PRODUCTIVE RESPONSE OF LACTATING DUAL-PURPOSE COWS GRAZING IN AN AGROSILVOPASTORAL SYSTEM DURING THE DRY SEASON SUPPLEMENTED WITH LOW LEVELS OF CRUDE PROTEIN †

[RESPUESTA PRODUCTIVA DE VACAS DOBLE PROPÓSITO EN LACTANCIA PASTOREANDO UN SISTEMA AGROSILVOPASTORIL EN LA ÉPOCA DE ESTIAJE SUPLEMENTADAS CON BAJOS NIVELES DE PROTEÍNA CRUDA]

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SUMMARY

Background. Cattle production in tropical regions of Mexico is in dual-purpose farms (DP) on unintended agrosilvopastoral systems (ASPS) with pastures dominated by tropical grasses with scattered shrubs and trees. During the dry season due to the forage diminished nutritional value and availability most farmers supplement their cattle to sustain milk production and weight gains of calves, without taking into consideration the wide variety of forages available in grazing lands to decide the composition of supplements offered to their cattle. **Objective/hypothesis.** The objective of the study was to determine the effect of low levels of crude protein (CP) content in supplements (S10 = 100, S11 = 110 and S12 = 120 g CP/kg of DM) on the performance of lactating Brown Swiss (BS) cows on an ASPS during the dry season. We hypothesize that low levels of CP in supplements of lactating grazing cows on an agrosilvopastoral system during the dry season would not affect animal performance. **Methodology.** The study was carried out in the dry season (March to June of 2012), in a commercial DP in the State of Mexico. Eighteen multiparous BS cows and their calves were used in the study, randomly allocated to three groups (six cows/group), to receive one of three supplements (4.5 kg DM/cow/day), consisting of 100, 110 or 120 g/kg DM of crude protein (CP) S10, S11 and S12, respectively. Data were analysed as a complete random experimental design with a mixed model. **Results.** There were no significant differences in most of the animal performance variables ($P > 0.05$), except for fat protein corrected milk (FPCM) where S12 (6.4) was significantly higher than S10 and S11 (4.7 and 4.5 kg/day, respectively). Milk protein yields (kg/day) significantly increased as the CP level increased in supplements. High levels of milk urea nitrogen (MUN) were detected (mean 14.0 mg/dL). **Implications.** In similar DP farms where cattle have access to other sources of forages like shrubs and trees, it is possible to reduce the CP content of supplements and to increase the efficiency of utilization of those alternative forages as well as a reduction in supplementation costs. **Conclusion.** There were no significant differences in the performance of BS cows grazing on an ASPS during the dry season receiving supplements with low crude protein levels. When correcting to fat-protein milk yields a significant difference was found in favour of supplement with 120 g CP/kg of DM.

Key words: Tropical cattle performance; supplementation; dry season.

RESUMEN

Antecedentes. La producción ganadera en las regiones tropicales de México se realiza en unidades de producción de doble propósito (DP) en sistemas agrosilvopastoriles no intencionados (SASP) con praderas dominadas por pastos tropicales, con arbustos y árboles dispersos. Durante la época de estiaje, debido a la disminución del valor nutritivo y disponibilidad del forraje, la mayoría de los ganaderos suplementan a su ganado para mantener la producción de leche y la ganancia de peso de los becerros, sin tener en cuenta la calidad del forraje disponible en las praderas para decidir la composición de los suplementos ofrecidos al ganado. **Objetivo/hipótesis**. El objetivo del presente estudio fue

