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Effects of dietary cowpea (*Vigna sinensis*) hay and protein level on milk yield, milk composition, N efficiency and profitability of dairy cows

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ABSTRACT

Thirty-two lactating Holstein cows were grouped by days-in-milk into 8 blocks of 4 cows and fed 4 diets containing either (dry matter (DM) basis) 0 or 125 g/kg of cowpea hay (*Vigna sinensis*), added at the expense of sorghum silage, with either low (155 g/kg) or high (170 g/kg) crude protein (CP) supplemented as soybean meal and distillers grains. Diets were balanced to be isoenergetic for cows weighing 540 kg and producing 29 kg/d of milk. Cows were milked and fed ad libitum 3 times a day. The experiment was conducted as a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments; periods were 21 days long with 14 days of adaptation and 7 days of data collection and sampling. Effects of cowpea, CP and their interactions were evaluated using the mixed procedures of SAS, with either pen (traits related to intake) or cow (traits related to production) as the experimental unit. No significant effects of treatment were observed for DM intake (DMI), body weight (BW) gain, and yield of milk and milk components. However, substituting cowpea hay for a portion of the dietary sorghum silage increased milk/DMI and milk N/N intake and decreased milk urea N (MUN) and fecal N excretion, tended to decrease urinary N excretion, reduced feed cost and increased income over feed cost. Moreover, feeding of cowpea hay increased apparent total tract digestibility of DM, organic matter, CP and neutral detergent fiber. Decreasing dietary CP from 170 to 155 g/kg increased Milk N/N intake and decreased MUN, urinary N, fecal N and urinary N/N intake, reduced feed cost and increased income over feed cost. Inclusion of cowpea hay in diets formulated under tropical conditions reduced the need to feed high-cost protein ingredients, improved feed and N efficiency, and reduced risk of N pollution.

1. Introduction

Tropical and subtropical grasses have high fiber content and low digestibility (Juarez Lagunes et al., 1999), which highlights the need for alternative forages to help achieve better productivity and profitability. Legumes such as alfalfa (*Medicago sativa)*, red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) are often fed in temperate regions to improve nutrient contribution, mainly protein, from forage. These species have been shown to increase dry matter (DM) intake and milk yield in dairy cows (Broderick et al., 2002; Dewhurst et al., 2003a) and digestibility (Dewhurst et al., 2003b). However, their cultivation and use in warmer climates

Abbreviations: BW, body weight; CP, crude protein; DM, dry matter; DMI, dry matter intake; FCM, fat-corrected milk; IOF, income over feed cost; MUN, milk urea N; NDF, neutral detergent fiber; OM, organic matter; TMR, totally mixed ration

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is not feasible because these forages are unadapted to tropical and subtropical pests and diseases. Ensiled forage from warm season legumes such as cowpea (*Vigna* spp.), perennial peanut (*Arachis glabrata*) and annual peanut (*Arachis hypogaea*) were evaluated in Florida for nutritive content by Foster et al. (2011) and, based on greater CP content and *in situ* DM and neutral detergent fiber (NDF) digestion compared to bahiagrass haylage, the authors concluded that these were promising forages for dairy cows in the sub-tropical US.

Studies addressing the optimization of dietary protein have shown that concentrations beyond 160 g/kg CP do not positively impact milk yield (Groff and Wu, 2005; Olmos Colmenero and Broderick, 2006). Salvadoran dairy farmers often overfeed CP: A survey in Central and Western El Salvador of 8 dairies using free-stall management of Holstein cows indicated that rations averaged 171 g CP/kg DM over both the dry and rainy seasons; a ration with 196 g CP/kg DM was fed on one farm during the rainy season (Zavala et al., 2012). Overfeeding CP reduces profit margins due to the relatively high cost of protein supplements and low efficiency of N use by dairy cows fed high protein diets (Broderick, 2003). Dairy cows excrete about 2–4 times more N in manure than they secrete in milk, which increases both cost of milk production plus environmental N pollution (Broderick, 2006).

Our hypotheses were that 1) reducing dietary CP below 170 g/100 kg DM, the typical concentration fed by Salvadoran dairy farmers, would not impair production, and 2) feeding a tropical legume forage would lower feed cost. This study evaluated feeding cowpea hay (*Vigna sinensis*), a tropical legume, at two levels of CP in the diet of lactating dairy cows under tropical conditions for yield of milk and milk components, nutrient efficiency and profitability.

