

Replacement of dry ground corn with reconstituted corn grain silage in the starter concentrate of dairy calves

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ABSTRACT. Thirty-six newborn Holstein calves were used in a randomized block design and allocated to the following groups: 1 – starter concentrate based on ground corn grain, soybean meal, wheat bran and premix of minerals and vitamins (Control); and 2 – the same diet composition, but ground corn replaced with reconstituted corn grain silage (RCGS). Calves were fed 6 l/day of whole milk and had free access to water and concentrate. Calves were weaned from day 57 to 63 and fed hay *ad libitum* until 70 days of age. The period from birth to 56 days of age was called pre-weaning and from day 57 to 70 – the transition period. On day 70, five animals/treatment were slaughtered to assess ruminal development. Dry matter intake (DMI) and health problems were recorded daily, while weight and blood samples were collected weekly. Ruminal fluid was collected at weeks 8 and 10. Replacing corn grain with RCGS resulted in greater feed efficiency (FE) at pre-weaning (0.71 vs 0.66). Concentrate DMI was higher for control during the transition period (303 vs 256 g/day). Control calves presented higher faecal scores throughout the study. The control diet resulted in higher concentrations of isovaleric and isobutyric acids and ammonia-N at week 8. RCGS increased glucose levels, but decreased total protein concentration during the whole evaluation period. Feeding RCGS was efficient during the pre-weaning period; however, it decreased intake during the transition period. Data from a longer feeding period after weaning are needed to evaluate the effects of a highly digestible starch source in the diet of young calves.

Introduction

The physical and metabolic ruminal development is essential for the transition from a preliminary to a functional ruminant state, mainly stimulated by solid diet intake (Khan et al., 2016). Gradually, glucose is replaced by short-chain fatty acids (SCFA) as the primary energy source for calves (Huntington, 1990). Starter concentrate intake and ruminal microbiota are responsible for the increase in SCFA concentration, with butyric and propionic acids involved in ruminal papillae

development (Khan et al., 2016). According to Quigley et al. (2019), when calves reached an accumulated intake of 15 kg of non-fibre carbohydrates (NFC) in the pre-weaning period, an adequate rumen development was achieved ensuring a smooth weaning process. According to Davis and Drackley (1998), calf starters should be composed of highly digestible ingredients because the rumen is under development. Therefore, according to the compiled data from the aforementioned studies, recommendations for crude protein (CP) should be about 20–22%, total digestible nutrients (TDN) – 80%,

neutral detergent fibre (NDF) between 15 and 25%, and acid detergent fibre (ADF) between 6 and 20%. The wide ranges of recommendations for NDF and ADF contents reveal the importance of dietary fibre in the concentrate, as the minimum levels are intended to ensure rumen epithelium health, and the maximum levels ensure the inclusion of highly digestible ingredients (Virgínio Júnior and Bittar, 2021). Incorporation of higher NFC levels does not reduce concentrate intake, animal performance or ruminal development when the NDF source is of high quality (Poczynek *et al.*, 2020).

The corn grain processing can be an alternative to increase propionic acid concentration in the rumen, improving not only energy availability, but also rumen development (Nussio *et al.*, 2003). Processing methods such as steam flaking or dry rolling and milling are typically applied to improve starter digestibility for pre-weaned calves (Nussio *et al.*, 2003; Lesmeister and Heinrichs, 2004). Reconstituted corn grain silage (RCGS) is a type of conservation and grain processing that can increase starch digestibility to levels similar to the steam flaking process (Owens *et al.*, 1986). RCGS is an excellent option for farmers located remotely from the corn processing industry, offering the advantage of being cheaper, more manageable and generating lower losses compared to dry grain storage. A study by Owens *et al.* (1997) suggested that supplying diets with reconstituted grain increased feed efficiency and metabolisable energy. However, no studies have evaluated the inclusion of RCGS in the diet of young calves.

