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REGULAR ARTICLES

Digestion, ruminal metabolism, and feeding behavior of buffaloes fed diets supplemented with soybean oil, whole and raw soybean, and calcium salts of fatty acids

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Abstract

This study aimed to evaluate the effects of the inclusion of unsaturated fatty acid (UFA) sources on the nutrient intake, apparent digestibility, ruminal fermentation, and feeding behavior in diets for buffaloes. Four castrated Murrah buffaloes with approximately 24 months of age and an initial average body weight of 351 ± 15 kg were randomly assigned to a 4 × 4 Latin square experiment, containing the following diets: (1) control (CON): control diet based on soybean meal and ground corn, (2) soybean oil (SO): dietary inclusion of 2.20% (DM basis), (3) whole raw soybean (WRS): dietary inclusion of 16.0%, and (4) calcium salts of FA (CSFA): dietary inclusion of 2.60%. There was an effect of diets in ether extract intake among buffaloes fed UFA and CON diets, and among buffaloes fed CSFA and WRS diets ($P < 0.05$). Diets containing UFA sources provided higher EE digestibility ($P < 0.05$). Buffaloes fed WRS had higher rumen pH values than animals fed the CSFA diet ($P < 0.05$). Supplementation of UFA sources decreased the molar concentrations of short-chain fatty acids ($P < 0.05$). Diets influenced the times spent in chewing, idling, and the rumination efficiencies of DM and NDF ($P < 0.05$). The supplementation with WRS, SO, and CSFA does not negatively affect intake, digestion, ruminal metabolism, and feeding behavior. The WRS as a fat supplement source decreases dietary costs by replacing ground corn and soybean meal simultaneously compared to other fat sources used. Nevertheless, whole and raw soybean in buffaloes' diet can reduce chewing and rumination activity.

Keywords Buffalo · Calcium salts · Rumen fermentation · Rumen pH · Protected fat

Introduction

The inclusion of unsaturated fatty acid (UFA) to ruminant diets has been considered an alternative to increase its energy value (Consôlo 2014; Oliveira et al. 2007a), as long as it does not compromise intake and, consequently, animal performance (Lourenço et al. 2010). The UFA sources in diets for ruminants can negatively affect DM intake (DMI) because the supplemented fatty acids decrease fiber ruminal digestibility, causing physical limitation in the intake. Furthermore, supplementation with fatty acids can affect the acceptability of diets, ruminal and intestinal motility, and the release of intestinal hormones (NRC 2001).

The use of vegetable oils as a source of UFA in ruminant diets can trigger undesirable effects on ruminal microbiota, such as the reduction in DM digestibility and the acetate: propionate ratio (Vargas et al. 2002). On the other hand, the use of inert UFA sources in the rumen has its benefits, such as increased energy density without compromising fiber digestion, increased energy utilization efficiency, and lipogenic effect, increasing the animals' glycogenic precursors (Ferguson et al. 1990).

The inclusion of calcium salts of fatty acids (CSFA) in diets for ruminants increases dietary UFA flow to the intestine to be absorbed. Oilseeds, such as whole raw soybean grain (WRS), represent a natural source of protected UFA with high energy and protein value (Palmquist and Conrad 1991). Its use is

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beneficial because it is a source of slow-released fatty acids inside the rumen. Furthermore, it can inhibit possible reductions in fiber digestibility since UFA sources have a detrimental effect on fibrolytic bacteria.

Studies have been conducted over the years evaluating the use of soybean in the diet of cattle through the inclusion of oil, and in the form of protected fats (soybean grain and calcium salts)(Gandra et al. 2014; Zanferari et al. 2018). Studies have been carried out evaluating the effect of supplementing different fat sources from soybean in cattle's diet. However, there are still few studies in the literature with buffaloes. The population of fibrolytic bacteria is higher in buffaloes (Wanapat and Cherdthong 2009), mainly *Ruminococcus albus*, when compared to cattle. Therefore, it could be speculated that supplemented UFA's ruminal effect would differ in buffaloes than cattle.

