Effect of carbohydrate source on productive performance, ruminal and systemic health of grazing cows

Efecto de la fuente de carbohidratos sobre el desempeño productivo, la salud ruminal y sistémica de vacas en pastoreo

Efeito da fonte de carboidratos sobre o desempenho produtivo, saúde ruminal e sistêmica de vacas em pastejo

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Abstract

Background: The nutritional limitations of *Cenchrus clandestinus* –i.e., high protein and low energy concentrations- make it necessary to supplement cows with non-fibrous carbohydrate (NFC) sources to improve productive performance. Nevertheless, such supplementation can lead to ruminal acidosis. **Objective:** To evaluate partial replacement of corn grain (*Zea mays*, ZM) with sorghum grain (*Sorghum vulgare*, SV), cassava root (*Manihot esculenta*, MES) or citrus pulp (*Citrus sp*., C) on milk yield and quality, ruminal pH and health of grazing cows. **Methods:** Eight Holstein cows were evaluated in a 4 x 4 Latin square design during the first 60 days of lactation. Treatments (isoenergetic rations, 1.45 ± 0.003 Mcal NE_L/kg DM) consisted of a mixture of grass and four concentrates with different NFC sources. **Results:** No differences in dry matter intake, feed efficiency, ruminal pH, hematological and metabolic profile were observed between treatments. Rumen pH was higher than 6.0, confirming the absence of ruminal acidosis. Milk yield (energy-corrected), protein, and total solids were higher for MES vs. C. **Conclusions:** None of the NFC sources tested compromised the ruminal or systemic health of the cows, while MES improved milk yield and quality.

Keywords: *cassava; cereal grains; citrus pulp; dairy cattle; energy source; feed efficiency; lameness; non-fibrous carbohydrates; ruminal acidosis; starch.*

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Resumen

Antecedentes: las limitaciones nutricionales del *Cenchrus clandestinus* (alta concentración de proteína y baja densidad energética) hacen necesario suplementar las vacas con fuentes de carbohidratos no fibrosos (NFC) para mejorar su desempeño productivo. Sin embargo, esta suplementación puede generar acidosis ruminal. **Objetivo:** evaluar el reemplazo parcial de maíz (*Zea mays*, ZM) por sorgo (*Sorghum vulgare*, SV), yuca (*Manihot esculenta*, MES) o pulpa cítrica (*Citrus sp*., C) sobre la producción de leche y su calidad, el pH ruminal y la salud de vacas en pastoreo. **Métodos:** ocho vacas Holstein fueron evaluadas empleando un diseño en cuadrado latino de 4 x 4 durante los primeros 60 días de lactancia. Los tratamientos (raciones isoenergéticas, 1,45 ± 0,003 Mcal NE_L/kg MS) consistieron de una mezcla de forraje y cuatro concentrados con diferentes fuentes de NFC. **Resultados:** no se observaron diferencias entre tratamientos en cuanto a consumo de materia seca, eficiencia alimenticia, pH ruminal, ni perfiles hematológico y metabólico. El pH ruminal fue mayor a 6,0; confirmando la ausencia de acidosis. La producción de leche (corregida por energía), proteína, y sólidos totales fue mayor para MES vs. C. **Conclusiones:** ninguna de las fuentes de NFC evaluadas comprometieron la salud ruminal o sistémica de las vacas, y MES mejoró la producción de leche y su calidad.

Palabras clave: *acidosis ruminal; almidón; carbohidratos no fibrosos; eficiencia alimenticia; fuente de energia; ganado lechero; granos de cereales; laminitis; pulpa cítrica; yuca.*

Resumo

Antecedentes: as limitações nutricionais do *Cenchrus clandestinus*-alta concentração de proteína e baixa densidade de energia- faz necessário suplementar às vacas com fontes de carboidratos não-fibrosos (NFC) para melhorar o desempenho produtivo. No entanto, essa suplementação pode gerar acidose ruminal. **Objetivo:** avaliar a substituição parcial do milho (*Zea mays*, ZM) por sorgo (*Sorghum vulgare*, SV), mandioca (*Manihot esculenta*, MES) ou polpa cítrica (*Citrus sp*., C) na produção e qualidade do leite, pH ruminal e a saúde de vacas em pastejo. **Métodos:** oito vacas Holandesas foram avaliadas empregando um delineamento em quadrado latino 4 x 4 durante os primeiros 60 dias de lactação. Os tratamentos (rações isoenergéticas, 1,45 ± 0,003 Mcal NE_L/kg DM) consistiram de uma mistura de forragem e quatro concentrados com diferentes fontes de NFC. **Resultados:** não foram observadas diferenças entre os tratamentos no consumo de matéria seca, eficiência alimentar, pH ruminal, perfil hematológico e metabólico. O pH ruminal foi superior a 6,0; confirmando a ausência de acidose ruminal. A produção do leite (corrigida para energia), proteína e sólidos totais foi maior para MES vs. C. **Conclusões:** nenhuma das fontes de NFC avaliadas comprometeu a saúde ruminal e sistêmica das vacas, embora o MES melhorou a produção e qualidade do leite.