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determinar el efecto de niveles bajos de proteína cruda (PC) en tres suplementos (S10 = 100, S11 = 110 y S12 = 120 g PC/kg de MS) en el desempeño productivo de vacas Pardo Suizo (PS) lactantes pastoreando en un SASP durante la estación seca. Nuestra hipótesis es que la suplementación con niveles bajos de PC vacas de doble propósito en lactación pastoreando en un SASP durante la estación seca no afecta su comportamiento productivo. **Metodología**. El estudio se realizó durante la época poco lluviosa (marzo a junio de 2012) en una unidad de producción comercial de DP del Estado de México. En el estudio se utilizaron dieciocho vacas de raza PS multíparas con sus crías, distribuidas aleatoriamente en tres grupos (seis vacas/grupo), para recibir uno de tres suplementos (4.5 kg MS/vaca/día), conteniendo 100, 110 o 120 g/kg MS de proteína cruda (PC) S10, S11 y S12, respectivamente. Los datos se analizaron mediante un diseño experimental de bloques completos al azar con un modelo mixto. **Resultados**. No hubo diferencias significativas en la mayoría de las variables de rendimiento animal (P > 0,05), excepto en la producción de leche corregida por grasa y proteína (LCGP), donde S12 (6.4 kg/vaca/día) fue significativamente mayor que S10 y S11 (4.7 y 4.5 kg/vaca/día, respectivamente). El rendimiento de proteína en leche (kg/día) aumentó significativamente a medida que aumentó el nivel de PC en el suplemento. Se detectaron niveles elevados de nitrógeno ureico en la leche (NUL) (14.0 mg/dL en promedio). **Implicaciones**. En unidades de producción DP de características similares a las de este estudio, donde el ganado accede a otras fuentes de forrajes como arbustos y árboles, se puede reducir el contenido de PC de los suplementos y aumentar la eficiencia de uso de esos forrajes alternativos y reducir los costos de suplementación. **Conclusión**. No hubo diferencias significativas en el desempeño de vacas PS pastoreando en un SASP durante la época de estiaje cuando fueron suplementadas con 120 g PC/kg de MS.

Palabras clave: Rendimiento de ganado en condiciones tropicales; suplementación; época de estiaje.

INTRODUCTION

The south of Estado de México, as well as the centre of the country, is dominated by high mountains with steep slopes where soil conditions are challenging to establish crops, therefore cattle production is the best way to use land resources. The steep slopes and the presence of stones in pastures avoid any forage conservation practices; therefore, for farmers the best way to preserve forages is on the pastures (unharvested forage) (Albarrán-Portillo *et al.*, 2015).

In the southwest subtropical region of the State of Mexico, cattle production is performed in dual-purpose farms (DP) (milk and beef) on unintended agrosilvopastoral systems (ASPS) where pastures are dominated by African Star grass (*Cynodon plectostachyus*) with scattered shrubs and trees at different stages of secondary vegetation. Grasslands are managed extensively with no fertilization, and neither sub-division nor rotations. At this time of year, cattle feed exclusively on grasses, forbs, shrubs and trees present in ASPS. While, in the dry season when forage is scarce and of low quality, farmers must supplement their cattle to sustain milk yields and body weight (BW), as well as the weight gain of calves (Albarrán-Portillo *et al.*, 2019).

During the dry season, farmers supplement lactating cows with concentrates that range from 4 to 9 kg/cow/day (fresh basis), depending on the forage availability on pastures, with crude protein (CP) levels that range from 14 to 18% of commercial concentrates (CC) (Albarrán-Portillo *et al.*, 2015).

Sources of crude protein like soybean meal, are the most expensive ingredients in the supplements offered to dairy cattle. Overfeeding with CP has economic implications increasing milk production costs, as well as negative environmental impacts (Jonker *et al.*, 2002). Dairy cattle excrete two to three times more N in faeces than in milk, resulting in low N efficiency; causing environmental pollution as ammonia and nitrous oxide volatilization into the environment, nitrate leakage to underground water, and N runoff in surface water (Tamminga, 1992). This low milk N efficiency is mainly due to overfeeding dietary crude protein. A positive relationship between crude protein intake and N excreted in urine has been established (Kebreab *et al.*, 2002). It has been well documented in confined intensive dairy systems that 14 vs 16% of CP levels in lactating cow´s diets do not affect dry matter intake, milk production, and milk composition even in high-yielding cows (Barros *et al.*, 2017; Zanton, 2019).

Dual-purpose cows in tropical and subtropical regions, reduce their nutritional intake due to heat stress, and therefore their nutritional requirements decrease too (Broderick, 2007). Esparza-Jiménez *et al.* (2020) found high levels of milk urea nitrogen (MUN) (25 ml/dL) in cows grazing on ASPS, receiving two levels of CP in supplements (14 and 16%). These high levels of NUL might have been the result of an additive effect of CP in supplements and CP from fodder legumes (shrubs and trees) consumed while grazing low-quality African Star grass (*Cynodon plechtostachyus*) during the dry season.

