PRODUCTIVE AND ECONOMIC PERFORMANCE OF BROWN SWISS COWS AT DIFFERENT STAGES OF LACTATION FED TWO CRUDE PROTEIN LEVELS †

[RESPUESTA PRODUCTIVA Y ECONÓMICA DE VACAS PARDO SUIZO EN DIFERENTES ETAPAS DE LACTACIÓN ALIMENTADAS CON DOS NIVELES DE PROTEINA CRUDA]

Arturo Álvarez-Sánchez[1], Anastacio García-Martínez[1] and Benito Albarrán-Portillo[1*]

1 Centro Universitario UAEM Temascaltepec, Universidad Autónoma del Estado de México. Carretera Toluca - Tejupilco Km. 67.5, Barrio de Santiago, 51300 Temascaltepec de González, México. Tel. (716) 2665209. Emails: alvarezsancheza@yahoo.com; agarciam@uaemex.mx, balbarranp@uaemex.mx
**Corresponding author*

SUMMARY

Background. Dairy cows convert between 24 to 32% of dietary nitrogen (N_2) into milk protein, the rest of the dietary N_2 is excreted in urine and feces which contributes to environmental N_2 pollution. Besides the N_2 excretions, crude protein (CP) represents up to 69% of the diet´s total cost. Therefore, the best way to reduce environmental pollution and increase dairy profits is the reduction of crude protein in the diet of cows. **Objective.** To determine the productive and economic performance of Brown Swiss cows at different stages of lactation fed two crude protein levels 14 vs 16%. **Methodology.** Twenty-three multiparous Brown Swiss cows stratified by stage of lactation as early (EL), mid (ML), and late (LL) were used and subjected to two CP levels 14 and 16% on a crossover design with two experimental periods (EP) of three weeks each. Cows on experimental CP 14% in the EP1 switched to 16% in the EP-2, whereas cows that received a 16% diet in the first EP switched to 14% in the EP2. Milk-to-feed price ratio and income over-feeding cost were estimated as indicators of profitability. **Results.** The were no significant differences in any response variable due to dietary crude protein ($P > 0.05$), except for milk protein yield (kg/day) ($P = 0.03$), where 16% CP had higher yields (0.57) than 14% CP (0.55, kg/day). Cows in early and mid-lactation stages had higher performance than in late lactation ($P < 0.05$), on most of the response variables. Income over feeding-costs were 0.24 and 0.21 ($ USD/kg) for CP 14 and 16%, respectively. Total income over feeding cost per treatment was $1,020 for CP 14%, and $917 for CP 16%. **Implications.** The reduction of crude protein in the diet of lactating cows will allow reductions if milk production cost, as well as reductions of N_2 excretions to the environment. **Conclusions.** The productive performance of the cows was not affected by a reduction of crude protein in the diet, but the reduction of crude protein resulted in lower milk production cost and higher income-over feeding cost.
Key words: reduced crude protein; economic and productive performance; milk production.

RESUMEN

Antecedentes. Las vacas lecheras convierten entre 24 y el 32% del nitrógeno (N_2) que contiene la dieta en proteína de la leche, el resto del N_2 de la dieta se excreta en la orina y heces, lo que contribuye a la contaminación ambiental por N_2. La proteína cruda representa hasta 69% del costo total de la dieta. Por lo tanto, una alternativa para reducir la contaminación ambiental y aumentar las ganancias de la producción de leche es la reducción de la proteína cruda en la dieta de las vacas. **Objetivo**. Determinar el efecto de dos niveles de proteína cruda en la dieta (PC; 14 y 16%) sobre el desempeño productivo y económico de vacas Pardo Suizo. **Metodología**. Se utilizaron 23 vacas Pardo Suizo multíparas estratificadas por etapas de lactancia temprana, media y tardía, y sometidas a dos niveles de proteína cruda (PC) 14 y 16%, en un diseño cruzado con dos periodos experimentales (PE) de tres semanas cada uno. Las vacas alimentadas con nivel de proteína cruda de 14% en el PE1 cambiaron al 16% en el PE2, mientras que las vacas que recibieron la dieta del 16% en el PE1 cambiaron al 14 % en el PE2. La relación precio de leche-alimento e ingreso sobre costo de alimentación fueron estimados como indicadores de rentabilidad. **Resultados**. No hubo diferencias significativas en ninguna variable de respuesta debido al nivel de proteína cruda de la dieta ($P > 0.05$), excepto en el

