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Effects of maternal nutrition on development of gastrointestinal tract of bovine fetus at different stages of gestation

M.S. Duarte [a,e,*,1], M.P. Gionbelli [a,b,1], P.V.R. Paulino [a], N.V.L. Serão [c], T.S. Martins [a], P.I.S. Tótaro [d], C.A. Neves [d], S.C. Valadares Filho [a], M.V. Dodson [e], M. Zhu [f], M. Du [e]

[a] Department of Animal Science, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil
[b] USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA
[c] Department of Animal Science, University of Illinois at Urbana-Champaign, IL, USA
[d] Department of Animal Biology, Universidade Federal de Viçosa, MG, Brazil
[e] Department of Animal Science, Washington State University, WA, USA
[f] School of Food Science, Washington State University, WA, USA

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ABSTRACT

This study was developed aiming to evaluate the effects of maternal feed-restriction on development of gastrointestinal tract (GIT) of bovine fetus at different gestational stages. Feed-restricted cows were fed 1.2 times the maintenance level while the control group was fed ad libitum. Pregnant cows were slaughtered at 136, 189, 239, and 269 days of gestation and gastrointestinal tracts of the fetuses were evaluated. No effects of maternal nutrition on body weight ($P=0.17$) and body length ($P=0.13$) of the fetuses were observed. No major effects of feed restriction on GIT mass of the fetuses were observed ($P=0.51$). However, the weight of small intestine per unit of body weight was 11.24% greater ($P=0.04$) in fetuses from restricted dams. Additionally, the length of small intestine and its villi were 12.93% and 16.44% respectively greater ($P<.001$) in fetuses from restricted dams compared to those from non-restricted dams. These data indicates that maternal feed-restriction does not affect the development of most of fetal gastrointestinal parts besides small intestine which in turn increases its surface area as a response of maternal feed restriction.

1. Introduction

During the early phase of fetal stage critical events for normal conceptus development occur including differentiation, vascularization, fetal organogenesis, and placental development (Funston et al., 2010). Several studies have shown that fetuses from dams subjected to nutrient restriction during early to midgestation have decreased growth of the gastrointestinal tract (Avila et al., 1989; Harding et al., 1985; Trahair et al., 1997; Wang et al., 2008), and even with postnatal nutritional intervention, the suboptimal growth causes permanent changes in gastrointestinal functions such as epithelial permeability (Trahair et al., 1997).

Fetal growth restriction due to maternal nutrition has been reported as a problem in livestock production (Du et al., 2010; Wu et al., 2006) since a variety of production conditions may lead to a scenario of fetal growth restriction. As an example, in tropical regions where beef cattle are raised mainly in grazing systems, pregnant cows usually experience feed restriction during the mid-gestation period which overlaps with a season of low quantity and quality of forage (Duarte et al., 2012). Therefore, since in cattle the absorption of intact macromolecules such as immunoglobulin across the intestinal

* Corresponding author. Tel.: +55 31 3891 2709.
E-mail address: marcio_dsouza@hotmail.com (M.S. Duarte).
[1] Contributed equally to this effort.

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epithelium is possible for approximately 24 h after the calf is born, the well development of gastrointestinal tract during intrauterine stages is crucial to reduce neonatal morbidity and mortality.

Moreover, since the gastrointestinal tract serves as the main site for nutrient absorption, the changes on the development of gastrointestinal tract at the fetal stage may permanently affect the offspring performance and efficiency of nutrient utilization (Wang et al., 2008; Wu et al., 2006) impairing efficiency of animal production. However, there is a very little information regarding this topic using beef cattle as a model (Meyer et al., 2010). The objective of this study was to evaluate maternal nutrition effects on the development of gastrointestinal tract of bovine fetus at different stages of gestation.

2. Material and methods

2.1. Animals and management

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Department of Animal Science of the Universidade Federal de Viçosa, Brazil.