2. Material and methods

2.1. Cows and design

The study was conducted at El Milagro dairy farm in Sonsonate Department, El Salvador, at an altitude of 425 m, Latitude 13.745°, Longitude −89.633°, with 26 °C average daily temperature and 1900 mm average annual rainfall. Twenty-two primiparous Holsteins with mean (± SD) 100 ± 4.7 days-in-milk, 531 ± 62 kg body weight (BW) and 27 kg ± 4.6 milk/d, plus 10 multiparous Holsteins with mean (± SD) parity 3.2 ± 1.5, 96 ± 59 days-in-milk, 585 ± 73 kg BW and 33 ± 4.3 kg milk/d. All cows used in the trial had body condition scores ranging from 3.5 to 3.75 and normal health histories. The cows had free access to water, were milked 3 times a day (07:00, 15:00 and 23:00) and fed after each milking. Heat stress was controlled using 91 cm fans and water sprinklers with continuous fan ventilation from 09:00 to 17:00 and with 2 min of sprinkling every 10 min. The pens were sand-floored free-stalls and cows had access to 95 cm of linear feed bunk space per animal. The experimental procedures applied to animals were approved by the Research Council of the University of El Salvador, which is the authority in this matter.

For data related to production and N-metabolism in which cow was the experimental unit, cows were blocked by parity and DIM into 8 squares of 4 cows (4 squares of primiparous cows, 2 squares of multiparous cows and 1 square with 2 primiparous cows and 2 s lactation cows). However, for data related to DMI in which pen was the experimental unit, 1 cow from each square was randomly assigned to 1 of the 4 pens (total 8 cows/pen). Treatments were applied in a 2 × 2 factorial arrangement in each Latin square; the 4 periods were 21 d long (total 84 d) with 14 d for adaptation and 7 d for data collection and sampling. Experimental diets were fed as total mixed rations (TMR) to all animals in a pen and diets were switched among pens at the end of each period. This arrangement of diets and pens over periods has yielded satisfactory results in previous studies (Aguerre et al., 2012; Swanepoel et al., 2014). Experimental diets were formulated to contain (DM basis) 500 g/kg of forage and 500 g/kg of concentrate plus mineral mix and either 0 of 125 g/kg of cowpea hay, which was added at the expense of sorghum silage. Furthermore, diets contained either low (155 g/kg) or high (170 g/kg) CP, with the additional CP from soybean meal and distillers grains added at the expense of wheat bran and corn meal. Compositions of the sorghum silage and cowpea hay are in Table 1 and of the 4 experimental diets are in Table 2. All diets were balanced to 11.0 MJ ME/kg DM using the CPM Dairy V3® program to meet the energy requirements for dairy cows weighing 540 kg and producing 29 kg/d of milk. Feeding rate was adjusted daily to yield refusals equivalent of about 100 g/kg of feed offered. The TMR were sampled every week to determine DM content of the as-fed TMR to compute DM intake (DMI).

2.2. Data collection and sampling

Daily amounts of feed offered and refused on a pen basis and milk production of each cow at every milking were recorded during

Table 1
Chemical composition of the cowpea hay (*Vigna sinensis*) and sorghum silage fed to the dairy cows.

Trait	Cowpea hay		Sorghum silage	
	Mean	SEM[a]	Mean	SEM
Dry matter, g/kg	874	5.0	285	10.8
Ash, g/kg DM	113	2.3	99	1.6
Organic matter, g/kg DM	890	2.3	901	1.6
Crude protein, g/kg DM	166	3.1	84	1.9
Neutral detergent fiber, g/kg DM	464	14.8	548	12.5

[a] Standard error of the mean.

Table 2
Ingredient and chemical composition of experimental diets fed to the dairy cows.

