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Effects of feeding tropical forage legumes on nutrients digestibility, nitrogen partitioning and performance of crossbred milking cows

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ABSTRACT

Eight crossbred (Brown Swiss × Creole) lactating cows were used in a replicated 4 × 4 Latin square design to test the effects of four diets on milk production, apparent total tract digestibility (aTTd), nitrogen balance and profitability. The diets differed in the main forage and nitrogen sources: 1) sorghum silage as main forage and soybean meal as main nitrogen source (control); 2) mixed sorghum + jackbean silage with reduced proportion of soybean meal in the diet (jackbean-silage diet); 3) sorghum silage and cowpea hay with reduced proportion of soybean meal in the diet (cowpea-hay diet); 4) sorghum silage as main forage and urea substituting a proportion of soybean meal in the diet (urea diet). The experiment lasted 84 days, with four periods including 14 days of adaptation and 7 days of data collection. Individual milk yield and dry matter intake were recorded. Total feces and urine excretions were estimated from spot samples, and samples of milk, feed, urine, and feces were collected and analyzed. Diets were designed to fulfil the requirements of cows producing 11 kg milk/d, but average production was 9.6 kg/d, therefore cows in this experiment were likely overfed on energy and protein. Dry matter intake was higher for the cowpea-hay diet compared with all other diets, but milk yield and composition was similar across all treatments. There were no differences in aTTd of dry matter and neutral detergent fiber between the diets. Crude protein aTTd was lowest for the jackbean-silage diet and highest for the urea diet; whereas aTTd of organic matter was lowest for the jackbean-silage diet and highest for the cowpea-hay diet. Milk urea-nitrogen concentration was highest with the control diet and lowest with the jackbean-silage diet. Similarly, nitrogen use efficiency (g milk nitrogen/100 g nitrogen intake) was highest when feeding jackbean silage. No effects of diet were observed for nitrogen excretion in urine, but nitrogen excretion in feces was highest when feeding both legumes. Both diets containing forage legumes had lower feed costs and resulted in a tendency for a higher benefit-cost ratio. Even though more research is needed to better understand the characteristics of forage legumes as sources of protein, and their effects on animal performance and nitrogen excretions, these results show that jackbean silage and cowpea hay at inclusion levels between 250 and 300 g/kg dry matter have potential to substitute soybean meal in diets of crossbred cows producing 10 kg milk daily.

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1. Introduction

Milk yields in tropical dairy farms are commonly increased by inclusion of large proportions of concentrate feeds in the cows' diet (Machado et al., 2014). These concentrate mixtures are rich in crude protein (CP) mainly to compensate for the low CP concentration of the forages most commonly used in dairy cattle feeding in the tropics and subtropics (e.g. grass hay, cereal straws, maize or sorghum silages) (Machado et al., 2014). Protein-rich ingredients (e.g., soybean meal, cottonseed meal) of such concentrate mixtures, however, are expensive and in many cases imported, leaving the production units susceptible to international price fluctuations and availability. The dependency on those CP sources could be reduced by maximizing the CP supply from the forage portion in the diet, for instance, by feeding forage legumes. However, due to the different CP characteristics (i.e. crude protein fractions, degradation characteristics) that forage legumes may have compared with those of the concentrate ingredients being replaced, changes in e.g. animal performance and nutrient partitioning may be expected upon feeding tropical forage legumes to lactating cows. Rumen degradability of CP from soybean is around 650 g/kg CP (Spiekers et al., 2009), it therefore provides a significant proportion of dietary amino acids to the duodenum; urea, on the other hand, is a source of inorganic nitrogen (N) and is rapidly solubilized and completely degraded in the rumen. Both feeds provide different scenarios of CP degradability and may serve as a guide for the understanding of the utilization of forage legumes as source of protein in tropical dairy. Therefore, the objectives of the current study were to investigate the effects of the supply of CP from forage legumes to lactating cows on feed intake, animal performance, nutrients' apparent total tract digestibility, N balance, and profitability.

2. Materials and methods

2.1. Experimental location, cows, and design

This study was performed at the Research Station of the Agricultural Faculty of the University of El Salvador located 13°28´30´´N and 89°5´43´´W, 42 km southeast of San Salvador city at 40 m above sea level between September and December 2015. Average ambient temperature and total monthly precipitation during the experimental period were 27 °C (min = 22 °C, max = 34 °C) and 200 mm (min = 110 mm, max = 345 mm), respectively (MARN, 2015).

Eight crossbred (Brown Swiss (0.5) × Creole (0.5)), multiparous lactating cows with (average ± standard deviation) 11.1 ± 2.31 kg/d milk yield, 444 ± 41.2 kg body weight (BW), and 125 ± 100.5 days in milk (DIM) at the beginning of the experiment were used for the study in a replicated 4 × 4 Latin square design testing four different diets within four periods of 21 days, each consisting of 14 days for adaptation and seven days for data and sample collection.

Throughout the experiment, the cows were housed in individual stalls, having 6 m^2 of concrete floor, 3 m^2 sand bedding, and 2.6 m of feeding space. Continuous airflow from 102-cm fans was provided from 10:00 to 16:00 h each day. Water and feed were offered ad libitum. Feed was provided as a total mixed ration (TMR). Milking was done using a portable milking machine (Euromovel, 450 EuroLatte, Sistemas de Ordenha, Cachoeirinha, Rio Grande do Sul, Brazil) at 7:00 and 16:00 h each day.

2.2. Experimental diets

Four diets were formulated differing in the main source of CP and forage using the CPM Dairy software (Version 3.0, CAHP software information) to have a 70:30 forage to concentrate ratio (dry matter (DM) basis) and to meet the protein and metabolisable energy (ME) requirements for cows of 450 kg BW and producing 11 kg milk/d (NRC, 2001). Therefore, all diets were designed to have a CP concentration of 130 g/kg DM and a ME concentration of 8.7 MJ/kg DM.