Salas-Reyes *et al.* (2022) documented that woody species like *Vachellia* (formerly *Acacia*) *farnesiana*, *Crescentia alata,* and *Pithecellobium dulce* contributed 20% to the CP requirements of lactating cows during the dry season in the same farm as Esparza-Jiménez *et al.* (2020). Based on this study, it was hypothesized that low levels of CP in the supplementation of lactating grazing cows on an agrosilvopastoral system during the dry season would not affect animal performance. Therefore, the objective of the study was to evaluate the effect of supplementing low levels of crude protein (CP) (100, 110 and 120 g CP/kg of DM) on the performance of lactating Brown Swiss cows on an agrosilvopastoral system during the dry season of the year.

MATERIALS AND METHODS

Description of the study area

The study was carried out during the dry season (March-June) of 2022 in the municipality of Zacazonapan, located in the southwest of the State of Mexico, at 19°04´48" North and 100°13'18" West, and an altitude of 1,470 meters above sea level. The main climate is subtropical (warm sub-humid), with an average annual temperature of 23°C, an average maximum of 31°C, and an average minimum of 15°C, and an average annual precipitation of 1,115 mm (SMN, 2020) (Figure 1).

Experimental production unit and cattle management

The experiment was performed on a dual-purpose farm with similar characteristics to farms in the region, with 100 ha of land, and 80 head of Brown Swiss (BS) cattle, composed of milking cows, replacement heifers (< 6 months old), and two Brown Swiss sires. The stoking rate was 0.8 cow/ha/year (Allen *et al.*, 2011). Infrastructure is limited to a perimeter fence, a barn to store the supplements, and a shed where cows are milked.

Milking was done by hand once a day (7:00 to 9:00 h), and while the cows were milked, they ate supplements. After milking, calves remained with their dams until 14:00 h, then separated into a fenced pasture until milking the following morning. After being separated from their calves, cows grazed until the next morning. Cows grazed in the ASPS 24 h a day throughout the year, except during milking and calf nursing 24 hours. Clean water and minerals were always available to the cattle. All cows are dried-off two months before the expected calving date and are reincorporated one month after calving to the milking herd. This management is the norm in the region to guarantee calf survival rate and weight gain.

Extensive grazing management is the norm under the agrosilvopastoral system (ASPS) in the region, where corn stubble, introduced and native grasses, shrubs and scattered trees in the paddock provided forage for livestock throughout the year. African Star grass (*Cynodon plectostachyus*) was the predominant grass in the pasture, which has been previously described by Albarrán-Portillo *et al.* (2019) and (Salas-Reyes *et al.*, 2022).

Grazing and pasture measurements

Herbage mass (HM) (kg DM/ha) in grazing areas was estimated at 2-week intervals for two consecutive days, by sampling a 0.5 x 0.5 (0.25 m^2) quadrat (n=5), adjacent to where cows were grazing. Botanical composition, live and dead material, leaves and stems present in the quadrats expressed as kg DM/ha. Pasture height was estimated by taking five measurements in each quadrat with a ruler.

Experimental cows and treatments

Eighteen cows and their calves were selected for this study. Experimental cows had mean body weight (BW) of 405 ± 50 kg (mean ± standard deviation), 3 ± 2 calving, and 98 ± 33 days in milk (DIM); while the calves had 99 ± 22 kg initial BW. Cows were randomly distributed into three groups (six cows/group), and each

