



Performance and development of gastric compartments of calves fed with cheese whey and transition milk

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ABSTRACT: *This study aimed to evaluate whether cheese whey associated with transitional milk or not, could serve as a suitable alternative to whole milk for calf rearing. For this, twenty-four male calves of crossbred Holstein-Zebu, were distributed in a completely randomized design with three treatments (8 calves per treatment) and fed for 60 days with one of the following liquid diets: i) 100% whole milk, ii) 50% whole milk and 50% cheese whey, and iii) 30% transitional milk and 70% cheese whey. There was no difference in food intake between treatments. The analysis showed that whole milk replacement did not alter the stomach weight or the ruminal cell proliferation when compared to treatment using 100% whole milk; however, the transitional milk/cheese whey group showed larger ruminal papillae. These results suggested that a transitional milk-cheese whey diet associated with solid food and proper nutrient complements might enhance the post-natal development of rumen papillae in calves, which might contribute to prepare them for weaning.*

Key words: Co-product, Liquid diet, Morphometry, Rumen.

Desempenho e desenvolvimento dos compartimentos gástricos de bezerros alimentados com soro de queijo e leite de transição

RESUMO: *Objetivou-se avaliar se o soro de queijo associado ou não ao leite de transição poderia servir como uma alternativa adequada ao leite integral para a criação de bezerros. Para isso, 24 bezerros machos, mestiços Holandês-Zebu, foram distribuídos em delineamento inteiramente casualizado com três tratamentos (oito bezerros por tratamento) e alimentados por 60 dias com uma das seguintes dietas líquidas: i) 100% leite integral; ii) 50% de leite integral e 50% de soro de queijo, e iii) 30% de leite de transição e 70% de soro de queijo. Não houve diferença na ingestão de alimentos entre os tratamentos. A análise mostrou que a substituição do leite integral não alterou o peso do estômago ou a proliferação das células ruminais quando comparado ao tratamento com 100% de leite integral. No entanto, o grupo de soro de queijo/leite de transição mostrou papilas ruminais maiores. Esses resultados sugerem que uma dieta de soro de queijo com leite de transição associada a alimentos sólidos e a complementos nutricionais apropriados pode aumentar o desenvolvimento pós-natal de papilas no rúmen de bezerros, o que pode contribuir para prepará-los para o desmame.*

Palavras-chave: Coproduto, Dieta líquida, Morfometria, Rúmen.

INTRODUCTION

The search for alternatives to reduce costs in cattle husbandry is important to ensure that producers receive a higher income for sustainability of dairy farming (OLIVEIRA et al., 2010). Early weaning and adoption of low-cost liquid diets are considerable alternatives for the management of breeding and caring of farm animals, as these practices can lower the costs for heifer replacement and contribute to the recovery of males for meat production as well.

After birth, animals ingest colostrum, the mammary gland secretion at the first milking after birth, which is a mixture of milk secretion

and constituents of blood serum. The secretions collected between 24 and 72 hours postpartum are called transitional milk, as their composition resembles milk (YANG et al., 2015). Colostrum differs from milk in its composition, mainly due to the high total protein content (14% with 6% (48 g/L) of immunoglobulins, solids (23.9%), and fat (6.7%) (GUERRA., 2017). Transition milk has approximately 15 g/L (2.4%) of immunoglobulins, whereas the secretion observed after 72 hours is considered milk and has only 0.6 g/L (0.09%) of immunoglobulins (LEWIS, 2007).

The energy contained in colostrum is 1.16 Kcal/g, which is approximately twice that supplied by

milk (COSTA, 2008). The energy from colostrum is provided by fat and lactose, and is considered essential for thermogenesis and regulation of body temperature in neonates. Some minerals and vitamins including calcium, magnesium, zinc, manganese, iron, cobalt, vitamin A, vitamin E, carotene, riboflavin, vitamin B12, folic acid, and selenium are reported at high concentrations in colostrum when compared to milk (GODDEN, 2008). In addition to these elements, colostrum contains a number of growth factors such as IGF-I and II, epidermal and nervous growth factor, insulin, cortisol, and thyroxine (COSTA, 2008). These substances stimulate mucosal growth, enzyme secretion, synthesis of intestinal deoxyribonucleic acid (DNA), and increased intestinal villi size, and glucose uptake (GODDEN, 2008).