Crude protein, g/kg	155	155	170	170
Cowpea hay, g/kg	0	125	0	125
Ingredients, g/kg DM[a]				
Sorghum silage	498	373	498	373
Cowpea hay	0	125	0	125
Soybean meal	90	50	140	100
Distillers dried grains	65.7	65.7	45.5	45.5
Corn hominy feed	123	123	123	123
Gluten	23.3	23.3	23.3	23.3
Wheat bran	83.8	123.3	33.7	73.2
Molasses	31.6	31.7	31.6	31.7
Yellow corn meal	48.9	48.9	69.2	69.2
Rumen inert fat[b]	12.1	12.1	12.1	12.1
Urea	3.3	3.3	3.3	3.3
Salt	5.5	5.5	5.5	5.5
Dicalcium phosphate	2.2	2.2	2.2	2.2
Calcium carbonate	4.4	4.4	4.4	4.4
Vitamin-mineral supplement[c]	3.3	3.3	3.3	3.3
Sodium bicarbonate	5.5	5.5	5.5	5.5
Composition				
Dry matter, g/kg	391	454	391	454
Crude protein, g/kg DM	158	157	171	168
Organic matter, g/kg DM	914	909	913	910
ME[d], MJ/kg DM	10.9	10.8	11.2	11.1
NDF[e], g/kg DM	378	376	354	353
Acid-insoluble ash, g/kg DM	33.1	25.8	33.0	25.9

[a] Dry matter.
[b] Lactomil®, GHAT Nutrition, LLC, Corcoran, CA 93212.
[c] Provided (/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, 17 mg Monensin sodium, 5116 IU vitamin A, 708 IU vitamin D3, 29 IU vitamin E.
[d] Metabolisable energy.
[e] Neutral detergent fiber.

wk-3 of each period. Sampling was performed on days 2, 4, and 6 of wk-3. Samples (0.5 kg) of concentrate mixes, sorghum silage and cowpea hay were collected. Two 200 ml samples of milk per cow were taken from milking machines at 2 of the 3 daily milkings (07:00 and 23:00) and composited for analysis. Spot fecal samples of about 0.5 kg were taken on d-2, 4 and 6 of wk-3 directly from the rectum of each cow after the 07:00 milking. On the same days, perineal massage was used to stimulate urination to obtain 3 spot urine samples from each cow during each period; 5 ml of 2 M hydrochloric acid was added to 200 ml urine to lower the pH below 4. Individual samples were held at 4 °C while being transported to the laboratory and stored at −20 °C until analysed. Body weights were recorded using a cattle scale at 08:00 on the day before the experiment began and on the last day of each period.

2.3. Laboratory analysis

Milk composition was determined using a Lactostar® milk analyzer (Funke Gerber, Labortechnik GmbH, Berlin, Germany). Milk urea N (MUN) was analysed by spectrophotometry using the diacetyl monoxime method (Merck, Darmstadt, Germany). Efficiency of N capture in milk N was estimated by dividing milk N output by N intake as described by Olmos Colmenero and Broderick (2006). Feed ingredients, TMR and fecal samples were weighed and dried at 60 °C for 48 h for DM determination. Dried samples were ground to pass a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) and analysed for total N (Kjeltec®, Foss, Denmark), NDF with the method of Van Soest et al. (1991) using an Ankom® 200 fiber analyzer (Ankom Technology, Macedon, NY), and for ash by combustion in a muffle furnace at 600 °C for 8 h. Acid Insoluble Ash in feed and feces was determined to estimate apparent digestibility of DM, organic matter (OM), N and NDF using the method of Van Keulen and Young (1977).

Urine samples were analysed for creatinine by spectrophotometry (Spinreact®, Girona, Spain) and for total N (Kjeltec®, Foss, Denmark). The daily amount of N excreted in urine was estimated as: Urine volume, L/d = BW, kg × 29 mg/(kg BW*d)/(creatinine concentration, mg/L) as described by Valadares et al. (1999).

2.4. Economic comparisons

Mean prices paid at El Milagro dairy farm over the course of the trial (June to September 2013) for the feed ingredients are in Table 3 (J. R. Castillo, personal communication). These prices were used to compute costs of the 4 diets, in US dollars (USD; USD/kg DM; Table 6) and of daily ration consumed (USD/d). Income over feed cost (IOF) was computed by subtracting the cost of the diet from the gross income from milk production using mean milk price received during the trial (0.553 USD/kg).

Table 3
Mean feed prices paid at El Milagro dairy farm, June 2013 to December 2013.

Feedstuff	USD[a]/ton, as fed	DM, g/100 g	USD/kg DM
Sorghum silage	41.90	28.0	0.150
Cowpea (*Vigna sinensis*) hay	176.40	87.0	0.203
Soybean meal	703.40	90.0	0.782
Distillers dried grains plus solubles	471.87	91.0	0.519
Corn hominy feed	291.06	88.4	0.329
Corn gluten	326.30	89.7	0.364
Wheat bran	248.06	88.8	0.280
Molasses	138.92	73.0	0.190
Corn Meal	436.59	88.0	0.496
Rumen Inert Fat (Lactomil®)	771.75	99.0	0.780
Urea	554.56	99.0	0.560
Salt	122.38	99.5	0.123
Dicalcium phosphate	882.00	99.5	0.886
Calcium carbonate	57.33	99.5	0.058
Mineral salt	1754.08	99.5	1.763
Sodium Bicarbonate	500.54	99.5	0.503

[a] US dollar.

