Lysozyme Transgenic Goats’ Milk Influences Gastrointestinal Morphology in Young Pigs

Dottie R. Brundige, Elizabeth A. Maga, Kirk C. Klasing, and James D. Murray

Abstract

Transgenesis provides a method of expressing novel proteins in milk to increase the functional benefits of milk consumption. Transgenic goats expressing human lysozyme (hLZ) at 67% of the concentration in human breast milk were produced, thereby enhancing the antimicrobial properties of goats’ milk. The objective of this study was to investigate the impact of pasteurized milk containing hLZ on growth, the intestinal epithelium, and an enteropathogenic Escherichia coli (EPEC) infection in young weaned pigs. Pigs were placed into 4 groups and fed a diet of solid food and either control (nontransgenic) goats’ milk or milk from hLZ-transgenic goats. Growth was assessed by weight gain. Nonchallenged pigs were necropsied after 6 wk, whereas the remaining pigs were necropsied at 7 wk following bacterial challenge. We determined the numbers of total coliforms and E. coli and examined small intestinal histology for all pigs. Complete blood counts were also determined pre- and postchallenge. Challenged pigs receiving hLZ milk had fewer total coliforms ($P = 0.029$) and $E. coli (P = 0.030)$ in the ileum than controls. hLZ-fed pigs also had a greater duodenal villi width ($P = 0.029$) than controls. Additionally, nonchallenged hLZ-fed pigs had fewer intraepithelial lymphocytes per micron of villi height ($P = 0.020$) than nonchallenged controls. These results indicate that the consumption of pasteurized hLZ goats’ milk has the potential to improve gastrointestinal health and is protective against an EPEC in young weaned pigs. These same benefits may occur in young children if they were to consume milk from hLZ-transgenic goats.

Introduction

Epidemiological studies have shown that human milk provides a number of benefits to nursing infants, including advantages in general health, growth, development, and considerable protection against a number of acute and chronic diseases (1–3). Both specific and nonspecific defense factors mediate these protective effects, with the antimicrobial protein lysozyme being an important nonspecific immune component in human breast milk (3,4). Lysozyme is a 1,4-$\beta$-N-acetylmuramidase that enzymatically cleaves a glycosidic linkage in the peptidoglycan component of bacterial cell walls, resulting in a loss of cell membrane integrity and cell lysis (5,6).

Lysozyme plays a role in defense against gastrointestinal pathogens and the decrease of gastrointestinal illness in breast-fed infants (4). In vitro, lysozyme has activity against several gastrointestinal pathogens, including Listeria monocytogenes and Clostridium perfringens (7,8). Lysozyme is more effective against gram positive bacteria (9) but has been demonstrated to kill gram negative bacteria in vitro (9–11). Breast-fed infants have a healthier commensal bacterial flora in the gastrointestinal tract than formula-fed infants (12), indicating lysozyme, along with other milk components, has a role in establishing an appropriate gastrointestinal bacterial environment. Studies indicate that the intestinal flora established by breastfeeding is protective against gastrointestinal illness and may contribute to the development and maturation of the intestinal tract (13,14).

Additional research implicates lysozyme in the modulation of the inflammatory response (15–18). Although the inflammatory response is important in clearing some types of infections, it is an abnormal response of the intestinal epithelium, and prolonged and inappropriate responses can result in tissue damage (19). Prolonged inflammation in the intestinal tract due to pathogens can cause substantial destruction of the intestinal epithelia leading to malnutrition and impairment in child growth (20,21).

Although lysozyme is found in the milk of cows (0.16 mg/L) and goats (0.23 mg/L), it is present at a concentration much lower than in human milk (400 mg/L) (22). The availability of milk high in lysozyme and other defense factors can be increased through the use of genetic engineering to generate dairy animals expressing human antimicrobial proteins in their milk. To investigate the potential of this application, our laboratory generated a line of dairy goats that express human lysozyme (hLZ) at

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3 University of California at Davis Academic Federation Committee on Research.
4 Dottie R. Brundige was supported by an Austin Eugene Lyons Graduate Fellowship.
5 Abbreviations used: C-Chal, nonchallenged + control milk feeding group; C+Chal, challenged + control milk feeding group; CAFHS, California Animal Health and Food Safety Laboratory; CBC, complete blood count; CFU, colony-forming unit; EPEC, enteropathogenic Escherichia coli; hLZ, human lysozyme; hLZ-Chal, nonchallenged + human lysozyme milk feeding group; hLZ+Chal, challenged + human lysozyme milk feeding group; LSM, least squares mean; SPF, specific-pathogen free; UC, University of California.
Materials and Methods