† *Submitted January 10, 2023 – Accepted May 26, 2023. http://doi.org/10.56369/tsaes.4721*

ISSN: 1870-0462.
Authors ORCID = Benito Albarrán-Portillo: https://orcid.org/0000-0001-9807-8452; **Anastacio García-Martínez**: https://orcid.org/0000-0001-8021-5412

rendimiento de proteína de la leche (kg/día) (P = 0.03), donde el 16 % de PC tuvo mayores rendimientos (0.57) que el 14% de PC (0.55 kg/día). Las vacas en la etapa de lactancia temprana y media tuvieron un mayor rendimiento que en la lactancia tardía (P < 0.05), en la mayoría de las variables de respuesta. Los ingresos sobre los costos de alimentación fueron 0.24 y 0.21 ($ dólares E.U./kg) para PC 14 y 16%, respectivamente. El ingreso total sobre el costo de alimentación por tratamiento fue de $1,020 para PCD 14% y $917 para PC 16%. **Implicaciones.** La reducción de proteína cruda en la dieta permitirá a los productores reducir costos de producción por concepto de alimentación, así como reducir las excreciones de N_2 al medio ambiente. **Conclusiones**. El desempeño productivo de las vacas no se vio afectado por un menor nivel de proteína cruda en la dieta, así como un menor costo de producción de leche y mayores ingresos sobre costo de alimentación.

Palabras clave: reducción de proteína cruda; respuesta económica y productiva; producción de leche.

INTRODUCTION

Dairy farms contribute to environmental nitrogen (N_2) pollution from forage production (fertilizer) and excretions from cows in manure and urine. Of the total N_2 farm input, only about 15% is transformed into animal protein (meat and milk) (Tamminga, 1992). Cattle manure contributes to nitrogen (N) pollution as ammonia and nitrous oxide volatilization into the atmosphere, nitrate leakage in groundwater, and N_2 runoff in surface water (FAO, 2002). The conversion of dietary N_2 into milk protein range between 24 to 32%, despite the continuous efforts during recent years of research to improve the efficiency of conversion of dietary N_2 into milk protein (Huhtanen *et al*., 2015; Uddin *et al*., 2020).

The concentration of dietary N_2 and N_2 intake are the main factors that determine N_2 excretions in dairy cow manure (Barros, 2017). Besides the environmental effect of overfeeding dairy cows with CP, there is an economic aspect since sources of proteins like soybean meal, canola or cottonseed are expensive. It has been reported that dietary crude protein accounts for 69% of the diet´s total cost (Hanigan *et al*., 2004), and has an important impact on milk production cost-reducing profit margins. A recently published study reported that 72% of dairy cow nutritionists in the USA were balancing diets with lower CP than they were doing in the past three to five years. The main reason for doing this was the higher and volatile cost of the main protein sources like soybean (Prestegaard-Wilson *et al*., 2021).

An early study assessing maximum crude protein CP levels in dairy cow´s diet was reported by Wu and Satter (2000), who evaluated milk production response to four different dietary crude protein (DCP) levels on complete lactations of Holstein cows. Their findings indicated that there was no milk yield increase when cows received above 17.4% of DCP. What is more, cows receiving DCP lower than 17.5% were more efficient using dietary nitrogen than cows receiving DCP above the mentioned level.

Afterwards, Ipharraguerre and Clark (2005), demonstrate that there were no significant differences in milk yield or milk components of cows receiving low (14), medium (16), or high (18%) CP in the diet. In this study, the authors highlighted the importance of a good balance between CP and energy in the diet to maximize milk yield without overfeeding CP.