2.5. Statistical analysis

Results from the trial were analysed using the mixed procedures of SAS (2013). Analyses of production data from individual cow measurements (n = 32) used the following model:

$$Y_{ijkl} = \mu + S_i + P_j + T_k + C_l(Si) + S_i{*}P_{ij} + S_i{*}T_{ik} + P_j{*}T_{jk} + E_{ijkl},$$

where Y_{ijkl} = dependent variable, μ = overall mean, S_i = effect of square i (i = 1–8), P_j = effect of period j (j = 1–4), T_k = effect of dietary treatment k (k = 1–4), $C_l(Si)$ = effect of cow l (l = 1–4) within square i (i = 1–8), $S_i{*}P_{ij}$ = interaction of square i and period j, $S_i{*}T_{ik}$ = interaction of square i and treatment k, $P_j{*}T_{jk}$ = interaction of period j and treatment k, and E_{ijkl} = residual error. All terms were considered fixed, except for $C_l(i)$ and E_{ijkl}, which were considered random. Contrasts were used to evaluate the effects of cowpea supplementation, CP concentration, and the interaction of cowpea and CP. No square-by-period, square-by-treatment or period-by-treatment interactions were significant ($P \geq 0.17$), except period-by-treatment interactions ($P = 0.01$) for fecal DM, N excretion in urine and feces, and apparent DM and OM digestibility. Analyses of data from pen-based measurements (n = 4) used the following model:

$$Y_{ijk} = \mu + Pen_i + Per_j + T_k + E_{ijk},$$

where Y_{ij} = dependent variable, μ = overall mean, Pen_i = effect of pen i (i = 1–4), Per_j = effect of period j (j = 1–4), T = effect of dietary treatment k (k = 1–4), and E_{ijk} = residual error. Treatment (Tk) was considered a fixed effect and Pen_i, Per_j and E_{ij}, were considered random. Contrasts were used to evaluate the effects of cowpea supplementation and CP concentration. Differences were declared significant at $P \leq 0.05$ and trends were considered when $0.05 < P \leq 0.10$.

3. Results

3.1. Effect of diet on production and nutrient utilization

Compositions of the 2 major forage components of the diet are in Table 1 and experimental diets are in Table 2. Content of CP in cowpea was considerably higher than in sorghum silage (166 versus 84 g/kg) while NDF content was lower (464 versus 548 g/kg). Thus, 40 g/kg DM more wheat bran and 40 g/kg DM less soybean meal and 20 g/kg less distillers grains were fed in the cowpea diets to maintain equal dietary NDF and CP contents (Table 2). Production data are in Table 4. Adding cowpea hay to the diet increased Milk/DMI (1.50 versus 1.43) and Milk N/N intake (28.2 versus 26.8 g/100 g), and tended to reduce DMI by 0.5 kg/d, compared with diets containing only sorghum forage. Decreased MUN was also observed when cowpea hay was fed. The only effects observed when dietary CP was reduced from 170 to 155 g/kg were decreased MUN (19.1 versus 16.8 mg/dL) and increased milk N/N intake (26.5 versus 28.5). A cowpea hay by CP interaction was detected for milk fat content; however, the cowpea hay by CP interaction was not significant for milk fat yield. No significant effects of dietary treatment were observed for DMI, BW gain, milk yield, milk composition or yield of milk components (Table 4). Milk content and daily yield of fat, protein and lactose were not altered by the addition of 125 g/kg of cowpea hay or with decreasing CP from 170 to 155 g/kg in the diet.

3.2. Effect of diet on excretion and apparent digestibility of nutrients

Effects of diet on excretion and apparent digestibility are reported in Table 5. The substitution of cowpea hay for dietary sorghum

Table 4
Effect of dietary crude protein concentration and partial replacement of sorghum silage with cowpea (*Vigna sinensis*) hay and on milk yield and composition from lactating dairy cows.