The control diet consisted of sorghum (*Sorghum bicolor*) silage as main forage and a concentrate mixture with soybean as main CP source (control). For the second diet, the sorghum silage was substituted by a mixed jackbean (*Canavalia ensiformis*) + sorghum silage, 40:60 ratio on DM basis,) and the proportion of soybean meal in the concentrate mixture was reduced (jackbean-silage). In a third diet, 45% (DM basis) of sorghum silage was substituted by cowpea (*Vigna sinensis*) hay and the proportion of soybean meal in the concentrate mixture reduced (cowpea-hay). A fourth diet was similar to the control diet, but urea substituted a proportion of soybean meal in the concentrate mixture (urea diet). All diets included the same amount of stargrass hay (*Cynodon plectostachius*).

The control and the urea diet served as comparison for the diets including legumes. Crude protein from soybean in the control was substituted by 57 and 65% by CP from jackbean silage and cowpea hay, respectively.

The chemical composition of the forages and concentrate mixtures are presented in Table 1. Dietary ingredients, diet composition, and the proportion of CP supplied by each main CP source are presented in Table 2.

Titanium dioxide (TiO_2) was used as external marker to estimate the total faecal output of nutrients. Each animal was administered 15 g TiO_2/d from day 10 until day 19 during each experimental period. For this, the powdered marker substance was mixed with a small amount of the respective concentrate mixture. A small amount of water was added to aggregate the mixture, which was then offered to each animal before morning feeding, ensuring that every cow consumed the complete amount.

2.3. Forage production

Sorghum, jackbean, cowpea, and stargrass were cultivated and prepared in the same location of the feeding trial.

Sorghum and jackbean were planted in March 2015. Sorghum CENTA S3 BMR was planted in rows at 0.8 m and 12 plants/m within rows. For the jackbean-sorghum silage, two 0.25-ha plots, one for each crop were simultaneously sown. Planting density of

Table 1
Chemical composition of the forages and concentrates (g/kg dry matter) used to feed crossbred lactating cows in El Salvador (n = 4).

	Forages[1]						Concentrates[2]					
	SS	SJS	CH	SGH	SEM	P-value	C1	C2	C3	C4	SEM	P-value
Dry matter (g/kg fresh matter)	239[b]	224[b]	852[a]	865[a]	81.3	< 0.01	883[ab]	886[a]	882[ab]	867[b]	0.262	0.02
Crude ash	110	97.6	118	92.3	6.71	0.55	93.9[a]	83.9[ab]	80.1[b]	86.7[ab]	1.727	0.01
Crude protein	80.3[c]	107[b]	143[a]	75.2[c]	7.14	< 0.01	263[a]	183[b]	161[b]	252[a]	12.03	< 0.01
Neutral detergent fiber	657[b]	615[b]	629[b]	756[a]	15.9	< 0.01	243[b]	276[a]	278[a]	244[b]	6.01	0.03
Acid detergent fiber	383[b]	393[ab]	453[a]	412[ab]	10.2	0.04	65.6	72.3	73.6	64.5	1.87	0.21

[1] SS = sorghum silage; SJS = sorghum + Jackbean silage; CH = Cowpea hay; SGH = Stargrass hay.
[2] C1 = concentrate for the control; C2 = concentrate for the jackbean-silage diet; C3 = concentrate for the cowpea-hay diet; C4 = concentrate.

Table 2
Ingredient and chemical composition of experimental diets fed to crossbred lactating cows in El Salvador.

Treatment	Control	Jackbean silage	Cowpea hay	Urea
Ingredients, g/kg dry matter				
Concentrate				
Soybean meal	103.1	43.2	35.3	68.5
Urea	0.0	0.0	0.0	5.2
Corn meal	53.0	75.2	65.1	67.6
Wheat bran	67.6	102.9	114.2	82.2
Molasses	29.4	29.2	36.2	29.5
Salt	3.0	3.0	3.0	3.0
Calcium phosphate	1.4	1.4	1.4	1.4
Calcium carbonate	2.9	2.9	2.9	2.9
Vitamin-mineral supplement[1]	3.0	3.0	3.0	3.0
Total concentrate	**263.4**	**260.7**	**261.1**	**263.2**
Forages				
Sorghum silage	650.8		358.6	650.9
Jackbean-sorghum silage		653.9		
Cowpea hay			294.3	
Stargrass hay	85.8	85.4	86.0	85.8
Total forage	**736.6**	**739.3**	**738.9**	**736. 8**
Chemical composition (g/kg dry matter)[2]				
DM, g/kg fresh matter	339	318	482	337
Crude protein	138[a]	126[bc]	121[c]	134[ab]
Crude ash	103	92.9	102	101
Neutral detergent fiber	535[AB]	528[B]	554[A]	538[AB]
Acid detergent fiber	285[b]	300[ab]	320[a]	286[b]
Metabolisable energy[3] (MJ/kg DM)	8.7	8.9	8.6	8.7
Proportion of crude protein from main ingredients (g/kg crude protein)				
Soybean meal	376	162	133	244
Sorghum silage	428	342	243	418
Jackbean silage		228		
Cowpea hay			348	
Urea				112

[1] Provided (per kg DM): 40 mg Zn, 25 mg Mn, 5.5 mg Fe, 16 mg Cu, 0.60 mg I, 0.30 mg Mg, 0.25 mg Co, 0.20 mg Cr, 0.45 mg Se, 5116 IU vitamin A, 708 IU vitamin D3, 29 IU vitamin E.
[2] Different superscripts within a row denote differences between means ($P < 0.05$), tendencies are denoted by capital letters ($P < 0.10$).
[3] Estimated from the CPM Dairy program. No statistical analysis done for this parameter.

sorghum was similar as described above, whereas jackbean was sown in rows at 0.8 m and 8 plants/m within rows.