Palavras-chave: *acidose ruminal; amido; carboidratos não fibrosos; eficiência alimentar; fonte de energia; gado leiteiro; grãos de cereais; laminite; mandioca; polpa cítrica.*

Introduction

Kikuyu grass (*Cenchrus clandestinus*) is widely used in Colombia as grazing forage for dairy cattle. As a forage base, kikuyu has nutritional limitations, such as excessive protein and low energy contents, which commonly result in milk yields lower than 11 kg/cow/d (Marais, 2001). Accordingly, non-fibrous carbohydrates (NFC) content should be increased in the diet of lactating cows in order to improve energy availability in the rumen.

Corn (*Zea mays*) is the most widely used energy source in dairy feeds because it is rich in starch, representing 72% of the grain (Tabasum *et al.*, 2018). Other NFC sources, such as sorghum (*Sorghum vulgare*), cassava (*Manihot esculenta*) and citrus pulp (derived from *Citrus* genus) are of interest because they do not compete with human food sources. In comparison to corn, starch content is similar in sorghum (Huntington *et al.*, 2006), higher in cassava (84%; Santana and Meireles, 2014), and lower in citrus pulp (2.3%); although the latter has higher sugar (24.1%) and pectin (22.3%) concentration (Bampidis and Robinson, 2006).

Rapidly-fermenting carbohydrate sources improve MY and quality, but it can compromise animal health by increasing the risk of subacute ruminal acidosis (SARA). In pasture-based production systems typical of the Colombian high tropics forage is offered *ad libitum* and cows are fed a concentrate supplement twice-a-day during milking, which increases SARA risk.

Researchers have studied this disorder by inducing sudden changes in the diet of non-lactating animals (Li *et al.*, 2012; Gao and Oba, 2016; Jing *et al.*, 2018), but the effects on yield and milk composition remain unclear. Therefore, it is important to evaluate the effect of NFC-rich ingredients on SARA incidence and performance of lactating cows. Therefore, the objective of this study was to evaluate a partial replacement of corn grain in the ration with sorghum grain, cassava root, or citrus pulp on MY and quality, ruminal pH, and animal health of grazing Holstein cows.

Materials and Methods

Ethical Considerations

This study was approved by the Ethics Committee for Animal Experimentation at Universidad de Antioquia (act 76, May 2012, Medellin-Colombia).

Location, animals and experimental design

The experiment was conducted in Santa Rosa de Osos municipality (Antioquia, Colombia). Eight Holstein-Friesian cows averaging 534 ± 34 kg body weight (BW) were used in a 4 x 4 replicated Latin square design, resulting in 32 experimental observations. The calving number effect was considered in the experimental design, with third-calving cows assigned to the first square and those with fourth and fifth calvings to the second square. The study was conducted during the first 60 days (d) postpartum. The duration of each period was 15 d, with 10 d for adaptation and 5 d for the experimental period.

Experimental diets and feeding

Animals were managed under strip-grazing with kikuyu grass (*Cenchrus clandestinus*), allowing 41-d regrowth periods. The area of the grazing strips was adjusted daily, providing 31 kg DM/cow/d. Grass dry matter (DM) concentration was 13.1 ± 0.5% and its chemical composition (as DM%) was as follows: CP: 24.7 ± 1.6%, ether extract (EE): 3.7 ± 0.6%, neutral detergent fiber (NDF): 52.9 ± 3.9%, acid detergent fiber (ADF): 29.8 ± 2.4%, lignin: 4.6 ± 0.5%, ash: 9.6 ± 1.0%, and NFC: 9.1 ± 0.9%. Nitrate concentration (NO_3) was 2,224.7 ppm. Energy density was 1.34 ± 0.08 Mcal net energy for lactation (NE_L)/kg DM.