Crude protein, g/kg		155	155	170	170		Contrasts[c]		
Trait Cowpea, g/kg	n[a]	0	125	0	125	SEM[b]	CP	Vig	CP*Vig
DMI[d], kg/d	4	19.6	19.6	20.5	19.6	0.25	0.14	0.12	.
BW[e] gain, kg	32	0.37	0.46	0.30	0.09	0.146	0.12	0.66	0.29
Milk yield, kg/d	32	28.0	29.3	29.1	29.1	0.77	0.35	0.19	0.23
3.5% FCM[f], kg/d	32	27.7	28.7	28.4	29.2	0.82	0.32	0.15	0.83
Milk/DMI	4	1.45	1.55	1.44	1.50	0.017	0.12	< 0.01	.
3.5% FCM/DMI	4	1.43	1.53	1.41	1.49	0.024	0.29	0.01	.
Fat, g/100 g	32	3.48	3.36	3.33	3.51	0.098	0.95	0.61	0.03
Fat yield, kg/d	32	0.96	0.99	0.98	1.02	0.035	0.38	0.17	0.78
Protein, g/100 g	32	3.13	3.11	3.15	3.15	0.028	0.15	0.65	0.46
Protein yield, kg/d	32	0.88	0.91	0.92	0.92	0.024	0.17	0.31	0.30
Lactose, g/100 g	32	4.98	4.91	4.95	4.99	0.046	0.47	0.64	0.12
Lactose yield, kg/d	32	1.39	1.43	1.44	1.45	0.038	0.23	0.36	0.58
MUN[g] mg/dL	32	17.4	16.1	19.6	18.6	0.33	< 0.01	< 0.01	0.54
Milk N/NI[h], g/100 g	4	28.1	30.1	25.9	27.3	0.39	< 0.01	< 0.01	.

[a] Number of observations per least squares mean.
[b] Standard error of the least squares mean.
[c] Probability of orthogonal contrasts: CP = dietary crude protein concentration; Vig = cowpea (*Vigna sinensis*) hay; CP*Vig = interaction of crude protein and cowpea (*Vigna sinensis*) hay.
[d] Dry matter intake.
[e] Body weight.
[f] Fat-corrected milk.
[g] Milk urea nitrogen.
[h] Nitrogen intake.

silage had no effect on estimated urine volume or urinary N as a proportion of N intake. Feeding cowpea also reduced fecal DM excretion by 1.4 kg/d and both amount and proportion of dietary N excreted in the feces, reflecting the higher nutrient digestibilities observed in this trial. Substitution of cowpea hay for dietary sorghum silage increased apparent N digestibility by 3.5 g/100 g and elevated apparent digestibility of DM, OM and NDF by 6–10 g/100 g. As expected, decreasing dietary CP from 170 to 155 g/kg (Table 5) decreased N intake (543 versus 496 g/d), excretion of urinary N (240 versus 199 g/d) and fecal N (152 versus 140 g/d), and trended to reduce urinary N as a proportion of N intake (44.9 versus 41.4 g/100 g). No changes were observed in apparent

Table 5
Effect of dietary crude protein concentration and partial replacement of sorghum silage with cowpea (*Vigna sinensis*) hay on N excretion and apparent digestibility in lactating dairy cows.

Crude protein, g/kg		155	155	170	170		Contrasts[c]		
Trait Cowpea, g/kg	n[a]	0	125	0	125	SEM[b]	CP	Vig	CP*Vig
N intake, g/d	4	490	479	560	528	6.1	< 0.01	0.01	.
Excretion, amount/d									
Urine volume, L/d	32	27.9	28.9	29.2	29.7	1.17	0.24	0.43	0.79
Urinary N, g/d	32	202	195	250	230	7.7	< 0.01	0.06	0.42
Fecal DM, kg/d	32	7.50	6.25	7.95	6.30	0.143	< 0.01	< 0.01	0.03
Fecal N, g/d	32	150	130	164	139	2.9	< 0.01	< 0.01	0.32
Excretion, g N/100 N intake									
Urinary nitrogen	4	41.6	40.3	44.5	44.5	1.47	0.05	0.70	.
Fecal nitrogen	4	30.5	27.1	29.4	26.2	0.63	0.15	< 0.01	.
Total nitrogen[d]	4	100.2	97.5	99.8	98.0	1.04	0.97	0.07	.
Apparent digestibility, g/100 g									
Dry matter	32	61.2	67.9	61.0	67.8	0.35	0.68	< 0.01	0.98
Organic matter	32	64.6	70.6	64.4	70.4	0.31	0.41	< 0.01	0.96
[e]NDF	32	42.4	52.1	41.5	52.5	0.68	0.68	< 0.01	0.31
Nitrogen	32	69.3	73.2	70.2	73.1	0.43	0.31	< 0.01	0.21