Sorghum and jackbean were irrigated and fertilized with 68 and 30 kg N/ha, respectively. Both crops were harvested 90 d after seeding, when sorghum grain was in milk to dough stage and jackbean started flowering, using a flail type harvester (Pecos 9004, São Pablo, Brazil) that cut the plant biomass to a particle size of 2.5 cm. Silages were prepared in trench silos. The material was compacted with a tractor and covered by plastic sheets until utilization (after approximately two months). The mixed jackbean-sorghum silage was prepared by combining the biomass from both 0.25-ha plots, yielding a silage with an approximate proportion of 400 g and 600 g/kg DM of jackbean and sorghum, respectively.

Cowpea was cultivated in four plots of 0.1 ha each, one for each experimental period. Plants were sown in rows at 0.8 m and with 8 plants/m within rows, irrigated, and fertilized with 30 kg N/ha. Cowpea was harvested 60 d after sowing at flowering stage when first green pods started to develop. The plant material was harvested by hand, mechanically chopped, and let to dry on a concrete floor under sunshine for three days before storage.

Stargrass hay was obtained from an established pasture with an area of 0.7 ha. The grass was harvested with a mowing board (Superior 394, Gribaldi & Savia, Rivarolo Canavese TO, Italy) at flowering stage after 40 d of regrowth, dried under sunlight for three days, and stored until feeding.

2.4. Data collection and sampling

At the beginning and at the end of each experimental period, cows were weighed at 9:00 h on two consecutive days using a livestock scale (743810-500 AG-tronic inc, Lakeshore, Ontario, Canada). If both BW differed by more than 10%, the animal was weighed again on a third day.

Sampling occurred between days 15 and 20 of each experimental period. Feed offered and refused by individual cows were weighed and recorded daily. Approximately 200 g each (as-fed basis) of sorghum silage, jackbean-sorghum silage, cowpea hay, stargrass hay, the four concentrate mixtures, and of the four TMR were collected and stored at 4 °C each day. At the end of every sampling week, samples of offered feed were pooled and a subsample of 200 g each (as-fed basis) was stored (4 °C) for further analyses. Similarly, approximately 200 g of feed refused (as-fed basis) were collected daily for every animal and stored. Feed refused was also pooled by animal at the end of each sampling period and 200 g of each was stored.

Milk samples were collected twice a day at milking time into 120-ml sterile plastic containers, transported in an ice box, and stored at 4 °C until further analyses.

Urine spot samples were collected by perineal massage once daily between days 15 and 20. Urine sample collection started at 10:00 h on day 15 and at 12:00, 14:00, 16:00, 18:00, and 06:00 h on days 16, 17, 18, 19, and 20, respectively, of each experimental period. A sample of 100 ml was collected for every cow and 8 ml of an aqueous solution of sulphuric acid 20% (v/v) was added to reduce urine pH to below 3. The pH was measured with a pH meter (Double Junction pH testr. 10 OAKTON instruments, Vernon Hills, IL, USA). Acidified urine samples were then filtered using P5 grade filter paper (09.801D, Fisherbrand, Waltham, MA, USA). From the filtrate, two aliquots were obtained, one of 20 ml for N determination, and one of 10 ml for creatinine determination. The latter subsample was further diluted by adding 40 ml of distilled water using a volumetric flask. From the filtered and diluted subsample, two 6-ml aliquots were stored in cap assay tubes for analysis of creatinine. A third aliquot of 15 ml was also collected as backup. All urine samples were stored at −20 °C until analyses.

Feces spot samples were collected following the same time schedule of urine sampling. Two-hundred grams of fresh feces were collected by rectal grab and stored at 4 °C until the end of the sampling week. Then, the six samples per animal were pooled and a subsample of 200 g fresh matter was stored at −20 °C for further analyses.

2.5. Laboratory analyses

All feed samples and orts were dried in a fan oven (100–800, Mermet GmbH and Co., Schwabach, Germany) at 60 °C for 48 h. Feces samples were dried at 50 °C for 72 h. The dried samples were ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas Company, Swedesboro, NJ, USA). Concentrations of DM were determined on dried feed, orts, and feces using an air-forced vacuum oven (100–800, Memmert GmbH and Co. KG, Schwabach, Germany) at 105 °C for 16 h. Nitrogen concentrations were determined by the Kjeldahl method (AOAC, 2012) using an automatic Kjeldahl digestion and distillation unit (DKL Series 20, UDK 129, VELP Scientifica, Usmate Velate, Italy) and the CP concentration was estimated by multiplying the N concentrations by 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations were analysed following the Van Soest et al. (1991) procedure using a fibre analyser (A 200, ANKOM technology, Macedon, NY, USA). Crude ash was determined by incinerating the samples at 550 °C for 5 h (AOAC, 2012) using a muffle furnace (L24/12/P320, Nabertherm, Bremen, Germany).

Milk samples were analysed daily during the sampling period for fat, protein, and lactose in a Lactostar device (NS3510, Funke-Gerber, Berlin, Germany). Milk urea-nitrogen (MUN) concentrations were determined by the diacetyl monoxime colorimetric assay as adapted by Broderick and Clayton (1997). Absorbance was measured at 525 nm using a spectrophotometer (4001, Thermo Genesys 20, Thermo Scientific, Waltham, MA, USA).

Total N in urine was analyzed by Kjeldahl (AOAC, 2012) using the same equipment as for the feed and feces samples. Creatinine was analysed by spectrophotometry using a commercial kit MI 1,001,111 (SPINREACT, Girona, Spain). Absorbance was recorded at 505 nm wavelength in a spectrophotometer (4001, Thermo Genesys 20, Thermo Scientific, Waltham, MA, USA). Titanium dioxide concentration was determined in feces according to the description of Brandt and Allam (1987) modified by Glindemann et al. (2009). Nitrogen balance was estimated as the difference between N intake and the sum of N excretions in milk, feces, and urine.

