

JOURNAL OF ANIMAL SCIENCE

The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Increased intestinal barrier function in the small intestine of formula-fed neonatal piglets

V. Huygelen, M. De Vos, S. Willemsen, B. Tambuyzer, C. Casteleyn, D. Knapen, S. Van Cruchten and C. Van Ginneken

J ANIM SCI 2012, 90:315-317.
doi: 10.2527/jas.53731

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://www.journalofanimalscience.org/content/90/Supplement_4/315



American Society of Animal Science

www.asas.org

Increased intestinal barrier function in the small intestine of formula-fed neonatal piglets

V. Huygelen,* M. De Vos,* S. Willemen,* B. Tambuyzer,* C. Casteleyn,* D. Knapen,† S. Van Cruchten,* and C. Van Ginneken*^{1,2}

*University of Antwerp, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Department of Veterinary Sciences, Laboratory of Applied Veterinary Morphology, 2610 Wilrijk, Belgium; and †University of Antwerp, Department of Veterinary Sciences, Veterinary Physiology and Biochemistry, 2610 Wilrijk, Belgium

ABSTRACT: Within-litter birth weight variation is adversely correlated to piglet survival and postnatal growth. A less efficient epithelial barrier function in light piglets may partly explain this inverse relationship between birth weight and zootechnical performance. A compromised epithelial barrier increases paracellular permeability; consequently, toxins, allergenic compounds, or bacteria may enter systemic circulation and induce inflammatory responses. Dietary effects on function of gut epithelium of piglet are largely unknown. This study investigated epithelial barrier function of the small intestine of normal birth weight (NBW) piglets (1.46 ± 0.10 kg) and low birth weight (LBW) piglets (<1 kg at birth) in relation to their diet. Sixteen pairs of 3-d-old LBW and NBW piglets were randomly assigned to 3 groups: a sow-fed control group euthanized at day 3 of age (SOW3), piglets sow fed until day 10 (SOW10), and formula-fed piglets fed formula from day 3 until

day 10 (FOR10). To measure gut permeability, piglets were dosed intragastrically with 0.75 g lactulose/kg BW and 0.3 g mannitol/kg BW 4 h before euthanasia. Urinary sugar excretion was measured using enzymatic spectrophotometry. Irrespective of birth weight, lactulose levels of FOR10 (4.4 ± 2.3 mmol/L) tended to be lower ($P = 0.07$) than SOW10 (26.4 ± 10.2 mmol/L) indicating a reduced paracellular intestinal permeability in FOR10. This reduction was associated with a 6-fold elevated ($P < 0.01$) protein expression of occludin, an important tight junction protein, in FOR10 compared to SOW10. Mannitol levels in FOR10 (31.0 ± 18.2 mmol/L) did not differ ($P = 0.28$) from SOW10 (61.1 ± 10.2 mmol/L). However, shorter villi ($P < 0.01$) in FOR10 indicated a reduced absorptive capacity. In conclusion, formula feeding caused minor symptoms of gastrointestinal dysfunction compared to sow-fed piglets irrespective of their birth weight.

Keywords: formula feeding, intestinal barrier, low birth weight, neonates, occludin, pig

© 2012 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2012.90:315–317
doi:10.2527/jas53731

INTRODUCTION

The use of hyperprolific sow breeds as a strategy to increase litter size will increase within-litter birth weight variation and reduce mean piglet birth weight. Low birth weight (LBW) in piglets correlates with decreased survival and lower growth rates (Milligan et al., 2002; Quiniou et al., 2002). Interfering by feeding these less competitive piglets a milk replacer ensures adequate milk intake and increases BW gain (Azain et al., 1996; Wolter et al., 2002). In this study, we examined postnatal developmental pattern of the

intestine in normal birth weight (NBW) and LBW piglets, which were either sow fed or formula fed, with a focus on intestinal barrier structure and function. The in vivo integrity of the intestinal barrier (associated with gut permeability) was determined using a functional lactulose–mannitol sugar absorption test. Occludin is an essential structural and functional component of tight junctions (Feldman et al., 2005; Moeser et al., 2007) and was quantified. Tight junctions are considered the most important components of the intestinal mucosal barrier, preventing paracellular diffusion of microorganisms, toxins, and other antigens across the epithelium.

¹Corresponding author: chris.vanginneken@ua.ac.be

²The authors acknowledge the excellent technical assistance of G. Vrolix and K. Huybrechts.