Thirty-two multiparous Nellore cows with average initial body weight of 451 ± 67 (mean $\pm$ SE) kg, age of 5.6 ± 1.9 years and body condition score of 4.6 ± 1.1 (from 1 to 9 scale) were used. Pregnancy was detected by ultrasound 25 days after mating and the day of mating was considered as day 0 of pregnancy. On day 27 of gestation cattle were confined in collective pens (48 m^2, 6 cows per pen) with individual electronic head gate system (Kloppen Soluções Tecnológicas, Pirassununga, SP, Brazil) for adaptation to individual feeders. At day 47 of gestation, cows were randomly assigned into two groups with different feeding levels where half of the cows ($n=16$) were fed at 1.2 times maintenance (NRC, 2000) and the other half were fed ad libitum ($n=16$). The restricted feeding level used was estimated to be enough to maintain the pregnancy of the dam throughout the experiment avoiding abortion at any period of gestation. Feed intake of feed-restricted dams was 10.8 ± 1.5 g of dry matter/kg of shrunk body weight (animal's equivalent weight after overnight fast without feed; NRC, 2000) while for dams fed at libitum the feed intake was 16.0 ± 2.0 g of dry matter/kg of shrunk body weight. Cows were fed the same diet with differences only in the feeding level.

Every 28 days cows were weighed in the morning before feeding and after a 16 h solid fast to obtain the shrunk body weight and the feed intake was adjusted based on values of shrunk body weight to maintain the feed-restriction throughout the entire gestational period.

Experimental diets consisted of 64.8% of total digestible nutrients and 13.5% of crude protein on dry matter (DM) basis and composed of corn silage (84.3% DM basis), ground corn (8.5% DM basis), soybean meal (5.1% DM basis), urea/ammonium sulfate (1.4% DM basis) and mineral mixture (0.7% DM basis). The mineral mixture was composed of 15% calcium, 9% phosphorus, 0.53% zinc, 0.13% manganese, 0.2% copper, and 100 mg/kg of cobalt.

To evaluate the effects of maternal feed-restriction on development of gastrointestinal tract at different stages of gestation, pregnant cows were slaughtered at four gestational periods. Each feeding level group (maintenance and ad libitum) was randomly divided into four groups with four cows in each group to be slaughter at 136, 189, 239, and 269 days of gestation. Cows were slaughtered at Universidade Federal de Viçosa abattoir using a captive bolt stunning and exsanguination. Pre-harvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997).

2.2. Tissue sample and data collection

After the exsanguination the gravid uterus was immediately collected and fetus was removed. The dissection of the fetus and isolation of the gastrointestinal tract was performed similarly to that described by Meyer et al. (2010). Briefly, Fetuses were dissected and the whole gastrointestinal tract was collected and gently stripped of fat and digesta. The stomach complex was isolated from the esophagus and the intestine at the pyloric valve and divided into reticulum-rumen, omasum, abomasum and each component was gently emptied and weighed. Small and large intestines were isolated and weigh and length was recorded separately. Then, small intestine was divided into duodenum, jejunum and ileum similarly to that described by Soto-Navarro et al. (2004) as it follows. The duodenum was identified as the segment from the pylorus to a point directly adjacent to the entry of the gastrosplenic vein into the mesenteric vein. The jejunum was the segment from the caudal end of the duodenum to the junction of jejunum and ileum. This junction was determined by measuring 15 cm up the mesenteric vein from the convergence of the mesenteric and ileocecal veins and then up the mesenteric arcade to the point of intestinal intersection. From this point, a 150-cm measurement was made caudally down the small intestine, which was identified as the terminal end of the jejunum and the beginning of the ileum. The ileum measurement was terminated at the ileocecal junction.

2.3. Small intestine villi morphology

Tissue samples from jejunum, duodenum and ileum were fixed in fresh 10% (w/v) formalin in phosphate buffer (pH 7.4) and embedded using the HistoResin Mounting Kit (Leica®, Heidelberg, BW, Germany). Fragments of small intestine were carefully embedded to allow the presence of great number of villi longitudinally oriented in each section. Sections were cut at 3 μm, stained with toluidine blue, and observed under light microscopy. For each segment of small intestine (duodenum, jejunum and ileum) of each animal only intact villus with evident lamina propria, base and top were selected to measure the villi length. Photomicrographs were taken with a CMOS digital camera (Biocam GmbH®, BAV, Germany) coupled to an Olympus BX50 light microscope (Center Valley, PA, United States). Ten fields and ten villi per field were randomly selected to measure the villus length.