Concentrate offer was based on weekly MY in a ratio close to 1:3.5 (1 kg concentrate per 3.5 kg milk). Four isoenergetic and isoprotein concentrates, corresponding to the experimental treatments, were compared. The *Zea mays* (ZM) treatment included corn grain as the main source of energy. In the remaining treatments, 50% approximately of the total digestible nutrients (TDN) contributed by corn were provided by sorghum grain (*Sorgum vulgare*: SV), cassava root (*Manihot esculenta*: MES), or dehydrated citrus pulp (*Citrus* sp.: C) (Table 1 and 2). Concentrates were offered twice-a-day during milking, at 04:00 and 14:00 h.

Chemical description of feed

Samples were analyzed for DM, CP, EE, ash, α-amilase NDF, ADF, Ca, P (AOAC, 2005; AOAC, 2007), lignin (Van Soest and Wine, 1968), gross energy (GE) (ISO, 1998), pectin (Hansen *et al.*, 2001), starch (AOAC, 2000), amylose and amylopectin (Yun and Matheson, 1990). TDN and NE_L were estimated with the model proposed by the NRC (2001). Condensed tannin content in sorghum grain was analyzed with the technique described by Dykes (2019). NO_3 in kikuyu was determined with photocolorimetry as described by Spörndly *et al.* (2016).

Dry matter intake

Total dry matter intake (DMI_T) corresponds to dry matter intake from pasture (DMI_P) and concentrate (DMI_C). The DMI_C was calculated by difference between the feed offered and rejected. Chromium oxide was used as an external marker, and indigestible dry matter (IDM) was the internal marker (Correa *et al.*, 2009) to determine DMI_p.

Milk yield and quality

Milk from the morning and afternoon milkings was weighed daily during the last three days of each experimental period (MM27BC automatic system, DeLaval International AB, Tumba, Stockholm, Sweden). The MY was corrected to 3.5% fat (3.5% FCM) and also for energy (ECM) (Boerman *et al.*, 2015). To evaluate milk quality, separate samples from morning and afternoon milkings were collected from each animal. A MilkoScan FT+ (Fourier Transform from Infrared; Foss, Hillerød, Denmark) was used to analyze samples for protein, fat, lactose, and total solids content (%). Somatic cell count (SCC; cells/mL) was assessed by flow cytometry (Fossomatic™ FC, Foss Electric, Hillerød, Denmark). Feed efficiency (FE), calculated from MY and DMI_T.

Table 1. Ingredients and chemical composition of the concentrates.

Treatment	ZM	SV	MES	C
Ingredient				
Corn	44.3	21.8	19.3	23.9
Sorghum*	-	22.5	-	-
Cassava	-	-	20.5	-
Citrus pulp	-	-	-	25.8
Corn forage	10.0	9.3	10.0	5.0
Wheat bran	11.2	10.0	11.8	10.0
Rice flour	8.6	10.8	11.7	7.6
Sunflower meal	8.0	8.0	5.0	8.0
Soybean meal	8.4	8.0	12.2	11.2
Molasses	4.4	4.4	4.4	4.4
Calcium carbonate	3.7	3.7	3.7	2.3
Sodium chloride	0.86	0.89	0.91	0.81
Sodium bicarbonate	0.30	0.30	0.30	0.30
Premix**	0.20	0.20	0.20	0.20
Tricalcium phosphate	0.17	0.10	-	0.58
*Chemical composition (%)****				
DM	89.7	89.5	91.5	89.4
CP	18.0	18.7	18.1	18.6
EE	4.3	4.3	4.3	4.6
Ash	8.6	8.8	9.5	8.4
Ca	1.8	1.9	1.8	1.9
Pt	0.7	0.7	0.7	0.7
NDF	17.0	16.7	19.0	18.5
ADF	8.4	9.0	8.4	12.6
NFC	52.2	51.6	49.1	49.9
Lignin	2.5	2.8	3.0	3.6
Pectin****	1.4	<1.0	<1.0	8.9
GE, Mcal/kg	4.4	4.4	4.3	4.4
NE_L, Mcal/kg	1.6	1.6	1.6	1.6
TDN	71.4	70.6	71.0	71.8

Zea mays (ZM), *Sorghum vulgare* (SV), *Manihot esculenta* (MES), and Citrus sp. (C).
* Sorghum with 954.5 mg catechins/100g sample.
** Composition per kg of premix: 2,500,000 IU of vitamin A; 750,000 IU of Vitamin D_3; 2,000 IU of Vitamin E; 12.5 g of Fe; 2.5 g of Cu; 10 g of Mn; 10 g of Zn; 0.15 g of I; 0.025 g of Co; 30 g of antioxidant (BHT).
***Expressed in 100% dry matter (DM): CP, crude protein; EE, ether extract; Ca, calcium; P_t, total phosphorus; NDF, neutral detergent fiber; ADF, acid detergent fiber; NFC, non-fibrous carbohydrates; GE, gross energy; NE_L, net energy for lactation; TDN, total digestible nutrients.

