Effect of feeding colostrum versus exogenous immunoglobulin G on gastrointestinal structure and enteric nervous system in newborn pigs

J. Wolinski, M. Slupecka, B. Weström, O. Prykhodko, P. Ochniewicz, M. Arciszewski, E. Ekblad, K. Szwiec, Ushakova, G. Skibo, T. Kovalenko, I. Osadchenko, K. Goncharova, J. Botermans and S. Pierzynowski

J ANIM SCI 2012, 90:327-330.
doi: 10.2527/jas.53926

Effect of feeding colostrum versus exogenous immunoglobulin G on gastrointestinal structure and enteric nervous system in newborn pigs[1]

J. Woliński,* M. Słupecka,* B. Weström,† O. Prykhodko,† P. Ochniewicz,* M. Arciszewski,§ E. Ekblad,# K. Szwiec,†§‡ Ushakova, G. Skibo,¶ T. Kovalenko,¶ I. Osadchenko,¶ K. Goncharova,¶ J. Botermans,‖ and S. Pierzynowski†‡$[2]**

*The Kielanowski Institute of Animal Physiology & Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland; †Department of Biology, Lund University, 223-62 Lund, Sweden; §University of Life Sciences, 20 – 950 Lublin, Poland; #Faculty of Medicine, Lund University, 223-62 Lund, Sweden; ‡SGPlus, 21 – 114 Malmö, Sweden; **Department of Biophysics and Biochemistry, DNU, 49050 Dnepropetrovsk; Ukraine; ¶Bogomoletz Institute of Physiology, 01024 Kiev, Ukraine; ‖Swedish University of Agricultural Sciences, 230 53 Alnarp, Sweden; and $Institute of Rural Health, 20 – 090 Lublin, Poland

ABSTRACT: Colostrum is an indispensable source of antibodies (IgG) protecting the newborn pig against infection. We studied the effect of feeding colostrum and purified IgG on early structure and development of the gastrointestinal tract (GIT). Newborn littermate pigs were fed either colostrum, an elemental diet (ED), or an ED supplemented with purified serum IgG (ED + IgG) for 24 h or then only ED up to 72 h. Afterwards, pigs were slaughtered. Colostrum-fed pigs or ED supplemented with IgG (ED + IgG) increased thickness ($P < 0.001$) of stomach mucosa and muscularis ($P < 0.05$) compared to the ED group not receiving IgG. Feeding an ED supplemented with IgG improved morphology of the GIT towards that of colostrum-fed piglets and indicates a beneficial effect of IgG on GIT development in neonatal pigs. Immunohistochemical studies indicate that ED feeding may influence the expression of nitric oxide synthase in jejunal myenteric (but not submucous) neurons of newborn pigs.

Key words: immunoglobulin G, elementary diet, colostrum, gut development

 J. Anim. Sci. 2012.90:327–330
doi:10.2527/jas53926

INTRODUCTION

Dietary bioactive peptides, growth factors, and hormones naturally present in colostrum and breast milk stimulate development of structure and function of the gastrointestinal tract (Woliński et al., 2003; Słupecka et al., 2012). The question is whether IgG in addition to their role in formation of systemic bacterial resistance may affect development of the intestine. According to Brambell (1970), absorption of IgG involves the fragment crystallizable region (**FcRn**) receptor. Indeed, Stirling et al (2005) demonstrated the presence of the FcRn receptor in enterocytes of young and adult pigs and its participation in absorption of IgG. Nitric oxide synthase was measured in the rat small intestine, because NO participates in vasoactive intestinal peptide-dependent adaptation and survival of enteric neurons (Lin et al., 2003). The aim of the study was to highlight the role of enteral IgG, the main component of colostrum, in the development of mucosa and enteric nervous system in newborn pigs.

MATERIALS AND METHODS

The Local Ethics Committee approved the study protocol.

