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Effect of Calcium Supplementation on Growth, Nutrient Digestibility and Fecal Lactobacilli in Dairy Calves

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Abstract: Problem statement: Based on earlier studies in veal calves and rats, the hypothesis tested was that high calcium intakes by ruminating dairy calves reduce fat digestibility, but do not affect growth performance due to enhanced colonization of the intestine with lactobacilli. **Approach:** In dairy calves that were fed on a combination of milk replacer, concentrate on grass hay, the effects of supplemental calcium on growth, nutrient digestibility and fecal lactobacilli were studied. Four concentrates with different levels of calcium were used. **Results:** Final body weight and weight gain were raised by the calcium level in the concentrate in a dose-dependent, linear fashion. Apparent digestibility of dry matter, organic matter, crude protein and crude fat were not influenced by the level of calcium in the concentrate. The number of fecal lactobacilli was significantly increased by higher dietary calcium levels, the effect having a linear trend. Calcium intake did not change the number of fecal *E. coli*. The apparent absorptions of calcium, phosphorus and magnesium were lowered in a linear, dose-dependent fashion by the calcium level in the concentrate. **Conclusion:** Increased calcium intakes stimulate weight gain in dairy calves fed a combination of milk replacer, concentrate and grass hay. This calcium effect may be related to an enhanced colonization of the intestine with lactobacilli.

Key words: Calcium, dairy calves, diet, growth, digestibility, lactobacilli

INTRODUCTION

In veal calves fed reconstituted milk replacers as the sole source of nutrition, it has been shown that high calcium intakes cause a reduction of fat digestibility (Xu *et al*., 1998; 2000; Yuangklang *et al*., 2004). Rat studies indicate that the inhibitory effect of calcium on fat digestibility can be explained by an increase in the amount of insoluble calcium phosphate sediment in the intestinal digesta (Brink *et al*., 1992). This sediment binds bile acids (Govers and van der Meer, 1993) so that the formation of biliary micelles is reduced, leading to impairment of fat digestion and re-absorption of bile acids (Beynen *et al*., 2002). This mechanism may extend to veal calves as high calcium intakes have been found to raise fecal bile acid excretion (Xu *et al*., 1998; Yuangklang *et al*., 2004).

For veal calves fed milk replacers only, fat represents about 40% of total energy intake. It would thus be expected that calcium-induced depression of fat digestibility would have a negative impact on growth. However, in veal calves subjected to a restricted feeding regimen there was no reduction in body-weight gain (Xu *et al*., 1998, 2000; Yuangklang *et al*., 2004). It could be suggested that the lowering of fat digestibility as mediated by calcium feeding is counteracted by an accompanying, positive effect. Such a positive effect of high calcium intake could be an increased growth of lactobacilli in the gut. In rats, the feeding of a high-calcium diet has been demonstrated to increase the numbers of ileal and fecal lactobacilli (Bovee-Oudenhoven *et al*., 1999). The effect of calcium may be explained by precipitating bile acids in the intestinal lumen thereby creating an environment that is

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less toxic to lactobacilli in particular (Govers and van der Meer, 1993). Enhanced growth of lactobacilli will improve their antagonistic action towards pathogenic bacteria. Veal calves are often affected by diarrhea and respiratory disease which not only determine morbidity and mortality, but also growth. When veal calves were fed probiotics in the form of different Lactobacillus species, the incidence of diarrhea and respiratory disease were reduced and weight gain and feed efficiency were enhanced (Timmerman *et al*., 2005).

In the present study we used dairy calves that were fed on a combination of milk replacer, concentrate on grass hay. In the light of the outcome of the studies with veal calves described above, the main questions addressed were as follows. Do high calcium intakes reduce fat digestibility, increase fecal numbers of lactobacilli and leave unchanged growth performance? Additional calcium was added to the concentrate in the form of calcium carbonate. In order to disclose possible dose-response relationships, four concentrates differing in the level of calcium were used in this study.

MATERIALS AND METHODS

Animals, diets and observations: Forty male Thai Friesian-Holstein calves, about 1 week of age, were purchased at a local market. Their body weight was 33 ± 1.7 kg (mean ± SD, n = 40). The calves were housed individually in metal stalls (80×175 cm) with rice straw as bedding.

On arrival, the calves were divided into four groups of 10 calves each so that body weight distributions of the groups were similar. The calves were fed twice a day, at 07.00 and 16.00 h, with a reconstituted milk replacer (Table 1) offered from a plastic bucket. The milk replacers were reconstituted in hot water (70°C) and presented at a temperature of about 42°C. The amount of reconstituted milk supplied was about 10% of body weight. As from the start of the experiment, the calves were fed one of four experimental concentrate diets that differed in the level of calcium (Table 2). The concentrates contained 0.7, 1.1, 1.7 or 2.2% calcium. Additional calcium was added in the form of calcium carbonate. The concentrates were fed at a level of 1% of body weight. When the calves were aged 2 weeks, they were given free access to pangola grass hay. Table 1 shows the analyzed composition of the grass hay.