Animals and diet. We chose young pigs for the animal model in this study, because they are monogastric animals and have a digestive system that is similar both anatomically and physiologically to humans. Because of these similarities, pigs are considered a relevant model for use in nutrition studies aimed toward human health (24–26). At 12 d of age, 24 (12 male, 12 female) crossbred, specific-pathogen free Yorkshire pigs from 3 litters at the University of California (UC)–Davis Swine Center were divided into 1 of 4 feeding groups: 1) nonchallenged-control milk (C-Chal); 2) nonchallenged-hLZ milk (hLZ-Chal); 3) challenged-hLZ milk (hLZ-Chal); and 4) challenged-control milk (C-Chal). Pigs were distributed so that each group was balanced in terms of sex, weight, and litter of origin. Each group was reared concurrently in adjoining pig nursery crates in a temperature-controlled environmental chamber under Association for Assessment and Accreditation of Laboratory Animal Care International approved conditions. The room temperature was kept between 25 and 27°C for the course of the trial. We monitored pigs twice daily for physical and general well-being and weighed them weekly.

Pigs were weaned onto Pig A2000 Peller Denagard/CTC starter diet (Akey) containing lactose, cereal food fines, soybean meal, oat groats, ground corn, animal plasma, poultry meal, fish meal, cheese meal, vegetable and animal fat, and the antibiotics tiamulin hydrogen fumarate and chlorotetracycline. This diet provided 21% crude protein, 8% crude fat, and 2% crude fiber. Pigs were switched to a standard grower diet (Associated Feed) after 2 wk. The grower diet contained wheat millrun, fat mixer, ground corn, blood meal, whole dried whey, soybean meal, Akey Swine Micro 4 mix, and Tylan 40 antibiotic. This diet provided 20% protein, 7% crude fat, 2% crude fiber, and ME of 13.6 MJ/kg. Pigs consumed solid feed and water ad libitum throughout the study. Two times per day, 48 oz (1.44 L) of pasteurized milk was fed to each group via lixit containers. Pigs were given 3 d after weaning to transition to consuming milk from nontransgenic goats in conjunction with the solid feed diet. After the transition period, the hLZ-Chal and hLZ-Chal groups were switched to pasteurized hLZ milk. All pigs remained on milk and solid food until time of necropsy (6 wk for C-Chal and hLZ-Chal pigs; 7 wk for C-Chal and hLZ-Chal). Eight hLZ transgenic dairy goats of Alpine and Toggenberg origin produced the hLZ milk used in this study. Mean expression for this line is 922 Brundige et al.

Bacteria challenge. Pigs in the hLZ-Chal and C-Chal groups were challenged with porcine-specific EPEC strain ECL1001 (Reference Laboratory for Escherichia coli, University of Montreal, Saint-Hyacinthe, Quebec, Canada). The strain was originally isolated from a 4-wk-old pig with postweaning diarrhea and was previously demonstrated to cause attaching/effacing lesions and inflammation in the small intestine of experimentally infected pigs (27,28). After 6.5 wk of feeding, challenged pigs were given a 1-mL dose of 1.0 × 1010 colony-forming units (CFU) ECL1001 E. coli diluted in 9 mL of trypticase soy broth via an orogastric tube. The next dose of EPEC was administered to each pig 24 h later and all pigs were necropsied 16 h after the 2nd dose of EPEC. Before each bacteria dose, pigs were given 10 mL of a 1.2% CaCO3 solution to neutralize stomach acid. Bacteria were prepared fresh before each dosing. Following the first dose of bacteria, pigs were observed at 6- to 8-h intervals until scheduled necropsy for physical signs of illness.