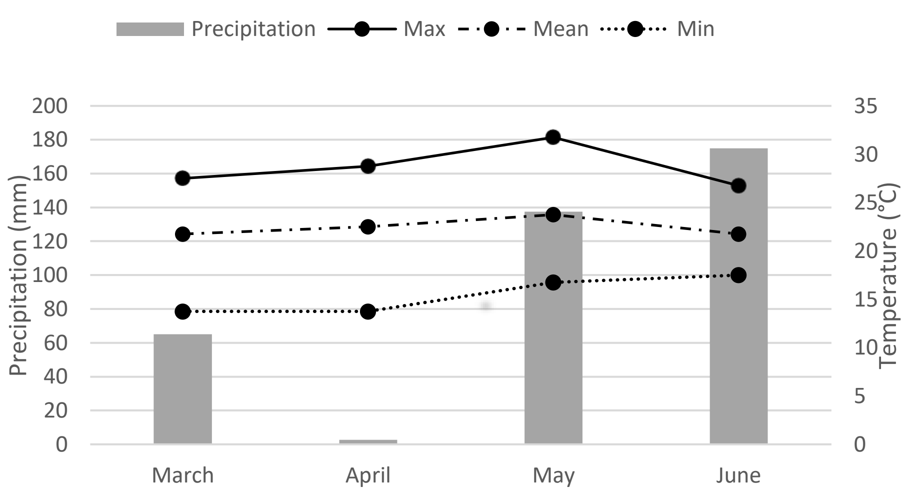

Figure 1. Rainfall and maximum and minimum temperatures (°C) in the southwest of the State of Mexico.

each group received one of three supplements, consisting of 4.5 kg DM/cow/day of a concentrate containing 100, 110 or 120 g CP/kg DM corresponding to treatments S10, S11 and S12, respectively. The ingredients used in the supplements are shown in Table 1. The experiment lasted 77 days (from March to June of 2012), divided into five experimental periods (EP). The first experimental period consisted of two weeks for the adaptation period and the third week for the first sampling; afterward, each EP was of two weeks.

Table 1. Proportion of ingredients (%) and nutritional composition of suplements with three levels of crude protein 100 (S10), 110 (S11) and 120 (S12) (g CP/kg DM).

Item	S10	S11	S12	Pastures
Cracked maize	93.8	92.2	88.3	
Soybean meal	6.2	7.8	11.7	
Urea	0.014	0.014	0.014	
Composition				
DM	941.2	945.3	943.0	580.0
CP	102.0	109.0	121.0	79.1
NDF	348.1	353.3	347.2	655.1
ADF	114.7	105.8	108.2	373.6
ADL	12.8	14.0	12.2	41.6
ME (MJ/kg/DM)	12.9	11.6	11.2	7.2

DM = Dry matter, CP= crude protein, NDF= Neutral Detergent Fibre, ADF = Acid Detergent Fibre, ADL = Acid Detergent Lignin, ME= Metabolizable Energy.

Measurements and samples

Milk yield (MY) was weighted on a 20 kg clock spring scale and recorded for two consecutive days in the second week of each EP. Cows were weighed on a portable electronic scale at the beginning of the experiment, and at the end of each EP. Body condition score (BCS) was determined on the same day of weighing using the 1 to 5 scale (Wildman *et al.*, 1982). Calves were weighed after their mothers were milked. Body weights and BCS records during the last week of each period were used in the analysis.

Milk samples were taken in two consecutive milking and milk components (g/kg) were determined with a portable automatic ultrasound analyser (Lactoscan Milk Analyzer ®). Afterward, milk subsamples were frozen in the laboratory for the determinations of milk urea nitrogen (MUN). The analysis of milk urea nitrogen was performed by enzymatic colorimetry. Fat and protein corrected milk (FPCM) was estimated by the following equation: FPCM = milk yield (kg/day) x 0.1226 x fat % + 0.0776 x true protein % + 0.253. This parameter allows a fair comparison between farms or between cows with different feeding regimes (IDF, 2010). The feed efficiency was estimated by dividing MY or FPCM by DMI.

Chemical analysis

Samples of supplements and grass were taken from on two consecutive days during the second week of every EP and dried at 55°C to constant weight to determine DM. Feed samples were also analysed for ashes, crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF). The *in vitro* gas production technique of Theodorou *et al.* (1994) modified by Mauricio *et al.* (1999), was used to estimate the *in vitro* DM digestibility (IVDMD). Estimated Metabolizable Energy (eME) was obtained from the IVOMD (AFRC, 1993): eME (MJ/kg DM) = 0.0157 *IVOMD.

Economic analysis

To determine the cost of milk production, an economic analysis was performed using partial budgets, considering the cost of supplementation, fuel and hired labour, according to Harper (2013).