Body weights and feed intakes were monitored. At various time intervals, the consistency of feces was scored on a scale from 1 (normal fecal consistency) to 4 (severe scour) as described (Quigley *et al*., 1997). The experimental period lasted 90 days.

Table 1: Analyzed composition of the milk-replacer powder and grass hay

Analyzed contents, g 100 g^{-1}	Milk replacer	Grass hay
Dry matter	95.30	94.10
Ash	6.60	4.50
Crude fat	5.10	1.70
Crude protein	21.00	4.10
NDF	0.00	73.80
ADF	0.00	45.70
Calcium	0.83	0.50
Phosphorus	0.62	0.31
Magnesium	0.16	0.47

Table 2: Ingredient and analyzed composition of the experimental concentrates

	Calcium level[1]			
	0.7%	1.1%	1.7%	2.2%
Ingredient (g)				
Constant components[2]	41.0	41.0	41.0	41.0
Cassava meal	59.0	58.0	57.0	56.0
Calcium carbonate	0.0	1.0	2.0	3.0
Total	100.0	100.0	100.0	100.0
Analyzed contents (g 100/g)				
Ash	7.5	7.7	7.9	8.8
Crude fat	3.0	3.4	3.5	3.6
Crude protein	14.3	14.1	14.0	14.1
NDF	19.7	19.6	18.9	19.5
ADF	8.0	8.0	8.0	8.0
Calcium	0.71	1.12	1.72	2.15
Phosphorus	0.68	0.67	0.72	0.68
Magnesium	0.38	0.37	0.41	0.40

[1]: Analyzed calcium levels; [2]:Constant components (g): soybean meal, 32.0; molasses, 3.5; tallow, 3.0; sodium chloride, 1.0; di-calcium phosphate, 0.5; vitamin premix, 0.5; mineral premix, 0.5

Collection of feces samples: Feces were collected quantitatively during the period of 85-90 days of the experiment using a plastic tray that was placed under stalls. Feces were removed from the trays daily and then weighed and homogenized with tap water (1:0.5, w/w). Fractions (5%) of the homogenates were pooled per calf and stored at-20°C until analysis.

Chemical analyses: Samples of reconstituted milk samples were freeze-dried and those of concentrates, hay and feces were oven-dried at 60°C to determine Dry Matter (DM), ash, crude protein, crude fat, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) as described (Jansen *et al*., 2000). Calcium and magnesium were determined by atomic absorption spectroscopy and phosphorus colorimetrically (Yuangklang *et al*., 2004). Fecal counts of *E. coli* and lactobacilli were determined according to Mathew *et al*. (1996) and Swanson *et al*. (2002), respectively.

Calculations and statistical analysis: The apparent nutrient digestibility was expressed as percentage of intake and computed as (intake-output with feces) × intake^{-1}×100%.

The data are presented as group means and SEM and were statistically analyzed using a computer program (SPSS for windows 9.0, SPSS Inc., Chicago, IL 1998). Linear, quadratic or cubic regressions were tested for the actual level of calcium in the concentrate. None of the variables showed statistically, significant quadratic or cubic effects of the level of dietary calcium, except for the cubic effect on the intake of milk replacer. The level of statistical significance was pre-set at $p<0.05$.

RESULTS

Growth performance: There was a statistically, significant, dose-dependent, linear effect of calcium in the concentrate on final body weight and weight gain (Table 3). The higher body weight with increasing calcium level in the concentrate was associated with higher intakes of the milk replacer. The intake of grass hay, which was freely available, was not influenced by dietary treatment.

Digestibility of nutrients: The amount of calcium in the concentrate did not affect the apparent digestibility of dry matter, organic matter, crude protein and crude fat (Table 4). The apparent absorptions of calcium, phosphorus and magnesium were lowered in a linear, dose-dependent fashion by the calcium level in the concentrate.

Fecal score and bacteria: Different calcium intakes did not affect fecal scores (Table 4). The number of fecal lactobacilli was significantly increased by higher dietary calcium levels, the effect having a linear trend. Dietary treatment did not change the number of fecal *E. coli*.

DISCUSSION

Earlier studies with veal calves fed a milk replacer as only source of feed have demonstrated that high calcium intakes reduced fat digestibility (Xu *et al*., 1998; Yuangklang *et al*., 2004). This study was carried out with dairy calves fed a ration consisting of milk replacer, concentrate and grass hay. Increasing the amount of calcium in the concentrate was found not to affect fat digestibility. The discrepancy between the previous studies with veal calves and the present study may be explained by a difference in the calcium concentrations in the whole ration. In the present study the calcium concentrations were lower.