[a] Number of observations per least squares mean.
[b] Standard error of the least squares mean.
[c] Probability of orthogonal contrasts: CP = dietary crude protein concentration; Vig = cowpea (*Vigna sinensis*) hay; CP*Vig = interaction of crude protein and cowpea (*Vigna sinensis*) hay.
[d] Total N recovered as milk N, fecal N plus urinary N as a proportion of N intake.
[e] Neutral detergent fiber.

Table 6
Effect of dietary crude protein concentration and partial replacement of sorghum silage with cowpea (*Vigna sinensis*) hay on feed cost, milk income and net profit in lactating dairy cows.

Crude protein, g/kg		155	155	170	170		Contrasts[c]	
Trait Cowpea, g/kg	n[a]	0	125	0	125	SEM[b]	CP	Vig
Cost, USD[d]/kg DM	.	0.305	0.291	0.329	0.316	.	.	.
Milk income, USD/cow/d	4	15.44	16.26	16.27	16.23	0.175	0.06	0.07
Feed cost, USD/cow/d	4	5.91	5.55	6.74	6.21	0.071	< 0.01	< 0.01
Feed cost, USD/kg milk	4	0.212	0.189	0.229	0.212	0.0022	< 0.01	< 0.01
IOF[e], USD/cow/d	4	9.54	10.72	9.53	10.02	0.148	0.05	0.01
Income/Feed cost ratio	4	2.62	2.94	2.41	2.62	0.030	< 0.01	< 0.01

[a] Number of observations per least squares mean.
[b] Standard error of the least squares mean.
[c] Probability of orthogonal contrasts: CP = dietary crude protein concentration; Vig = cowpea (*Vigna sinensis*) hay.
[d] US dollar.
[e] Income Over Feed Cost.

digestibility of DM, OM, NDF and N due to altering dietary CP content.

3.3. Effect of diet on economic parameters

Differences were observed with the addition of 125 g/kg of cowpea hay on several economic parameters (Table 6). Replacing dietary sorghum silage with cowpea hay decreased feed cost per cow and per kg of milk produced and increased IOF by 0.83 USD/d. A trend for reduced milk income was detected when CP was reduced; however, milk income on the diet containing 155 g CP/kg DM plus cowpea hay was nearly identical with that on the 2 higher CP diets. Decreasing dietary CP from 170 to 155 g/kg DM improved the economic outcome: feed cost/cow (6.48 versus 5.73 USD/d) and feed cost per kg of milk (0.221 versus 0.201 USD) were both decreased, while IOF was improved (9.78 versus 10.13).

4. Discussion

In this study, substituting cowpea hay for 125 g/kg of dietary DM from sorghum silage (which was 250 g/kg of the dietary forage DM) improved milk/DMI, FCM/DMI and Milk N/N intake (Table 4). The study also indicated that decreasing dietary CP from 170 g/kg to 155 g/kg improved Milk N/N intake without reducing yield or altering milk composition; MUN was also reduced. Thus, ration cost could be reduced without negative impact on production variables in Holstein cows under tropical conditions. Replacing lower CP, higher NDF sorghum silage with cowpea hay made possible the reduction of dietary soybean meal and distillers grains; as mentioned earlier, wheat bran, a non-forage fiber source, was also added to the diet to equalize NDF. It is noted that cowpea forage was fed as hay, rather than silage, in the current trial at the expense of sorghum silage, which may help explain the improved milk/DMI and Milk N/N intake observed. Utilization of CP from hay is often greater than utilization of CP from silage. For example, Broderick (1995) observed in one of two trials that yield was 1.9 kg milk/d and 100 g milk protein/d greater when dairy cows were fed alfalfa forage as hay rather than silage. However, there were additional changes in protein supply in the present study when cowpea hay was substituted for sorghum silage: diets containing cowpea hay had 10 g/kg and 20 g/kg less CP from, respectively, sorghum silage and soybean meal, and 21 g/kg and 8 g/kg more CP from, respectively, cowpea hay and wheat bran. At DM intake = 4 kg/100 kg BW, tabulated RUP values for sorghum silage, soybean meal, mid-maturity legume hay (as a proxy for cowpea hay) and wheat bran were listed at, respectively, 42, 43, 19 and 21 g/100 g CP (National Research Council, 2001). This suggested that dietary alterations would have reduced RUP supply and the improved productivity observed in this trial was due to other factors.