Urine volume was estimated from the creatinine concentration using a constant of creatinine excretion of 24.04 mg/kg BW (Chizzotti et al., 2008). Total feces excretion was estimated from the concentration of titanium dioxide in feces as described by Myers et al. (2004).

2.6. Economic evaluation

Feed costs were calculated using mean feedstuffs prices in El Salvador over the course of the experiment (September to December 2015). Similarly, forage production costs were estimated using mean prices of inputs (i.e. seeds, fertilizers, agrochemicals) during the preparation period, including labor and rental of machinery needed for forage cultivation and processing (April to November 2015). Cost of land rental was not included. Income over feed cost (IOFC) was computed by subtracting the costs of the diets from the gross income from milk production using mean milk price received during the trial (0.553 USD/kg) as calculated by Buza et al. (2014).

Benefit-cost ratio summarizes the overall value of money incurred and is expressed as ratio of gross income from milk production to gross costs of feed (Buza et al., 2014).

2.7. Statistical analysis

Data was analyzed using the MIXED procedure of SAS (2013) with underlying model:

$$Y_{ijk} = \mu + T_i + \beta_j + (T_i \times \beta_j) + \alpha_k + \varepsilon_{ijk}$$

where, Y_{ijk} is the response variable; μ is the overall mean; T_i is the effect of experimental diet *i*; β_j is the effect of period *j*; $(T_i \times \beta_j)$ is the interaction between the *i*-th treatment and the *j*-th period; α_k is the random effect of cow *k*; and ε_{ijk} is the random residual error. The appropriate variance component for each variable was chosen by running the analyses with all variance components (i.e. compound symmetry, autoregressive, unstructured, and variance component) and choosing the variance component with the lowest AIC value. Mean differences were determined by Tukey test with a significance level of 0.05. Tendencies were declared for P between 0.05 and 0.10.

3. Results

3.1. Effect of diet on production and nutrient utilization

Of the forages, CP concentration was highest for the cowpea hay, intermediate for the jackbean-sorghum silage, and lowest for the sorghum silage and stargrass hay ($P < 0.01$, Table 1). Neutral detergent fiber was highest for the stargrass hay and similar between the other three forages ($P < 0.01$), but ADF was highest for the cowpea hay, and lowest for the sorghum silage ($P = 0.04$). Concentrate feeds for both diets including legumes had, by design, lower CP and higher NDF concentration, mainly as a result of substitution of soybean meal by wheat bran (Table 2).

Crude protein concentration of both legume-containing diets were lower than that of the control diet ($P < 0.05$, Table 2), due to a lower actual CP concentration of both legumes than the concentrations assumed during diet formulation. Acid detergent fiber concentration was higher in the cowpea-hay diet than in both the control and the urea diet ($P < 0.05$, Table 2). Estimations based on literature data (Spiekers et al., 2009) using the ingredients (or similar ones) from each diet showed rumen undegradable crude protein (RUP) concentrations of 270, 287, 243 and 229 g/kg CP for the control, the cowpea-hay diet, the jackbean-silage diet, and the urea diet, respectively. Highlighting that the RUP concentration of the cowpea-hay diet was closer to the control and that of the jackbean-silage diet was closer to the urea diet. The forage legumes also substituted 40 and 45% of the sorghum silage (on DM basis) for the jackbean-silage and cowpea-hay treatments, respectively, which increased the proportion of corn meal and wheat bran for the legume-containing diets. Because of the high ADF concentration of the cowpea hay, concentrations of ADF were also higher in the cowpea-hay diet compared with the control ($P < 0.05$, Table 2).

Compared with the control diet, the cowpea–hay diet caused a higher dry matter intake (DMI) ($P < 0.01$), whereas jackbean-silage diet and the urea diet did not affect DMI (Table 3). However, owing the lower CP concentration of the legume-containing diets, N intake was lower in the jackbean-silage diet by approximately 6% compared with all other diets ($P = 0.02$, Table 4).

There were no effects of dietary treatments on BW and milk yield ($P > 0.23$, Table 3). Similarly, no effects of the diets were observed on milk composition or on milk fat, protein and lactose yield ($P > 0.19$). Concentrations of MUN were considerably lower in the jackbean-silage diet than in the control, with the cowpea-hay diet and the urea diet being intermediate ($P = 0.04$).

Table 3
Effect of partial replacement of sorghum silage and soybean meal with jackbean silage, cowpea hay, or urea on milk yield and composition in lactating crossbred cows (n = 8).

	Control[1]	Jackbean Silage	Cowpea hay	Urea	SEM	P-value
Dry matter intake (kg/d)	14.3[b]	14.2 [b]	15.6 [a]	14.4 [b]	0.22	0.01
Body weight (kg)	443	446	444	442	7.18	0.77
Milk yield (kg/d)	9.55	9.52	9.31	9.82	0.51	0.83
ECM (kg/d)[2]	8.96	9.65	8.66	9.16	0.35	0.23
Milk yield (kg/kg HEP[3])	11.7[c]	18.0[a]	17.1[a]	14.3[b]	0.93	< 0.01
Milk fat (g/kg milk)	40.4	44.0	39.3	39.3	1.74	0.25
Milk protein (g/kg milk)	30.6	31.4	30.9	30.9	0.41	0.19
Lactose (g/kg milk)	46.1	47.0	46.4	46.6	0.61	0.41
Fat yield (kg/d)	0.360	0.410	0.342	0.365	0.014	0.32
Protein yield (kg/d)	0.290	0.296	0.286	0.300	0.015	0.82
Milk urea-nitrogen (mg/dL)	15.2 [a]	13.3 [b]	14.1 [ab]	14.0 [ab]	0.58	0.04

[1] Different superscripts within a row denote differences between means (P < 0.05).
[2] ECM = Energy-corrected milk = Milk yield * (0.383 * Milk fat (g/100 g) + 0.242 * Milk protein (g/100 g) + 0.7832)/3.1138).
[3] HEP = Human-edible protein (estimated from the edible proportion of crops and by-products of the Council for Agricultural Science and Technology as reported by Wilkinson, 2011).