Table 3: Body Weight (BW), Average Daily Gain (ADG) and feed intakes in calves fed the experimental diets

	Calcium level					
Item	0.7%	1.1%	1.7%	2.2%	SEM	L
Initial BW, kg	32.6	34.8	34.6	30.6	1.27	NS
Final BW, kg	97.8	109.2	113.2	114.0	1.44	*
ADG, g/day	724.0	827.0	873.0	927.0	12.60	*
Feed intake, g dry matter day^{-1}						
Grass hay	390.0	323.0	321.0	297.0	21.30	NS
Concentrate	287.0	383.0	368.0	371.0	14.50	NS
Milk replacer	2081.0	2303.0	2356.0	2328.0	27.40	*
Total feed	2758.0	3009.0	3045.0	2996.0	51.30	NS

[1]: Statistical analysis; L: Linear effect; NS: Not Significant; *: $p<0.05$

Table 4: Nutrient digestibility, fecal scores and fecal bacteria counts of *E. coli* and lactobacilli in calves fed the experimental diets

	Calcium level					
Item	0.7%	1.1%	1.7%	2.2%	SEM	L
Digestibility, (%) of intake						
Dry matter	89.00	87.60	90.00	90.90	0.93	NS
Organic matter	90.80	89.90	93.40	91.10	0.62	NS
Crude protein	96.80	96.40	97.40	96.60	0.27	NS
Crude fat	86.50	85.70	84.80	84.50	1.66	NS
Calcium	76.70	73.40	65.60	60.70	0.31	*
Phosphorus	85.20	83.10	78.50	72.60	0.36	*
Magnesium	45.60	44.80	41.70	40.20	0.28	*
Fecal score						
Day 30	2.56	2.51	2.56	2.52	0.01	NS
Day 60	2.26	2.28	2.26	2.26	0.01	NS
Day 90	1.30	1.30	1.25	1.25	0.01	NS
Fecal bacteria, $\log_{10}$ CFU g^{-1}						
E. coli	8.24	8.39	8.36	8.20	0.05	NS
Lactobacilli	7.68	7.71	7.83	8.12	0.07	*

[1]: Statistical analysis; L: Linear effect; NS: Not Significant; *: $p<0.05$

Final body weight and weight gain were raised by the calcium level in the concentrate in a dose-dependent linear fashion. The stimulatory effect of calcium on weight gain cannot be explained by increased feed intake. The voluntary intake of freely available grass hay was not influenced by dietary treatment. Milk-replacer intake was raised with increasing amounts of calcium in the concentrate, but this was a secondary rather than a primary effect. The amount of milk replacer supplied to the calves was a fixed fraction of body weight. Thus, higher body weights led to higher intakes of milk replacer rather than vice versa. Apparent digestibilities of dry matter, organic matter, crude protein and crude fat were not influenced by the level of calcium in the concentrate, indicating that the calcium-induced increase in weight gain was not related to an improved utilization of macronutrients.

The calcium-induced enhanced weight gain of the calves may relate to the observed increase in fecal lactobacilli. This effect of calcium on lactobacilli was specific as the *E. coli* numbers in feces were not affected. The number of bacteria in feces reflects the degree of their colonization in the gut. Thus, it can be suggested that calcium feeding had caused greater populations of lactobacilli in the gut. Lactobacilli are known to contribute to resistance against colonization of pathogenic bacteria, thus protecting the host. It has been shown that the feeding of a mixture of lactobacilli species to calves produced an increase in growth (Timmerman *et al*., 2005). It is thus likely that the calcium-induced increase in intestinal lactobacilli had promoted growth in the calves. However, the mechanism by which calcium feeding strengthens the lactobacillus flora is not clear. Based on rat studies there is evidence that high calcium intakes stimulate proliferation of the endogenous lactobacilli through binding of bile acids to the calcium phosphate sediment in the intestinal lumen (Bovee-Oudenhoven *et al*., 1999). However, a decrease in bile acid solubility should be associated with diminished fat digestibility, but this was not seen in the present study.

Increasing levels of calcium in the concentrate were associated with a decrease in the apparent absorption of calcium, phosphorus and magnesium. Similar effects have been observed earlier in veal calves (Xu *et al*., 1998; Yuangklang *et al*., 2004) and ruminating calves (Fielding *et al*., 1985). The calcium-induced decrease in calcium absorption can be explained by endocrine-controlled down-regulation of the absorptive efficiency (Field, 1981). As mentioned above, high versus low calcium intake induces extra formation of insoluble calcium phosphate in the small intestinal digesta so that phosphorus becomes unavailable for absorption (Brink *et al*., 1992). The fact that magnesium also is a component of the sediment (Brink *et al*., 1992) could explain the calcium-induced decrease in magnesium absorption. However this explanation does not hold if a substantial part of magnesium absorption takes place in the rumen as is the case in adult cows (Field, 1981).

CONCLUSION

This study with dairy calves fed a combination of milk replacer, concentrate and grass hay shows that high calcium intakes stimulate weight gain. This calcium effect probably is related to an enhanced colonization of the intestine with lactobacilli.

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