Blood analysis. Blood was collected from all challenged pigs (hLZ-Chal and C-Chal) 1 wk prior to EPEC administration and postchallenge immediately before necropsy. Samples were collected via venupuncture into EDTA blood collection tubes (Vacutainer, BD). A standard research complete blood count (CBC) measuring 32 components was performed on each sample by IDEXX Veterinary Services. All samples were automatically analyzed by an ADVIA hematology analyzer (Siemens Medical Solutions Diagnostics). The 32 measured components included: total white blood cell count, total RBC count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, nucleated RBC, neutrophil seg, neutrophil band, relative lymphocytes, relative monocytes, relative eosinophils, relative basophils, relative metamyelocytes, relative myelocytes, relatives promyelocytes, unclassified cell types, platelet estimate, polychromasia, anisocytosis, poikilocytosis, heinz bodies, absolute neutrophil seg, absolute neutrophil band, absolute lymphocyte, absolute monocyte, absolute eosinophil, absolute basophil, absolute metamyelocytes, absolute myelocytes, and absolute promyelocytes.

Necropsy, sample collection, and analysis. An American College of Veterinary Pathologists (ACVP) board-certified animal pathologist at the California Animal Health and Food Safety (CAFHS) laboratory (UC-Davis) necropsied all pigs. Pigs were killed via injection of Beuthanasia and then inspected for macroscopic signs of illness and inflammation. Samples of the contents of the duodenum and ileum were collected for bacterial analysis from a section of intestine 30 cm distal to the pyloric valve (duodenum) and 30 cm proximal to the ileal secum (ileum). A bacteriologist at CAFHS used Petrifilm Coliform Count Plates (3M Microbiology) per the manufacturer’s directions to determine the number of CFU of total coliforms and E. coli in 2-g samples of digesta from each pig. Briefly, dilutions from 10−1 to 10−6 were made in phosphate-buffered formalin, embedded in paraffin blocks, thin-sectioned, and stained with hematoxylin–eosin by CAFHS technicians for histological analysis. Two sections were prepared for each sample and mounted on the slide. Estimates of villi height, width, lamina propria thickness, crypt depth, villi intraepithelial lymphocytes, and goblet cells were made on 5–7 villi per slide. Villi height, villi width, lamina propria thickness, and crypt depth slides were visualized at 10× magnification, photographed, and measured using Spot Advanced Software (v3.4, Diagnostic Instruments). The numbers of goblet cells and intraepithelial lymphocytes per villi were visualized at 40× magnification and manually counted.

Statistics. We analyzed the histology and CBC data using 2-factor ANOVA (SAS) to determine the main effects of diet and challenge, and interactions between diet and challenge. When the interaction between main effects was significant, we used Tukey’s means comparisons for post hoc testing. Because of unequal variances between nonchallenged and challenged animals, 2-factor ANOVA is not considered an appropriate statistical analysis for bacteria counts. We divided the bacteria data into challenged and nonchallenged subsets and compared only hLZ-fed and control-fed animals within the same subset to each other. Bacteria counts (CFU/g) were log-transformed prior to analysis using single-factor ANOVA. We also used single-factor ANOVA to analyze pig weights. Values presented in the text are means ± SEM. Repeated measures of weight were not accounted for in the analysis. We considered a P-value of 0.05 significant for all statistical analysis.
Results

**Weight and growth.** To examine the effect of hLZ transgenic milk on growth, we weighed all pigs once per week during the 6-wk study. Neither weight nor mean daily growth differed at any point in the trial between hLZ milk-fed and control-fed pigs. Growth curves for the 2 feeding groups were identical and both groups had the same final mean daily growth (0.26 ± 0.015 kg/d). The final mean weights for hLZ milk-fed and control milk-fed pigs were within 1 kg (13.4 ± 0.7 and 14.2 ± 0.6 kg).

**Bacteria counts.** hLZ-Chal pigs did not differ in total coliform numbers or *E. coli* in either the duodenum or ileum compared with C-Chal pigs (Table 1). In challenged pigs, there were fewer numbers of both total coliforms (P = 0.029) and *E. coli* (P = 0.030) in the ileum of hLZ-Chal pigs compared with C-Chal pigs (Table 1). Neither total coliform nor *E. coli* numbers in the duodenum of challenged pigs differed significantly (Table 1).

**Blood analysis.** As a measure of the effect of hLZ milk on blood composition and immune response, we had a standard CBC run on all C-Chal and hLZ-Chal pigs both pre- and postbacterial challenge (Table 2). The 2-factor diet × challenge interaction was significant for both relative (P = 0.015) and absolute monocyte counts (P = 0.025) (Table 2). None of the pre- or postchallenge relative monocyte means for either treatment group significantly differed from one another (Table 2); however, the absolute number of monocytes was higher (P = 0.045) prechallenge than postchallenge in hLZ-Chal pigs (Table 2). The absolute monocyte count was also higher (P = 0.018) prechallenge in hLZ-Chal pigs than postchallenge (Table 2). Pre- and postchallenge means for C-Chal pigs did not differ (Table 2). We did not observe any significant effect of diet, challenge, or diet by challenge interaction for the other 30 blood components measured in the CBC (data not shown).