Stage of lactation has been reported as a factor that determines the cow´s response to DCP (Hristov *et al*., 2005). Olmos-Colmenero and Broderick (2006), determined the productive response of dairy cows in early lactation (120 days in milk) to five different DCP levels (13.5, 15.0, 16.5 17.9 and 19.4%), concluding that milk yield and protein did not increase by feeding above 16.5% of DCP level. More recently, Barros *et al*. (2017), evaluated the productive performance of dairy cows on late lactation (224 days in milk) fed with diets with 11.8, 13.1, 14.4, and 16.2% of DCP. Milk yields of cows that received 14.4 and 16.2% of DCP were not different. The lack of reduction in MY yield was explained by the existence of a labile N_2 pool that cows rely upon temporarily to sustain steady productivity.

A recent publish research, reported significant interactions between DCP and stage of lactation. In this study a range from 16.3 to 17.4% of DCP would maintain dry matter intake and milk net energy of lactation (NE_L) of cows in early to mid-early lactation; whereas, a range from 15.7 to 17.1% could be used in late lactation cows (Letelier *et al*., 2022).

As far as is known, little attention has been paid in the diet of lactating cows or the effects of overfeeding crude protein in Mexico, based on the reduced publications in Mexican scientific journals. Therefore, the study aimed to determine the productive and economic performance of Brown Swiss cows at different stages of lactation fed two crude protein levels 14 vs 16% in the diet.

MATERIALS AND METHODS

Experimental farm

The study was performed during July and August of 2018 in a dairy farm located in Telpintla, Temascaltepec in the southwest of Estado de México, between 100° 02' 50.9” west longitude, and 19° 03'

48.5 north latitudes, at an altitude of 1,798 meters above sea level. The weather is classified as Cwb, with average temperature of 15.7 °C, with an average annual precipitation of 2,446 mm. The farm is 50 ha of extension of which 10 were destined for maize crops during the spring-summer, and during autumn, and winter oat was established and preserved as silage.

Cows and experimental diets

Twenty-three multiparous Brown Swiss cows of 600 ± 58 body weight (BW) (mean ± SD), 2.0 ± 0.4 body condition score (BCS), and 4 ± 2 parity, were used in the study. Cows were stratified by stage of lactation as early (EL) (42 ± 30), mid (ML) (161 ± 33), and late (LL) lactation (321 ± 28; days in milk), and randomly distributed into two groups (n = 12 and 11, respectively) allocating similar number of cows in each lactation stage (Table 1). The experimental groups were housed in a separate free stall barn.

Table 1. Days in milk (DIM) of cows grouped according to lactation stage, assigned to two crude protein (DCP) levels 14 and 16 %.

	14		16	
Lactation stage	DIM	n	DIM	n
Early	44	4	40	3
Mid	154	3	168	3
Late	313	5	334	5
Average	194		196	

The experimental diets were balanced using the NRC (2001) program. The nutritional composition of feeds and the average cow´s characteristics by group were used as inputs into the program. The assigned experimental diets (ED) contained ~64% forage mix of oat silage and alfalfa hay, and 36% concentrate mix (sorghum grain and soybean meal) (Table 2). After milking, the cows returned to the barn where received the concentrate mix offered in two meals at 9:00 am and 4:00 pm., and afterwards had access to the forage mix. Experimental diets containing 14 or 16% of dietary crude protein (DCP) were randomly assigned to the groups of cows.

The dry matter intake per cow (kg of DM/day) was also estimated using the NRC (2001). The individual characteristics of each cow included in the experiment were used as inputs into the program to estimate individual DMI according to each experimental period. The cow´s characteristics used to feed the program were: days in milk (DIM), age in months, age at first calving in months, body weight (BW), body condition score (BCS), days pregnant, milk yield (kg/day) and, milk components (fat, protein and lactose; g/kg).

Table 2. Percentages of inclusion of ingredient in the experimental diets with two crude protein levels 14 and 16%.

