Available online at www.sciencedirect.com
ScienceDirect
Livestock Science 109 (2007) 14–18
LIVESTOCK SCIENCE
www.elsevier.com/locate/livsci

Bacterial colonization affects early organ and gastrointestinal growth in the neonate ☆

R.H. Siggers [a], T. Thymann [a], J.L. Siggers [a], M. Schmidt [b], A.K. Hansen [c], P.T Sangild [a,*]

[a] Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, DK-1870 Frederiksberg, Denmark
[b] Department of Reproduction, Faculty of Life Sciences, University of Copenhagen, DK-1870 Frederiksberg, Denmark
[c] Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, DK-1870 Frederiksberg, Denmark

Abstract

Enteral nutrition coupled with bacterial colonization has been shown to have major functional and developmental effects during the postnatal period of neonates. In this study, we developed a highly sensitive premature pig model to elucidate the specific developmental impact of initial bacterial colonization on premature neonates by comparing germ-free and conventionally reared pigs. Thirty-eight preterm pigs (93% gestation) were delivered via caesarean section and reared in either germ-free or conventional isolators for 40–48 h. Pigs were fed either infant milk formula or sow's colostrum. Enteral feeding for two days had trophic effects on gastrointestinal weights, particularly for pancreas, stomach and small intestine (SI). The absence of bacteria in formula fed pigs resulted in a mucosa that appeared more robust and had higher weights for both SI and pancreas, compared to conventional formula fed pigs ($P<0.05$). Colostrum fed pigs also had markedly increased mucosal SI proportions, lung and spleen weights compared to conventional formula fed pigs. Colostrum fed pigs and germ-free pigs were similar although stomach, pancreas and distal SI weights were highest for germ-free pigs. The results demonstrate that the initial bacterial colonization interacts with diet to modulate the early neonatal organ development, particularly of the GIT.

Keywords: Germ-free; Preterm; Gastrointestinal tract; Organ; Development

1. Introduction

At birth, there is a dramatic shift from the highly protected, sterile condition *in utero* to a more demanding *ex utero* condition that includes an immediate acquisition of colonizing bacteria derived from the mother and external environment. During the growth and development phases of different animal species, bacterial colonization affects a number of physiological variables, particularly in the gastrointestinal tract (Gordon and Pesti, 1971). The presence of bacteria may also affect organ function in the immediate neonatal period, as suggested by the colonization-dependent intestinal dysfunction in preterm pigs fed diets other than mother's milk (Sangild et al., 2006). However, it is unclear whether bacterial colonization affects the marked changes in organ growth normally occurring in response to first enteral feed. Such diet-bacteria interactions could be most pronounced in

☆ This paper is part of the special issue entitled "Digestive Physiology in Pigs" guest edited by José Adalberto Fernández, Mette Skou Hedemann, Bent Borg, Jensen, Henry Jørgensen, Knud Erik Bach Knudsen and Helle Nygaard Lærke.

* Corresponding author. Tel.: +45 35 28 26 98; fax: +45 35 28 24 69.
E-mail address: psa@life.ku.dk (P.T. Sangild).

1871-1413/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.livsci.2007.01.025

preterm neonates, which commonly develop inappropriate inflammatory responses to bacterial colonization and formula feeding (Sangild et al., 2006). Therefore, the objective of this study was to compare organ and gastrointestinal development in preterm pigs fed milk formula reared in a germ-free environment, with conventionally reared preterm pigs fed either milk formula or sow's colostrum. Results on mucosal morphology and enzyme activities have already been reported (Sangild et al., 2006).

2. Materials and methods

2.1. Preparation of germ-free isolator

A germ-free isolator (model type 13366, Maintenance Isolator, Harlan Isotec, Indianapolis, ID) capable of housing eight individual premature piglets (1–1.5 kg) was used during the experiment. All autoclavable isolator components and experimental materials were sterilised (autoclaved at 120 °C for 45 min) and placed inside the isolator. All isolator surfaces and materials were thoroughly sprayed and sterilised with a potent oxidising agent (1:10 Virkon S:water, Pharmaxim, Skörpinge, Denmark). The isolator was sealed and ventilated for approximately 48 h, and maintained under positive pressure with incoming and outgoing air forced through high efficiency particulate filters.

2.2. Animals and treatments

Thirty-eight piglets obtained from three sows (Large White × Danish Landrace, term 115 ± 2 days) were delivered preterm via caesarean section at approximately 106 days (93% gestation). Preparation of sows for surgery was performed as previously described with minor modifications (Sangild et al., 2002). Briefly, prior to caesarean section sows were sedated with azaperone (0.05 ml/kg, i.m.; Janssen, Beerse, Belgium). Anaesthesia was subsequently induced and maintained with thiopental sodium (5–10 mg/kg, i.v.; Abbott, North Chicago, IL). Fetuses from a single uterine horn were removed following ligation and transection of the umbilical cord and passed through a 1% sodium hypochlorite submersion dip tank (1:100 dilution for 1000 ppm of chloride; Johnson Diversey A/S, Nivå, Germnay) connected to the main entry port of the germ-free isolator. Piglets were recovered from the dip tank into the germ-free isolator before breathing began. Fetuses from the second uterine horn were removed as above, however following umbilical ligation and transection, they were placed in individual conventional infant incubators (Air-Shields, Hatboro, PA). All piglets were supplied with extra oxygen at 2–3 L/min for approximately 24 h postpartum. While still anaesthetized, the piglets were weighed and fitted with an orogastric tube (infant feeding tube 6F; PharmaPlast) to be used for enteral feeding. Piglets were reared in either a conventional infant incubator (CV) or germ-free isolator (GF), and fed either a sterile infant milk formula (CV–FORM, GF–FORM) or sows' colostrum (CV–COL). All piglets were fed enteral boluses (15 ml/kg bodyweight/3 h) for 40–48 h. All piglet housing and experimental procedures were performed in the gnotobiotic experimental unit (Royal Veterinary and Agricultural University Experimental Unit, Copenhagen, Denmark). The experimental room temperature was maintained at 37 °C starting at day 0, and decreased 1 °C every 24 h. All procedures were approved by the National Committee on Animal Experimentation, Denmark.

2.3. Feed collection and preparation

Porcine colostrum was collected from multiparous sows (Large White × Danish Landrace, Research Station Sjælland III, Denmark) within 6 h of completed farrowing. The formula diet was prepared as described previously (Bjornvad et al., 2005), kept in 50 ml polypropylene tubes (Sarstedt, Germany) and subjected to 1 × 10 kGy of gamma irradiation (Sterigenics Denmark, Copenhagen, Denmark). Sterility of the formula was confirmed by conventional microbiology plating on blood agar media at 37 °C under aerobic and anaerobic conditions.

2.4. Tissue collection

Piglets were sedated with zolazepam and tiletamine HCl (0.02 mL/kg body weight (BW), Zoletil, Virbac, France) and then euthanized with sodium pentobarbital (200 mg/kg) via cardiac puncture prior to tissue collection. Tissue collection was performed as previously described (Bjornvad et al., 2005). Briefly, the entire gastrointestinal tract (GIT) was quickly excised and placed on ice. The GIT was divided in three regions; stomach, small intestine (SI), and colon, and the SI was further divided equally into three segments representing the proximal, middle, and distal SI. Weights of the heart, spleen, liver, kidneys, pancreas, lungs and the empty stomach, SI and colon were recorded. Relative mucosa proportions for SI locations were determined from 10 cm segments and reported on a dry matter basis after drying the mucosa and muscularis layers at 60 °C for 72 h. Sections of distal intestine were fixed in