Animals

One-day-old piglets were divided into 6 groups: (i) colostrum fed for 24 h (n = 6), (ii) elemental diet (**ED**) fed for 24 h (n = 6), (iii) ED + IgG fed for 24 h (n = 6), (iv) colostrum fed for the first 24 h and then ED up to 72 h (n = 6), (v) ED fed for 72 h (n = 6), and (vi) ED + IgG fed for the first 24 h and then ED up to 72 h (n = 6). All feedings were completed via gastric tube in 2-h intervals. The IgG (33.5 mg/mL) was prepared

[1]Financial support of this work by the FORMAS and SGPlus, Sweden, is acknowledged.
[2]Corresponding author: Stefan.Pierzynowski@biol.lu.se

Table 1. Villus height, crypt depth, and mucosa and muscularis thickness of pigs fed colostrum, elemental diet, or elemental diet + IgG for 24 h. Means are based on 6 observations per diet

Item	Diet		
	Colostrum	Elemental	Elemental + IgG
Duodenum			
Villus height, μm	430 ± 9.9[a]	251 ± 13.9[b]	425 ± 17.9[a]
Crypt depth, μm	98 ± 0.9[a]	74 ± 2.3[b]	111 ± 2.1[c]
Mucosa thickness, μm	515 ± 10.4[a]	322 ± 16.7[b]	526 ± 18.5[a]
Muscularis thickness, μm^2	134 ± 2.8[a]	159 ± 5.9[b]	137 ± 4.8[a]
Jejunum			
Villus height, μm	628 ± 17.7[a]	572 ± 13.8[ab]	535 ± 17.2[b]
Crypt depth, μm	108 ± 1.3[ab]	112 ± 2.1[a]	104 ± 1.6[b]
Mucosa thickness, μm	717 ± 17.9[a]	661 ± 14.1[ab]	623 ± 17.0[b]
Muscularis thickness, μm^2	90 ± 2.4[a]	116 ± 3.1[b]	84 ± 4.3[c]
Ileum			
Villus height, μm	498 ± 12.1[a]	631 ± 24.3[b]	440 ± 17.0[c]
Crypt depth, μm	112 ± 4.0[a]	116 ± 1.5[b]	114 ± 1.5[b]
Mucosa thickness, μm	583 ± 12.4[a]	732 ± 24.2[b]	537 ± 17.1[a]
Muscularis thickness, μm^2	143 ± 2.9[a]	154 ± 3.8[b]	126 ± 2.7[c]

[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

Table 2. Villus height, crypt depth, and mucosa and muscularis thickness of pigs fed colostrum, elemental diet, or elemental diet + IgG for 24 h and then elemental diet up to 72 h. Means are based on 6 observations per diet

Item	Diet		
	Colostrum	Elemental	Elemental + IgG
Duodenum			
Villus height, μm	358 ± 9.7[a]	200 ± 8.9[b]	258 ± 9.8[c]
Crypt depth, μm	94 ± 1.1[a]	87 ± 2.4[b]	96 ± 1.8[a]
Mucosa thickness, μm	437 ± 10.6[a]	274 ± 10.8[b]	340 ± 10.9[c]
Muscularis thickness, μm^2	148 ± 4.1[a]	126 ± 4.4[b]	147 ± 3.3[a]
Jejunum			
Villus height, μm	478 ± 11.5[a]	282 ± 6.7[b]	276 ± 6.6[b]
Crypt depth, μm	112 ± 0.9[a]	98 ± 1.1[b]	105 ± 1.2[c]
Mucosa thickness, μm	578 ± 11.7[a]	376 ± 7.0[b]	367 ± 6.7[b]
Muscularis thickness, μm^2	89 ± 1.7[a]	98 ± 2.3[b]	107 ± 2.6[c]
Ileum			
Villus height, μm	509 ± 8.1[a]	411 ± 13.1[b]	327 ± 9.9[c]
Crypt depth, μm	99 ± 0.9[a]	119 ± 1.0[b]	120 ± 1.2[b]
Mucosa thickness, μm	585 ± 8.2[a]	508 ± 13.1[b]	421 ± 10.1[c]
Muscularis thickness, μm^2	134 ± 1.7[a]	183 ± 4.4[b]	169 ± 3.7[b]

[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

from pig blood in our lab. Upon completion, pigs were slaughtered to dissect the gastrointestinal tract.

Intestinal Histometry

Stomach, duodenum, jejunum (middle part), and ileum were dissected, weighed, and measured. Samples of each section were collected and immediately fixed in 10% neutral formalin solution. Paraffin-embedded samples were processed for histometry and then stained with hematoxylin and eosin. Villus height, crypt depth, and thickness of mucosa and muscularis were measured using a light microscope. Data were expressed as means and SEM. The Kruskal–Wallis test followed by the Dunn's Multiple Comparison post hoc test was used to indicate the statistical differences (GraphPad Prism version 5.00; GraphPad Software, San Diego, CA). In analysis, $P < 0.05$ was considered significant.

