BRIEF COMMUNICATION: The effect of arginine supplementation and milk allowance on small intestinal development in pre-weaning calves

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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 in pre-ruminant calves are particularly important because it is the primary site of digestion and absorption (Blum 2006). Understanding factors that influence intestinal development is essential to inform calf feeding practices to optimise growth performance and health. Increasing milk allowance increases calf growth, but suppresses solid feed intake before weaning and, therefore, delays rumen development (Khan et al. 2011). In comparison to the rumen, development of the small intestine has received little attention in calf nutrition (Steele et al. 2016) and the effect of increased milk intake on small intestine development in calves is largely unknown (Khan et al. 2016).

Arginine (Arg) is a conditionally essential amino acid for the growth of neonates (Wu et al. 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 signalling role in intestinal cell proliferation (Tan et al. 2010). Supplementation of Arg in milk-fed piglets enhanced the small intestinal absorption area as a consequence of increased villus height (Wang et al. 2012). It is unknown, however, whether this effect also occurs in calves.

The objective of this study was to determine the effect of dietary Arg supplementation and milk allowance on intestinal development in pre-weaning calves. It was hypothesised that supplementary Arg and an increased milk allowance would enhance the development of the small intestine.

Material and methods

All animal manipulations in this study including welfare, husbandry and experimental sampling were reviewed and approved (AE13831) by the Animal Ethics Committee of AgResearch Grasslands, Palmerston North, New Zealand (NZ).

Forty mixed-sex Friesian×Jersey calves (4±1 d) were sourced from two local commercial farms, weighed and randomly allocated to four treatments in a 2×2 factorial design (n=10/treatment). Mean arrival body weight (BW) was 29.5±0.6 kg, and was similar among the treatments. Calves were individually fed whole milk powder (24% protein, 25% fat, NZAgbiz, mixed at 125 g/L) using automatic-feeders (CalfSmart, Palmerston North, NZ). Calves were offered either a control diet without supplementation at low (10% arrival-BW/d; LC) or high (20% arrival-BW/d; HC) milk allowance or with supplemental L-arginine-HCl (Merck, Darmstadt, Germany) included at 1% of milk DM in combination with low (LA) or high (HA) milk allowance (i.e., calves were offered 0.12 and 0.22 g supplemental Arg/kg BW per day in LA and HA, respectively). Meal (22% protein, 14 MJ ME/kg DM, SealesWinslow, Morrinsville, NZ) and water were offered *ad libitum.*

Animals were slaughtered at 35±1 d of age and duodenum samples (one sample per animal) were collected 10 cm from the pylorus and fixed in 10% buffered formalin for later histological analysis of intestinal morphology. The tissues were dehydrated through graded alcohols, embedded in paraffin, and 4 μm thick sections (two sections per animal) were stained using the haematoxylin-eosin method (Histology Laboratory, Massey University, Palmerston North, NZ). Morphometric analysis involved villus height and width, crypt depth, villus:crypt (V:C) ratio, goblet cell count, and muscle layer and epithelium thickness. Ten measurements were taken from each section using a light microscope (BH2, Olympus, Tokyo, Japan) coupled with a digital camera (ProgRes C14, Jenoptik, Jena, Germany) to a computer with image processing software (Image-Pro 7.0, Media Cybernetics, Rockville, MD, USA). The histological analysis was performed by an investigator who was unaware of the dietary treatments affiliated with the sections.

Histological measurements were pooled per animal and average values were analysed with a linear mixed model using REML in GenStat (18th edition, VSN International, Hemel Hempstead, UK) for the response variables, with main and interaction effects of dietary treatment factors (arginine and milk allowance) as fixed effects and calf parameters (source farm, gender, age, and weight) as random effects. Values are presented as least squares means ± SEM, and effects were considered significant at $P \leq 0.05$.

Results and discussion

The hypothesis that supplementary Arg would enhance intestinal development was supported through increased

villus height (P<0.01) and width (P<0.01) in calves fed a high allowance of Arg supplemented milk, but not at a low milk allowance (Table 1). The number of goblet cells per villus was higher (P<0.05) in Arg supplemented calves, regardless of milk allowance. Increasing milk allowance increased (P<0.01) the V:C ratio, irrespective of Arg supplementation.

Milk intake was 80% higher (P<0.01) in high milk allowance (HC and HA; 4.5±0.4 L/d) than low milk allowance groups (LC and LA; 2.5±0.2 L/d) and not influenced by Arg supplementation (Fig. 1). An Arg supplementation by milk allowance interaction was observed, whereby HA had enhanced villi height (+33%) and width (+24%) compared with the other three treatments, which in turn did not differ from each other (Table 1). Our observations on Arg supplementation in calves are consistent with the positive effect of supplementary Arg (0.6% of milk DM) on villus development reported in milk-fed neonatal piglets (Wang et al. 2012, Xu et al. 2012). The positive effect of Arg on villus growth reported by Wang et al. (2012) and Xu et al. (2012) was observed with an average supplementary dose rate of 0.39 and 0.30 g/kg BW/d, respectively. An average supplementary Arg intake of 0.16 g/kg BW/d, in combination with a higher milk intake, resulted in a positive effect on villus growth in the current study with calves, but not at a dose rate of 0.10 g/kg BW/d. While a full dose response study has not been conducted in calves, these results provide some insight into the potential dietary concentration required to elicit a positive effect on villi development. Villus growth is an important parameter in increasing intestinal nutrient absorption and, therefore, important to support calf growth (Hammon & Blum 1997).