According to Quigley *et al.* (2018), starch level range from $\leq 25\%$ for pelleted starters to $\geq 40\%$ for texturised feeds, normally associated with an increased proportion of higher NDF ingredients, such as soybean hulls and wheat middlings. However, feeding a starter concentrate with a higher content of NFC such as processed corn starch, increases the starch fermentation rate, total SCFA production and lactic acid proportion, but decreases fibre digestion, ammonia levels and the acetate:propionate ratio (Oba and Allen, 2003). Usually, lactate is not present in high concentrations in the rumen; however, when a sudden high supply of NFC occurs, lactic acid builds up (Owens *et al.*, 1998) and ruminal pH decreases. In these situations, significant variations or decreased starter intake are common, probably due to subacute ruminal acidosis. As grain processing methods increase starch availability, and thus its fermentability, RCGS may lower ruminal pH.

Ensiling of reconstituted corn can increase starch rumen digestibility, resulting in improved animal performance and rumen development; however, the risk of acidosis should also be taken into account. Considering all these aspects, we believe that the inclusion of reconstituted corn grain silage in the starter would improve performance and ruminal development in the pre- and immediate post-weaning period. Therefore, this study aimed to evaluate the inclusion of reconstituted corn grain silage as a replacement for ground corn in the starter concentrate, taking into account the performance, metabolism, and ruminal development of Holstein dairy calves.

Material and methods

Animals and feed management

The experiment was carried out at the Experimental Calf Facility “Evilásio de Camargo” of the Animal Science Department at ESALQ/USP, located in Piracicaba – SP. The institutional Animal Care and Use Committee of the “Luiz de Queiroz” College of Agriculture at the University of São Paulo approved all animal procedures in this study (Protocol No. 4279180620).

Thirty-six newborn Holstein calves (12 females and 24 males) were used in this study. At birth, all calves were immediately separated from their dams, weighed, identified, and navels were sanitized with 10% iodine. High-quality colostrum (> 60 g/l IgG) was provided at 10% of birth weight volume in the first 6 h after birth (Godden, 2008); only calves with serum protein levels above 5.5 g/dl at 48 h of life were included in this study.

An experimental design of randomized blocks was applied, using sex, birth weight (34.84 ± 1.32 kg) and date of birth of the calves (calves in the same block were born within 7 days) as blocking factors. The animals were assigned to the following treatment groups: 1 – starter concentrate based on ground corn, soybean meal, soybean cone, and mineral and vitamin premix (Control); and 2 – starter concentrate with RCGS replacing ground corn on a dry matter (DM) basis (Silange).

Both starter concentrates were formulated to meet the requirements of pre-weaned dairy calves (NRC, 2001) with the inclusion of, g/kg: DM of ground corn grain or RCGS 560; DM soybean meal 270; DM wheat bran 130; and DM mineral and vitamin premix 40. Premix composition was as follows, %: Ca 20, W 6.5, In 4, K 1, Mg 7, S 0.7; mg/kg: F 650, Co 25, Cu 800, Cr 20, I 40,

Fe 1 400, Mn 1 500, Si 18, Zn 3 200; IU/kg: vitamin A 140 000, vitamin D₃ 50 000, vitamin E 1 500; mg/kg: vitamin B₁ 250, vitamin B₂ 250 000, vitamin B₆ 250, vitamin B₁₂ 250, niacin 400, pantothenic acid 500, folic acid 20, biotin 10; mg/kg: butylated hydroxytoluene 800 and sodium monensin 900.

RCGS was prepared prior to the experiment in 18-litre buckets. Dried and ground corn grain was rehydrated to 35% moisture using a mixer (Cirelli, Descalvado, Sao Paulo, Brazil) to ensure homogeneity. The buckets were sealed to prevent air from entering the silos and remained closed for at least 60 days to achieve corn fermentation stability and increased degradability effects (Fernandes et al., 2021). For the RCGS-containing concentrate, a pre-mix was prepared with all ingredients except silage, which was mixed in daily just before feeding. The control concentrate was mixed into 100 kg batches and stored in closed buckets.