Feeding cowpea hay led to greater N utilization at least partly because of improved forage quality and improved digestibility. When cowpea hay replaced one-fourth of the dietary sorghum silage apparent digestibility increased about 4 units for CP, 6 units for DM and OM, and more than 10 units for NDF (Table 5). Fecal N excretion is influenced by DMI and fecal DM output per unit of intake (Dorgeloh et al., 1998), which were both reduced when cowpea hay was fed in the current trial; this likely explains part of the improvement in apparent CP digestibility. Sawar et al. (1998) reported higher rate and extent of *in situ* DM digestibility for the legumes *Trifolium alexandrinum, Medicago sativa* and *Vigna sinensis* compared to the grasses *Zea mays, Panicum milliaceum* and *Sorghum vulgare*. Tropical legumes such as cowpea have been shown to have greater *in vivo* ruminal DM digestion (Foster et al., 2009a) and *in situ* DM and NDF digestibility compared to bahiagrass (Foster et al., 2011). Cowpea had higher *in vitro* OM degradability and gave rise to higher average daily gain in grazing beef calves compared to bahiagrass (Vendramini et al., 2012); cowpea fed to lambs as haylage also increased N apparent digestibility and N retention compared to Bahiagrass and Pigeonpea (Foster et al., 2009b). Although we found no reports of feeding cowpea hay to lactating dairy cows, the improved apparent digestibility we observed may be related to increased ruminal OM fermentation stimulating microbial protein growth and, thus, increasing metabolizable protein supply (National Research Council, 2001).

Increasing dietary CP has been shown to have positive effects on milk production until a point is reached beyond which yield and efficiency may decline. Broderick (2003) reported that increasing dietary CP from 151 to 167 g/kg increased milk yield from 33.0 to

34.1 kg/d, but further increase to 183 g/kg CP reduced DM and N efficiency without altering production. A similar limit to the positive effects of dietary CP on production was shown by Olmos Colmenero and Broderick (2006), who observed that milk yield increased from 36.3 to 38.8 kg/d when the diet was increased from 135 to 165 g CP/kg DM, but milk yield declined at 179 and 194 g CP/kg DM. Nadeau et al. (2007) also found no differences in milk yield or N utilization when diets containing 160 and 170 g CP/kg DM were compared in Swedish Red dairy cattle. In a study with dairy cows fed forage at 4 ratios of alfalfa silage to corn silage ranging from 100:0 to 25:75 (proportion of forage DM), and 4 protein levels ranging from 150 to 188 g CP/kg DM, Groff and Wu (2005) also did not observe consistent differences in milk composition or yield due to dietary composition. Castillo et al. (2001) reported an increase in N utilization for milk secretion from 23.6 to 26.1 g milk N/100 g N intake when dietary CP was reduced from 190 to 150 g/kg, leading to a comparable decrease in urinary N excretion. Results from these studies and our observation of improved N utilization when reducing dietary CP indicate that Salvadoran dairy farmers can substantially reduce dietary CP without losing production (Zavala et al., 2012).

Dietary CP concentration was without effect on apparent nutrient digestibility in the current trial (Table 5). Sufficient amounts of degradable protein are required for microbial activity in the rumen (National Research Council, 2001), indicating that 155 g CP/kg DM was adequate for ruminal microbial digestion. In a study with 3 levels of NDF (28, 32 and 36 g/100 g) and 3 levels of CP (15.1, 16.7 and 18.4 g/100 g), Broderick (2003) found that DM and OM digestibility did not change with dietary CP but, as expected, that DM and OM digestibility decreased with increased dietary fiber.

As expected, MUN increased with dietary CP content, reflecting reduced N efficiency when feeding higher CP. Mean MUN levels were elevated on all diets in this study (16.6 mg/dL at 155 g CP/kg DM and 19.1 mg/dL at 170 g CP/kg DM) compared to values at similar levels of CP from other reports (Nousiainen et al., 2004; Olmos Colmenero and Broderick, 2006). In feeding trials with high producing cows, Olmos Colmenero and Broderick (2006) found that increasing dietary CP from 135 to 194 g/kg linearly increased MUN from 7.7 to 15.6 mg/dL. The high MUN values observed in the present study are probably related to the average consumption of 68 g/d of urea on all 4 diets (Table 2). It is well known that urea is an NPN source that is often poorly utilized in the rumen of dairy cows and its lower efficiency likely contributed to elevated MUN. A potential benefit of monitoring MUN is to identify diets in which dietary protein supplementation can be reduced, which would lower feed costs and improve profitability (Godden et al., 2001). Both Nousiainen et al. (2004) and Groff and Wu (2005) observed reduced MUN and higher milk N efficiency at lower dietary CP concentrations, which is consistent with our findings.