Table 2. Starch composition of ingredients and concentrates.

	Nutrient		
	Starch*	Amylose**	Amylopectin**
In the ingredient			
Zea mays	60.9	25.7	74.3
Sorghum vulgare	59.1	25.0	75.0
Manihot esculenta	69.0	20.7	79.3
Citrus sp.	2.4	9.3	90.7
In the concentrate			
ZM	30.4	21.7	78.3
SV	31.8	17.3	82.7
MES	25.6	19.9	80.1
C	21.2	18.0	82.0

* Expressed as a percentage of DM.
** Expressed as a percentage of starch.
Zea mays (ZM), *Sorghum vulgare* (SV), *Manihot esculenta* (MES) and *Citrus* sp. (C).

Ruminal pH, rectal temperature and locomotion score

Ruminal pH was measured in the last 3 d of each experimental period using intraruminal boluses (eCow Ltd, Exeter, Devon, UK) (Mottram *et al.*, 2008). The pH was measured every 60 seconds, taking average values every 15 minutes. The average, minimum, and maximum ruminal pH values were determined for 24-h periods. Rectal temperature was measured on the last 3 d of each period at 09:00 and 15:00 h, and data were averaged per day. Locomotion score (LS) was assessed using a 1 to 5 scale (Sprecher *et al.*, 1997) on the last day of each period. The animals were weighed on day 11 of each experimental period, and body condition score (BCS) was evaluated simultaneously.

Hematology, blood chemistry, and milk urea nitrogen

Blood samples were collected from the coccygeal vein on the 15th d of each period (at 09:00 h). Parameters evaluated were: red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW), white blood cells (WBC), and WBC differential (neutrophils, lymphocytes, monocytes, eosinophils, neutrophil: lymphocytes ratio) (Cell-Dyn system 3500 automated hematology analyzer; Abbott Laboratories, Abbott Park, IL, USA). Blood chemistry analysis included urea, creatinine, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), Ca, P, Mg, total plasma proteins (TPP), albumin (ALB) and globulins (GLOB) (Vitros 250 Chemistry Analyzer, Ortho-Clinical Diagnostics, Markham, ON, Canada). Likewise, milk urea nitrogen (MUN) was determined with a MilkoScan FT+ (Fourier transform infrared; Foss, Hillerød, Denmark) using the same samples analyzed for quality.

Statistical analysis

Response variables were analyzed under a 4x4 replicated Latin square design using the MIXED procedure in SAS/STAT® 14.1 software (SAS, 2015). A subsampling design was used to analyze rectal temperature and pH (Zamudio-Sanchez and Alvarado-Segura, 1996). Significant differences were declared at $p<0.05$, and a tendency was declared at $0.05 \leq p \leq 0.10$. The BCS and LS were evaluated with Friedman's two-dimensional ANOVA by ranges for related samples (5% statistical significance).

Results

Table 3 presents BW, BCS, DMI and forage: concentrate ratio (F:C ratio). No statistical difference was observed between treatments ($p>0.05$). MY, 3.5% FCM, and lactose tended to differ ($p<0.10$) with highest and lowest values for MES and C, respectively, which differed ($p<0.05$) in ECM, protein, and total solids in favor of MES. Protein was also different ($p<0.05$) between MES and SV (Table 4).

Table 5 presents the effect of treatment on ruminal pH, rectal temperature, and LS. No differences were found between treatments ($p>0.05$).

The pH dynamics is shown in Figure 1.

Table 3. Body weight (BW), body condition score (BCS), dry matter intake (DMI), and forage: concentrate ratio (F:C ratio) of lactating cows supplemented with different carbohydrate sources.