**Gross pathology.** At necropsy, all nonchallenged pigs appeared to be in good health and did not display any gross pathological abnormalities or signs of illness, with 1 exception. One hLZ-fed pig had splenomegaly and pulmonary petechiae.

In challenged pigs, 3 of the 6 hLZ+Chal pigs displayed mild signs of illness. Animal 6–3 showed a slight increase of fluid in the cecal and colon contents. Animal 8–4 had a slight increase of fluid in the jejenum and ileum and animal 9–1 had a minimal increase of fluid in the ileum. Two of the 6 C+Chal pigs displayed pathological signs of illness. Animal 6–5 showed a minimal increase of fluid in ileal content and animal 9–7 had a moderate increase of fluid in the jejenum and ileum, a mild increase of fluid in the cecum and colon, and also displayed mild mesenteric lymph node edema.

**Histology.** In the duodenum, diet did not affect villi height, lamina propria thickness, or crypt depth (Table 3). Diet affected (P = 0.029) villi width; pigs reared on hLZ milk had significantly wider villi than those reared on control milk (Table 3). In the ileum, diet did not affect villi width, lamina propria thickness, or crypt depth (Table 3). The diet by challenge interaction was significant (P = 0.029) for villi height in the ileum, indicating that hLZ increased villi height in the nonchallenged but not the challenged pigs. However, villi height did not differ between any of the 4 experimental groups (Table 3).

Challenge status had a significant (P = 0.0065) effect on the number of goblet cells per micron of villi height in the duodenum; challenged pigs had fewer goblet cells than nonchallenged pigs (Table 3). The diet by challenge interaction was significant (P = 0.042) for goblet cells/micron of height (Table 3). hLZ+Chal pigs had fewer (P = 0.009) goblet cells/micron of villi height than hLZ-Chal pigs (Table 3). In contrast, the number of goblet cells/micron of villi height did not differ between C-Chal and C+Chal pigs (Table 3). In the ileum, the number of goblet cells/micron of height did not differ between any of the groups (Table 3).

With respect to number of lymphocytes/micron of villi height in the duodenum, both the main diet effect and the diet by challenge interaction were significant (P = 0.018 and P = 0.047, respectively; Table 3). The mean number of lymphocytes/micron of villi height was lower (P = 0.020) for hLZ-Chal pigs than for C-Chal pigs (Table 3). Challenge status also affected lymphocytes/micron of villi height in the duodenum (P = 0.033; Table 3). C+Chal pigs had fewer (P = 0.022) lymphocytes than C-Chal pigs (Table 3). In contrast, hLZ-Chal and hLZ+Chal pigs did not differ (Table 3). In the ileum, neither diet nor challenge had a significant effect on lymphocytes per micron of villi height between any of the feeding groups (Table 3).

**Discussion**

Genetic engineering provides a means for increasing the availability of milk containing effective levels of antimicrobial proteins advantageous for human health. In these experiments, we demonstrate that pasteurized milk from transgenic goats containing hLZ can improve several measures of gastrointestinal health in weaned pigs and protect against gastrointestinal infection with an EPEC.

Overall weight gain and mean daily growth did not differ between pigs reared on hLZ or control milk. These results duplicate those found in a previous trial in which pigs were reared on hLZ or control milk for 3 wk (23) and demonstrate that feeding hLZ milk to young pigs does not negatively impact growth.

Studies in other species show an effect of antimicrobial proteins, including lysozyme, on weight gain and feed efficiency. Kid goats reared on hLZ milk had higher weight gain during wk 3–5 of a 6-wk feeding trial but displayed overall growth rates similar to control-fed goats (23). Chicks fed a diet containing 15.2 mg/kg of transgenic hLZ produced in rice had significantly improved feed efficiency over chicks reared on a diet containing neither the transgenic protein nor antibiotics (29). However, as with the pigs in this study, body weight gain did not differ significantly from controls. The use of antibiotics in livestock feed can improve growth rates in several species, including swine (30–32); however, the effectiveness of these antibiotics is diminished in clean