Given the context, this study was carried out to test the hypothesis that supplemented dietary fatty acids fed either as oil or whole grain will increase the energy intake of buffalo without reducing DMI, digestibility, and the acetate:propionate ratio. This study aimed to evaluate the effects of the inclusion of unsaturated fatty acid sources on nutrient intake, apparent digestibility, ruminal fermentation, and feeding behavior in diets for buffaloes.

Material and methods

The study was carried out at the Experimental Farm of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia, located in Entre Rios municipality, BA, Brazil.

Four castrated Murrah buffaloes with approximately 24 months of age and 351 ± 15 kg of BW at the beginning of the experiment, ruminally fistulated with 4-inch silicone cannulas (Kehl®) which were assigned in a 4 × 4 Latin square design. The experiment consisted of four periods of 21 days consisting of 15 days for adaptation of animals to diets and management, 6 days for data collection, and 3 days of washout. Buffaloes were fed one of the following experimental diets: control (CON): control diet, soybean oil (SO): inclusion of 2.20% of soybean oil in the diet (DM basis), whole raw soybean (WRS): inclusion of 16.0% of whole raw soybean in the diet, and calcium salts of FA (CSFA): inclusion of 2.60% of calcium salts (Megalac-E, Church & Dwight Co. Inc., Trenton, NJ) in the diet.

Buffaloes were housed in individual stalls, provided with *ad libitum* feed and free access to water. To provide greater comfort and well-being, the animals were released into a paddock in the morning, 1 h before feeding, throughout the experimental period. Besides, they were submitted daily to sprinkler baths for thermal comfort. Buffaloes were fed twice daily in a total mixed ration, at 08:00 and 13:00 h, with four experimental diets formulated for animals with approximately 380 kg of body weight, following the recommendations of Paul and Lal (2010). Transvala hay (*Digitaria decumbens* cv. Transvala) was used as the roughage source, and the roughage:concentrate ratio used was 70:30 (Table 1).

At the beginning of each experimental period, samples of the ingredients and diets were collected, whereas the samples of refusals were collected daily during the data collection period, totaling four samples composed per period. Composite samples were obtained at the end of each period by performing a pool of daily samples for each animal and period. All samples of ingredients, diets, and refusals were frozen at − 20 °C for further analysis.

A half of representative sample of ingredients, refusals, and fecal samples were dried in a forced-air circulation ventilation (55 °C for 72 h), ground through a Willey mill (Wiley mill, Arthur H. Thomas, Philadelphia, USA) to pass through a 1-mm screen, and analyzed for dry matter (DM; Method: 950.05), mineral matter (MM; Method: 942.05), crude protein (CP; method 984.13), and ether extract (EE; Method: 920.39) contents according to the Association of Official Analytical Chemists (AOAC 2000) methods. Organic matter was calculated by subtracting ash content from DM.

Contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991). The acid detergent lignin was determined according to AOAC (2012) method 973.18, in which the ADF residue was treated with 72% sulfuric acid. Non-fibrous carbohydrates (NFC) were calculated as described by Hall (2000) and the total digestible nutrients (TDN) of the ingredients according to NRC (2001).

During the experimental periods, samples of forage and concentrate provided and refusals of each buffalo were weighed daily to calculate feed intake, allowing up to 5 to 10% refusals on the fed basis. Amounts of feed offered and refusals were recorded daily for each animal to calculate feed intake. In addition, samples of diets and refusals of each animal were also collected every 5 days of the experimental period.

Apparent total tract digestibility of nutrients was measured on the 18th day of each experimental period, by the total collection method for 24 h. Feces were collected after spontaneous defecation and stored in a plastic container. At the end of each collection period, the total weight of the homogenized fecal contents was determined. The digestibility coefficients were calculated as follows: DC = [(kg of the portion ingested − kg of the portion excreted)/(kg of the portion ingested)] ×100.