Item	ZM	SV	MES	C	SEM	p-value
BW (kg)	537.87	534.50	535.62	526.25	5.96	0.24
BCS	3.03	3.03	3.03	2.94	0.04	0.59
DMI_T						
kg/d	19.36	20.77	20.47	19.20	0.57	0.77
$g/kg^{0.75}/d$	174.28	187.28	184.34	181.27	5.33	0.79
%BW	3.63	3.90	3.84	3.79	0.11	0.79
DMI_P						
kg/d	11.58	12.18	12.35	11.62	0.54	0.93
$g/kg^{0.75}/d$	104.28	110.12	111.36	105.53	5.05	0.94
%BW	2.17	2.29	2.32	2.20	0.11	0.94
DMI_C						
kg/d	7.79	8.58	8.13	8.30	0.22	0.16
$g/kg^{0.75}/d$	70.00	77.16	72.98	75.74	2.00	0.17
%BW	1.46	1.61	1.52	1.58	0.04	0.17
F:C ratio	59.2:40.8	57.9:42.1	60.14:39.86	57.19:42.81	1.27	0.78
Intake, kg/day						
NDF	7.45	7.88	8.08	7.68	0.05	0.37
NFC	5.12	5.54	5.11	5.20	0.04	0.53

Treatments: *Zea mays* (ZM), *Sorghum vulgare* (SV), *Manihot esculenta* (MES), and *Citrus* sp. (C).
BW, body weight; BCS, body condition score; DMI_P, DMI_C and DMI_T, pasture, concentrate and total dry matter intake; F:C ratio, forage:concentrate; NDF, neutral detergent fiber; NFC, non-fiber carbohydrates.
SEM, Standard error of the mean.

Hematological parameters (Table 6) showed no differences between treatments (p>0.05).

Metabolic profile (Table 7) showed no differences between treatments (p>0.05).

Discussion

The chemical composition of kikuyu fits the values described by Correa *et al.* (2008). Despite different starch levels (Table 2), our concentrates were not different in NFC because citrus pulp is rich in sugar and pectin (Bampidis and Robinson, 2006).

No decrease in ruminal pH was found (Table 5), which explains the similar DMI_T, DMI_P, and DMI_C observed between treatments (Table 3). The absence of ruminal acidosis could be due to the intake of NDF and NFC, and the F:C ratio, close to the 60:40 recommended in the literature (Mertens, 2009). Total NDF intake represented 38.5, 37.9, 39.4, and 38.6% of DMI_T for treatments ZM, SV, MES and C, respectively, while NDF consumption from forage was 31.6, 31.0, 31.9, and 30.9% of DMI_T, correspondingly. Therefore, NDF from forage fluctuated between 80.0 and 82.2% of total NDF intake. According to Mertens (1997), ruminant diets must contain at least 25% NDF, 76% from forage. Krause and Oetzel (2006) suggest restricting NFC to 35-40% of DMI_T to prevent SARA. In our study, NFC intake represented 26.4, 26.7, 25.0, and 26.1% of the DMI_T for ZM, SV, MES and C, respectively. In conclusion, the lack of difference in DMI between treatments reflects its similarity in nutritional characteristics without adversely affecting ruminal fermentation conditions.

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Table 4. Effect of carbohydrate source on yield and quality of milk, and feed efficiency.

Item	ZM	SV	MES	C	SEM	p-value
MY, kg/d	32.15	31.33	33.55	30.63	1.11	0.07
3.5% FCM, kg/d	32.12	32.18	35.19	31.33	1.10	0.06
ECM, kg/d	31.76 [ab]	31.42 [ab]	34.52 [a]	30.56 [b]	1.20	0.03
Fat, %	3.50	3.67	3.83	3.72	0.14	0.52
Protein, %	2.89	2.83	2.94	2.81	0.05	0.26
Fat: protein ratio	1.20	1.30	1.31	1.33	0.05	0.55
Lactose, %	4.77	4.76	4.73	4.71	0.03	0.19
Total solids, %	11.98	12.08	12.37	12.07	0.05	0.29
Fat, kg/d	1.14	1.15	1.27	1.12	0.05	0.28
Protein, kg/d	0.93 [ab]	0.87 [b]	0.97 [a]	0.85 [b]	0.03	0.01
Lactose, kg/d	1.53	1.48	1.60	1.45	0.05	0.06
Total solids, kg/d	3.85 [ab]	3.77 [ab]	4.13[a]	3.67 [b]	0.13	0.02
SCC (10^3 cells/mL)	150	231	269	309	52.8	0.63
FE						
MY, kg/kg DMI	1.66	1.51	1.64	1.54	0.06	0.64
3.5% FCM, kg/kg DMI	1.66	1.55	1.72	1.63	0.07	0.82
ECM, kg/kg DM	1.64	1.51	1.68	1.59	0.07	0.74

Treatments: *Zea mays* (ZM), *Sorghum vulgare* (SV), *Manihot esculenta* (MES), and *Citrus* sp. (C).
MY, milk yield; 3.5% FCM, 3.5% fat-corrected milk; ECM, energy-corrected milk; SCC, somatic cell count; FE, feed efficiency; DMI, dry matter intake.
SEM, Standard error of the mean.
Treatment means with different letters (a, b) within the same row differ significantly ($p<0.05$).