Prediction of animal response variables

The NASEM Dairy-8 (2021) program was used to predict dry matter intake (DMI) from the performance of S10, S11 and S12 groups of cows, using the nutritional composition of forage and supplement, as well as averages of BW, BCS, MY, and milk fat and protein composition. Results obtained from the NASEM Dairy-8 program net energy of lactation (NE_L, MJ/day), metabolizable protein (MP, g/day), diet crude protein content, milk allowed by NE_L and milk allowable by MP were compared to cow nutritional requirements and diet nutrients supply as a function of supplements CP levels.

Experimental design

For the study, a completely randomized experimental design was implemented to evaluate the effects of supplements on animal response variables. Data were analysed using SAS mixed model procedures (SAS, 2021), using the following model.

$$Y_{ijkl} = \mu + S_i + EP_j + C_k + \varepsilon_{ijk}$$

where:
μ = overall mean, S_i was the fixed effect of supplements offered (i = 100, 110, and 120 g CP/kg DM), EP_j was the fixed effect of experimental evaluation periods (j = 1, 2…5), C_k was the random effect of cows (k = 1, 2…18) and ε_{ijk} was the residual effect of the random term. Significant differences

between means were compared by Tukey´s test (P < 0.10).

RESULTS

A total rainfall of 820 mm was recorded during the experiment. Mean, maximum and minimum temperatures were 22.4, 28.7 and 15.4 °C. The highest temperature was 31.7°C which was recorded during May (Figure 1).

The available HM in grazing areas showed variations depending on the grazing site on the day of sampling (Table 2). Of the amount of HM (1,882 kg/DM/ha) 64% was senescent herbage, whereas green herbage and leaves accounted 36 and 35%, respectively, with a mean grass height of 2.8 cm. The botanical composition of the grazing area was composed of 95% African Star grass (*C. plectostachyus*) and 5% Signal Grass (*Urochloa plantaginea*).

Table 3 shows the response of the animals to the supplements. There were no significant differences (P > 0.05) for the effect of supplements, except for FPCM (P = 0.01) and protein yield (P < 0.001). Fat-protein-corrected milk (FPCM) of S10 (4.7) and S11 (4.5 kg/day) were statistically similar but lower than S12 (6.0 kg/day). Protein yield increased as CP in supplements increased from 0.175 to 0.186 and 0.205 (kg/day) in S10, S11 and S12, respectively.

The mean DMI and milk yield were 12.4 and 5.8 (kg/day), respectively. Milk fat yield (k/day) showed a tendency to increase (P = 0.06) as CP in the supplements increased. Likewise, milk protein content (g/kg) (P = 0.09), lactose (g/kg) (P = 0.07) and milk lactose yield (kg/day) (P = 0.09) showed similar trends.

Table 2. Herbage mass and morphological composition of pastures (kg DM/ha) by experimental period (EP).

Item	EP1	EP2	EP3	EP4	EP5	Average	%
Herbage mass	848	3,904	1,792	1,208	1,656	1,883	
Green herbage	611	519	199	760	1,142	686	36
Dead herbage	237	3,385	1,393	448	514	1,195	64
Leaves	507	393	936	667	737	653	35
Stems	340	3,511	829	540	918	1,228	65
Height (cm)	2.2	3.8	2.4	2.6	2.8	2.8	

Table 3. Response variables of lactating Brown Swiss cows to supplemented with three levels of crude protein 100 (S10), 110 (S11), and 120 (S12) (g CP/kg DM), grazing in an agrosilvopastoral system.

Item	S10	S11	S12	P =	SEM
DMI (kg/day)	11.8	13.0	12.5	0.08	0.30
Milk (kg/day)	5.3	5.8	6.4	0.20	0.42
FPCM (kg/day)	4.7[a]	4.5[a]	6.0[b]	0.01	0.39
Fat (g/kg)	32.4	36.5	37.8	0.42	3.09
Fat (kg/day)	0.167	0.191	0.242	0.06	23.8
Protein (g/kg)	30.4	28.3	30.5	0.09	0.51
Protein (kg/day)	0.175[a]	0.186[ab]	0.205[b]	<0.001	7.61
Lactose (g/kg)	43.2	40.7	43.2	0.07	0.70
Lactose (kg/day)	0.234	0.224	0.277	0.09	8.84
Fat/Pro ratio	1.1	1.3	1.2	0.35	0.09
Fat/Lac ratio	1.1	0.8	0.9	0.76	0.15
MUN (mg/dL)	13.7	14.5	13.9	0.92	1.23
BW (kg)	391	428	396	0.34	23.19
BW change (kg/day)	0.05	0.17	0.25	0.82	0.24
BCS	2.6	2.5	2.5	0.33	0.06
CBW (kg)	115	112	115	0.96	5.4
CBWc (kg/day)	0.41	0.27	0.23	0.60	0.13
FE (MY/DMI)	0.57	0.48	0.53	0.69	0.07
FE (FPCM/DMI)	0.53	0.38	0.50	0.85	0.10

[a,b,c] = Means with different literals between columns show significant differences (P > 0.05).