	Crude protein, % of DM	
Item	14	16
Ingredient, % of DM		
Oat silage	29.1	23.4
Alfalfa hay	34.8	40.8
Sorghum grain grounded	32.4	28.4
Soybean meal	3.7	7.4

DM = dry mater

To determine the amount of feed offered to each group the dry matter intake (DMI) (kg/cow/day) was estimated by using the NRC (2001). The average DMI was 20.4 (kg/cow/day) being multiplied by the number of cows in each group, plus an extra 10% the feed to avoid feed restrictions.

Milk sampling

Cows were milked twice daily (7:00 am and 3:00 pm), and milk yield (MY) was recorded from individual cows from four consecutive milkings by using an electronic hanging scale, on Thursday and Friday of the last week of the EP. Milk components fat, protein and lactose (g/kg) were determined immediately after milking with a portable ultra-sound (Lactoscan Milk Analyzer®, serial 9414, Milkotronic, Bulgaria, 2008). Fat and protein-corrected milk (FPCM) and energy-corrected milk (ECM) were calculated according to IDF (2015), by using the following equation: FPCM (kg/yr) = production (kg/yr) x [0.1226 x Fat% + 0.0776 X true protein% + 0.2534].

Body weight and body condition score measurements

Cows were weighed after morning miking on Thursday and Friday on the last week of the of the first and second experimental periods on a Smart Scale 200 (Gallagher®) of 1,500 kg capacity. The body condition score (BCS) of cows was determined on a 1 to 5 points scale in the same days (Wildman *et al*., 1982). Body weight and body condition scores change was estimated subtracting the BW and BSC at the start of the experiment from the BW and BCS of EP-1, and then divided into the number of days between the two. The same procedure was performed for the BW and BSC of EP-1 and EP-2.

Feed analysis

The nutritional composition of the ingredients used in the diets before the start of the experiment was determined in the laboratory of nutrition of Centro

Universitario UAEM Temascaltepec. The nutritional composition of the ingredients was introduced into the NRC (2001) program to balance the experimental diets according to average cows' characteristics by group (parity, yield and composition milk, body weight, body condition score, days in milk, etc.). Subsequently, during the third week of the first and second EP, dietary ingredients were sampled for three consecutive days to determine the nutritional composition.

Diet ingredients were subject to dry matter determination by drying at 60°C in a forced-air oven and ash was obtained by incineration at 550°C for 6 h. Crude protein (CP) was estimated by the Kjeldahl method (AOAC, 1995), and neutral detergent fibre (NDF) and acid detergent fibre (ADF) by the Ankom micro-bag technique (ANKOM Technology, Macedon, New York, USA).

Experimental design and statistical analysis

The experimental design was a crossover with two experimental periods (EP) of three weeks, the first two weeks were for adaptation to the diets and the third week was for measurements and sampling. Cow´s response variables (i.e. milk yield, milk composition, body weight etc.) were analysed using the Mixed procedures of SAS (OnDemand, 2021), using the following equation:

$$Y_{ijkl} = \mu + T_i + P_j + LS_k + T{*}P_{ij} + T{*}LS_{ik} + C_l + e_{ijkl}$$

where Y_{ijk} = cow´s performance variables, μ = overall mean, T_i = effect of treatment (dietary CP; i = 14 vs 16), P_j = effect of the period (j = 1 to 2), LS_k = effect of lactation stage (k = early, mid and late), $T{*}P_{ij}$ = interaction of treatment and period, $T{*}LS_{ik}$ = interaction treatment and lactation stage, C_l = effect of the cow and e_{ijk} was the random residual error. All terms were considered fixed effect, except for C_l which was considered random.

Income over feeding cost

Milk-to-feed price ratio and income over-feeding cost were estimated as indicators of profitability according to Wolf (2010).

Milk-to-feed ratio was estimated as: $= \frac{\text{Milk sales price (\$/kg)}}{\text{Feed cost (\$/kg)}}$

Income over feeding cost was estimated as: $MSP - FC * (\frac{1}{FE})$

where: MSP = milk sales price ($/kg), FC = Feed cost ($/kg), and FE = Feed efficiency.