The development of ruminal papillae is directly related to the increase in the ability of ruminal epithelium to metabolize fermentation products and is stimulated by the presence of volatile fatty acids in the ruminal environment. In animals consuming only milk, there is no increase in the development of the rumen papillae, whereas animals fed a concentrated and bulky diet besides milk, showed a larger size of the ruminal papillae, indicating that the composition of the food and not the age of the animal is the main factor that contributes to the development of ruminal papillae (FONTES et al., 2006).

Cheese whey and transitional milk retain some nutrition properties of whole milk and may be suitable alternatives for whole milk replacement in feeding and managing calves (FONTES et al., 2006; LIMA et al., 2011). Combination of both cheese whey and colostrum as part of a balanced diet for newborn calves is possible because cheese whey is known as a rich source of lactose and colostrum can provide the necessary immunonutrients (LIMA et al., 2012).

The functional capacity of the calf stomach varies with age. The main morphological change is accelerated ruminal growth as calves grow. After birth, the abomasum is the main digestive unit in calves and the reticulum-rumen is nonfunctional, incompletely developed, and represents only 30% of the complex stomach in calves. The major postnatal development changes of the rumen in growing calves involve colonization by different types of anaerobic bacteria and fungi, development of muscle layers and ruminal papillae, and an increase in enzymatic production for ruminal fermentative digestion (CARVALHO et al., 2003). These ruminal changes are a consequence of solid feed intake, which promotes the growth of ruminal mucosa and stimulates its motility leading to muscle layer development (COELHO, 2005).

Evaluation of the rumen response to different diets helps optimize the feeding management used in calf husbandry systems. This study aimed to verify the combination of liquid diets with transitional milk and cheese-whey or whole milk and cheese-whey that is suitable as a whole milk substitute, and the effects of such a diet on rumen mucosa.

METHODS AND MATERIALS

Animals, diets, and experimental design

Twenty-four male calves from crossbred Holstein-Zebu, were distributed in a completely randomized design, into three different groups (8 calves per group) according to the following feeding treatments: group i) whole milk (WM); group ii) 50/50 whole milk/cheese-whey (MCW); group iii) 30/70 colostrum/cheese whey (CCW). All animals received colostrum after birth. At 2 d calves were fed with 4 L of WM per day (2 L each time at morning and afternoon) for 10 days. At 12 d of age were distributed in the respective treatments, weighing on average 32.9 ± 0.85 kg. Subsequently, animals were dewormed and received the ADE vitamin complex. The experimental diet was introduced gradually (10% per day) to avoid gastrointestinal disorders. The animals were kept in individual stalls with access to water, commercial pellets concentrate, and Tifton hay (*Cynodon sp.*) *ad libitum* (Table 1).

Dry matter intake (DMI) was calculated using the offer/leftover method, where the amount of feed offered was based on the hay and concentrate consumption on the previous day added with 10% surplus. Performance evaluation was conducted by weighing and measuring the thoracic girth, wither height, hip height, and body length at birth and weekly until weaning.

Slaughter and sampling procedures

The calves were slaughtered at 60-days of age, after 16 hours of solid fasting and were weighed afterwards to determine the fasting slaughter weight (FSW). Calves were slaughtered in compliance with the current legislations (BRASIL, 2000).

After slaughter, the stomach compartments were weighed together and separated; later, all compartments of rumen (*atrium ruminis*, *saccus dorsalis*, *saccus cecus caudodorsalis*, and *saccus ventralis*) and abomasum were emptied and weighed again to obtain the weight of the full and empty compartments, the difference was considered as the weight of the contents. The results were expressed in absolute values (kg), relative to empty body weight (% EBW), and relative to stomach weight (% FSW).

