The effect of arginine or glutamine supplementation and level of milk allowance on small intestinal development in pre-weaning calves

P van Keulen\textsuperscript{a,b}, MA Khan\textsuperscript{a}, J. Dijkstra\textsuperscript{b}, FW Knol\textsuperscript{a}, and SA McCoard\textsuperscript{a,*}

\textsuperscript{a}Animal Nutrition and Physiology Team, AgResearch Grasslands, Palmerston North 4442, New Zealand; \textsuperscript{b}Animal Nutrition Group, Wageningen University & Research, 6708 WD, Wageningen, the Netherlands

*Corresponding author. E-mail: sue.mccoard@agresearch.co.nz

Abstract

The objective was to evaluate the effect of 1\% fortification of milk with arginine (Arg) or glutamine (Gln) at two levels of milk allowance (20\% or 10\% of arrival body weight) on the histomorphological development of the small intestine in pre-weaning calves. Sixty mixed sex Friesian x Jersey calves (3 days of age) were offered reconstituted whole milk powder (125 g/L, 26\% fat, 26\% protein) at either high or low milk allowance with or without Arg or Gln fortification in a 2 x 3 factorial design (n=10/group). Post-mortem small intestine samples were collected at day 35 for histomorphometric evaluation. The results indicate that Arg or Gln supplementation of milk enhances intestinal development through increasing the surface area at a high (6 g/d with high milk allowance) but not at a lower supplementation level (3 g/d with low milk allowance). Milk allowance alone had no effect on intestinal development. This may have important implications for feed digestion and nutrient absorption, and subsequently calf performance. Furthermore, Arg and Gln supplementation had a positive effect on goblet cell numbers independent of milk allowance indicating improved intestinal integrity and potentially pathogenic defence barrier which may be beneficial for calf health.

Keywords
calf nutrition; L-arginine; L-glutamine; feeding level; intestinal development

Introduction

The gastrointestinal tract is an organ system crucial in feed digestion, nutrient absorption and protection against external pathogens. In this respect, changes in the structure of the small intestine (SI) in pre-ruminant calves are particularly important because it is the primary site of digestion and absorption of milk (Blum 2006). Increasing milk allowance enhances pre-weaning growth, but suppresses solid feed intake before weaning and, therefore, delays rumen development (Khan et al. 2016). In contrast to the rumen, the effect of increased milk intake on SI development in calves is largely unknown (Steele et al. 2016).

Arginine (Arg) and Glutamine (Gln) are conditionally essential AA for the growth of neonates (Wu 2009). In addition to the role Arg plays as a building block for protein synthesis, it is also a common substrate for nitric oxide and polyamine synthesis, thereby fulfilling a key role in intestinal cell proliferation (Tan et al. 2010). In neonatal piglets, supplementation of Arg increases the intestinal absorptive area (Wang et al. 2012; Xu et al. 2012). Furthermore, Gln is used by enterocytes as a source
of energy as well as for the endogenous synthesis of Arg (Wu 2009). Dietary Gln-supplementation enhances villus growth (Jiang et al. 2009) and reduces the severity of villus atrophy due to diarrhea in weaned pigs (Wu et al. 1996). The effect of Arg or Gln on SI development in calves, however, is unknown. The objective of this study was to determine the effect of dietary Arg- or Gln-supplementation at two levels of milk allowance on SI development in pre-weaning calves.

Material and Methods

This study was reviewed and approved by the Animal Ethics Committee of AgResearch Grasslands (Approval #13831). Sixty mixed-sex Friesian×Jersey calves (4 ± 1.1 d) were sourced from two local commercial farms, weighed and randomly allocated to six treatments in a 2×3 factorial design (n=10/treatment). Mean arrival body weight (BW) was 29.3 ± 0.64 kg, and was similar among the treatments. Calves were individually fed whole milk powder (26% CP, 26% fat; NZAgbiz; mixed at 125 g/L) using automatic-feeders (CalfSmart, NZ). Calves were either offered a control diet without supplementation at low milk (LM; 10% of arrival-BW/d) or high milk (HM; 20% of arrival-BW/d) allowance or with supplementary L-Arg or L-Gln (Merck NZ). The amino acids were included at 1% of milk DM (i.e., calves were offered 3.0 or 6.0 g/d in LM-Arg/LM-Gln and HM-Arg/HM-Gln, respectively). Pelleted calf starter (22% CP; SealesWinslow) and water were offered ad libitum.

Animals were slaughtered at 35 ± 2.4 d of age and duodenum, jejunum, and ileum samples were collected, fixed in formalin, and processed by the Histology Laboratory of Massey University (Palmerston North, NZ). In short, the tissues were dehydrated through graded alcohol baths, embedded in paraffin, and 4 μm thick sections were stained using the haematoxylin-eosin method. Morphometric analysis involved villus height, width, and density, whereupon the total absorptive surface area was calculated according to Kisielinski et al. (2002). Villus height (VH) and crypt depth (CD) were measured and VH:CD ratio calculated, and goblet cells were counted. Analysis was performed using a light microscope coupled with a digital camera (ProRes C14, Jenoptik) to a computer with image processing software (Image-Pro 7.0, Media-Cybernetics).