On the 16th day of each experimental period, ruminal digesta samples were collected from five different sites (cranio-dorsal, cranio-ventral, ventral, caudo-ventral, and caudo-dorsal ruminal regions) through the ruminal cannula shortly before and 2, 4, 6, 8, 10, and 12 h after the morning

Table 1 Ingredients and chemical composition of experimental diets

Item	Diets			
	CON	SO	WRS	CSFA
% of DM				
Transvala hay (*Digitaria decumbens* cv. Transvala)	70.0	70.0	70.0	70.0
Ground corn	20.5	18.1	11.4	17.5
Soybean meal	6.05	6.22	----	6.49
Soybean oil	----	2.20	----	----
Whole raw soybean	----	----	16.0	----
CSFA	----	----	----	2.60
Urea	0.85	0.85	----	0.85
Salt	0.61	0.61	0.61	0.61
Mineral[a]	2.00	2.00	2.00	2.00
Chemical composition (% DM)				
Crude protein	10.4	10.3	10.3	10.3
Ether extract	2.26	4.37	4.00	4.39
Neutral detergent fiber	44.3	39.5	44.4	43.8
Non-fibrous carbohydrates	21.4	19.1	18.3	13.7
Total digestible nutrients	56.0	59.0	60.0	59.0

CON: control, SO soybean oil, WRS: whole raw soybean, CSFA: calcium salts of fatty acids

[a] Contained per kilogram: calcium 128 g, phosphorus 44 g, sodium 178 g, sulfur 12 g, magnesium 5 g, cobalt 107 mg, copper 1.25 g, iodine 50 mg, manganese 750 mg, selenium 12 mg, zinc 3.7 g, iron 1.4 g, and fluorine 440 mg

feeding, totaling eight times. Ruminal fluid samples were collected, combining straining subsamples from the different sites inside the rumen. Then, the digesta collected was strained in cheese cloth to extract ruminal fluid. Rumen pH was determined immediately after collecting 200 mL of samples using a pH meter (AZ instrument model: 8651 PH & ORP Meter). Subsequently, 20 mL of ruminal fluid were collected, stored in plastic containers with lid, and immediately frozen at − 20 °C for further analysis.

Analysis of volatile fatty acids were determined using a High-performance Liquid Chromatograph (Shimadzu, Japan) coupled to an ultraviolet detector (SPD-10AVP Columbia, USA) and column (HPX-87H—30 cm × 4.5 mm, Bio-Rad Laboratories Ltd.), according to the method described by Mathew et al. (1997). Ammonia nitrogen concentration (NH_3-N) in the ruminal fluid was analyzed using a spectrophotometer (Spectrophotometer Mono Beam SP-22, Curitiba, Biospectro, Brazil®) (Broderick and Kang 1980). Methane production (CH_4) was calculated according to Moss et al. (2000).

On the 17th day of each experimental period, the buffaloes were observed for 24 h at 5-min intervals to evaluate their ingestive behavior, which comprised the times spent in feeding, rumination, and idling activities. In these periods, the numbers of chews per bolus and the spent to ruminate each cud were also recorded in three different periods of the day (morning, afternoon, and night). During the nocturnal observation of the animals, the environment was maintained with artificial lighting for 2 days so that they could adapt to the luminosity. The eating and rumination efficiencies of DM and NDF were calculated as feed intake (in g) divided by the time taken to consume the feed (in min) and were calculated according to the methods described by Burger et al. (2000).

The data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC 2002) in a 4 × 4 Latin square design, according to the model described below:

$$Y_{\mathrm{ijk}} = \mu + a_i + p_j + D_k + e_{\mathrm{ijk}}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, a_i is the random effect of the animal, p_j is the random effect of the period, D_k is the fixed effect of diet, and e_{ijk} is the residual error.

