Effect of replacing soybean meal by corn ground + urea-ammonium sulphate blend on milk production and composition, digestibility and N balance of dairy Murrah buffaloes

Tobias Tobit Melo, Leilson Bezerra, Viviany Santos, Marcelo Ferreira, Valdi Lima Junior, Luana Andressa Silva, Migson Menezes, Francisco Silva Filho and Ronaldo Oliveira

SUPPLEMENTARY FILE

Supplementary Materials & methods

All animal procedures were conducted in accordance with the regulations of the animal ethics committee of the Federal University of Rio Grande do Norte, Natal, Brazil (5°37'5" South latitude and 33°35'44" West longitude) with Protocol Nº 078/2015 (Brazil, 2008).

Animals, diets and general procedures

Twelve Murrah, pluriparous, lactating buffaloes with an average of 100 ± 4 days of lactation and weight of 650 ± 45 kg, were treated to control internal and external parasites.

The experimental phase lasted 72-d and was divided into four periods of 18-d each.

The animals were adapted to the diets during the first 13-d of each period, and data were collected during the last 5-d. The animals were housed in a covered shed in 6 × 3-m² individual pens and were provided with access to water and feed *ad libitum*.

The experimental diets were formulated to meet the nutritional requirements of lactating buffaloes to produce milk at 10 kg/d with 7.0% fat according to the recommendations of Paul and Lal (2010).

The diets were composed by ingredients (Table S1) soybean meal, ground corn, urea + ammonium sulfate blend and mineral mixture as concentrates and sugarcane + cactus pear as roughage concentrated in a ratio concentrate: roughage of 60:40%. The animals were randomly distributed in a triple Latin square (4 × 4) comprising four supplementations concentrate with corn ground (CG) added urea + ammonium sulfate (U-S) blend replacing soybean meal at levels 0 inclusion or control, 8.0, 16.4 and 24.1 g/kg total DM (Table S2). The cactus material was chopped by a modified machine into pieces approximately 5.0 to 10 cm in size. Sugarcane (*Cynodon* sp.) was cut in a stationary forage machine with a 5.0 cm sieve.

The total mixed ration was fed twice daily at 6:00 and 15:30 h; afterwards, at the time the buffaloes were milked, adjusting the offer to allow 10% refusals.

Intake, digestibility and nitrogen (N) balance

Dry matter intake (DMI) and nutrients intake were obtained through the records of the feed offered and refusals and the collection of diet and refusals samples performed during the last five days of each experimental period.

Feed refusals were weighed in the morning, and 10% was sampled, packed in plastic bags with appropriate identification of the animals, treatments and collect period and then frozen at –20ºC. Then, daily fecal samples were mixed into a single sample, weighed, identified and stored at −15°C. After removal from storage, the fecal samples were immediately oven dried at 55°C for 72 h, ground in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, PA), and passed through a 3-mm screen.

Feces were collected directly in the final portion of the rectum from the 06:00, 08:00, 10:00, and 12:00 h between 1-d and 5-d of the trial period according Casali *et al.,* (2008). Indigestible neutral detergent fiber (iNDF) was used as an indicator to estimate fecal excretion (Van Soest *et al.*, 1991; Casali *et al.,* 2008; Valente *et al.*, 2011).

Thus, diet, fecal and refusal samples (100 mg DM/cm^2) were placed in polypropylene bags nonwoven (Valente *et al.,* 2011) and incubated for 288 h in the rumen of two adult buffaloes (± 650 kg), previously adapted to the diet for seven days. The residues remaining after the incubation were removed, washed until the water became transparent, and dried under forced ventilation at 55°C for 72 h. The iNDF content was determined according to Van Soest *et al.,* (1991). The fecal output of dry matter (FODI) was calculated using the following equation: FODI (kg) = (indicator intake (kg)/ % of fecal indicator) × 100.

To calculate digestibility coefficients (DC) dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), total carbohydrates (TC), non-fiber carbohydrates (NFC) and crude energy (CE). The TDN intake followed the suggestions of Sniffen *et al.,* (1992) using following equation: DC = [(kg of the portion ingested – kg of the portion excreted)/(kg of the portion ingested)] × 100.