Table 4
Effect of partial replacement of sorghum silage and soybean meal with jackbean silage, cowpea hay or urea on nitrogen (N) excretion and nutrient digestibility in lactating crossbred cows (n = 8).

	Control[1]	Jackbean silage	Cowpea hay	Urea	SEM	P-value
N intake (g/d)	304 ^{a}	286 ^{b}	301 ^{a}	308 ^{a}	3.88	0.02
N secretion in milk (g/d)	45.7	46.6	43.8	47.2	2.39	0.62
Milk N/NI[2] (g/100 g)	13.1 ^{B}	15.0 ^{A}	13.1 ^{B}	14.2 ^{AB}	0.81	0.23
N excretion in urine (g/d)	109	97.7	104	109	3.59	0.62
N excretion in feces (g/d)	119.4 ^{ab}	122.2 ^{a}	121.7 ^{a}	109.3 ^{b}	2.98	0.10
N balance (g/d)	19.6 ^{B}	27.5 ^{AB}	37.7 ^{A}	42.3^{A}	6.58	0.06
Urine N (g/100 g NI)[2]	35.7	34.3	34.9	35.5	1.17	0.96
Feces N (g/100 g NI)[2]	39.1 ^{ab}	42.8 ^{a}	40.8 ^{a}	35.6 ^{b}	1.13	0.08
Apparent total tract digestibility coefficients						
Dry matter	0.593	0.567	0.609	0.600	0.008	0.36
Crude protein	0.625^{ab}	0.572^{c}	0.596^{bc}	0.650^{a}	0.011	0.04
Organic matter	0.620^{AB}	0.591^{B}	0.645^{A}	0.629^{AB}	0.009	0.16
Neutral detergent fiber	0.527	0.514	0.560	0.532	0.011	0.59
Acid detergent fiber	0.500	0.481	0.530	0.523	0.013	0.55

[1] Different superscripts within a row denote differences between means (P < 0.05), tendencies are denoted by capital letters (0.05 < P < 0.10).
[2] NI, N intake.

3.2. Effect of diet on N balance and apparent total tract digestibility of nutrients

There were no differences in N secretion in milk between all dietary treatments (P = 0.02), but the N use efficiency (in g N secreted in milk per g of N intake) tended to be higher in the jackbean-silage diet and lowest in both the control and the cowpea-hay diet (P < 0.10). The excretion of N in urine did not vary between treatments, neither when expressed as g/d nor relative to N intake (P > 0.62). However, the N excretion in feces was highest for both legume-containing diets and lowest for the urea diet, with the control being intermediate (P < 0.05). Apparent total tract digestibility of DM (aDMd), NDF (aNDFd), and ADF (aADFd) did not differ between dietary treatments (P > 0.36). However, apparent total tract digestibility of CP (aCPd) clearly differed between diets (P = 0.04) with the following order: urea diet > control > cowpea-hay diet > jackbean-silage diet. The aOMd tended to differ between diets (P < 0.10), particularly between both legume-containing diets, with the highest aOMd coefficient observed for the cowpea-hay diet and the lowest for the jackbean-silage diet.

3.3. Effect of diet on economic parameters

Income from milk sale did not differ between dietary treatments (P = 0.83), but feeding costs were clearly lower for the cowpea-hay diet and the jackbean-silage diet compared with both, the control and the urea diet (P < 0.01, Table 5). However, only a tendency for a higher benefit-cost ratio was observed when cowpea hay was fed compared with the control (P < 0.10). No differences were observed for the IOFC between the diets.

4. Discussion

As explained in Section 2.2 all diets were designed to fulfil the requirements of cows weighing 450 kg BW and producing 11 kg milk/d. However, the production expectation were not met during the trial and milk yield averaged 9.6 kg/d. Therefore, an oversupply of CP and ME likely occurred in this experiment, which could have limited the capability of the diets to cause observable effects on the studied parameters. Additionally, unexpected differences were observed for milk production between the animals once the experimental periods started. The differences were not related to the variation in the DIM of the animals and we can only assume that the genetic potential of some of these animals was maximized with the high feeding level during the trial. Milk yield among

Table 5
Effect of partial replacement of sorghum silage and soybean meal with jackbean silage or cowpea hay, and partial replacement of soybean meal with urea on feed cost, milk income and net profit in lactating crossbred cows (n = 8).

	Control[1]	Jackbean Silage	Cowpea hay	Urea	SEM	P-value
Diet cost (USD/kg DM)	0.28	0.26	0.22	0.27	.	.
Milk income (USD/cow/d)	5.73	5.71	5.59	5.89	0.309	0.83
Feed cost (USD/cow/d)	4.05^{a}	3.66^{b}	3.46^{c}	3.92^{a}	0.066	< 0.01
IOFC[2] (USD/cow/d)	1.68	2.05	2.13	1.97	0.302	0.36
Benefit-cost ratio	1.40^{A}	1.57^{AB}	1.63^{B}	1.49^{AB}	0.083	0.17

[1] Different superscripts within a row denote differences between means (P < 0.05), tendencies are denoted by capital letters (P < 0.10).
[2] IOFC = Income over feed cost.

animals was uniform and no strong decline or increase was observed for any animal across the experiment. Nevertheless, additional to the high feeding level, the large variation in milk production between experimental animals may also limit the ability to detect differences between treatments for production, efficiency and economic parameters.