RESULTS

Nutritional composition of the diets

The nutritional characteristics of the experimental diets predicted by the NRC (2001) are presented in Table 3. The diet of 16% was 1% higher in rumen degradable protein (RDP) and rumen undegradable protein (RUP) compared with the diet of 14%. The ME and NE_L (MJ/kg/DM) contents in both diets were similar with 10.2 and 10.3 and 6.7 and 6.8, respectively. The rest of the nutrition characteristics of the diets were also similar.

Table 3. Predicted nutritional composition of diets with of 14 and 16% of crude protein according to National Research Council (NRC) 2001.

	Crude protein, % of DM	
Item	14	16
DM, % as fed	64.5	68.1
Forage, % DM	63.9	64.1
CP	14.1	16.1
RDP	9.0	10.1
RUP	5.1	6.1
ME, MJ/kg	10.2	10.3
NE_L, MJ/kg	6.7	6.8
NDF, % DM	32.3	32.9
ADF, % DM	23.8	24.4
Forage NDF, % DM	29.6	29.7

DM = dry mater; CP = crude protein; RDP = rumen degradable protein; RUP = rumen undegradable protein; ME = metabolizable energy; NE_L = net energy of lactation; NDF = neutral detergent fibre and ADF = acid detergent fibre.

Cow performance and feed efficiency

The were no significant differences in any response variable due to dietary crude protein (P > 0.05), except milk protein yield (kg/day) (P = 0.03), where 16% DCP had higher yields than 14% DCP. There was a trend towards higher lactose yield (P = 0.08), for cows that received 16% compared with 14% DCP. Fat, protein and lactose average concentrations were 44.2, 29.9, and 42.1 (g/kg), respectively. Mean BW and BSC were 613 (kg) and 2.2, respectively. Body weight change (BWc) and body condition score change (BCSc) averages were 0.6 and 0.2, respectively. Average feed efficiency (FE) (MY/DMI) and fat-protein corrected milk feed efficiency were 0.92 and 0.95, respectively. The interactions treatment and experimental period and treatment and lactation stage (not shown) were not significant (P > 0.05).

Experimental periods

Milk yield cows was significantly higher (P < 0.05) in the first EP compared to the second for most of the response variables, except fat concentration and yield (g/kg), BW (kg), and feed efficiencies (MY/DMI and FPCM/DMI) (P < 0.05) (Table 2).

Lactation stages

Significant differences were observed in most performance variables (Table 4), except for DMI (kg/day), fat concentration and yield (kg/day), protein and lactose yield (kg/day), BWc (kg/day) and BCSc (P > 0.05). The Milk yield of EL cows (21.2) was significantly higher than of ML (18.1) and LL (16.6 kg/day) cows (P < 0.01). Energy-corrected milk and FPCM (kg/day) tended to be higher (P = 0.06) in EL cows decreasing as lactation progressed. Fat content and yield were not affected by the lactation stage (P > 005), whereas protein and lactose contents (g/kg) were significantly lower in EL lactation than in ML and LL lactation. Protein and lactose yields (kg/day) were not significant as a function of the lactation stage (P > 0.05). Body weight was higher in LL cows, than EL and ML cows which were not different among the last two. Body weight and BSC change were not affected by the lactation stage (P > 0.05). Finally, feed efficiencies (MY/DMI and PFCM/DMI) were significantly higher for EL than for ML and LL (P < 0.019.

Feeding cost and returns

The 16% crude protein dietary supplement was 11% more expensive than 14% CP dietary supplement (Table 5). The amount of milk produced by treatment by the 16% treatment was 3% higher than that of the 14% treatment. Milk-to-feed price ratios were 2.5 and 2.2 for CP 14 and 16%. Income over-feeding costs were 0.24 and 0.21 ($/kg) for DCP 14 and 16%, respectively. Total revenue over feeding cost per treatment was $1,020 for DCP 14%, and 917 for DCP 16%.