Table 5. Rumen pH, rectal temperature and locomotion score (LS) of lactating cows supplemented with different carbohydrate sources.

Ítem	ZM	SV	MES	C	SEM[2]	p-value
Ruminal pH						
Mean	6.50	6.38	6.51	6.37	0.03	0.44
Minimum	6.16	6.12	6.20	6.00	0.03	0.52
Maximum	6.82	6.68	6.80	6.71	0.03	0.41
Rectal temperature	38.5	38.7	38.6	38.6	0.07	0.29
LS	1.38	1.13	1.13	1.13	0.07	0.39

Treatments: *Zea mays* (ZM), *Sorghum vulgare* (SV), *Manihot esculenta* (MES), and *Citrus* sp. (C).
SEM, Standard error of the mean.

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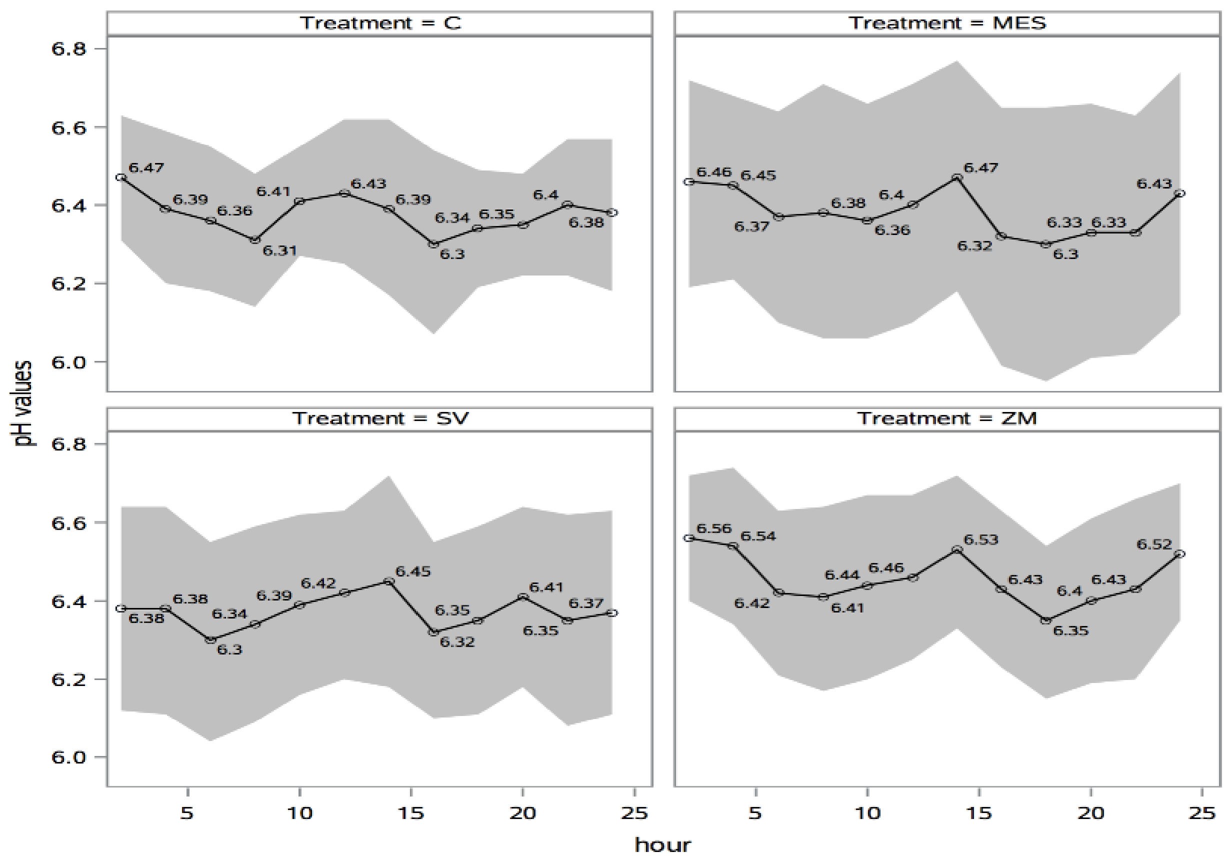

Figure 1. pH dynamics of lactating cows supplemented with different carbohydrate sources (Treatments: *Zea mays* (ZM), *Sorghum vulgare* (SV), *Manihot esculenta* (ME), and *Citrus* sp. (C)). The solid line represents the average pH obtained at 2-hour intervals. The gray shadow represents the standard deviation of the observations.