Facilities

The calves were individually housed in suspended cages (1.13 × 1.40 m) in a ventilated barn with a rubber mat and sawdust bedding until ten days of age. Then the calves were moved to individual wooden stalls (height 1.35 m, width 1 m and depth 1.45 m), secured with a chain strap attached to a thin chain (2-m-long). The wooden stalls were changed daily to avoid faeces accumulation and to keep the calves in a clean and dry environment. During the study period, the calves had *ad libitum* access to solid diet and water from the first day of life. Whole milk was provided from the 2nd feeding at a volume of 6 l/day, divided into two meals (7:00 and 17:00), initially in bottles and after training in open buckets.

The starter concentrate was fed every morning andorts were weighed the next day to calculate individual intake. When solid feed intake exceeded 1 kg/day, the diet was offered three times a day: 1/3 with milk supply, 1/3 2 h after milk supply and 1/3 in the afternoon before milk supply. This management was adopted to avoid wasting solid feed by the calves, since this behaviour was noticed.

The experimental period consisted of 70 days, of which 56 days were the pre-weaning period. Weaning started at 57 days of age, by reducing the ration by 1 l/day (57 days = 5 l, 58 days = 4 l, 59 days = 3 l, 60 days = 2 l, 61 days = 1 l, 62 days = 0), dividing the total supplied volume into two meals until complete weaning. After completion of weaning, the calves were fed chopped Tifton-85 hay with free-choice access during the following week, when the experimental period ended. The transition period lasted from 57 to 70 days of age, which included the

weaning process (57–63 days) and the subsequent week (64–70 days).

Feed analysis

Feed samples were periodically collected during the experimental period to determine the chemical composition (Table 1). The samples were ground in a Wiley mill (Marconi, Piracicaba, Brazil) to a size of 1.0 mm and analysed for: DM by drying the samples in an oven (Marconi, Piracicaba, Brazil) at 105 °C for 24 h; ash by incinerating the samples in a muffle furnace at 550 °C for 4 h (AOAC International, 2012); total nitrogen using a Leco FP528 apparatus (Leco Corporation, St. Joseph, MI) according to Thiex et al. (2002), with crude protein (CP) calculated by multiplying total nitrogen by 6.25. Neutral detergent fibre (NDF) was determined according to Van Soest et al. (1991), using thermostable α -amylase and sodium sulphite. Acid detergent fibre (ADF) analysis was conducted as described by Goring and Van Soest (1970). Ether extract (EE) analysis was performed according to AOAC International (2012). The content of non-fibrous carbohydrates (NFC) was estimated using the following equation: $NFC (\% DM) = 100 - (CP + EE + NDF + Ash)$.

Table 1. Chemical composition of experimental diets

Composition, g/kg	Control	RCGS
DM	879	770
Ashes	69	59
CP	269	259
NDF	148	133
ADF	76	69
EE	35	32
NFC	478	516

RCGS – reconstituted corn grain silage, DM – dry matter, CP – crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, EE – ether extract, NFC – non-fibrous carbohydrates

Performance and blood parameters

Calves were weighed weekly before the morning feeding on a mechanical scale (ICS-300, Coimma Ltda, Dracena-SP, Brazil) to calculate average daily gain (ADG) and feed efficiency (FE). FE was calculated as the ratio of average daily gain (ADG) and kg of total DMI. In addition, hip width and wither height were measured every two weeks using a ruler with a scale in centimetres, and heart girth with a measuring tape with a scale in centimetres. The evaluation of faecal score, according to Larson et al. (1977), was registered daily.

Blood samples were collected weekly 2 h after the morning feeding, using three evacuated tubes (Vacuette do Brasil, Campinas, SP, Brazil)

containing: sodium fluoride as antiglycolytic agent and potassium EDTA as an anticoagulant to obtain plasma; a clot activator to obtain serum and anticoagulant (EDTA) for haematocrit analysis. Plasma and serum were obtained after centrifugation (Universal 320R, Hettich, Tuttlingen, Germany) at 2 000 g for 20 min at 4 °C. Blood aliquots were used to analyse capillary haematocrit, which was centrifuged in a microhaematocrit centrifuge (SPIN 1000, MICROSPIN, USA) at 12 000 g for 10 min. After centrifugation, capillaries were read using a haematocrit ruler.