Table 1 - Chemical composition of the experimental diets.

	Concentrate	Hay	WM	MCW	CWC
Dry matter	94.78	94.64	12.67	10.73	9.22
Mineral matter	9.86	7.85	-	-	-
Crude protein	18.98	7.33	24.43	27.26	20.50
Ether extract	5.62	1.77	-	-	-
Neutral detergent fiber	36.78	75.01	-	-	-
Acid detergent fiber	6.00	36.78	-	-	-
Lactose	-	-	40.92	46.36	48.64

WM: whole milk (control); MCW: 50% whole milk + 50% cheese whey; CCW: 30% Colostrum + 70% cheese whey.

Histological analyses

To analyze the ruminal papilla height, tissue samples from *atrium ruminis*, *saccus dorsalis*, *saccus cecus caudodorsalis*, and *saccus ventralis* were collected and histologically processed according to the methodology of Teófilo et al. (2007) as described next. Tissue fragments were fixed by immersion in 10% formaldehyde solution with 0.1 M sodium phosphate buffer (pH 7.4), for 18 h at 4°C. Fragments were dehydrated in ethanol, cleared in xylene, and embedded in paraffin; next, 5.0 µm-thick sections were placed on Meyer's albumin-coated glass slides and stained with hematoxylin and eosin. Coverslips were placed on the slides and analyzed using a photomicroscope (Olympus BX51®, Olympus Optical Co., Japan) equipped with a digital camera DP72 CCD.

Cell proliferation kinetics were assessed by counting AgNOR/nucleus (PLOTON et al., 1986) *in situ*. To do so, 5.0 µm thick slices of the ruminal epithelium (dorsal and ventral regions) were post-fixed in ethanol and acetic acid solution (75/25) at room temperature for 30 min. Silver staining was performed with two solutions prepared separately: 2.0% gelatin in 1.0% aqueous solution of formic acid (Solution A) and 50.0% silver nitrate aqueous solution (Solution B). The staining solution was prepared by mixing solution A with B (1:2), and the sections were immediately stained in a dark room for 30 min. Sections were washed and treated with 5.0% sodium thiosulfate for 5 min. Slides were covered with coverslips and double-blind analyzed for AgNOR counting. The count was made in 2000 nuclei lining epithelial cells covering the top and basal region of ruminal papillae. The number of AgNOR/nucleus was calculated and expressed as mean ± standard error of the mean.

Statistical analysis

The design adopted was completely randomized, and data were tested with analysis of variance followed by the Tukey's test at 5% significance level, using the SAS statistical software package (SAS Institute Inc., NC). The statistical model used was: $Y_{ij} = \mu + i + \epsilon_{ij}$, where: Y_{ij} = dependent variable; μ = overall mean; i = liquid diet; j = repetition (animal); ϵ_{ij} = residual error.

RESULTS

The daily dry matter intake (concentrate (CDMI) or hay (HDMI) dry matter intake), weight gain, and body measurements did not show significant differences ($P > 0.05$) among groups (Table 2). However, a trend ($P = 0.09$) suggesting higher intake of concentrate by the animals receiving the experimental diets (MCW, CCW) compared to the control (WM) was observed.

Mean values for absolute and relative stomach compartment weights were similar ($P > 0.05$) among treatments; however, calves fed with whole milk (WM) showed heavier abomasum content than the others ($P < 0.05$) (Table 3).

The effect of the experimental diets on ruminal papillae height is shown in table 4. The papillae from the *atrium ruminis* and the *saccus dorsalis* were similar in height ($P > 0.05$) among groups. However, the papillae from the *saccus caudodorsalis* were larger than those reported in the papillae from the *atrium ruminis* and *saccus dorsalis* ($P < 0.05$) and smaller papillae were reported in calves fed with MCW. The papillae from the *saccus ventralis* were larger in calves fed with CCW compared to the other groups ($P < 0.05$).