Ruminal fermentation data (0, 2, 4, 6, 8, 10, or 12 h after feeding), pH, NH_3-N, and VFA were statistically evaluated as repeated measures over time using the MIXED procedure of SAS, according to the following model:

$$Y_{\mathrm{ijk}} = \mu + a_i + p_j + D_k + c_{k(\mathrm{iJ})}\, T_l + (T \times D)_{\mathrm{lk}} + e_{\mathrm{ijkl}}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, a_i is the random effect of animal, p_j is the random effect of period, D_k is the fixed effect of the diet, $c_{k(\mathrm{iJ})}$ is the error associated with diet nested with animal period, T_l is the fixed effect of time, $(T \times D)_{\mathrm{lk}}$ is the fixed effect of the interaction between

diet and time, and e_{ijkl} is the residual error.

To evaluate the effects of treatments, orthogonal contrasts were used to compare the effect of fat supplementation (C1; control versus fat sources), the effect of soybean oil versus protected fat (C2; soybean oil versus calcium salts of fatty acids plus (WRS), and the effect between the protected sources (C3; WRS versus calcium salts of fatty acids).

The Kenward and Roger (1997) option was used to correct the degrees of freedom, and Akaike's information criterion was used to select the best covariance structure. For the analyses over time, such as the concentration of ruminal fermentation parameters, all diets were confronted within each time through the PDIFF option, with the means obtained by the LSMEANS command. Significance was declared at $P < 0.05$.

Results

There was no effect ($P > 0.05$) of the supplemented UFA sources on DM, NDF, CP, TDN, and NFC intakes. On the other hand, buffaloes fed diets containing additional UFA sources showed a higher intake of EE compared to animals fed the control diet (C1; $P < 0.05$). There was an improvement in the intake of EE for buffaloes fed a diet containing CSFA when compared to those fed WRS (C3; $P < 0.05$). No effect of diets ($P > 0.05$) on the total apparent digestibilities of DM, OM, CP, NDF, NFC, and the TDN was observed. However, diets containing UFA sources provided higher values of EE digestibility ($P < 0.05$) compared to the control diet (Table 2).

Buffaloes fed diets containing CSFA showed lower ruminal pH compared to animals fed WRS ($P < 0.05$). There was an effect of time on ruminal pH values ($P < 0.05$)(Fig. 1). Animals fed diets containing UFA sources showed lower concentrations of total VFA and propionate when compared to animals fed the control diet ($P < 0.05$). Higher concentrations of VFA and acetate were observed for buffaloes fed diets containing CSFA compared to the WRS treatment ($P < 0.05$). The animals fed diets containing sources of UFA presented lower concentrations of acetate and butyrate compared to animals fed the control diet ($P < 0.05$). On the other hand, there was no effect ($P > 0.05$) of UFA sources on the concentrations of N-NH_3, acetate, propionate, butyrate, acetate:propionate ratio, and methane (Table 3).

Buffaloes fed diets containing protected UFA spent longer total chewing times ($P < 0.05$) and idling ($P < 0.05$) compared to animals fed soybean oil. The feeding efficiencies of DM and NDF (g/h), and efficiencies related to the variables of the number of chews/bolus, numbers of ruminated bolus/day, and chewing time/bolus (s) were not influenced by experimental diets ($P > 0.05$). There was an effect of fat sources on rumination efficiency (grams of DM and NDF consumed per hour)($P < 0.05$). When analyzing contrast 1, lower rumination efficiencies of DM and NDF were observed in buffaloes fed diets containing fat sources in comparison to animals fed the control diet (Table 4).

Discussion

The inclusion of UFA sources in buffaloes diets increased EE intake and digestibility. Since no effect of diets on DMI was observed consequently, the other organic fractions' intake and digestibility were also not influenced. Therefore, the inclusion of UFA sources in free and complex (protected) forms and their dietary levels might not have any adverse effect on the rumen fibrolytic bacteria population.