Dietary protein content is the most important factor determining milk N efficiency, urinary N losses, and consequently, ammonia emissions from dairy cow manure (Powell et al., 2011; Hristov and Giallongo, 2014). Our data showed that reducing dietary CP from 170 to 155 g/kg did not impair milk or component yield but decreased N excretion as well as feed costs. In previous studies, increased loss of N to the environment was observed as dietary CP increases. When dietary CP decreased from 180 to 165 g/kg, Wattiaux and Karg (2004) found similar milk yield and fecal N excretion but a significant reduction in urinary N. Similarly, Olmos Colmenero and Broderick (2006) evaluated 5 diets with different levels of CP from 135 to 194 g/kg and showed that amounts of urinary N that rose linearly from 113 to 257 g/d.

In the present study, N efficiency (milk N/N intake) ranged from 0.26 to 0.29 and N excretion in feces and urine ranged from 0.71 to 0.74 of N intake. Powell (2014) reported average milk N utilization of 0.23 (range 0.181–0.325) in Wisconsin dairy herds, which was very much higher than that reported for Sub-Saharan Niger systems of 0.041 (range 0.015–0.18). The N utilizations observed in our trial may be interpreted as evidence that acceptable N efficiency can be achieved in dairies in the tropics when conditions exist for balancing diets and controlling heat stress.

In part because less dietary soybean meal and distillers grains were needed to equalize CP, replacing sorghum silage with cowpea hay lowered ration cost and feed cost per kg of milk and, thus, IOF improved at similar milk yield. Reduced expenditures for soybean meal and distillers grains also is why feed costs were reduced and IOF increased when dietary CP was reduced from 170 to 155 g/kg DM. Adding legume forages to the diet has been shown to be valuable under a number of conditions. In temperate European countries, feeding legumes such as white clover, red clover, alfalfa and lotus species increased both profitability and production (Wilkins, 2001). Incorporating tropical and subtropical legumes such as *Dollichus, Trifolium* and *Leucaena* into the diet increased nutrient intake, although they were most effective when the basal diet was of lower digestibility (Poppi and Norton, 1995). Feed is the principal cost component in dairy herds for both heifers (Gabler et al., 2000) and cows (Salfer, 2010; Buza et al., 2014); therefore, diet optimization is essential for satisfactory economic outcomes. Although gross income did not increase in the present study with the feeding of the legume forage, its addition significantly reduced ration cost and increased IOF.

Roseler (1990) estimated a yearly cost of $23.6 million ($0.09/cow per day) for the dairy industry in New York State because of excess of protein feeding. Concentrations of MUN were reduced in the current trial when N utilization was improved due to lowering dietary CP content and feeding cowpea hay. Roseler (1990) suggested that MUN concentrations could be used to identify diets with excessive CP and, using this approach, a potential payback of as much as $3.96/cow could be obtained. However, Buza et al. (2014) collected IOF data from 95 dairy herds in Pennsylvania from 2009 to 2012 and found that high feed costs were also associated with increased production and suggested that optimal formulation rather than low-cost strategies was the key to improved production and profitability. Thus, IOF and profit margins may be affected more by feed quality than by ingredient cost alone.

5. Conclusions

Inclusion of cowpea hay as a dietary forage ingredient under tropical conditions reduced the need for high-cost protein ingredients without lowering DM intake and milk production or altering milk composition in dairy cows in this experiment. Feeding cowpea hay also improved feed efficiency, apparent digestibility of DM, OM, NDF and N, and IOF, while reducing urinary and fecal N excretion. Decreasing CP from 170 to 155 g CP/kg did not alter milk yield, milk composition, feed efficiency or nutrient digestibility, but decreased MUN, fecal and urinary N excretion and increased N efficiency and IOF. These results show that optimizing dietary forage and CP content will have positive economic and environmental impacts on dairy production under tropical conditions.

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