The fat-to-protein (Fat/Pro) and fat-to-lactose (Fat/Lac) ratios were 1.2 and 0.93 ($P > 0.05$). Milk urea nitrogen mean was 14.02 (mg/dL) ($P > 0.05$), while body weight, body weight change, and BSC of cows were 405 (kg), 0.16 (kg/day) and 2.5, respectively ($P > 0.05$). Body weight of calves and BW change means were 114 (kg) and 0.30 (kg/day), respectively ($P > 0.05$). Feed efficiency was 0.53 kg for MY/DMI, and 0.47 kg for FPCM/DMI, respectively ($P > 0.05$).

There were significant differences ($P < 0.001$) due to experimental periods in some of the variables analysed except for DMI ($P = 0.42$), Fat/Lac ratio ($P = 0.48$) and BW and BCS of cows ($P > 0.05$). In general, the values of the response variables tended to increase as the experiment progressed, with few exceptions. Feed efficiency was not significantly affected by the level of CP in the supplement, averaging 0.54 and 0.49 kg of MY/DMI and FPCM/DMI, respectively. However, feed efficiency was numerically but not significantly higher ($P > 0.05$) in EP5 with 0.77 for MY/DMI and 0.79 for FPCM/DMI. Interactions between supplement and EP were not significant except for milk fat and protein yield ($P < 0.001$).

Economic analysis

Table 4 shows the partial economic analysis, where the estimated cost of milk production was 0.38 (S10), 0.36 (S11) and 0.33 (S12) (USD $/kg). The structure of the cost of milk production indicates that supplements represented ~66% of the production cost, in second place were the cost of hired labour with 27% and fuel 9%. Cows eating supplements S11 and S12 produced 2 and 4% higher milk production than S10, respectively. Similarly, milk sales revenue was 1 and 6% higher when cows ate the S11 and S12 supplements, respectively, than when supplemented with S10. Milk production cost for the S11 and S12 supplementation strategies were 5 and 14% lower than S10.

Nutrient prediction using the NASEM program

Table 5 shows the predictions of nutrients required according to cow performance at the three CP levels supplemented. These calculations did not consider milk consumed by the calves. The NE_L balance was positive in the three supplementation strategies, averaging 13.3 (MJ/day). Similarly, there was a positive MP balance for the three supplements with a mean of 197.3 (g/day).

The predicted CP in the diet was 10.4, 10.4, and 10.9%, of which 76% was rumen degradable protein (RDP) and remaining 24% was rumen undegradable protein (RUP). The predicted net energy of lactation (NEL) for milk production averaged 10.6 (kg/day) which is 4.8 (kg/day) more than the average of 5.7 (kg/day) of MY observed. As for allowable milk MP the average was 10.5 (kg/day) which is 4.8 kg above MY.

DISCUSSION

Environmental conditions were hot and dry from during March, April and May, which had a negative impact on response variables, but at the end of May and June there were some rains that lowered temperatures improving the availability of green forage (Table 2), which explain the increases in animal response at the end of the experiment.

In general, the available herbaceous mass in the grazing areas was scarce, of which 64% was senescent and a smaller proportion was green material (36%). Also, there was a low leaf proportion (35%) in the HM, which corresponded to the dominant stoloniferous growing grass *C. plectostachyus*. This low proportion

Table 4. Milk production cost (USD $) of dual-purpose cows supplemented with three levels of crude protein, 100 (S10), 110 (S11) and 120 (S12) (g CP/kg DM) during dry season.