The nitrogen (N) balance (N_B) was obtained by the difference between the N-intake and that present in the urine (Reed *et al.,* 2015), feces and milk.

Sniffen *et al.,* (1992). using the equation TDN = [(CP_I – CP_f) + 2.25 (EE_I – EE_f) + (TC_I – TC_f)], where "I" is respectively nutrient intake and "f" is respectively fecal excretion. Concentrations of dietetic TDN were calculated with the equation TDN = (TDN intake/DM intake) × 100.

Chemical analysis

The samples were stored in plastic jars with lids, labeled, and subjected to analyses to determine dry matter (DM; method 967.03), ash (method 942.05), crude protein (CP; method 981.10), and ether extract (EE; method 920.29) contents (AOAC, 2012).

Chemical composition was determined from the samples of diets, refusals, and feces were pre-dried in a forced-air ventilation oven at 55°C for 72 h (Table 1 and 2). Then, samples of the diets and refusals were ground in a Wiley knife mill with a sieve size of 1 mm.

The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methodology of Van Soest *et al.,* (1991) using thermostable α-amylase, without sodium sulfite, in F57 filter bags (Ankom^{200}®- Technology Corporation, 140 Turk Hill Park - Fairport, New York 14450, USA). The autoclave temperature was maintained at 110°C for 40 min. The NDF residue was incinerated in an oven at 600°C for 4 h, then ash and protein correction were determined (Licitra *et al.,* 1996).

The content of non-fiber carbohydrates (NFC) (Hall, 2000) = 100 – [(CP – CP from urea + urea) + NDF + EE + Ash, and total carbohydrate (TC) was calculated according to Sniffen *et al.*, (1992), where: TC = 100 - (CP + EE +ash).

Blood collection

Blood samples were collected from all animals from the jugular venipuncture in the morning before feeding on experimental day 18 of each experimental period following the protocols for blood collection of Nexus Academic Publishers (2013) and Uhart (2016). For biochemical determinations, blood collection (5 mL) procedure was performed by jugular venipuncture from each animal in tubes using a vacuum system (Becton, Dickson and Co., São Paulo, SP, Brazil) without anticoagulant, left at temperature environment for clot retraction and centrifuged at 2.500 × *g* for 10 minutes. The obtained sera were frozen at -20°C until performing the analyses. No hemolyzed or icteric samples were processed. Finally, the serum samples (2 mL) were frozen stored at –20 °C in Eppendorf Tubes® (Sigma–Aldrich, São Paulo, Brazil) for 1–3 days prior to analysis using a semiautomatic biochemical analyzer (BioPlus 2000®, São Paulo, Brazil). The serum metabolites were measured using commercial kit tests to measure total protein, albumin, blood urea nitrogen (BUN), and cholesterol from chemical methods, including sensitivity and specificity for each parameter (Nexus Academic Publishers, 2013).

Production and chemical composition of buffaloes milk

Animals were manually milked twice a day (at 6:00 and 15:30 h) after feeding. Buffaloes' teats were washed with water and dried with paper towels. The first jets of milk were eliminated, and the teats were disinfected with a glycerin solution (Cerón-Muñoz *et al.*, 2002).

Milk production correction at 6% fat ($MPC^{6\%Fat}$) was performed according to Rice *et al.*, (1970). $MPC^{6\%Fat}$ (kg/d) = 0.308 × total milk yield (kg) + 11.54 × total fat yield (kg). The feeding efficiency (FE) was determined using the formula FE (g/k) = DMI/ $MPC^{6\%\ Fat}$.

Milk production (MP) was obtained by weighing (Filizola® Balance Platina 5 kg, São Paulo, Brazil) the milk production between days 13 and 18, with daily production corresponding to the sum of two milkings for each animal. No drugs were administered to induce milking.

Milk samples (morning and afternoon) per animal were mixed to form a single sample, which was placed in plastic containers with Bronopol® preservative and stored in a freezer at −20°C until analysis of the chemical composition (100 mL sample).