4.1. Feed intake and milk performance

There were no differences in the DMI of cows fed the control diet or the urea diet, in line with the review of Kertz (2010) who stated that feeding urea decreases DMI only at daily urea supply above 135 g/cow, which is much higher than the 75 g urea/d fed to each cow in the present study. Similarly, replacing soybean meal and sorghum silage with jackbean silage did not affect DMI of lactating cows. Feeding forage legumes is normally associated with an increased DMI due to their faster passage rate and therefore a faster rumen emptying as compared with diets based on grasses (e.g. Dewhurst, 2013). However, such observations have been derived from studies with temperate forage legumes. A recent review of (Castro-Montoya and Dickhöfer (2018) on the effects of tropical legume silages found that replacing grasses or whole-crop silages with legume silages does not affect DMI for proportions of forage legumes in ruminant diets up to 400 g/kg DM. The findings of Castro-Montoya and Dickhöfer (2018) were confirmed by results of the present study, in which jackbean was only included in the diet at 262 g/kg DM. Furthermore, compared with the control DMI increased when cowpea hay was included in the diet. This increase in DMI is in contrast to a previous study by Corea et al. (2017) who found no effects on DMI of lactating cows when substituting sorghum silage by cowpea hay. However, a main difference between the current study and that of Corea et al. (2017) is the rate of substitution of sorghum silage by cowpea hay, with 45% of sorghum silage (DM basis) being replaced in the present study and only 25% (DM basis) in the trial of Corea et al. (2017). The increased DMI with the higher rate of cowpea hay inclusion, despite the high ADF concentration in the cowpea-hay diet, may be related to the substitution of silages by hay, an observation previously made by e.g. Broderick (1995) and Beauchemin et al. (1997), which may be due to the presence of fermentation products such as acetate and ammonia from *in silo* conservation (see. e.g. review of Huhtanen et al., 2007).

Some authors have reported an increase in milk yield associated with feeding forage legumes (Dewhurst. 2013; Phelan et al., 2015), which is generally associated with higher DMI and CP intake when feeding forage legumes in substitution of grasses. In the present study, milk production did not differ between dietary treatments, likely because of the similar levels of intake that were never limiting for the animals along with the already mentioned oversupply of ME in the diet, which could have masked any possible effect on an improved efficiency by a diet. Similarly, concentrations of milk fat, protein, and lactose were not affected by the diets, in agreement with findings of Castro-Montoya and Dickhöfer (2018) with tropical legume silages, resulting in similar yield of energy corrected milk. The lack of effects of the diets on milk protein may indicate a similar supply of metabolic protein to the duodenum, while the similar fat concentration may be a reflection of the similar fiber digestibility observed for all diets in the current study.

Milk urea nitrogen (MUN) concentrations were high across all treatments. At low performance levels, like the ones in the present study ($\sim$ 10 kg milk/d), MUN concentrations above 12 mg/dl can be associated with excess supply of dietary protein, excessive rumen CP degradation, and an imbalance between protein and energy in the rumen (Hof et al., 1997). The high MUN observed in this study likely reflected an excessive supply of dietary CP to the cows, as mentioned above. In this line, MUN level was lowest and N use efficiency in milk (g milk N/100 g N intake, Table 4) highest for the jackbean-silage diet, in which daily N intake was on average 18 g/cow lower than in the other diets.

Interestingly, based only on the RUP concentration of the diets, higher MUN would have been expected with the jackbean-silage diet and the urea diet and lowest with the cowpea-diet and the control. Conversely, MUN was highest for the control diet, albeit numerically, compared with the cowpea-hay and the urea diet, despite their similar N intakes, which could indicate a less efficient use of ammonia-N for rumen microbial protein synthesis (Hristov and Sandev, 1998) for the control diet. A higher rumen degradability of CP, like the one attributed to urea, would typically lead to a higher postprandial ammonia-N concentration in the rumen, and therefore higher blood and milk urea concentrations. The lower MUN observed with the urea diet, despite the similar N intake, might indicate that the N recycled into the rumen might have been better utilized in-between meals due to different patterns of carbohydrates fermentation in the rumen in the cowpea-hay diet and the urea diet compared with the control, as suggested by Cole and Todd (2008). This might also be an additional explanation for the lowest MUN in the jackbean-silage diet. Compared with the control, all other diets included a higher proportion of wheat bran and corn meal, both ingredients with a lower rate of carbohydrate rumen degradation compared with the carbohydrates from the soybean substituted (Spiekers et al., 2009). Additionally, wheat bran and corn meal will also have a lower rate and extent of carbohydrate degradation in the rumen compared to the fermentable carbohydrates in the sorghum silage (Hoffman et al., 2011). Therefore, the lower MUN observed with the jackbean-silage and the urea diets, compared with the control, may also be due to a better synchronization between N and energy sources in the ration. A better synchronization of N and carbohydrate sources upon substituting soybean by urea and maize meal has been previously reported in dairy cows (Gonçalves et al., 2014).

4.2. Apparent total tract digestibility

Compared with the control only the jackbean-silage diet showed a decrease in aCPd, in agreement with recent findings of Castro-Montoya and Dickhöfer (2018) who found that diet's aCPd decreased with increasing proportion of tropical legume silages in the diet. On the other hand, there were no differences between the aCPd of the control and the cowpea-hay diet, even though a numerical decrease was observed when feeding the cowpea hay. In contrast to our findings, Corea et al. (2017) found an increased aCPd when cowpea hay replaced about one quarter of sorghum silage DM. Besides the lower rate of inclusion of cowpea hay in the study of Corea et al. (125 g/kg DM) compared with the present study (294 g/kg DM), there was a marked difference in quality of both cowpea hays: the cowpea hay of the study of Corea et al. (2017) had CP and NDF concentrations of 166 and 464 g/kg DM, respectively, whereas the cowpea hay in the present study had 143 and 629 g/kg DM of CP and NDF, respectively. An increase in NDF concentrations is typically associated with an increase in fiber-bound N (Rinne et al., 1997) which could have reduced the aCPd of the cowpea-hay diet compared with the control in the present study and could explain the differences between our findings and those of Corea et al. (2017).