The higher EE intake by animals fed diets containing SO, WRS, and CSFA was already an expected response since these diets had higher EE levels (Table 2). Also, the higher intake of EE observed for the CSFA, compared to those fed the WRS, can also be justified by the level of EE in these diets, as well as their chemical composition (Table 1). Therefore, the results of this study corroborate what was described by Palmquist (1991), that an increase in EE intake tends to dilute the effect of endogenous losses of EE in feces, causing greater apparent digestibility.

Similar to this study, Oliveira et al. (2007b) evaluated the inclusion of lipid sources in the form of oil and complexed in the diet of lactating buffaloes on the nutrient intake and digestibility. The authors also observed an effect of lipid sources only on the EE intake and digestibility. The absence of effect of lipid sources in ruminants has been reported when the diets provided are constituted by large proportions of forages, mainly as hay, and was also noticed in the current study, as expected.

Studies evaluating the effect of lipid supplements on nutrient intake and digestibility have shown different results, such as reduced fiber digestibility (Boerman and Lock 2014), increased (Granja-Salcedo et al. 2017), or no influence (Lima et al. 2014). In general, the studies conducted evaluating lipid sources were conducted with cattle in different categories, and as a result, the observations ended up being contradictory. Species, level of inclusion of the fat source, and the type of forage used in diets are other factors that interfere with the results. Different responses are expected with different lipid supplements, as the effects of supplementation are inherent in the specific physical and chemical characteristics of supplemented fatty acids (Allen 2000). According to Onetti et al. (2001), this response may also be associated with the quality of forage NDF, forage type, and roughage:concentrate ratio.

In addition to the factors mentioned above, the diets' acceptability can lead to divergent results between the forms of supply of lipid sources. Fat sources can decrease fiber digestibility due to the formation of films covering the food particle hindering microbial adhesion or, toxic effects on cellulolytic gram-positive bacteria, mainly responsible for fiber digestion

Table 2 Nutrient intake and apparent digestibility of diets supplemented with fatty acids sources fed to buffaloes

Item	Diets				SEM	*P* value[a]		
	CON	SO	WRS	CSFA		C1	C2	C3
Intake (kg/day)								
Dry matter	5.8	4.3	4.9	5.7	0.27	0.171	0.134	0.265
Organic matter	5.6	4.1	4.6	5.3	0.26	0.135	0.159	0.317
Crude protein	0.60	0.40	0.50	0.60	0.03	0.167	0.127	0.389
Ether extract	0.10	0.19	0.19	0.27	0.02	< 0.001	0.237	0.029
Neutral detergent fiber	2.8	2.1	2.4	2.8	0.12	0.186	0.109	0.221
Non-fibrous carbohydrates	1.9	1.3	1.5	1.7	0.09	0.053	0.244	0.523
Total digestible nutrients	2.7	1.5	2.3	2.6	0.28	0.350	0.219	0.678
Intake (% BW)								
Dry matter	1.5	1.3	1.6	1.4	0.08	0.727	0.260	0.394
Neutral detergent fiber	0.7	0.6	0.7	0.8	0.03	0.702	0.200	0.396
Total apparent digestibility (%)								
Dry matter	65.1	63.6	66.1	60.3	1.46	0.634	0.920	0.256
Organic matter	67.1	64.9	67.5	61.1	1.55	0.486	0.882	0.232
Crude protein	64.8	57.7	69.4	53.4	2.85	0.411	0.525	0.058
Ether extract	47.2	60.8	80.2	77.1	4.74	< 0.001	0.142	0.179
Neutral detergent fiber	56.7	54.7	69.7	57.2	3.45	0.667	0.373	0.281
Non-fibrous carbohydrates	60.3	60.9	65.9	49.2	2.42	0.113	0.529	0.737

CON: control, SO: soybean oil, WRS: whole raw soybean, CSFA: calcium salts of fatty acids