Intestinal response variables were analysed using a linear mixed model (R Core team 2017) with main and interaction effects of dietary treatment factors (AA supplementation and milk allowance level) as fixed effects and calf parameters (sex and farm source) as random effects. Values are presented as LS means ± SEM and effects were significant at P≤0.05.

Results and Discussion

There was an interaction (P<0.01) between milk volume and AA supplementation whereby duodenal and jejunal absorptive surface area was increased (P<0.01) in HM-Arg/HM-Gln calves by 63/40% and 36/45%, respectively (Fig. 1), compared to all other groups which in turn did not differ. Furthermore, there was no treatment-effect on ileal morphology. Villus growth and absorptive surface area of the intestinal epithelium are important parameters in increasing intestinal nutrient absorption to support calf growth. Our observations on Arg- and Gln-supplementation in the HM groups (i.e. at the higher level of AA supplementation of 6 vs 3 g/d) are consistent with the positive effect of supplementary Arg (0.6% of milk DM) on villus development, particularly in the duodenum and jejunum, reported in milk-fed neonatal piglets (Wang et al. 2012; Xu et al. 2012). Furthermore, Wu et al. (1996) and Jiang et al. (2009) showed that the supplementation of 1% Gln of milk DM prevented jejunal atrophy in diarrheic pigs. In this experiment, supplementary Arg or Gln intake of 1% of milk DM,
only at a higher milk intake, resulted in a positive effect on villus growth. The absence of an effect of milk volume per se on villi development suggests that the increase in absorptive surface area in the HM-Arg and HM-Gln but not the LM-Arg and LM-Gln groups is a response to the greater intake of the supplemented AA. While a full dose response study has not been conducted in calves, these results provide initial insight into the potential dietary concentration required to elicit a positive effect on villi development.

Fig. 1. Intestinal surface area of 35-d-old pre-weaning calves fed a non-supplemented control diet or supplemented (at 1% of milk DM) with arginine (+Arg) or glutamine (+Gln) in combination with a low (10% BW/d; LM) or high milk allowance (20% BW/d; HM). Values with different superscripts differ significantly (P≤0.05).

New intestinal cells form in the base of the crypt and migrate up along the epithelial surface to the top of the villus. Thus, an increased VH:CD ratio is an indicator of enhanced intestinal cell proliferation and/or reduced cell apoptosis (Tan et al. 2010). Therefore, the interaction between AA supplementation and milk volume with a greater VH:CD ratio observed in the duodenum of the HM-Arg calves relative to all other groups (P<0.01), and in the jejunum of the HM-Arg and HM-Gln calves relative to HM-Ctrl calves (P=0.04), is indicative of greater intestinal cell growth/proliferation and/or reduced apoptosis in response to Arg (duodenum) and to Arg or Gln (jejunum) at the higher supplementation level (Table 1).

Goblet cells are intestinal mucins secreting cells creating a physical barrier at the mucosal surfaces of the intestine, which serve as the front line of innate host defence (Kim & Ho 2010). Similar to other intestinal epithelial cells (Tan et al. 2010), goblet cells may also depend on Arg, Gln, and their metabolites for proliferation and differentiation. An interaction between AA supplementation and milk feeding level on the number of duodenal goblet cells was observed where Arg increased (P<0.01) and Gln tended to increase (P=0.08) the number of jejunal goblet cells compared to controls, regardless of milk allowance (Table 1). These results are similar to the results of Wu et al. (2010), where dietary Arg-supplementation increased the number of goblet cells throughout the SI of pigs.

Table 1. Small intestinal histomorphology of 35-d-old pre-weaning calves fed a non-supplemented control diet or supplemented (at 1% of milk DM) with arginine (+Arg) or glutamine (+Gln) in combination with a low (10% arrival BW/d) or high milk allowance (20% arrival BW/d).
**VH:CD = Villus height to crypt depth ratio; goblet cells are counted per 100 μm of intestine.**

The effect of supplementation (S), milk allowance (M) and their interaction (S × M) are presented.

a, b, c Values within a row with different superscripts differ significantly (P≤0.05).

In summary, the results indicate that Arg- or Gln-fortification of milk enhances intestinal development through increasing surface area when supplemented at 1% in milk diets offered at a greater allowance (20% of BW), but not at lower level milk allowance (10% of BW). Furthermore, milk allowance alone had no effect on intestinal development. This may have important implications for feed digestion and nutrient absorption, and subsequently calf performance. Furthermore, the positive effect of Arg- or Gln-supplementation on goblet cells is important for intestinal integrity and potentially improving the pathogenic defence barrier and calf health.

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**References**