All metabolic parameters were determined using an Automatic System for Biochemistry (SBA-200, CELM, Barueri, SP, Brazil). Commercial kits (Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) were used to determine plasma glucose (Ref. 133-1/500), total serum protein (Ref. 99), urea (Ref. 104) and lactate (Ref. 116). In addition, the determination of B-hydroxybutyrate (BHB) concentrations was performed using the RANBUT enzymatic kit (Ref. RB1007) (RANDOX Laboratories – Life Sciences Ltd. Crumlin, UK; imported by RANDOX Brasil Ltda., São Paulo, SP, Brazil).

Ruminal parameters

At 8 and 10 weeks, ruminal fluid samples were collected, 2 h after the morning feeding. Samples were collected through an oro-esophageal tube, using a flexible tube (150-cm-long, 1.3 cm in internal diameter and 0.2 cm in wall thickness) connected to a vacuum pump (TE-0581, Tecnal Ltda., Piracicaba, SP, Brazil). The initial portion of ruminal fluid was discarded to avoid contamination with saliva. After filtering through a cotton fabric, a 50-ml sample was used to measure the pH with a digital pH meter (Tec-5, Tecnal Ltda, Piracicaba-SP, Brazil); three aliquots (2.0 ml each) were stored in plastic tubes, in a freezer at –20 °C for SCFA and ammonia nitrogen (NH₃-N) determination according to de Paula *et al.* (2017). The concentration of total SCFA (mM), acetic, propionic and butyric acids (mM/100 mM) was measured. The C2:C3 variable was obtained from the acetic acid to propionic acid ratio. The C2:(C3 + C4) variable was obtained based on the ratio of acetic acid to the sum of propionic and butyric acids (Poczynek *et al.*, 2020).

Ruminal morphology

At 10 weeks of age, five calves from each group were randomly slaughtered. The digestive tract was separated and weighed before and after washing out the content under running water. Samples (approx.

1.0 cm²) of ventral sac, ventral blind sac, dorsal sac and dorsal blind sac were collected and stored in 70% alcohol. For the morphological evaluation of the rumen epithelium, the mean number of papillae per cm² was determined by counting the papillae in each rumen fragment and calculating the mean result of three independent evaluators.

Statistical analysis

Statistical analyses were performed using the MIXED procedure implemented in the SAS statistical package. For interpretation and discussion of the results, a *P*-value ≤ 0.05 was adopted as statistical significance. All data were analysed for normality of residuals using the Shapiro-Wilk test, homogeneity of variances by Levene's test and removal of outliers based on Student's *t*-value.

The following statistical model was used for the variables analysed as repeated measures over time (week of age), (DMI, ADG, FE, body measurements and blood parameters), considering diet (Control or RMGS), age (age at which the variable was measured) and the interaction of both as a fixed effect:

$$Y_{ijk} = \mu + D_i + b_j + e_{ij} + A_k + (bD)_{jk} + (DA)_{ik} + e_{ijk},$$

where: μ – general mean, D_i – fixed effect of the diet, b_j – random block effect, e_{ij} – residual error A, A_k – fixed age effect, $(bA)_{jk}$ = random effect of block × age interaction, $(DA)_{ik}$ = fixed effect of the diet × age interaction, and e_{ijk} – residual error B.

The covariance matrices “compound symmetry, heterogeneous compound symmetry, autoregressive, heterogeneous autoregressive, unstructured, banded, variance components, Toeplitz, independence and heterogeneous Toeplitz” were tested and defined according to the lowest value obtained for “Akaike's Information Criterion corrected” (AICc).

Non-repeating data (initial weight, weaning weight, final weight, rumen papillae data) were evaluated using the following statistical model:

$$Y_{ij} = \mu + D_i + b_j + e_{ij},$$

where: μ – general mean, D_i – fixed effect of the diet, b_j – random block effect, and e_{ij} – residual error. The block was included as a random effect.

The means for all response variables were obtained using the LSMEANS command. Comparison between treatments was performed using Tukey's test if the analysis of variance was significant. If there was an interaction between diet and age, the split was performed using the SORT procedure.