The total number of AgNOR/nucleus in the epithelial cells of the ruminal mucosae was statistically similar at the top and base of the papillae

Table 2 - Concentrate dry-matter intake (CDMI), hay dry-matter intake (HDMI), daily weight gain (DWG), total weight gain (TWG), thoracic perimeter (TP), body length (BL), hip height (HH) and withers height (WH) of calves fed with different liquid diets.

Variable	Liquid diets			P-value	SEM
	WM	MCW	CCW		
CDMI (kg per day)	0.42	0.65	0.59	0.09	0.01
HDMI (kg per day)	0.07	0.05	0.07	0.64	0.07
DWG (kg per day)	0.35	0.31	0.46	0.20	0.02
TWG (kg)	17.27	15.32	22.48	0.20	0.01
TP (cm)	80.81	80.31	87.06	0.21	0.09
BL (cm)	72.12	76.62	77.62	0.33	0.02
HH (cm)	81.37	81.37	84.37	0.83	0.62
WH (cm)	77.87	78.37	79.87	0.93	0.13

^aMeans with the same superscript letters in a row are not different ($P>0.05$) by Tukey's test; WM: Whole milk (control); MCW: 50% whole milk + 50% cheese whey; CCW: 30% colostrum + 70% cheese whey; SEM: standard error of the mean.

from the ventral and dorsal ruminal regions ($P>0.05$), when compared between and within the same group, despite the diet consumed (Table 5).

DISCUSSION

Whole milk is a complete diet for calves as it contains all the nutrients required for their proper development. However, considering the high cost of using whole milk to feed calves, alternate diets are always in consideration.

In the present study, we observed a trend toward high concentrate consumption in calves fed with CCW, probably because this diet had a lower amount of protein than WM, inducing the animal to find nutritional compensation. According to D'AVILA (2006) the animals fed with milk replacement consume more concentrate than animals fed with only whole milk, as diets with whole milk have all the elements for the correct nutrition of the calf; however, in the production of cheese whey, most of these nutrients, mainly casein, are retained

Table 3 - Stomach compartments weight (kg), relative percentage of empty body weight (%EBW), relative percentage of stomach weight (%SW), and stomach content (kg) of calves fed with different liquid diets.

Variable	Liquid diets			P-value	SEM
	WM	MCW	CCW		
-----kg-----					
Rumen-reticulum	0.74 ^a	0.80 ^a	1.02 ^a	0.19	1.11
Omasum	0.20 ^a	0.21 ^a	0.26 ^a	0.39	0.67
Abomasum	0.34 ^a	0.32 ^a	0.35 ^a	0.79	1.31
-----%EBW-----					
Rumen-reticulum	1.61 ^a	1.97 ^a	2.08 ^a	0.13	0.06
Omasum	0.44 ^a	0.54 ^a	0.54 ^a	0.33	0.01
Abomasum	0.75 ^a	0.83 ^a	0.71 ^a	0.42	0.01
-----%SW-----					
Rumen-reticulum	57.07 ^a	57.95 ^a	62.28 ^a	0.18	0.26
Omasum	16.01 ^a	15.96 ^a	16.37 ^a	0.97	0.02
Abomasum	26.90 ^a	26.08 ^a	21.33 ^a	0.24	0.08
-----Gastric contents (kg)-----					
Rumen-reticulum	2.30 ^a	2.09 ^a	3.09 ^a	0.17	0.21
Omasum	0.03 ^a	0.05 ^a	0.09 ^a	0.06	0.01
Abomasum	0.60 ^a	0.20 ^b	0.32 ^b	0.05	0.07

^{a,b}Means with different superscript letters in a row are significantly different ($P<0.05$) by Tukey's test; WM: Whole milk (control); MCW: 50% whole milk + 50% cheese whey; CCW: 30% colostrum + 70% cheese whey; SEM: standard error of the mean.