Performance differences between MES and C (Table 4) can be caused by differences in the ruminal degradation rate of carbohydrates and fermentation profile. Santos-Silva *et al.* (2016) suggested that pectin can interfere with ruminal fermentation because of its slightly lower rate and extent of degradation when compared to starch. According to Gómez *et al.* (2016), higher digestibility of cassava starch vs. starch from corn and sorghum is due to the lack of pericarp and protein matrix, less protein-starch association, lower proportion of corneous endosperm, lipids and amylose. Pectin increases the acetate molar proportion and reduces propionate molar proportion (Bampidis and Robinson, 2006). In contrast, starch fermentation tends to increase the propionate molar proportion (Ørskov, 1986), which is associated with an increase in glucose supply and MY (Khalili and Sairenen, 2000).

The greater milk protein and solids in MES vs. C resulted from greater MY and, to a lesser extent, a slight increase in their concentration (Table 4). Theurer *et al.* (1999) found increased MY and milk protein from greater ruminal digestion of starch. Lower milk protein in SV vs. MES (Table 4) can be explained by its lower starch fermentation (Offner *et al.*, 2003) and high tannin content. Adetunji *et al.* (2013) reported catechins concentrations ranging between 0.09-0.33% in tannin-free sorghum and 0.37-1.34% in tannin sorghum. Sorghum catechins concentration in this study was 0.95%.

According with milk fat results (Table 4) none of the treatments had a negative effect on ruminal pH or fiber digestibility. The fat:protein ratio (F/P; Table 4) was within the optimal range (1.2 to 1.4) reported by Čejna and Chládek (2005), again, confirming SARA absence (G/P<1.2).

Table 6. Hematological parameters of lactating cows supplemented with different carbohydrate sources.

Ítem	ZM	SV	MES	C	SEM	p-value
RBC (10^6 x □L)	6.11	6.08	6.20	6.10	0.14	0.90
Hb (g/dL)	10.74	10.01	10.94	10.75	0.26	0.12
HCT (%)	28.93	28.46	29.23	28.71	0.66	0.75
MCV (fL)	47.37	46.75	47.12	47.11	0.57	0.61
MCH (pg)	17.57	17.31	17.65	17.62	0.19	0.49
MCHC (%)	37.06	36.96	37.52	37.50	0.18	0.55
PLT (x 10^3)	259.37	328.87	335.37	263.50	22.40	0.07
PCT (%)	0.19	0.23	0.24	0.19	0.01	0.22
MPV (fL)	7.13	7.14	7.17	7.30	0.11	0.78
PDW (%)	34.77	33.94	34.15	34.91	0.50	0.61
WBC (10^9 x L)	9.36	9.09	10.31	10.81	0.67	0.26
WBC differential (% WBC)						
neutrophils (Neu)	39.4	28.3	35.6	39.9	4.57	0.28
lymphocytes (Lym)	55.5	66.0	59.7	55.5	4.82	0.39
monocytes	0.12	0.63	0.25	0.13	0.28	0.57
eosinophils	5.03	5.05	4.32	4.51	0.89	0.91
Neu:Lym ratio	0.84	0.51	0.67	0.82	0.16	0.44

Treatments: *Zea mays* (ZM), *Sorghum vulgare* (SV), *Manihot esculenta* (MES), and *Citrus* sp. (C).
RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; MCV; mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; PCT, plateletcrit; MPV, mean platelet volume; PDW, platelet distribution width; WBC, white blood cells.
SEM, Standard error of the mean.

The SCC values were below the limit (400 × 10^3 cells/mL) set in Europe, New Zealand and Australia (Sharma *et al.*, 2011).

Under optimum conditions, FE should fluctuate between 1.4 and 1.8 kg of 3.5% FCM per kg DMI (Hutjens, 2005), which is in line with our results (Table 4).