[a] Orthogonal contrasts: C1 = CON vs. fat-supplemented diets (SO, WRS, and CSFA), C2 = SO vs. WRS + CSFA, and C3 = WRS vs. CSFA (significance was declared at $P < 0.05$)

(Jenkins 1993). The lack of effect of WRS and CSFA on nutrient digestibility may be associated with slow lipid release in the ruminating environment, not exceeding biohydrogenation capacity, preventing the adverse effects of lipids on ruminal microorganisms (Coppock and Wilks 1991; Palmquist and Conrad 1991). Also, at the levels evaluated, diets containing unprotected lipid sources (soybean oil) and protected (soybean grain and CSFA) did not promote harmful effects on the digestibility of fibrous fractions (NDF), possibly because they did not exceed the recommended maximum inclusion level. As

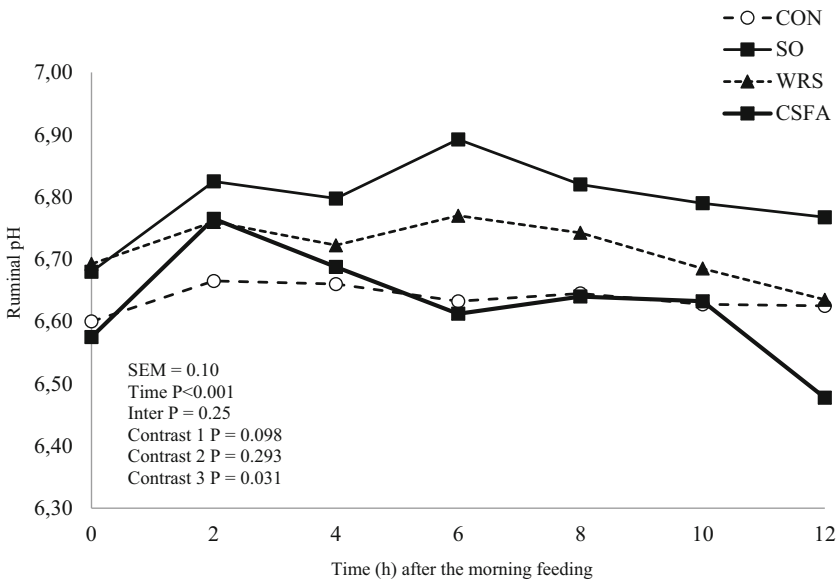

Fig. 1 Ruminal pH values in buffaloes fed diets containing fat sources (CON = control, SO = soybean oil, WRS = whole raw soybean, CFSA = calcium salts of fatty acids)

Table 3 Effects of unsaturated fatty acids on ruminal fermentation of buffaloes

Item	Diets				SEM	*P* value[a]				
	CON	SO	WRS	CSFA		Time	Inter**	C1	C2	C3
pH	6.6	6.7	6.8	6.6	0.10	< 0.001	0.25	0.098	0.293	0.031
NH_3-N (mg/dL)	11.1	13.4	9.9	13.6	0.30	0.27	0.35	0.516	0.430	0.148
Total VFA (m*M*)	45.2	41.1	37.9	42.8	0.60	0.43	0.37	< 0.001	0.581	0.031
mol/100 mol										
Acetate	55.2	55.2	56.3	56.6	0.61	0.88	0.84	0.360	0.161	0.786
Propionate	21.0	21.5	21.2	21.0	0.21	0.75	0.65	0.660	0.392	0.768
Butyrate	23.6	23.2	22.4	22.4	0.35	0.92	0.34	0.206	0.317	0.948
C2:C3	2.6	2.6	2.6	2.7	0.42	0.76	0.84	0.885	0.380	0.691
$CH_{4\%}$[b]	28.5	28.2	28.5	28.7	0.26	0.74	0.74	0.835	0.264	0.590

CON: control, SO: soybean oil, WRS: whole raw soybean, CSFA: calcium salts of fatty acids

[a] Orthogonal contrasts: C1 = CON vs. fat-supplemented diets (SO, WRS, and CSFA), C2 = SO vs. WS + CSFA, and C3 = WRS vs. CSFA (significance was declared at $P < 0.05$)

[b] CH_4 = (acetic acid × 0.45) − (propionic acid × 0.275) + (butyric acid × 0.40)(Moss et al. 2000)

Bateman and Jenkins (1998) described, the supply of diets containing adequate levels of NDF plus up to 7% of soybean oil has a slight effect on ruminal fermentation.