	Supplements crude protein		
Item	S10	S11	S12
Supplement cost (USD $/kg)	0.29	0.30	0.31
Total supplement cost (USD $)	593	609	625
Fuel	79	79	79
Hired labour	254	254	254
Production cost	926	942	958
Milk production (kg/cow/day)	5.4	5.8	6.4
Milk production per treatment (kg)	11,727	11,929	12,132
Milk sale price (USD $/kg)	0.47	0.47	0.47
Milk sales incomes (USD $)	1,152	1,295	1,365
Net profit margin (USD $)	225	295	407
Milk production cost (USD $/kg)	0.38	0.36	0.33

S10 = 110, S11 = 110 and S12 = 120 g CP/kg DM

Table 5. NASEN (2021) model predictions[1] and observed performance of cows supplemented with three dietary crude protein levels, 100 (S10), 110 (S11), and 120 (S12) (g/kg DM), grazing in an agrosilvopastoral system.

Item	S10	S11	S12
NE_L, MJ/day			
Supplied	75.1	81.8	79.7
Required	59.5	67.8	69.5
Balance	15.6	14.0	10.2
Metabolizable Protein			
Supplied	810	885	901
Required	607	679	718
Balance, g/day	203	206	183
CP (%)	10.4	10.4	10.9
RDP	7.9	7.9	8.3
RUP	2.5	2.5	2.7
Milk yield, kg/day			
NE_L allowable milk[2] (A)	11.1	10.8	9.9
MP allowable milk[3] *(B)	10.1	10.8	10.6
MY Observed (C)	5.3	5.6	6.3
Observed – predicted[4] (C - A)	-5.8	-5.2	-3.6
Observed – predicted[5] (C - B)	-4.8	-5.2	-4.3

[1]Predicted based on the NASEM (2021) model using treatment means of DMI, milk yield and composition, BW, and BCS. [2]NASEM (2021)-predicted milk production based on the supply of NE_L. [3]NASEM (2021)-predicted milk yield based on the supply of MP.

of leaves in the forage coincides with López-González *et al.* (2015). Similarly, the nutritive value of the grasses in terms of CP (7.9%) and ME (7.2 MJ/kg of DM) was low.

However, there were also other sources such as shrubs and trees that contributed nutrients to the cow´s diet but were not considered in this study. In any case, the botanical composition of the diet of grazing cows was previously documented by Salas-Reyes *et al.* (2022), indicating that during the dry season fodder from trees contributed with 9% of the dry matter intake of cows, providing 20% of CP and 9% of the cow´s metabolizable energy requirements.

Hunter and Kennedy (2016) demonstrated that the provision of readily available energy sources such as molasses improved the rumen degradability of Angleton grass (*Dichanthium aristatum*) and the growth rate of steers. Therefore, the presence of forages in the paddocks where cows grazed, and the supplementation of energy sources may contribute to improving the rumen environment and the degradability of the *C. plectostachyus*. In our study, the nutrient content of *C. plectostachyus* was low: CP (79.1 g/kg DM) and energy (7.2 MJ/kg DM), while NDF and ADF presented high values 655 and 373.6 g/kg DM, respectively.

The lack of a positive response to supplementation of different CP levels is congruent with that by Jado Chagas *et al.* (2021), who also evaluated low, mid and high concentrations of CP in supplements (7.9. 15.4 and 20.5%, respectively), offered to cows grazing good quality intensively manage tropical pasture 16.4% of CP. They found significant increments in milk fat and protein yields at increasing levels of CP in the supplement, which coincides with our results, where milk protein yield was significantly higher ($P < 0.001$) for S12 compared with S10 0.205 vs. 0.175 kg/day, respectively. Moreover, in our study, there were numerical but no statistically significant increments in fat concentration and fat yield when CP was increased in supplements.

Olmos-Colmenero and Broderick (2006) evaluated the effect of increasing CP content from 13.5 to 14.0, 16.5, 17.9 and 19.4% in the diet of Holstein cows of 120 days in milk under intensive management. Their results showed no-significant differences in MY among treatments. On the contrary, they found a significant increase in milk fat content with increasing CP in the diets, which was like the results in our study.