Other factors like a short digesta retention time in the rumen, as attributed to temperate legumes (Dewhurst, 2013; Phelan et al., 2015), could decrease aCPd. However, this explanation may not be entirely valid, at least for the cowpea-hay diet, which had the highest aOMd and numerically highest aDMd, aNDFd, and aADFd among all diets (by trend), all parameters that are generally negatively affected by a shorter retention time. The presence of tannins could also affect aCPd. However, even though tannins were not analyzed, both, cowpea and jackbean have typically low concentrations of total tannins (between 1.8 and 4.0 g/kg DM for both forages) (Heuzé et al., 2015; Heuzé and Tran, 2015). Therefore only minor, if any, effects would be expected on aCPd in this study.

The jackbean-silage diet and the cowpea-hay diet greatly differed in their aOMd. These contrasting results between both legumes are difficult to explain, particularly knowing that the NDF concentration of the cowpea-hay diet was higher than that of the jackbean-silage diet. Nevertheless, the process of ensiling is often recognized as a factor decreasing aDMd and aOMd, even though contradictory results are often presented regarding the effects of *in silo* conservation on nutrients digestibility (Yahaya et al., 2001).

4.3. Nitrogen balance

Nitrogen excretion in urine in the present study was not affected by the dietary treatments, but N excretion in feces (both, in g/d and g/100 g N intake) was higher when forage legumes were fed compared with the urea diet, and was intermediate for the control. The greater fecal N excretion with inclusion of forage legumes in the diets is in accordance with results of a recent review that found a higher daily excretion of N in feces by small ruminants fed diets containing between 100 and 400 g/kg of dietary DM of legume silages compared with diets without forage legumes (Castro-Montoya and Dickhöfer, 2018). Fecal N excretion comprises undigested dietary N, microbial N, and endogenous N (Mason, 1969), the reasons for the differences observed in this study may be explained by the higher excretion of undigested dietary N with both forage legumes-containing diets. Some contribution of microbial growth in the hindgut may be likely in the case of the jackbean-silage diet, as aOMd decreased, but would not be a plausible explanation for the cowpea-hay diet.

4.4. Economic evaluation

Feed conversion efficiency (kg milk/kg DMI) was similar for all diets and varied between 0.61 and 0.67 (data not shown). Hence, the financial benefits of feeding forage legumes arise mainly from reduced feeding costs. Only a trend was observed for a higher benefit-cost-ratio when including jackbean silage and cowpea hay in the diets in substitution of soybean and sorghum silage (0.17 and 0.23 more USD for each USD invested, respectively). Similarly, the IOFC increased by 0.37 and 0.45 USD when including jackbean-silage and cowpea hay, respectively, compared with the control, but the differences were not statistically different. The oversupply of nutrients discussed above may also limit the ability of our design to detect statistical differences in the profitability of using forage legumes. Nevertheless, the increase in those economic parameters indicates the probability that substituting sorghum silage and soybean meal with jackbean silage or cowpea hay will increase economic benefits with a low risk of a decreased milk yield and profitability. Still, the access to machinery, labor and storage facilities should be considered for a more detailed analysis of the economic benefits of using forage legumes as hay or silage.

Another important aspect associated with the utilization of legume forages is taking advantage of the ability of ruminants to use fiber-rich plant materials, reducing the competition in the use of high-quality plant biomass as livestock feed or human food. In this regard, substituting soybean by legume forages increased the milk produced per kg of human-edible protein from 11.7 kg (control) to 17.1 and 18.0 kg for jackbean-silage diet and cowpea-hay diet, respectively (Table 3).

Higher milk yields per unit of human-edible protein and slightly higher economic benefits were also observed when soybean meal was substituted by urea. This increase in profit is not entirely surprising as previous studies have demonstrated a positive economic return upon urea feeding (Golombeski et al., 2006; Kertz, 2010), mainly by decreasing the feed costs associated with soybean meal utilization. The use of urea to increase the output of milk per unit of human-edible protein may need a more detailed revision considering other factors and consequences of using urea, for example the need for fossil fuels for its manufacture or transport, which could compromise the benefits mentioned here.

5. Conclusions

Tropical forage legumes can substitute soybean meal and sorghum silage in the diet of lactating crossbred cows while maintaining dry matter intake and milk production, and milk composition. However, the apparent total tract digestibility of crude protein is lower in diets including forage legumes, an important factor to take into consideration when designing diets with these forages. The digestibility of other nutrients may also be compromised depending on the legume species or the conservation form of the forage, but more research is needed to elucidate the individual effects of those factors. There was no indication of a change in the efficiency of protein utilization in the rumen upon feeding forage legumes, but the adjustments in the diets that change the sources and amount of carbohydrates in the overall diet may have an effect on the utilization of ammonia-N in the rumen. Finally, using forage legumes decreases feeding costs and therefore, upon the maintenance of the same milk production level, may increase the profitability of the dairy enterprise.

Declarations of interest

The authors state that there is no conflict of interest associated with the development of this original research article.

