of PEMS-challenged poults were necessary to ensure adequate numbers of PEMS-challenged poults 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 poults 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 poults 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 poults were maintained and handled separately. These control and PEMS-inoculated poults were not used again but were returned to the general population of test animals.

The feed-deprived poults were weighed and were given a dose of 5% D-Xylose[3] 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.[4] 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[3] 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[5] 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 $P \leq 0.05$. 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 poults at 1 d PI. At 4, 7, and 11 d PI, the poults in the PEMS-challenged groups absorbed significantly less D-xylose than did the control poults (Table 1). The difference between plasma xylose concentrations of control and PEMS-inoculated poults varied as a function of days PI (Table 1). The difference between control and PEMS poults 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 poults, even though PEMS-infected poults 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

[3]Sigma Chemical Co., St. Louis, MO 63178-9916.
[4]Fisher Scientific, Fairlawn, NJ 07410.
[5]Ciba-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[1]	D-Xylose	Control vs. PEMS	
			Numerical	Percentage
		(mg/dL)	(mg/dL)	(%)
1	Control (10)	36.6 ± 2.2[a]	0.5	1.4
	PEMS (10)	36.1 ± 2.2[a]		
4	Control (10)	30.4 ± 1.9[a]	10.3	34.0
	PEMS (10)	20.1 ± 1.9[b]		
7	Control (10)	37.6 ± 2.5[a]	20.3	54.0
	PEMS (10)	17.3 ± 2.6[b]		
11	Control (10)	29.0 ± 2.4[a]	10.5	36.3
	PEMS (10)	18.5 ± 2.5[b]		
15	Control (10)	41.8 ± 3.0[a]	–4.6	–11.0
	PEMS (10)	46.4 ± 3.0[a]		

[a,b]Within Days Postinoculation, means (± SEM) with unlike superscripts differ significantly ($P \leq 0.05$).
[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-

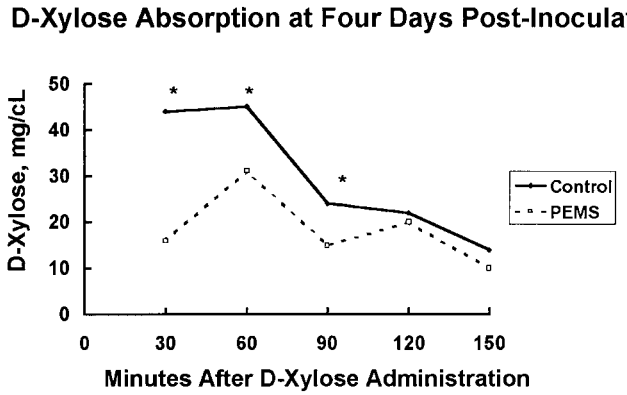

FIGURE 1. D-Xylose absorption in poult enteritis and mortality syndrome (PEMS)-infected poults at 4 d postinoculation. The asterisk (*) associated with means for different times indicates a significant difference ($P \leq 0.05$) between control and PEMS-infected groups.

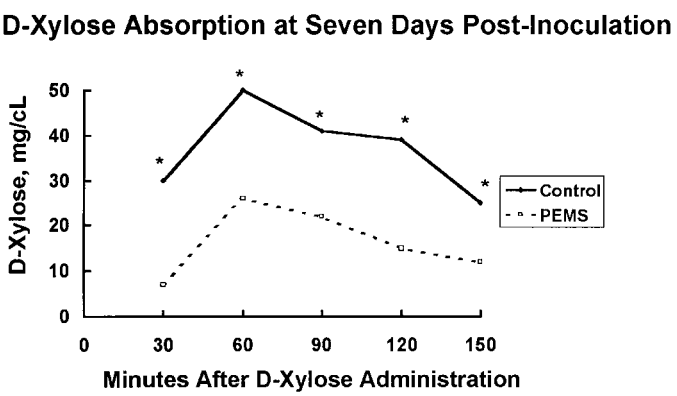

FIGURE 2. D-Xylose absorption in poult enteritis and mortality syndrome (PEMS)-infected poults at 7 d postinoculation. The asterisk (*) associated with means for different times indicates a significant difference ($P \leq 0.05$) between control and PEMS-infected groups.