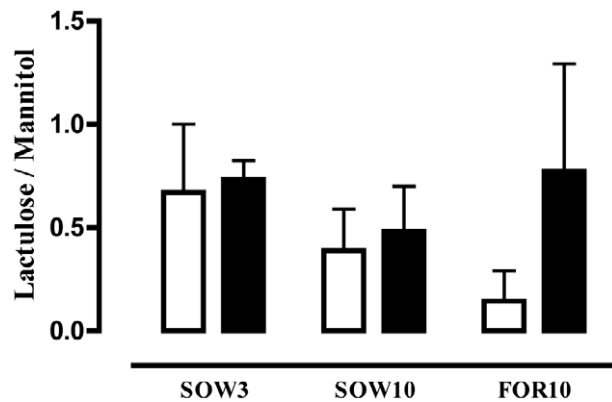


Figure 1. Urinary lactulose:mannitol ratio of a sow-fed control group euthanized at day 3 of age (SOW3), piglets sow fed until day 10 (SOW10), and formula-fed piglets until day 10 (FOR10) piglets of low birth weight (LBW) and normal birth weight (NBW). Bars represent mean $\pm$ SEM. Open bars represent LBW piglets and dark bars represent NBW piglets.

MATERIALS AND METHODS

Experimental Design

The Ethical Committee of Animal Experimentation, University of Antwerp, Belgium, approved the experimental design. A total of 16 pairs of 3-day-old LBW and NBW crossbred piglets [(Finnish Yorkshire $\times$ Belgian Landrace) $\times$ Piétrain] obtained from a commercial farm were randomly assigned in 3 experimental groups: a sow-fed control group euthanized at day 3 of age (SOW3; $n = 12$), piglets sow fed until day 10 (SOW10; $n = 10$), and piglets with ad libitum access to formula (28 g/L protein, 23 g/L lipid, and 56 g/L lactose; 2,470 kJ/L GE) and water using a semiautomatic dispenser from day 3 until day 10 (FOR10; $n = 10$).

Permeability Measurements

To measure *in vivo* gut permeability, piglets were dosed intragastrically with 0.75 g lactulose/kg BW and 0.3 g mannitol/kg BW (Sigma-Aldrich, Steinheim, Germany) 4 h before euthanasia. Lactulose passes the intestinal epithelium paracellular when intestinal barrier function is compromised. Mannitol passes the intestinal epithelium by unmediated diffusion and therefore provides a measure of the absorptive surface. Urinary lactulose and mannitol concentrations collected by cystocentesis at the time of euthanasia were measured using an enzymatic spectrophotometric method (Behrens et al., 1984; Blood et al., 1991).

Intestinal Barrier Structure

Occludin expression was determined using SDS-PAGE and western blotting analysis. Frozen small intestinal samples were homogenized in lysis buffer (50 mM Tris, 150 mM NaCl, 1% Nonidet P40, and 0.5% deoxycholate) supplemented with complete protease inhibitor cocktail tablet (Roche Applied Science,

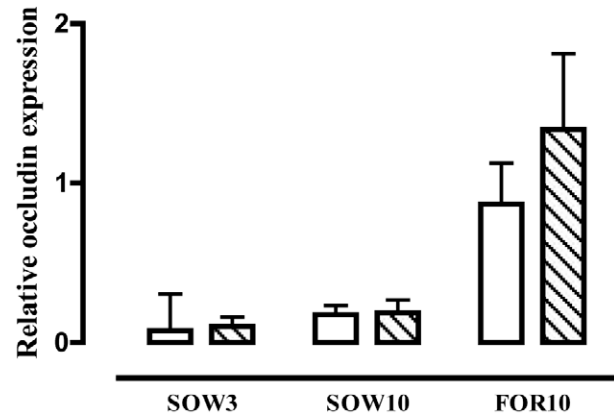


Figure 2. Relative occludin expression in small intestine of a sow-fed control group euthanized at day 3 of age (SOW3), piglets sow fed until day 10 (SOW10), and formula-fed piglets until day 10 (FOR10) piglets of low birth weight (LBW) and normal birth weight (NBW); β -actin was used as a loading control. Occludin expression was less ($P = 0.04$) in LBW vs. NBW piglets across diets. Furthermore, the occludin expression differed ($P = 0.001$) among diets. Bars represent mean $\pm$ SEM. Open bars represent LBW piglets and dark bars represent NBW piglets.

Mannheim, Germany). Ten micrograms of protein was loaded on a 4 to 15% precast gradient gel (Biorad Labs, Hercules, CA). Nonspecific protein binding on blots was blocked with 5% nonfat dry milk in Tris buffer with Tween-20. Blots were incubated with a rabbit anti-occludin antibody (1/1000; Invitrogen, Merelbeke, Belgium) and subsequently incubated with a biotin-conjugated anti-rabbit antibody (1/1000; Dako, Glostrup, Denmark) and streptavidin-horse reddish peroxidase (1/1000; Dako). Detection was performed using a chemiluminescence system (SuperSignal West Femto Maximum Sensitivity Substrate; Thermo Scientific, Rockford, IL). Band intensities were quantified by densitometry using GeneSnap and GeneTools software (Syngene, Cambridge, UK).