### Table 1

<table>
<thead>
<tr>
<th>Tissue and bacteria</th>
<th>Nonchallenged</th>
<th>Challenged</th>
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<tbody>
<tr>
<td></td>
<td>C-Chal</td>
<td>hLZ-Chal</td>
</tr>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total coliforms</td>
<td>2.50 ± 0.71</td>
<td>1.82 ± 0.50</td>
</tr>
<tr>
<td>E. coli</td>
<td>2.12 ± 0.97</td>
<td>1.07 ± 0.53</td>
</tr>
<tr>
<td>Ileum</td>
<td>4.83 ± 0.88</td>
<td>4.16 ± 1.07</td>
</tr>
<tr>
<td>E. coli</td>
<td>4.72 ± 0.88</td>
<td>3.43 ± 1.37</td>
</tr>
</tbody>
</table>

* Values are log-transformed means ± SEM; n = 6. Nonchallenged and challenged subsets were not compared. *Different from C-Chal, P < 0.05.
or germ-free environments (33). The pigs used in this study were raised in a specific-pathogen free facility and were also fed a diet designed for optimal growth that contained antibiotics. It is possible that under these conditions the hLZ was unable to have an additional impact on enhancing growth.

We previously demonstrated the ability of milk from hLZ transgenic goats to affect the microflora of young pigs in a manner similar to that in breast-fed infants (23), which tend to have a microflora lower in coliforms and higher in beneficial species (34). In this study, nonchallenged pigs fed hLZ milk did not have significantly different numbers of total coliforms or E. coli in the duodenum or ileum, which contrasts with the previous study in which both were lower in the duodenum and total coliforms were reduced in the ileum (23).

The pigs used in this study were fed for 3 wk longer than those in the previous study. The amount of milk given each day was not increased as the pigs grew and thus made up a smaller percent of the pigs’ diet over time. In this study, hLZ milk may have had a less significant impact on the microflora than in the previous study. Furthermore, neutrophil counts did not increase postchallenge. These results indicate a protective effect of hLZ milk against EPEC infection. Several studies show that breast-feeding is associated with a decreased chance of contracting diarrhea caused by E. coli (37–39). In 1 study involving 50 infant/mother pairs in urban Mexico, infants on a strict formula diet were 3 times more likely than exclusively breast-fed infants to have diarrhea caused by pathogenic E. coli (40).

Diet did not have a significant effect on any of the 32 components measured in the CBC analysis. We observed a significant interaction between diet and challenge for both relative and absolute monocyte counts; however, relative monocyte counts did not differ between either feeding group pre- or postchallenge. Although we did observe significant differences in absolute monocyte numbers, these counts were well within published normal ranges for pigs of the age used in this study (41) and therefore probably do not represent a significant biological effect; however, this is an observation that warrants further study. Furthermore, neutrophil counts did not increase postchallenge, indicating there was no systemic immune response to the EPEC challenge. That eosinophil counts were not affected by diet suggests the hLZ milk-fed pigs are not responding in an overall positive effect of lysozyme on the microbiial milieu is indicated by fewer numbers of intraepithelial lymphocytes in the nonchallenged pigs. Further work is needed to more completely characterize and understand the full impact of hLZ milk on gastrointestinal microflora, including potential positive effects on beneficial bacteria.

In challenged pigs, both total coliforms and E. coli were significantly higher in the ileum in pigs receiving control milk. These results indicate a protective effect of hLZ milk against EPEC infection. Several studies show that breast-feeding is associated with a decreased chance of contracting diarrhea caused by E. coli (37–39). In 1 study involving 50 infant/mother pairs in urban Mexico, infants on a strict formula diet were 3 times more likely than exclusively breast-fed infants to have diarrhea caused by pathogenic E. coli (40).

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### Table 2

<table>
<thead>
<tr>
<th>CBC component</th>
<th>Prechallenge</th>
<th>Postchallenge</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C+Chal</td>
<td>hLZ+Chal</td>
</tr>
<tr>
<td>Absolute monocytes, n/mL</td>
<td>342 ± 166&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1010 ± 166&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relative monocytes, % of total white blood cell/L</td>
<td>2.00 ± 0.76</td>
<td>4.33 ± 0.76</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are least squares mean (LSM) ± SEM; n = 6. Means in a row with superscripts without a common letter differ, P < 0.05.