Fat, protein, lactose, casein, urea, total solids, and nonfat dry extract were assessed using the infrared spectroscopy method (Bentley 1995; Bentley Instruments Incorporated®, USA) and expressed in g/d.

Statistical Analysis

Data were analyzed using a triplicated 4 × 4 Latin square design, with four periods, four levels of ground corn and urea + ammonium sulfate blend [0 (control); 8.0, 16.4 and 24.1 g/kg in total DM] and 12 animals. Urea + ammonium sulfate blend level (1 to 4) and square (1 to 3) were included in the statistical model as fixed effects, and period (1 to 4) and buffalo nested within square were the random effects. Polynomial contrasts evaluated the linear, quadratic and cubic effects of substitution of soybean meal by ground corn added U + S blend. Results are presented as least square means ± SEM. All data were analyzed using the MIXED procedure of SAS® (v.9.0; 2003; SAS Inst., Inc., Cary, NC). Differences were considered to be significant when $P \leq 0.05$ and tendencies were discussed when $P < 0.10$.

The mathematical model used was:

$$Yijkl = \mu + Ai + Pj + Sk + Tl + (A \times S)ik + (T \times S)lk + eijkl$$

where: $Yijkl$ is the observed variable, μ is the mean, Ai is the effect of the animal; Pj is the effect of the period; Sk is the effect of the square; Tl is the effect of the corn ground + urea-ammonium sulphate blend at levels 0 (control); 8.0, 16.4 and 24.1 g/kg in total DM; $(A \times S)ik$

is the interaction between animal and square; (T × S)*lk* is the interaction between treatment; and square and *eijkl* is the experimental error.

Supplementary Table S1

Chemical composition of ingredients used in experimental diets

Composition (g/kg DM)	Ground corn	Soybean meal	Cactus pear	Sugarcane
Dry matter (g/kg, as fed)	892	897	75.3	37.8
Crude protein	82.0	455	32.1	32.0
Ether extract	72.1	34.0	23.1	21.0
Neutral detergent $Fibre_{ap}$[a]	73.2	72.1	354	455
Acid detergent fibre	42.1	56.3	171	233
Total carbohydrates	821	431	792	888
Non-fibrous carbohydrates	748	358	438	433
Total digestible nutrients	857	810	701	632

[a]NDF_{ap} = corrected for ash and protein content.

Supplementary Table S2.

Ingredients and composition of dairy buffalo diets containing a blend of ground (GC) together with urea-ammonium sulphate (U-S) replacing soybean meal

Itens	CG + U-S blend[a] (g/kg DM total)			
	0.0	8.0	16.4	24.1
Ingredients dietary				
Sugarcane	300	300	300	300
Cactus pear	300	300	300	300
Soybean meal	170	114	56.0	0.00
Ground corn	199	247	296.6	344.9
Urea (U)	0.00	7.20	14.8	21.7
Ammonium sulphate (S)	0.00	0.80	1.60	2.40
Mineral mixture[a]	31.0	31.0	31.0	31.0
Chemical composition (g/kg DM)				
Dry matter (g/kg, as fed)	496	497	498	500
Crude protein	120	120	120	120
Ether extract	33.3	35.2	37.1	38.2
Neutral detergent Fibre$_{ap}$[b]	269	269	268	268
Acid detergent fibre	139	138	137	136
Total carbohydrates	741	757	773	788
Non-fibrous carbohydrates	470	488	504	520
Total digestible nutrients	708	705	700	699

[a]Guaranteed levels (per kilogram, in active elements): calcium (max.), 220 g, and calcium (min.), 209 g; phosphorus (min.), 163 g; copper (min), 45 mg; iron (min), 150 mg; iodine (min.), 2.7 mg; manganese (min.), 120 mg; selenium (min.), 1.8 mg; cobalt (min.), 3 mg; zinc (min.), 120 mg; fluorine (max.), 100 mg; lasalocid (min.), 200 mg; virginiamycin (min.), 180 mg; vitamin A (min.), 2400 IU; vitamin D3 (min.), 6000 IU; vitamin E (min.), 140 IU. Solubility of phosphorus citric acid: 2 to 95%.

[b]NDF$_{ap}$ = corrected for ash and protein content.