Statistical Analysis

Data were analyzed using GLM procedures (SPSS statistics 18.0; IBM, New York, NY). A P value < 0.05 was considered significant. Results are presented as mean $\pm$ SEM.

RESULTS

Following an oral lactulose-mannitol bolus, the urinary excretion ratio did not differ ($P = 0.31$) between NBW or LBW pigs in the 3 groups (Figure 1). In FOR10, the lactulose:mannitol ratio did not differ ($P = 0.15$) between the SOW3 and SOW10 groups. Irrespective of birth weight, lactulose levels of FOR10 (4.4 ± 2.3 mmol/L) tended to be lower ($P = 0.07$) compared to SOW10 (26.4 ± 10.2 mmol/L). In contrast, protein expression of occludin was increased ($P < 0.01$) in FOR10 compared to SOW10 (Figure 2). Urinary mannitol levels in the FOR10 group

did not differ ($P = 0.28$) compared to SOW10 (31.0 ± 18.2 versus 61.1 ± 10.2 mmol/L).

DISCUSSION

Intestinal permeability and absorption were investigated using differently sized marker molecules. Lactulose levels tended to be lower in formula-fed piglets compared to sow-fed piglets regardless of their birth weight. These results indicate a reduction in paracellular permeability of the intestinal mucosa of FOR10, which might be caused by the observed higher milk intake (data not shown). Indeed, lactulose levels in urine of piglets and feed intake were negative correlated recently (Wijtten et al., 2011). To further elucidate underlying molecular changes in intestinal barrier structure causing reduced gut permeability, occludin protein expression was determined. Formula feeding increased occludin expression in the small intestine compared to sow-fed piglets. This indicates a structural increase in mucosal integrity of the small intestine of formula-fed piglets irrespective of birth weight. In addition, mannitol levels did not differ between sow-fed and formula-fed piglets. However, villi were shorter in formula-fed piglets compared to sow-fed piglets (data not shown) indicating a reduced absorptive capacity of mucosal surface of the small intestine.

In conclusion, formula feeding was associated with structural small intestinal differences as indicated by a reduced villus length and increased occludin expression. However, these findings were not associated with physiological permeability tests; therefore, we conclude

that formula feeding resulted in minor symptoms of gastrointestinal dysfunction.

LITERATURE CITED

- Azain, M. J., T. Tomkins, J. S. Sowinski, R. A. Arentson, and D. E. Jewell. 1996. Effect of supplemental pig milk replacer on litter performance: Seasonal variation in response. *J. Anim. Sci.* 74:2195–2202.
- Behrens, R. H., H. Docherty, M. Elia, and G. Neale. 1984. A simple enzymatic method for the assay of urinary lactulose. *Clin. Chim. Acta* 137:361–367.
- Blood, J., A. R. Ingle, N. Allison, G. R. Davies, and P. G. Hill. 1991. Rapid enzymatic method for the measurement of mannitol in urine. *Ann. Clin. Biochem.* 28:401–406.
- Feldman, G. J., J. M. Mullin, and M. P. Ryan. 2005. Occludin: Structure, function and regulation. *Adv. Drug Deliv. Rev.* 57:883–917.
- Milligan, B. N., D. Fraser, and D. L. Kramer. 2002. Within-litter birth weight variation in the domestic pig and its relation to pre-weaning survival, weight gain, and variation in weaning weights. *Livest. Prod. Sci.* 76:181–191.
- Moeser, A. J., K. A. Ryan, P. K. Nighot, and A. T. Blikslager. 2007. Gastrointestinal dysfunction induced by early weaning is attenuated by delayed weaning and mast cell blockade in pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 293:G413–G421.
- Quiniou, N., J. Dagorn, and D. Gaudre. 2002. Variation of piglets birth weight and consequences on subsequent performance. *Livest. Prod. Sci.* 78:63–70.
- Wijtten, P. J. A., J. J. Verstijnen, T. A. T. G. van Kempen, H. B. Perdok, G. Gort, and M. W. A. Verstegen. 2011. Lactulose as a marker of intestinal barrier function in pigs after weaning. *J. Anim. Sci.* 89:1347–1357.
- Wolter, B. F., M. Ellis, B. P. Corrigan, and J. M. DeDecker. 2002. The effect of birth weight and feeding of supplemental milk replacer to piglets during lactation on preweaning and postweaning growth performance and carcass characteristics. *J. Anim. Sci.* 80:301–308.

References

This article cites 9 articles, 4 of which you can access for free at:
http://www.journalofanimalscience.org/content/90/Supplement_4/315#BL