DISCUSSION

The levels of CP tested in the present study did not affect DMI (kg/day) of cows at any stage of lactation, which is in agreement with Yang *et al*. (2022) and Law *et al*. (2009) who found no differences in DMI of cows consuming diets of 14.4 *vs* 17.3% of DCP. Olmos-Colmenero and Broderick (2006) also evaluated several levels of DCP in dairy cow diets, from 13.5 to 19.4% CP with no effects on DMI of early and mid-lactation cows. A negative effect of low DCP was reported by Barros *et al*. (2017) by reducing DCP below 13.1% in late-lactation cows.

Reductions in DMI occur due to low DCP by impairing ruminal microbial activity due of deficient levels of RDP (Allen, 2000). According to NASEM Dairy-8, (2021), the RDP level for early, mid and late lactation cows should be 10%. The RDP of the 14% diet was 9.0% below NASEM (2021) guidelines. In this regard, Yang *et al*. (2022), evaluated the effect of three different CP levels (low 11.4, medium 14.4 and high 17.3%), on the performance of dairy cows throughout lactation. The low and medium CP levels contained 7.8 and 9.5% of RDP without affecting DMI, as in the present study. NASEM (2021) recommendations were established for high-yielding dairy cows producing from 33 to 55 kg of milk/day, with BW of 570 kg, which could explain the differences between the results of the present study and the report by Yang *et al*. (2022), who also used cows of lower MY and BW. Liu and VandeHaar (2020) mentioned that low yielding cows which have also lower nutritional requirements, which could explain the differences between the present study and NASEM (2021) recommendations.

The productive performance of cows was not different by the effect of DCP content which was in line with other reports in which similar levels of CP were offered (Olmos-Colmenero and Broderick, 2006; Barros *et al*., 2017; Yang, Ferris and Yan, 2022). The average milk yields, milk protein and lactose in the present study were lower than those reported by Barros *et al*. (2017), at similar DCP levels using late-lactating Holstein cows, with MY of 30.4 kg/day and 34.9 and 47 (g/kg) of protein and lactose, respectively. The milk fat concentration in the present study (44 g/kg) was higher than that reported by Barros *et al*. (2017) (42 g/kg). Contrary to the present study, Cabrita *et al*. (2011) reported lower MY (34.6 kg/day) and fat-corrected milk (FCM) (33.6 kg/day) of Holstein cows around mid-lactation (134 ± 45 DIM) when feeding 14.3 compared with 15.7% DCP t (35.8 kg/day of MY and 34.7 of FCM). The lack of differences among DCP levels on protein content (g/kg) in the present study, indicates that 14% CP is sufficient to meet the metabolizable protein requirements of the cows under the conditions of the present study.

The results of the present study, contribute to the knowledge of feeding dairy cows with low CP levels under conditions different from those usually reported in the literature, with the objective of increasing dietary N_2 utilization as well as reducing N_2 excretions to the environment. However, as pointed out by Yang *et al*. (2022), results of short-term studies such as the present report should be interpreted with caution before putting them into practice on commercial farms and for full lactation.

Table 4. Effect of dietary crude protein content (14 vs 16%), experimental period and lactation stage (E = early, M = mid and L = late) on Brown Swiss cow.