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 V:C ratio is an indicator of enhanced intestinal cell proliferation and/or reduced cell apoptosis. In this study, crypt depth was unaffected by Arg supplementation, which is consistent with prior studies in piglets (Wang et al. 2012, Xu et al. 2012). Similarly, crypt depth was not affected by milk allowance level. However, calves offered a high milk allowance had a greater (+25%) V:C ratio than did calves offered a low milk allowance, regardless of Arg supplementation (Table 1). This was consistent with the positive relationship between intake levels of bovine milk and V:C ratio in neonatal pigs reported by Pluske et al. (1996). These results suggest that the balance between cell loss from the villus top and cell division in the crypts can be enhanced by feeding level.

Arginine supplementation increased (+56%) the number of goblet cells in the intestinal villi, irrespective of milk allowance (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), and vascular endothelial cells (Zhan et al. 2008), goblet cells may also depend on Arg and its metabolites for proliferation and differentiation. The observed increase in goblet cell number in the current study is similar to the results of Wu et

Figure 1 Mean daily milk intake per calf in litres. Values are shown for calves offered either a control diet at low (10% arrival-BW/d; LC) or high (20% arrival-BW/d; HC) milk allowance without supplementation, or with arginine supplemented at 1% of milk DM in combination with low (LA) or high (HA) milk allowance. The dashed black lines are the fitted curves and dotted grey lines are the 95% confidence interval limits.

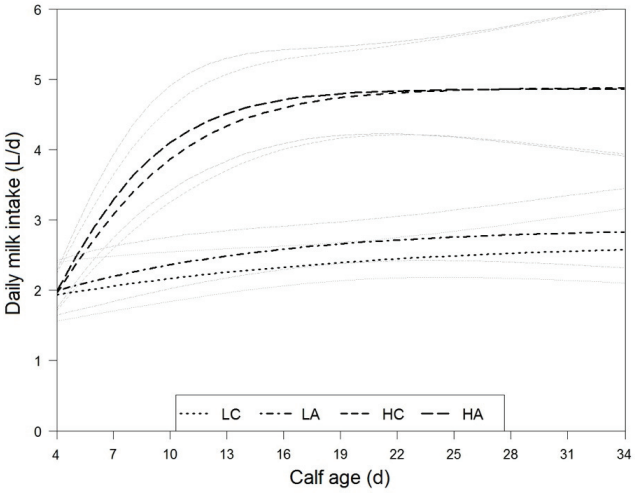

Table 1 Effect of arginine (Arg) supplementation (Control, no Arg supplementation of calves; 1%Arg, Arg supplemented at 1% of milk DM) and milk allowance (High, 20% of arrival-BW per day; Low, 10% of arrival-BW per day) on small intestine (duodenum) morphology. Data presented as least squares means and averaged SEM.

	High milk allowance		Low milk allowance			P-value[1]		
	Control	1%Arg	Control	1%Arg	SEM	Arg	Milk	A × M
Villus height, µm	329[b]	439[a]	339[b]	324[b]	17	0.01	0.01	0.01
Villus width, µm	135[b]	168[a]	141[b]	138[b]	6	0.01	0.03	0.01
Crypt depth, µm	359	419	414	400	23	0.31	0.42	0.13
Villus:Crypt ratio	0.92[ab]	1.11[a]	0.81[b]	0.82[b]	0.08	0.30	0.01	0.25
Goblet cells/villus	5.2[b]	7.7[a]	5.3[b]	8.5[a]	0.8	0.05	0.90	0.87
Muscle layer, µm	1226	1095	1181	1195	71	0.41	0.72	0.32
Epithelium, µm	36.2	38.6	39.6	39.6	1.3	0.33	0.09	0.35

[1] The effect of Arg supplementation (Arg), milk allowance (Milk) and their interaction (A × M) are presented.

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

al. (2010), who reported that dietary Arg supplementation increased the number of goblet cells throughout the small intestine in pigs. In addition, muscle layer and epithelium thickness were not affected by Arg supplementation or milk allowance (Table 1).

Nitric oxide (NO) and polyamines are physiologically important metabolites of Arg degradation and play an essential role in the mammalian target of rapamycin (mTOR) pathway activation and, therefore, cell proliferation and apoptosis (Tan et al. 2010). The results of the current study suggest that Arg and its products might have a local signalling effect in small intestine enterocytes of the intestinal villi and crypts, which was previously proven in pigs (Flynn & Wu 1996). Whether increased synthesis of mTOR, NO and/or polyamines mediated the positive effects of Arg supplementation in bovine intestinal villi warrants further investigation.

In summary, the results of the current study indicate that dietary Arg supplementation of pre-weaning calves enhanced intestinal development through increasing villus height and width at high milk allowance, which might enhance intestinal nutrient absorption and growth performance. The differentiation of goblet cells was increased by supplementary Arg, positively influencing intestinal integrity and potentially ameliorating the pathogenic defence barrier and calf health. Moreover, a higher plane of nutrition positively affected V:C ratio indicating enhanced cell proliferation and/or reduced cell apoptosis.

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