Immunohistochemistry

In all pigs of both control and experimental groups, a midline incision of the abdomen was made. The middle part of the jejunum was visualized and dissected out. Specimens from the jejunum (3 cm long) were fixed with Stefanini's solution (4°C for 48 h). After fixation, tissue was processed for immunohistochemistry. The percentages of neurons that are immunoreactive to nitric oxide synthase (**NOS-IR**) were calculated.

Statistical Analyses

Between homologous and nonhomologous groups, ANOVA was performed followed by Bonferoni's post hoc test and $P < 0.05$ was considered significant. Pig was the experimental unit.

RESULTS

Pigs fed ED instead of colostrum for 24 h had a reduced ($P < 0.05$) villus height, crypt depth, and mucosa thickness in the duodenum (Table 1) without changes in the jejunum and ileum. Pigs fed ED had increased ($P < 0.05$) muscularis thickness in all segments. Adding IgG to ED to piglets for 24 h increased ($P < 0.05$) villus length, crypt depth, and thickness of the mucosa up to values similar to those of colostrum-fed piglets and reduced ($P < 0.05$) thickness of muscularis in the duodenum. Adding IgG to ED reduced ($P < 0.05$) crypt depth in the jejunum and reduced ($P < 0.05$) villus height and increased ($P < 0.05$) mucosa thickness in the ileum.

Pigs fed ED instead of colostrum for 24 h had a reduced ($P < 0.05$) villus height, crypt depth, and mucosa thickness in the duodenum and jejunum at 72 h (Table 2) with similar changes in the ileum. Adding IgG to ED to piglets for 24 h increased ($P < 0.05$) villus length, crypt depth, and thickness of mucosa and muscularis in the duodenum at 72 h, increased ($P < 0.05$) crypt depth and muscular thickness in the jejunum, and reduced ($P < 0.05$) villus height and mucosa thickness in the ileum.

Nitric Oxide Synthase Expression in Enteric Neurons

The ED- and ED + IgG-fed piglets had higher ($P < 0.05$) expression of NOS-IR after 24 h in myenteric neurons than colostrum-fed piglets. The NOS-IR levels in submucosal neurons did not differ after 24 h and were lower ($P < 0.05$) after 72 h but remained similar among groups.

DISCUSSION

Colostrum and exogenous IgG had a trophic effect on structure of the gut in the duodenum but not consistently in the jejunum and ileum. Gut development might be related to immune system development and IgG transmission via gut to the offspring. Feeding of ED may influence the expression of nitric oxide synthase in jejunal myenteric (but not submucous) neurons of newborn pigs. In conclusion, IgG administered to newborn piglets are indispensive factors and may reverse the negative effects of ED enteral feeding or total parentelal nutrition by increasing the morphometric parameters to the values observed in colostrum feeding piglets, but these effects are limited to the duodenum. We suggest also that IgG, similarly to colostral factors, may be an important factor in maintaining the activity of the small intestine nitroergic myenteric neurons.

LITERATURE CITED

Brambell, F. W. R. 1970. The Transmission of Passive Immunity from Mother to Young. North-Holland Publishing, Amsterdam, The Netherlands.

Lin, Z., K. Sandgren, and E. Ekblad. 2003. Increased expression of vasoactive intestinal polypeptide in cultured myenteric neurons from adult rat small intestine. Auton. Neurosci. 29:9–19.

Słupecka, M., J. Woliński, and S. G. Pierzynowski. 2012. The effects of exogenous ghrelin on the remodeling of the small intestinal mucosa in neonatal piglets. Regul. Pept. 174:38–45.

Stirling, C. M., B. Charleston, H. Takamatsu, S. Claypool, W. Lencer, R. S. Blumberg, and T. E. Wileman. 2005. Characterization of the porcine neonatal Fc receptor-potential use for trans-epithelial protein delivery. Immunology 114:542–553.

Woliński, J., M. Biernat, P. Guilloteau, B. Weström, and R. Zabielski. 2003. Exogenous leptin controls the development of the small intestine in neonatal piglets. J. Endocrinol. 177:215–222.