Item	Dietary crude protein (%)				Experimental period				Lactation Stage				
	14	16	P =	S.E	1	2	P =	S.E.	E	M	L	P =	S.E.
DMI (kg/day)	20.1	20.4	0.12	0.31	20.7	19.8	< 0.01	0.31	20.4	19.5	20.9	0.19	0.54
MY kg/day	18.4	18.9	0.14	0.59	19.0	18.3	0.04	0.59	21.2^{a}	18.1^{ab}	16.6^{b}	< 0.01	1.0
ECM (kg/day)	20.7	21.4	0.11	0.67	21.5	20.5	< 0.01	0.67	23.3	20.4	19.3	0.06	1.14
FPCM (kg/day)	18.8	19.5	0.11	0.61	19.6	18.6	< 0.01	0.61	21.2	18.6	17.6	0.06	1.04
Fat (g/kg)	44.0	44.5	0.68	0.12	44.4	44.3	0.97	0.12	42.5	44.2	46.3	0.34	0.19
Fat (kg/day)	0.81	0.84	0.15	0.03	0.84	0.84	0.09	0.03	0.90	0.79	0.77	0.16	0.05
Protein (g/kg)	29.8	30.0	0.39	0.02	30.5	29.5	< 0.01	0.02	28.9^{a}	30.5^{b}	30.6^{b}	< 0.01	0.03
Protein (kg/d)	0.55	0.57	0.03	0.01	0.58	0.54	< 0.01	0.01	0.61	0.55	0.51	0.05	0.03
Lactose (g/kg)	42.1	42.2	0.78	0.02	42.9	41.5	< 0.01	0.02	40.7^{a}	42.9^{b}	43.0^{b}	< 0.01	0.04
Lactose (kg/d)	0.77	0.79	0.08	0.02	0.81	0.76	< 0.01	0.02	0.86	0.78	0.71	0.05	0.04
BW (kg)	610	616	0.24	10.95	617	609	0.14	10.95	599^{a}	573^{a}	667^{b}	< 0.01	18.8
BWc (kg/day)	0.44	0.75	0.24	0.24	0.95	0.25	0.01	0.24	0.71	0.60	0.50	0.90	0.37
BCS (1-5)	2.2	2.3	0.47	0.10	2.0	2.5	< 0.01	0.10	1.9^{a}	2.2^{ab}	2.6^{b}	0.02	0.27
BCSc	0.18	0.22	0.47	0.06	-0.003	0.41	< 0.01	0.06	0.11	0.16	0.35	0.22	0.11
FE[1] (MY/DMI)	0.92	0.93	0.44	0.02	0.92	0.93	0.82	0.02	1.04^{a}	0.93^{ab}	0.80^{b}	< 0.01	0.03
FE[2] (FPCM/DMI)	0.94	0.96	0.20	0.02	0.95	0.94	0.64	0.02	1.03^{a}	0.95^{ab}	0.84^{b}	< 0.01	0.04

MY = Milk yield; ECM = Energy corrected milk; FPCM = Fat-protein corrected milk; BW = body weight; BWc = BW change; BCS = Body condition score; BCSc = BSC change and FE = Feed efficiency.

performance.

Table 5. Feeding costs and returns (USD $) for milk production of supplements.

Item	Dietary crude protein (%)	
	14	16
Feed cost ($/kg)	0.17	0.19
Total supplement cost/treatment ($)	792	905
Milk yield/treatment (kg)	4,252	4,366
Milk selling price ($/kg)	0.42	0.42
Milk sales income ($)	1,789	1,838
Milk production cost ($/kg)	0.19	0.21
Cost/returns ratio	0.44	0.49
Milk-to-feed price ratio	2.5	2.2
Income over feeding cost ($/kg)	0.24	0.21
Total income over feeding cost	1,020	917

In this regard, Barros *et al*. (2017) reported that reducing DCP from 16.2 to 13.1%, they observed a reduction in MY after the fourth week of lactation, and with a more pronounced reduction of DCP (from 16.2 to 11.8%) MY dropped one week after. Therefore, the ability of cows to temporarily maintain milk yields with poor DCP is related to the ability to increase urea N_2 recycling to the gastrointestinal tract (Mutsvangwa *et al*., 2016).

A recent report showed that there are cows that can maintain milk production when fed low protein diets, due to higher metabolic efficiency than their contemporaries (Liu and VandeHaar, 2020). They defined these cows as having low dietary protein resilience, which is a trait that is correlated with protein efficiency i.e. protein capture in milk or both milk and body tissue per unit of consumed intake. They also mentioned that there are low yielding cows that need less protein to meet their requirements, which does not mean that these cows are more efficient using DCP.

There were small non-significant numerical differences in milk protein between the two treatments with 14 and 16% DCP. It is possible that these slight differences, in association with the small numerical differences in milk yields could have contributed to the significant differences detected in protein yield (kg/day) among treatments, rather than to the effect due to DCP. This situation could also have occurred with milk lactose yield which showed a tendency to increase with16% DCP.