When FPCM was included in the analysis, significant differences were observed indicating that the highest yields were achieved with S12. The higher volume and concentration of milk fat may have resulted in this significant difference comparing S10 and S11. Surprisingly, the milk fat content observed in our study (35.5 g/kg) was higher than that of the other reports (mean 32.0 g/kg) conducted in different years on the same farms, in 4th calving cows, with BW and days in milk similar to those of the presents study (Salvador-Loreto *et al.*, 2016; Salas-Reyes *et al.*, 2019; Esparza-Jiménez *et al.*, 2020) and from other farms in the same region reported by Morales *et al.* (2011) of 26.7 (g/kg). Although the milk fat content values mentioned here are in the normal range reported in the literature (Daley *et al.*, 2022).

According to NASEM (2021) predicted NE_L-allowable milk was 11.1, 10.8 and 9.9 kg/day but MP-allowable milk was 10.1, 10.8 and 10.6 for cows in S10, S11 and S12, respectively. In both cases, estimates were higher than the observed MY. However, NASEM predictions do not consider the milk consumed by the calves. Our research team (unpublished data) has estimated that calves consumed on average 3 kg of milk a day during the five hours they stayed with their mothers´, which could partially explain the difference between expected and observed MY.

Differences in animal response variables as a function of EP could be due to poor pasture quality, lower forage availability, and high temperatures above 23°C cause heat stress in cows, which reduces forage intake, milk yield by 17%, milk protein by 4%, milk protein yield by 19%, fat-corrected milk by 23%, and fat content by 19% (Gao *et al.*, 2017). On the contrary, heat stress tends to increase MUN by 24.5% (Gao *et al.*, 2017). This could explain the significantly lower levels in MY, FPCM, milk protein, and lactose contents, BW change and feed efficiency of cows in EP3 and EP4 when the maximum temperatures were 28.8 (in April) and 31.7°C (in May) (Figure 1). In addition, consistent with that reported by Gao *et al.* (2017), higher MUN levels during EP3 and EP4 was a result of the higher temperatures and of the higher heat stress in cows.

Some authors (Kohn *et al.*, 2002) have established that MUN values above 12 mg/dL have been taken as a reference to determine if cows have been overfed with crude protein or if the protein-energy balance in the diet is adequate. The possible explanation for these MUN levels in this study could be the combination of excess crude protein in the diet from leguminous forages not accounted in this study, and of the higher temperatures during some EPs as explained above.

If grazing cows were fed with more energy-dense diets, the efficiency of utilization of the crude protein (nitrogen) from supplementation and forages as grasses, shrubs and trees present in pastures, the latter two usually legumes, could be improved. Increased energy in the diet could also increase milk production, body weight, and improve BSC of the cows.

In the economic aspect, Posadas-Domínguez *et al.* (2014) mentioned that because the cost of family labour does not represent a cash expense, the family members receive economic benefits from milk and cattle sales as profits of the system. Therefore, if 18% of the estimates family labour cost is excluded the cost of milk production becomes profitable for this type of production system, and then the feed cost is the factor that most influences in the cost of milk production.

The milk production cost (USD $/kg) in the S10 group of cows was 5 and 13% higher than that of cows housed in the S11 and S12 treatment, respectively. Thus, although there were no statistical differences in milk yield between treatments due to supplementation of different CP in the diet, milk yield was numerically higher in the S11 and S12 treatment compare with S10, which translated into 24 and 45% higher net profit, respectively.

CONCLUSIONS

Supplementation with 110, 111 and 112 g CP/kg DM did not affect milk production of Brown Swiss cows grazing an agrosilvopastoral system with scattered trees in paddocks during the dry season of the year. However, milk yield corrected for fat and protein is higher when CP is increased. These results indicate that cows could benefit if more energy-dense supplements that make more efficient use of dietary crude protein, reducing MUN levels. The supplement with 120 g/kg CP had the lowest milk production cost and generated the greater net profit margins in the dual-purpose system with agrosilvopastoral management.

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Compliance with ethical standards. The research adhered to the standard farm practices and did not involve additional direct work with animals. It followed research guidelines accepted by Autonomous University of the State of Mexico.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contribution statement (CRediT). B. Albarrán-Portillo – Conceptualization, Methodology, Investigation, Writing an original draft., **I.G. Salas Reyes** – Investigation, Project administration, Data curation., **A. García-Martínez** – Formal analysis

Writing – review & editing., **C.M. Arriaga-Jordán** - Writing – review & editing.

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