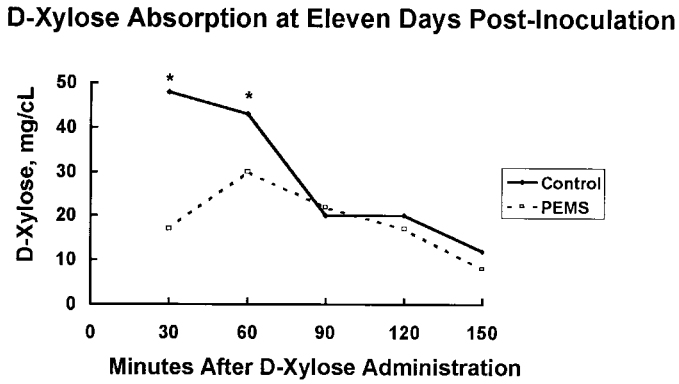

FIGURE 3. D-Xylose absorption in poult enteritis and mortality syndrome (PEMS)-infected poults at 11 d postinoculation. The asterisk (*) associated with means for different times indicates a significant difference ($P \leq 0.05$) between control and PEMS-infected groups.

nal absorption and malabsorption" (Goodwin et al., 1984b; Perry et al., 1991). Decreased D-xylose absorption as a method to indicate malabsorption has been demonstrated in several cases of virus-induced intestinal dysfunction in poults, including rotavirus (Yason and Schat, 1986; Yason and Schat, 1987a,b; Shawky et al., 1993), reovirus (Goodwin et al., 1984b), and astrovirus (Reynolds and Saif, 1986).

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 et al. (1997) demonstrated both of these malabsorptive conditions in the intestinal tract of PEMS-infected poults and further demonstrated that the microvilli and mitochondria within the enterocytes were also severely damaged by PEMS infection. These malabsorptive conditions in the turkey poult are characterized by decreased villus surface epithelium and, subsequently, a loss of absorptive surface area (Perry et al., 1991; Edens et al., 1997).

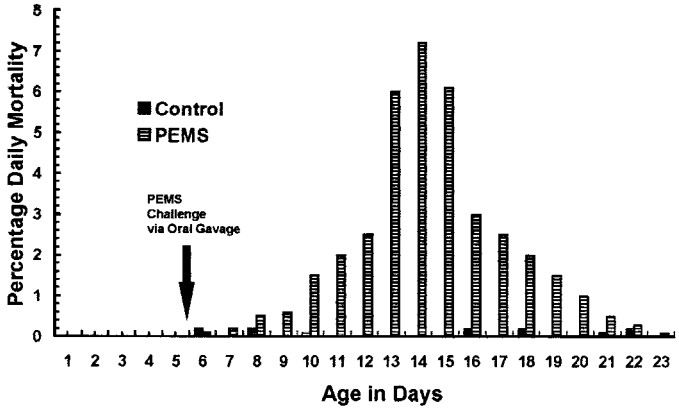

FIGURE 4. Daily mortality profile due to poult enteritis and mortality syndrome (PEMS) (adapted and updated from Doerfler et al., 1998).

Poults hatch with a relatively immature intestinal tract (Moran, 1985; Sell et al., 1989; Uni et al., 1999), and during the early posthatch period, poults have a limited absorptive capacity for carbohydrates, proteins, and lipids (Phelps et al., 1987). Therefore, an insult to the poult digestive tract, such as that associated with PEMS during this critical period, can provoke an especially severe negative impact subsequently on poult survival and performance. Although malabsorption of D-xylose was evident in our experiment, the pathogenesis causing decreased plasma D-xylose absorption in PEMS-infected poults remains equivocal at this time. However, Edens and Doerfler (1997a,b) have suggested that in PEMS-infected poults, the 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 active transport is dependent upon the presence of an Na^+-dependent mobile-carrier mechanism (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).

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). When monosaccharides such as glucose and xylose are absorbed across the brush-border on enterocytes, that absorption is dependent upon the interaction between the monosaccharide and sodium. If the monosaccharide is a nonmetabolizable sugar such as D-xylose, the transport of both D-xylose and sodium can be greatly depressed if the cell is not provided energy in the form of glucose. Because turkeys with PEMS infections have been reported to have impaired glucose metabolism and tend to be hypoglycemic (Edens and Doerfler, 1997b; Doerfler et al., 1998), decreased absorption of D-xylose in the PEMS-infected poults would be expected and possibly be related to decreased cotransport of sodium into the enterocytes of the intestinal tract. On the other hand, the deficit could be related to reduced movement of fluids through the cell.

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, dehydration 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).

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