Results

Intake, performance, body measurements and faecal score

The replacement of corn grain with RCGS in the concentrate did not influence BW and total DMI (Table 2). However, total DMI as a percentage of BW was higher in control calves in the pre-weaning and transition periods ($P < 0.02$). The starter DMI during the pre-weaning and transition periods increased with age (Figure 1; $P < 0.001$). However, calves fed a control diet had a higher starter intake during the transition period ($P < 0.04$). The pre-weaning ADG increased with age ($P < 0.001$), but no age effect in the transition period was observed. In contrast, FE during the pre-weaning period was higher for RCGS-fed calves (Table 2; $P = 0.02$), which did not occur during the transition period.

Table 2. Performance, intake and body growth of animals fed the control diet or reconstituted corn grain silage

Item	Diets		SEM	P-value		
	Control	RCGS		Diet	Age	Diet*Age
Preweaning						
initial BW, kg	34.98	34.71	1.320	0.68		
BW at 8 th week, kg	72.27	73.95	2.043	0.38		
total DMI ¹ , g/d	1.042	1.003	23.25	0.29	<0.001	0.63
total DMI, % BW	1.99	1.84	0.048	0.02	<0.001	0.22
starter DMI, g/d	303	256	27.44	0.22	<0.001	0.16
ADG, kg	0.69	0.71	0.021	0.52	<0.001	0.53
FE	0.66	0.71	0.017	0.02	0.02	0.47
Transition period²						
BW at 10 th week, kg	81.8	81.6	1.54	0.86		
total DMI ³ , kg/d	2.11	2.08	0.081	0.83	<0.001	0.92
total DMI, % BW	2.00	1.74	0.075	0.02	<0.001	0.37
starter DMI ³ , g/d	1.638	1.432	68.10	0.04	<0.001	0.35
hay DMI, g/d	81.2	77.7	7.71	0.16	0.65	0.72
ADG, kg	0.80	0.70	0.047	0.15	0.39	0.56
FE	0.53	0.50	0.039	0.54	<0.001	0.24
Body measurement gain in the whole period, cm/week of age						
hip width	0.13	0.14	0.007	0.28	0.17	0.48
withers height	0.23	0.27	0.013	0.08	0.02	0.26
heart girth	0.37	0.39	0.022	0.41	0.02	0.93
faecal score	1.21	1.07	0.060	0.05	<0.001	0.37

Diet – diet effect, Age – age effect, Diet*Age – diet effect and age effect, RCGS – reconstituted corn grain silage, BW – body weight, DMI – dry matter intake, starter DMI – starter concentrate dry matter intake, ADG – average daily gain, FE – feed efficiency, hay DMI – hay dry matter intake; ¹intake of concentrate + milk (12.5% solids). Milk dry matter intake was of 750 g/d for all calves since there were no refusals; ²period of gradual weaning (57 to 63 days) and the subsequent week (64 to 70 days); ³intake of concentrate + milk + hay. Milk dry matter intake was an average of 268 g/day for all calves since there were no refusals during the weaning week; SEM – standard error of the mean; $P < 0.05$

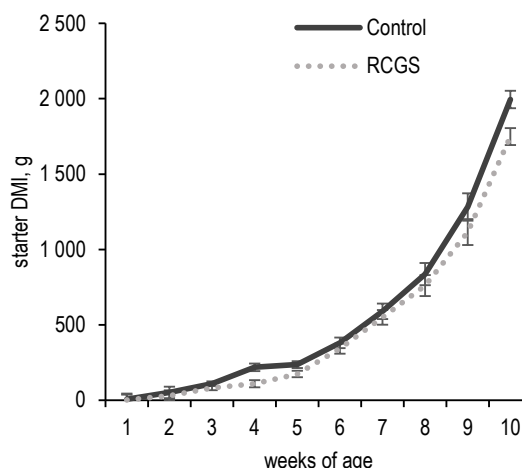


Figure 1. Starter dry matter intake (g/day) in calves receiving control diet or reconstituted corn grain silage diet (RCGS) during the 10-week experimental period. During pre-weaning: $P < 0.22$ for diet effect, $P < 0.001$ for age effect and $P < 0.22$ for the interaction of diet and age effect. During the transition period: $P < 0.04$ for diet effect, $P < 0.001$ for age effect and $P < 0.35$ for the interaction of diet and age effect

Supplementing RCGS had no effect on the increase in body measurements throughout the experimental period, but wither height and heart girth increased with age (Table 2; $P < 0.02$ and $P < 0.02$, respectively).