### Table 3

<table>
<thead>
<tr>
<th>Tissue and variable</th>
<th>Nonchallenged</th>
<th>Challenged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-Chal</td>
<td>hLZ-Chal</td>
</tr>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villi height, μm</td>
<td>419.52 ± 39.12</td>
<td>470.00 ± 42.86</td>
</tr>
<tr>
<td>Villi width, μm</td>
<td>102.82 ± 4.82</td>
<td>110.25 ± 5.28</td>
</tr>
<tr>
<td>Crypt depth, μm</td>
<td>93.84 ± 18.94</td>
<td>70.55 ± 20.74</td>
</tr>
<tr>
<td>Lamina propria thickness, μm</td>
<td>259.70 ± 17.95</td>
<td>208.23 ± 19.66</td>
</tr>
<tr>
<td>Intraepithelial lymphocytes, n/μm villi height</td>
<td>0.13 ± 0.014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.068 ± 0.015&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Goblet cells, n/μm villi height</td>
<td>0.010 ± 0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.017 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villi height, μm</td>
<td>287.77 ± 21.11</td>
<td>363.87 ± 21.11</td>
</tr>
<tr>
<td>Villi width, μm</td>
<td>104.33 ± 5.53</td>
<td>108.89 ± 5.53</td>
</tr>
<tr>
<td>Crypt depth, μm</td>
<td>80.93 ± 11.49</td>
<td>61.40 ± 11.49</td>
</tr>
<tr>
<td>Lamina propria thickness, μm</td>
<td>226.31 ± 15.41</td>
<td>180.36 ± 15.41</td>
</tr>
<tr>
<td>Intraepithelial lymphocytes, n/μm villi height</td>
<td>0.100 ± 0.015</td>
<td>0.091 ± 0.015</td>
</tr>
<tr>
<td>Goblet cells, n/μm villi height</td>
<td>0.017 ± 0.004</td>
<td>0.027 ± 0.004</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are LSM ± SEM, n = 6 for all groups and tissues except for nonchallenged, hLZ-fed, duodenum, n = 5. Means in a row with superscripts without a common letter differ, P < 0.05.
allergic way to the presence of either goat milk proteins or the hLZ protein in milk. These results indicate that the consumption of hLZ milk did not adversely affect the CBC profile or cause an allergenic response.

Although there were individual anomalies in gross pathology results, there are no clear differences between groups that are attributable to treatment. None of the pigs showed severe signs of illness; however, the bacteria strain used for the challenge has been shown to be more effective in immunosuppressed or newborn gnotobiotic pigs (27,28).

High levels of hLZ in the diet of chicks increased villi height in the duodenum and decreased lamina propria thickness in the ileum (29). In this study, hLZ milk did not have a significant effect on crypt depth or lamina propria thickness in either intestinal segment but did affect villi width in the duodenum; pigs reared on hLZ milk had a significantly greater width. hLZ also tended to increase villi height in nonchallenged pigs, although this was not observed in challenged pigs. Increased villi height and width corresponds to an increased surface area and, therefore, absorptive capacity in the duodenum and is an indicator of improved gastrointestinal health in pigs reared on hLZ milk.

Decreased absorptive capacity in the intestine and other damaging morphological changes such as villous shortening and destruction of villi is associated with prolonged cases of EPEC infection (20,42,43). This damage can lead to or worsen malnutrition in affected children (21). The challenged pigs in this study showed no signs of histological damage; however, we gave the pigs an acute bacterial challenge rather than a prolonged infection.

As an additional indicator of increased gastrointestinal health, the number of intraepithelial lymphocytes per micron of villi height was significantly lower in the duodenum of nonchallenged hLZ milk-fed pigs. This lower count is likely indicative of fewer microbial challenges in the duodenum of nonchallenged pigs receiving hLZ milk.

Taken together, the results of this study demonstrate that pasteurized hLZ milk can be beneficial toward improving gastrointestinal health in a young monogastric animal receiving solid feed without adverse effects. hLZ-containing goat milk had no detrimental effect on weight gain, growth, or blood composition while improving several measures of gastrointestinal health, including increased absorptive surface area and decreased microbial challenges, as indicated by a reduction in intraepithelial lymphocytes in the duodenum. Also, the hLZ milk was protective against an acute challenge with EPEC. Additional studies should be completed to extend these observations to other situations and species, including humans.

Acknowledgments

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Literature Cited


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