Table 4 - Ruminal papillae height of 60-day-old calves fed with different liquid diets.

Regions	Liquid diets			P-value	SEM
	WM	MCW	CCW		
<i>Atrium ruminis</i>	2.99 ^{a,B}	2.64 ^{a,B}	3.11 ^{a,B,C}	0.23	0.01
<i>Saccus dorsalis</i>	2.05 ^{a,C}	1.76 ^{a,C}	2.13 ^{a,C}	0.06	0.01
<i>Saccus caudodorsalis</i>	2.89 ^{b,B}	2.25 ^{c,B}	3.53 ^{a,B}	<0.0001	0.01
<i>Saccus ventralis</i>	6.83 ^{b,A}	6.02 ^{b,A}	8.48 ^{a,A}	<0.0001	0.02
P ⁴	<0.001	<0.001	<0.001	-	-
CV ⁵ (%)	32.16	35.02	32.30	-	-

^{a,b,c}Means with different superscript letters in a row are significantly different ($P < 0.05$) by Tukey's test; ^{A,B,C}Means with different superscript letters in a column are significantly different ($P < 0.05$) by Tukey's test; WM: Whole milk (control); MCW: 50% whole milk + 50% cheese whey; CCW: 30% colostrum + 70% cheese whey; SEM: standard error of the mean.

in cheese, resulting in a nutritionally poor product. Thus, the animals received lower amounts of protein when compared to the control diet, and tend to supply the deficit with solid food. This nutritional compensation was probably the reason that generated higher consumption of concentrate by animals that received cheese whey replacing partially the whole milk. This explains why there is no difference in the weight gain of animals despite different net diets. This also contributed to understanding why there was no difference in animal weight gain among the groups in this experiment, even among calves fed only with liquid diets. The average weight gain per day was 0.46 kg, which is similar to that observed by other authors when using colostrum as a substitute (MODESTO et al., 2002; CASTRO et al., 2004).

Interestingly, the biometric data showed no difference among groups suggesting that our experimental diet, specially the CCW for its low cost, did not interfere with the development of the calves. It was observed that 60-day-old calves presented a relative stomach weight with an average of 2.82% to that observed by CARVALHO et al. (2003).

Diet can influence the stomach size or weight (CASTRO et al. 2004). However, we observed that calves fed with whole milk or substitute diets did not show significant variation in rumen-reticulum weight.

The abomasum showed a heavier content in calves fed with whole milk than that in those fed with substitute diets, possibly due to a larger casein coagulum; it is known that this coagulum remains within the stomach until gastric emptying is complete (SILVA & CAMPOS, 1986).

At birth, the rumen-reticulum compartment of calves is not fully developed and is nonfunctional,

corresponding to 30.0 % of the complex stomach; this is transient since the development of the digestive tract occurs as the calves grow (COSTA et al., 2003). The development of the rumen depends on the intake of solid diet and the consequent rate of short-chain fatty acids (SCFA) production. Differences in the gastric compartment weights reflect variations in food consumption and bacterial fermentation rate (BALDWIN et al., 2004; BITTAR et al., 2009). AZEVEDO et al. (2013) evaluated two different feeding systems and reported a direct correlation between gastric compartment size and the consumption of total dry matter. In our study, daily consumption of hay did not differ among the groups and it is reasonable to suggest that the milk replacements tested might not impair the body performance of calves. Nutritional deficiency inherent to liquid diets could be adjusted with suitable supplementation; here we reported a slightly higher consumption of concentrate by experimental groups.

Although, calves do not efficiently metabolize non-dairy diets in the early days of life due to their physiological limitations, increasing the intake of solid food and the consequent production of SCFA by microbial flora is accompanied by a higher rate of rumen papillae development. We reported that the average papillae height in the rumen of calves fed with the tested milk replacements was similar to that observed in other studies (HILL et al., 2005; ROTH et al., 2009; SILVA et al., 2009). Calves fed with whole milk for 8 weeks exhibited ruminal papillae of 5.0 to 7.0 mm height (HUBER et al., 1969). It is expected that calves provided with a whole milk-based diet during their growth phase will exhibit the largest ruminal papillae among those fed with milk replacers (GÓRKA et al., 2011).