Given the results obtained of nutrient intake and digestibility, although an effect on intake was observed and, consequently, on EE digestibility, it is essential to highlight that the level of inclusion of fat sources in the diet was not sufficient to promote a negative effect on the digestibility of the other fractions and total digestible nutrients. Thus, there was no impairment and/or deleterious effect on microbial activity due to the use of fatty acid sources provided as free oil, as WRS, or in calcium salt form.

Ruminal pH values in buffaloes were influenced by diets containing different sources of fatty acids. Animals fed diets

Table 4 Effects of unsaturated fatty acids on ingestive behavior of buffaloes

Item	Diets				SEM	*P* value[a]		
	CON	SO	WRS	CSFA		C1	C2	C3
Time spent (min/day)								
Feeding	164.5	140.0	218.2	260.0	22.03	0.334	0.055	0.602
Rumination	404.9	402.5	458.7	517.5	60.92	0.376	0.204	0.642
Idling	870.6	897.5	763.1	662.5	47.35	0.193	0.040	0.994
Chewing								
Number/day	19,420	21,293	25,240	25,832	1,481.5	0.183	0.258	0.893
Total chewing time (h/day)	9.5	9.0	13.0	12.9	0.78	0.194	0.040	0.980
Feeding efficiency								
Grams DM/hour	1,849.9	2,031.4	1,553.6	1,376.7	333.79	0.650	0.203	0.708
Grams NDF/hour	1,981.2	1,015.6	625.7	677.9	303.66	0.103	0.628	0.954
Rumination efficiency								
Grams DM/hour	968.5	648.7	526.6	667.9	71.01	0.032	0.746	0.468
Grams NDF/hour	468.7	325.2	258.1	331.8	33.62	0.036	0.690	0.429
Number chewing/bolus	42.12	34.2	53.7	45.8	3.99	0.794	0.141	0.523
Number of ruminated bolus/day	501.3	627.5	513.0	613.3	49.7	0.505	0.630	0.538
Chewing time/bolus	53.3	38.8	69.4	55.4	4.97	0.908	0.061	0.332

CON: control, SO: soybean oil, WRS: whole raw soybean, CSFA: calcium salts of fatty acids

[a] Orthogonal contrasts: C1 = CON vs. fat-supplemented diets (SO, WRS, and CSFA), C2 = SO vs. WS + CSFA, and C3 = WS vs. CSFA (significance was declared at $P < 0.05$)

containing WRS presented higher ruminal pH than buffaloes fed diets containing calcium salts of fatty acids (Megalac-E). This result can be attributed to the higher inclusion of corn meal and soybean meal in the diet containing Megalac-E (Table 3). These ingredients present faster fermentation compared to carbohydrates contained inside the soybean, serving as a substrate for the ruminal fermentation process and, consequently, an effect on hydrogen availability (Macedo et al. 2016). Similar behavior was verified in a study developed by Freitas Júnior et al. (2018) when evaluating the supply of fat sources in diets for lactating cows. As verified by the author, this behavior on ruminal pH was already predicted due to increased food intake. Thus, there is also an increase in the substrate for ruminal fermentation. The observed ruminal pH values corroborate with the data from Franzolin (2010). According to the authors, higher buffering capacity in buffaloes than cattle results in a lower diet effect on ruminal pH. The adaptation of ruminal microorganisms to different levels of energy and protein in the diet can justify this effect. Ruminal pH of buffaloes usually reaches its lowest level at 2 to 6 h after feeding, depending on the source of nutrients and the diet intake rate (Alves et al. 2009).