A higher faecal score was observed for control calves ($P < 0.045$; Table 2) and there was an age effect for this variable.

Blood parameters

Higher levels of total serum protein were observed in control calves as compared to RCGS-fed calves ($P < 0.001$; Table 3). Albumin and lactate concentrations decreased with age, while BHB concentrations increased with age ($P < 0.001$, $P = 0.003$ and $P < 0.001$, respectively; Table 3). Urea concentrations did not change irrespective of age, treatment applied or the interaction of these factors.

Table 3. Blood parameters of animals fed the control diet or reconstituted corn grain silage during the entire experimental period

Item	Diets		SEM	P-value		
	Control	RCGS		Diet	Age	Diet*Age
Total serum protein, g/dl	7.19	6.96	0.079	0.01	0.24	0.09
Albumin, g/dl	3.67	3.66	0.026	0.81	<0.001	0.27
Urea, mg/l	22.1	22.3	1.642	0.93	0.68	0.81
Glucose, g/dl	56.23	57.11	2.921	0.76	<0.001	0.02
Lactate, mg/dl	9.05	8.83	0.253	0.54	0.003	0.18
BHB, mmol/l	0.24	0.26	0.013	0.45	<0.001	0.12

Diet – diet effect, Age – age effect, Diet*Age – diet effect and age effect, RCGS – reconstituted corn grain silage, BHB – beta-hydroxybutyrate; SEM – standard error of the mean; $P < 0.05$

There was an interaction effect between treatment and age for plasma glucose levels ($P = 0.021$; Figure 2); calves fed RCGS showed higher values of this parameter at weeks 4, 6 and 8, with a decrease observed from week 9 onwards. Glucose also showed an age effect ($P < 0.001$).

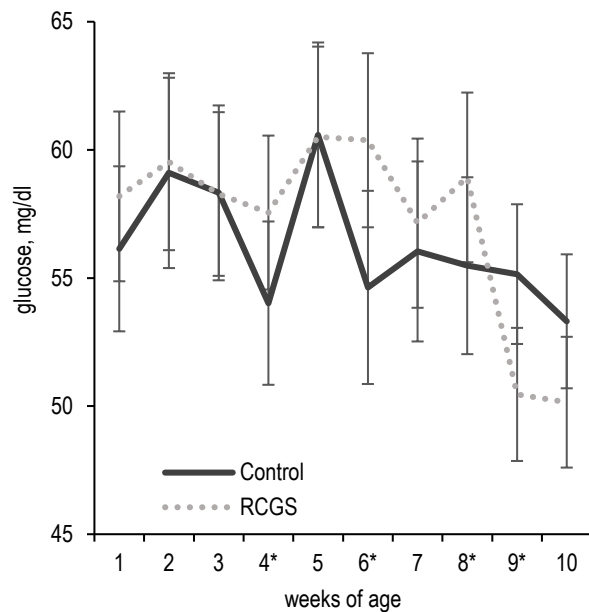


Figure 2. Plasma glucose concentrations in calves receiving control diet or reconstituted corn grain silage (RCGS) diet during the 10-week experimental period. During the whole evaluation period: $P < 0.76$ for diet effect, $P < 0.001$ for age effect and $P < 0.02$ for the interaction of diet and age effect

Ruminal fermentation parameters

Ruminal pH was not affected by the replacement of corn grain with RCGS in the concentrate of calves at both 8 and 10 weeks of age (Table 4). Only molar concentrations of isovaleric and isobutyric acids were affected by the source of corn in concentrates, with higher values in control-fed calves ($P = 0.045$ and $P = 0.044$, respectively; Table 4) at week 8. Other SCFA were not affected by the treatments at 8 or 10 weeks of age.