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References

AOAC, 2012. Official Methods of Analysis of AOAC International, 19th edition. AOAC International, Gaithersburg, MD, USA.
Beauchemin, K.A., Rode, L.M., Eliason, M.V., 1997. Chewing activities and milk production of dairy cows fed alfalfa as hay, silage, or dried cubes of hay or silage. J. Dairy Sci. 80, 324–333.
Brandt, M., Allam, S.M., 1987. Analysis of titanium dioxide in intestine content and feces using the Kjleldahl method. Arch. Anim. Nutr. 37, 453–454.
Broderick, G.A., 1995. Performance of lactating dairy cows fed either alfalfa silage or alfalfa hay as the sole forage. J. Dairy Sci. 78, 320–329.
Broderick, G.A., Clayton, M.K., 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J. Dairy Sci. 80, 2964–2971.
Buza, M.H., Holden, L.A., White, R.A., Ishler, V.A., 2014. Evaluating the effect of ration composition on income over feed cost and milk yield. J. Dairy Sci. 97, 3073–3080.
Castro-Montoya, J.M., Dickhöfer, U., 2018. Effects of tropical legume silages on intake, digestibility and performance in large and small ruminants: a review. Grass Forage Sci. 73, 26–39.
Chizzotti, M.L., Valadares Filho, S., Valadares, R.F.D., Chizzotti, F.H.M., Tedeschi, L.O., 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livest. Sci. 113, 218–225.
Cole, N.A., Todd, R.W., 2008. Opportunities to enhance performance and efficiency through nutrient synchrony in concentrate-fed ruminants. J. Anim. Sci. 86, E318–E333.
Corea, E.E., Aguilar, J., Alas, N., Alas, E., Flores, J.M., Broderick, G.A., 2017. Effects of dietary cowpea (*Vigna sinensis*) hay and protein level on milk yield, milk composition, N efficiency and profitability of dairy cows. Anim. Feed Sci. Technol. 226, 48–55.
Dewhurst, R., 2013. Milk production from silage: comparison of grass, legume and maize silages and their mixtures. Agr. Food Sci. 22, 57–69.
Glindemann, T., Tas, B.M., Wang, C., Alvers, S., Susenbeth, A., 2009. Evaluation of titanium dioxide as an inert marker for estimating fecal excretion in grazing sheep. Anim. Feed Sci. Technol. 152, 186–197.
Golombeski, G.L., Kalscheur, K.F., Hippen, A.R., Schingoethe, D.J., 2006. Slow-release urea and highly fermentable sugars in diets fed to lactating dairy cows. J. Dairy Sci. 89, 4395–4403.
Gonçalves, G.D.S., Pedreira, M.D.S., Azevedo, J.A.G., Del Rei, A.J., Silva, H.G.O., Silva, F.F., 2014. Replacement of soybean meal by conventional and coated urea in dairy cows: intake, digestibility, production and composition of milk. Acta Sci. Anim Sci. 36, 71–78.
Heuzé, V., Tran, G., 2015. Jackbean (*Canavalia ensiformis*). Feedipedia, a Programme by INRA, CIRAD, AFZ and FAO. Last updated on September 7, 2015, 13:09. (Accessed 14 February 2018). https://www.feedipedia.org/node/327.
Heuzé, V., Tran, G., Nozière, P., Bastianelli, D., Lebas, F., 2015. Cowpea (*Vigna unguiculata*) forage. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. Last updated on October 20, 2015, 16:20. (Accessed 14 February 2018). https://www.feedipedia.org/node/233.
Hof, G., Vervoorn, M.D., Lenaers, P.J., Tamminga, S., 1997. Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. J. Dairy Sci. 80, 3333–3340.
Hoffman, P.C., Esser, N.M., Shaver, R.D., Coblentz, W.K., Scott, M.P., Bodnar, A.L., Schmidt, R.J., Charley, R.C., 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94, 2465–2474.
Hristov, A.N., Sandev, S.G., 1998. Proteolysis and rumen degradability of protein in alfalfa preserved as silage, wilted silage or hay. Anim. Feed Sci. Technol. 72, 175–181.
Huhtanen, P., Rinne, M., Nousiainen, J., 2007. Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. Animal. 1, 758–770.
Kertz, A.F., 2010. Urea feeding to dairy cattle: a historical perspective and review. Prof. Anim. Scientist. 26, 257–272.
Machado, S.C., McManus, C.M., Stumpf, M.T., Fischer, V., 2014. Concentrate:forage ratio in the diet of dairy cows does not alter milk physical attributes. Trop. Anim. Health Prod. 46, 855–859.
MARN, 2015. Boletín climatológico mensual, anual 2015, San Salvador. El Salvador 1–16.
Mason, V.C., 1969. Some observations on the distribution and origin of nitrogen in sheep faeces. J. Agric. Sci. 77, 91–98.
Myers, W.D., Ludden, P.A., Nayigihugu, V., Hess, B.W., 2004. Technical Note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. J Animal Sci. 82, 179–183.
NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th Revised Edition, Subcommittee on Dairy Cattle Nutrition, Committee on Animal Nutrition, Board on Agriculture and Natural Resources. National Research Council, National Academy Press, Washington, D.C.
Phelan, P., Moloney, A.P., McGeough, E.J., Humphreys, J., Bertilsson, J., O'Riordan, E.G., O'Kiely, P., 2015. Forage legumes for grazing and conserving in ruminant production systems. Criti. Rev. Plant. Sci. 34, 281–326.
Rinne, M., Jaakkola, S., Huhtanen, P., 1997. Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. Anim. Feed Sci. Technol. 67, 1–17.
Spiekers, H., Nussbaum, H., Potthast, V., 2009. . Erfolgreiche Milchviehfütterung (Succesful Dairy Cattle Feeding), 5 edition. DLG-Verlag, pp. 560–562.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Wilkinson, J., 2011. Re-defining efficiency of feed use by livestock. Animal 5, 1014–1022.
Yahaya, M.S., Kimura, A., Harai, J., Nguyen, H.V., Kawai, M., Takahashi, J., Matsuoka, S., 2001. Effect of length of ensiling on silo degradation and digestibility of structural carbohydrates of lucerne and orchardgrass. Anim. Feed Sci. Technol. 9, 141–148.