The diet supply without the inclusion of fat promoted higher values of total VFA, acetate, propionate, and butyrate, possibly because the control diet had higher carbohydrate levels (Table 3). Like the results of ruminal pH, the significant effect of the VFA production can also be justified by the availability of substrate in the rumen environment and the presence of non-fibrous carbohydrates available. Also, there was an influence of the type of complexation of fat sources used in the concentration of total VFA and butyrate. Thus, lower values were verified for buffaloes fed diets containing WRS. This result can be justified by the changes that occurred in the chemical composition of the diets provided.

In the current study, there was no effect of diets on the intake of DM and NDF. Therefore, the lowest production of VFA may have occurred due to more carbohydrate available in the ruminal environment of the diet containing CSFA compared to a diet containing WRS. With this lower availability, there is less fermentation resulting in lower VFA production. The diet containing CSFA had higher inclusion of corn and soybean, thus with a greater amount of carbohydrates. The inclusion of fat sources beyond the effects described above can also decrease ruminal fermentation and fiber digestibility, causing greater ruminal filling and a reduction in the ruminal passage rate (Palmquist and Jenkins 1980).

There was no effect of fat sources on most of the characteristics of buffaloes' ingestive behavior. The lack of effects on buffaloes' intake can be related to the absence of differences in the DM and NDF intakes. The supplemented fat did not substantially decrease the gram-positive bacteria community. Therefore, no decrease in DMI and the NDF digestibilities were observed. Another aspect to consider is that apparently, the added fat sources were palatable or at least did not negatively alter the appetite of animals (Davis 1993; NRC 2001).

Animals fed diets containing WRS and CSFA spent more time chewing, demonstrating that these sources possibly decrease rate passage. Freitas Júnior et al. (2019) evaluated the ruminal kinetics with the inclusion of the same fat sources used in the current study. The authors observed a reduction in the passage rate of diets containing fat sources compared to the control diet. As highlighted by the authors, the observed effects can be attributed primarily to whole raw soybean and soybean oil.

Buffaloes were fed diets containing soybean grain, which is considered a naturally complex fat source. Consequently, animals spent more time in activities related to rumination than those with fat source treatment used causes greater difficulty in the fractionation of particles (Gandra et al. 2016; Haraki et al. 2018). Previously studies were carried out with cattle using levels of inclusion of the same sources of fatty acids evaluated in the current study (Freitas Júnior et al. 2010; Barletta et al. 2016; Bettero et al. 2017; Freitas Júnior et al. 2018). Thus, similar results on the food intake with the use of whole soybean grain were attributed to this passage rate or acceptability. Therefore, the form of protection of unsaturated fatty acids, although it helps in the maintenance of the rumen environment because of the lower release and effect of these on the microbiota, can interfere in the ingestive behavior and time spent on activities mainly related to rumination, and, to a lesser extent, in chewing and idling by animals.

Conclusion

The supplementation with fat sources using whole raw soybean, soybean oil, and calcium salts of fatty acids does not negatively affect intake, digestion, ruminal metabolism, and feeding behavior in buffaloes. The whole raw soybean as a fat supplement source decreases dietary costs by replacing ground corn and soybean meal simultaneously compared to other fat sources used. Nevertheless, whole raw soybean in buffaloes' diet can reduce chewing and rumination activity. For this reason, the use of whole raw soybean in buffaloes' diet should be included with higher monitoring, especially about the feeding behavior.

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Declarations

Statement of animal right All procedures involving the animals were approved by the Ethics Committee on Animal Use from the School of Veterinary Medicine and Animal Science of Federal University of Bahia (UFBA), Salvador, Brazil (permit number: 35/2016).

Conflicts of interest We declare that there is no conflict of interest in this project.

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