Animals fed the control diet had higher concentrations of ammonia-N at 8 weeks of age ($P = 0.015$; Table 4); however, no effect was observed at 10 weeks.

Morphometric parameters of the ruminal epithelium

The replacement of ground corn with RCGS had no effect on the mean number of rumen papillae per cm^2 , nor on their mean number in individual ruminal sites (ventral, dorsal and ventral and dorsal

blind sacs; Table 5). However, parakeratosis onset points (Figure 3) spread over the rumen were found in two of the five slaughtered calves (40%) fed the RCGS concentrate.

Table 4. Ruminal fermentation parameters in animals fed the control diet or reconstituted corn grain silage

Item	Diets		SEM	P-value Diet
	Control	RCGS		
Week 8				
Ruminal pH	5.60	5.54	0.092	0.62
N-NH ₃ , mg/dl	18.34	14.85	11.33	0.015
Total SCFA, mM	111.02	105.63	9.013	0.68
C2, mM/100 mM	45.07	45.05	2.642	1.00
C3, mM/100 mM	39.54	41.31	2.869	0.67
C4, mM/100 mM	9.81	8.88	0.885	0.46
C2:C3, mM/100 mM	0.96	0.97	0.081	0.93
C2:C3+C4, mM/100 mM	1.20	1.24	0.117	0.83
C5, mM/100 mM	3.25	3.20	0.311	0.90
Iso-C4, mM/100 mM	0.99	0.75	0.081	0.04
Iso-C5, mM/100 mM	1.27	0.91	0.125	0.05
Week 10				
Ruminal pH	5.92	5.73	0.125	0.22
N-NH ₃ , mg/dl	15.95	12.55	1.504	0.09
Total SCFA, mM	114.74	106.25	8.930	0.35
C2, mM/100 mM	48.69	46.95	0.868	0.09
C3, mM/100 mM	38.46	39.76	1.296	0.43
C4, mM/100 mM	8.80	8.15	0.776	0.56
C2:C3, mM/100 mM	1.32	1.21	0.064	0.13
C2:C3+C4, mM/100 mM	1.04	0.99	0.035	0.13
C5, mM/100 mM	2.76	3.34	0.334	0.23
Iso-C4, mM/100 mM	0.68	0.77	0.068	0.35
Iso-C5, mM/100 mM	0.69	0.84	0.095	0.25

Diet – diet effect, RCGS – reconstituted corn grain silage, N-NH₃ – ammonia nitrogen, Total SCFA – total short-chain fatty acids, C2 – acetic acid, C3 – propionic acid, C4 – butyric acid, C2:C3 – acetic to propionic acid ratio, C2:C3+C4 – acetic to propionic plus butyric acid ratio, C5 – valeric acid, Iso-C4 – isobutyric acid, Iso-C5 – isovaleric acid; SEM – standard error of the mean; $P > 0.05$

Table 5. Number of rumen papillae of 10-week-old calves fed the control diet or reconstituted corn grain silage

Item	Diets		SEM	P-value Diet
	Control	RCGS		
Papillae, N/cm ²	220.29	222.08	17.223	0.94
VS	181.55	214.83	22.528	0.33
DS	213.78	186.33	11.028	0.12
VBS	247.00	280.00	11.028	0.53
DBS	238.89	207.17	16.980	0.23

Diet – diet effect, RCGS – reconstituted corn grain silage, VS – ventral sac, DS – dorsal sac, VBS – ventral blind sac, DBS – dorsal blind sac; SEM – standard error of the mean; $P > 0.05$



Figure 3. Occurrence of parakeratosis in calves fed concentrate containing reconstituted corn grain silage