Table 5 - Number of AgNOR/nucleus in epithelial cells of ruminal papillae from the ventral and dorsal regions of calves fed with different liquid diets.

Site	Liquid diets			P-value	SEM
	WM	MCW	CCW		
-----Dorsal region-----					
	2.94 ^{a,A}	2.64 ^{a,A}	2.82 ^{a,A}	0.33	0.06
Top	2.76 ^{a,A}	2.61 ^{a,A}	2.82 ^{a,A}	0.33	0.02
P	0.34	0.85	0.89	-	-
CV (%)	11.13	9.31	9.11	-	-
-----Ventral region-----					
Base	2.94 ^{a,A}	2.64 ^{a,A}	2.79 ^{a,A}	0.33	0.02
Top	2.83 ^{a,A}	2.96 ^{a,A}	2.86 ^{a,A}	0.76	0.01
P	0.63	0.13	0.96	-	-
CV (%)	10.99	6.67	27.98	-	-

^{a,A}Means with the same superscript letters in a row and column are not different ($P>0.05$), Tukey's test; WM: Whole milk (control); MCW: 50% whole milk + 50% cheese whey; CCW: 30% colostrum + 70% cheese whey; SEM: standard error of the mean.

In this study, a significant difference in papillae height among groups was observed; although, LIMA et al. (2013) showed no negative effect of cheese whey-based diets on the ruminal papillae height. The milk cheese whey diet showed smaller papillae when compared to other diets, which may be caused by increased intestinal transit due to diet lactose content leading to a decrease in rumen retention time, thus restricting the amount of available nutrients for microbial fermentation (VALADARES et al. 2002). Colostrum cheese whey diet showed bigger papillae than the other groups, possibly due to the higher epidermal growth factor (EGF) higher in colostrum when compared to whole milk (DZIK et al., 2017). Nevertheless, the colostrum cheese whey diet may form a firm casein coagulum in the abomasum that provides a nutritional supply for longer time, and decreases the lactose flow toward the intestine (BURRIN et al., 1995; BLUM & HAMMON, 2000; BERGE et al., 2009).

Based on our findings, we suggested that calves fed with colostrum cheese whey might be physiologically more prepared for weaning than the others, based on the morphological features observed, which reflect a great capacity for absorption of nutrients and microbial fermentation products by their gastric mucosa.

The adaptive mechanism of the ruminal mucosa is related to physical stimulation caused by food ingestion (QUIGLEY, 1996; BITTAR et al., 2009), the amount of SCFAs produced, and the necessity for efficiently high blood flow to support the

gastric absorptive function induced by trophic agents, hormones, and increased proliferative activity in the ruminal papillae (FURLAN et al., 2011). AgNOR is considered a reliable tool to assess the doubling speed of cells (RICI et al., 2011; MANÇANARES et al., 2012). Although, we reported morphological differences in papillae height, we did not find significant proliferation variation in the epithelial cells of the ruminal mucosae regardless of the type of diet and/or replacement provided. The animals fed with whey showed a good papillary development and; therefore, were prepared for the weaning. Thus, the use of this tested diet is a physiologically viable alternative for feeding calves.

CONCLUSIONS

Cheese whey and transitional milk are an alternative for use as whole milk replacements for feeding calves as partial or total substitutive diets, without impairing their gastric development. Furthermore, the combination of cheese whey and transitional milk in a feeding system can improve the ruminal papillary development; consequently, providing proper physiological contribution for the weaning process.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was carried out in accordance with the ethics principles in animal experimentation, and with the

approval of the Ethics Committee on the use of Animals of the Universidade Federal Rural do Semiárido, Brazil (protocol # 23091.002083/2011-66).

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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