Once more, our results confirm the absence of SARA. It is also known that acidosis increases the heat produced per unit of digestible energy, resulting in lower net energy available for production (Casper and Mertens, 2007). SARA occurs when ruminal pH lower than 5.5 to 5.8 persists for several hours a day (Danscher *et al.*, 2015). According to this, none of our treatments resulted in SARA (Table 5; Figure 1). Other factors, besides those discussed (F:C ratio, NDF and NFC consumption), can explain the absence of SARA in this work, namely, dietary CP and forage NO_3. Based on BW, MY and DMI_T, dietary CP should have been 15.6% to meet NRC (1988) requirements. However, it was 22.1 ± 0.08%, with 66.2 ± 1.3% from kikuyu. It is known that more than 30% of kikuyu CP is rapidly and completely degraded in the rumen (Correa *et al.*, 2008). High CP degradation releases ammonia, which captures protons (H^+) due to its p*K*a (9.2 dissociation constant) increasing pH (Bates and Pinching, 1949), which explains the buffer effect of CP in the present experiment. In our study, NO_3 in kikuyu was between the range reported by Correa *et al.* (2008). As an electron acceptor, NO_3 is reduced to NH_4^+ (Leng, 2008), buffering the ruminal pH. Acidosis leads to gram-negative bacteria lysis, increasing lipopolysaccharide, which passes from rumen to blood causing fever, hypoperfusion of hoof capillaries, and laminitis (Kleen *et al.*, 2003). No evident alterations in LS or rectal temperature were observed in our study, confirming once more the absence of SARA.

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Table 7. Metabolic profile of lactating cows supplemented with different carbohydrate sources.

Ítem	ZM	SV	MES	C	SEM	p-value
Metabolic function						
Urea (mmol/L)	7.27	6.68	6.84	6.34	0.48	0.59
Creatinine (□mol/L)	79.1	77.4	75.8	79.7	2.0	0.54
Hepatic function						
GGT (U/L)	21.8	24.3	22.2	24.9	1.5	0.38
ALP (U/L)	40.9	42.2	37.9	47.7	2.7	0.10
Endocrine function						
Ca (mmol/L)	2.12	2.11	2.16	2.29	0.09	0.61
P (mmol/L)	1.71	1.85	1.91	1.77	0.11	0.64
Mg (mmol/L)	0.98	0.96	0.86	0.95	0.05	0.43
TPP (g/dL)	6.84	6.60	6.82	6.83	0.09	0.19
ALB (g/L)	3.42	3.17	3.32	3.31	0.08	0.19
GLOB (g/L)	3.41	3.43	3.54	3.52	0.07	0.50
FBG (mg/dL)	275	300	288	262	0.42	0.93
ALB: GLOB ratio	1.02	0.94	0.97	0.95	0.04	0.49
(TPP-FB)/FBG	5.84	5.60	5.81	5.83	0.09	0.19
MUN (mg/dL)	16.6	18.6	18.7	19.1	0.57	0.19

Treatments: *Zea mays* (ZM), *Sorghum vulgare* (SV), *Manihot esculenta* (MES), and *Citrus* sp. (C).
GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; Ca, calcium; P, phosphorus; Mg, magnesium; TPP, total plasma proteins; ALB, albumin; GLOB, globulins; FBG, fibrinogen; MUN, milk urea nitrogen.
SEM, Standard error of the mean.

Hematological parameters and metabolic profile were within reference values (Coroian *et al.*, 2017; Botezatu *et al.*, 2014; Radkowska *et al.*, 2014), with the exception of MUN (Table 7). Optimal MUN in lactating cows fluctuates between 7 and 12 mg/dL (Hulsen *et al.*, 2014), markedly lower than that observed in this work. As previously stated, CP consumption exceeded NRC (1988) requirement, and NFC was below the limit described by Krause and Oetzel (2006).

In conclusion, partial substitution of corn grain by sorghum grain, cassava root, or citrus pulp did not affect rumen pH, DMI, or FE. Cows had a high intake of CP and NDF, while NFC intake was lower than that reported in the literature. These dietary characteristics protected the cows against SARA. The higher availability of energy from cassava increased MY, protein and total solids, contrary to the effect of citrus pulp inclusion.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Sandra Lucia Posada-Ochoa and Ricardo Rosero-Noguera performed substantial contributions to the conception, design of the work, analysis and interpretation of data. Luis Miguel Gómez-Osorio carried out the experiment, performed the interpretation of data and drafting the work. Martha Olivera-Angel contributed with the veterinary approach of the work. All authors discussed the results and contributed to the final manuscript.

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