Discussion

As expected, age affected all performance parameters, as differences in DMI, weight and ADG occurred with animal growth. Even though replacing ground corn with RCGS did not influence starter concentrate intake, TDMI or ADG during the pre-weaning period in the present study, it resulted in higher FE. However, control calves had higher TDMI, as evidenced by the BW percentage during the pre-weaning and transition periods. We expected a positive effect on ADG, as ensiled grain was shown to improve the ruminal digestibility of finely ground corn (Andrade Filho et al., 2010). Owens et al. (1997) reported in their study that DM intake was generally 6% lower in animals fed high-moisture corn compared to those fed dry corn. We observed a lower starter intake in RCGS-fed calves during the transition period, probably due to higher homeostatic energy as a result of increased starch digestibility of this ingredient (Harrelson et al., 2009). For this reason, starter intake during the transition period was likely lower in calves fed RCGS, with no effect on total DMI. After weaning was completed, the animals were fed hay, causing a decrease in FE and faecal score. Toledo et al. (2020) observed that calves fed a diet with a higher NFC content had more liquid stools (higher faecal score) due to the supply of whole corn grains at will. The authors believed that this was due to the escape of corn from the rumen into the large intestine, causing caecal acidosis. In the present study, the RCGS concentrate did not cause the same effect, probably due to the more significant degradation of this component in the rumen associated with a smaller particle size, minimizing leakage into the large intestine.

Replacing ground corn with RCGS also affected metabolic parameters. González et al. (2000) argued

that a decrease in total serum protein concentrations could be related to diseases or could be used as a parameter verifying the efficiency of diets. Accordingly, we concluded that the efficiency of protein utilisation was higher for the control concentrate. The lower plasma glucose concentrations in the control starter were probably due to the higher starter DMI, which stimulated rumen fermentation (Khan et al., 2008) and the use of propionic acid in gluconeogenesis for glucose production. The reversal of plasma glucose concentrations, favouring RCGS in the transition period, suggested more advanced ruminal development during this period in the calves from this treatment. However, the different starters did not influence the concentrations of BHB, a better indicator of rumen development (Bittar and Ferreira, 2016). Age effects on blood parameters have been previously described in response to rumen development and metabolic changes (Toledo et al., 2020).

Contrary to our expectations, feeding RCGS did not affect rumen fermentation by increasing propionic acid level and decreasing pH. Molar concentrations of isovaleric and isobutyric acids in the rumen were higher in calves fed a control diet, which could indicate amino acid fermentation (Vargas et al., 2001), as confirmed by increased $\text{NH}_3\text{-N}$ concentrations. Amino acid fermentation by ruminal microorganisms contributes to protein synthesis or its use as an energy source, releasing $\text{NH}_3\text{-N}$ into the rumen environment (Ribeiro et al., 2001). This could be associated with improved ruminal microbial efficiency, since calves fed the control feed exhibited higher values of total protein and isoacid concentrations.

Toledo et al. (2020) also found no effect of diets on the number of rumen papillae and reported similar values to those obtained in the current study. In addition, we found a total of twenty-six parakeratosis sites in the rumen reticulum of two animals

fed RCGS, and both animals consumed more than 1.5 kg/day of starter at 10 weeks of age. Jensen *et al.* (1958) stated that parakeratosis was a classic consequence of diets rich in rapidly fermentable concentrates. According to Barker *et al.* (1993), ingestion of diets rich in carbohydrates, easily fermentable in the rumen, resulted in the accumulation of SCFA, and thus induced ruminal acidosis. In addition to negatively affecting animal performance and health, excess acid accumulation in the rumen are also capable of causing deleterious effects on ruminal motility, fibre fermentation, total feed consumption and rumen wall morphology (Bernardes *et al.*, 2007).

Conclusions

Reconstituted corn grain silage was an excellent grain processing and storage option that was efficient during the pre-weaning period. However, its use in the transition period decreased starter intake, probably as result of acidosis. We recommend that calves fed reconstituted corn grain silage should receive hay or alternatively diet should be supplemented with other ingredients to minimize the risk of acidosis. Consequently, performance would not be adversely affected by a reduction in total DMI and ADG. Data from a longer feeding period are needed to better understand the effects of a highly digestible starch source in the diet of young calves.

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Conflict of interest declaration

The Authors declare that there is no conflict of interest.

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