The lack of interaction between DCP and lactation stages indicates that the significant differences in the response variables were due to the stage of lactation advancement. As lactation stage progressed, changes in nutrition partitioning occurred, in addition to which, gestation becomes a priority over milk production (NRC 2001). Protein and lactose content in milk was lower at the early lactation stage than during mid and late lactation, as MY decreases in late-lactation, and milk components show a tendency to increase (Stanton *et al*., 1992). However, this trend was not significant for milk fat content, despite the numerical differences observed as lactation phase increased.

The economic analysis shows that the 14% DCP treatment allowed lower production cost of ~11% ($/kg), improved milk/feed price ratio (2.5 vs 2.2), and income over feed cost (IOFC) (0.24 *vs* 0.21; $/kg) than the 16% DCP. Total income over feeding cost was 10% higher for the 14% DCP treatment than for the 16% DCP treatment. Buza *et al*. (2014) reported an average IOFC ($/cow/day) of 7.71 ranging from -0.33 to 16.60, on dairy farms in the US. In the present study IOFC ($/cow/day) were 4.02 and 3.74, for 14 and 16% DCP, respectively, lower than those mentioned above.

Currently, with the high volatility of raw material prices such as feed and fertilizers and the reduced profit margins for dairy farmers the dairy industry is focused on reducing CP in dairy cow diets, rather than being concerned about the environment (Prestegaard-Wilson *et al*., 2021).

In the present study, alfalfa hay and soybean meal were the main sources of protein in the experimental diets which represented 58% of the supplement cost of the diet at 14% CP, while in the diet 16% crude protein represented 68%. These main sources of protein are imported in most of the dairy farms in México and Latin America, a situation that increases the cost of the carbon footprint due to transportation (Velarde-Guillén *et al*., 2022). Thus, reducing protein in the cow diet to reduce milk production costs is as important as the efficient use of external inputs such as soybean meal, and to as well as producing feed with a reduced environmental impact.

CONCLUSION

There are no differences in milk yields of Brown Swiss cows with a crude protein reduction of 16 to 14%. Differences found are the influence of lactation stage rather than a diet effect. Supplementation with 14% of crude protein reduced milk production cost and higher income-over feeding cost.

Acknowledgments
The authors thank the cooperating participating farmer for his participation in the project. Our gratitude to the following institutions for their financial support: Autonomous University of the State of Mexico, SEP-PROMEP and the National Research Council for Science and Technology – CONACYT support. México.

Funding. Autonomous University of the State of Mexico, SEP-PROMEP and the National Research Council for Science and Technology – CONACYT.

Conflicts of interest/competing interests. The authors declare that they have no conflict of interest.

Compliance with ethical standards. The research did involve direct work with farm animals without any management modification that is normally performed by the personnel in the participant farm. The research followed the Institutional Guidelines for the handling of laboratory, teaching, research and production of Centro Universitario UAEM Temascaltepec; 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). A. Álvarez Sanchez Investigation, Project administration, Data curation., **B. Albarrán-Portillo** – Conceptualization, Methodology, Investigation, writing original draft., **A. García-Martínez** – Formal analysis Writing – review & editing.

REFERENCES

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *Journal of Dairy Science*, [online] 83(7), pp. 1598–1624. https://doi.org/10.3168/jds.S0022-0302(00)75030-2.

AOAC, 1995. *Official Methods of Analysis. 15th ed., Association of Official Analytical Chemists, Arlington, VA.*

Barros, T., 2017. *Nitrogen efficiency in late lactation dairy cows*. Ph. D. Thesis. Department of Dairy Science. University of Wisconsin-Madison.

Barros, T., Quaassdorff, M.A., Aguerre, M.J., Colmenero, J.J.O., Bertics, S.J., Crump, P.M. and Wattiaux, M.A., 2017. Effects of dietary crude protein concentration on late-lactation dairy cow performance and indicators of nitrogen utilization. *Journal of Dairy Science*, [online] 100(7), pp. 5434–5448. https://doi.org/10.3168/jds.2016-11917.

Buza, M.H., Holden, L.A., White, R.A. and Ishler, V.A., 2014. Evaluating the effect of ration composition on income over feed cost and milk yield. *Journal of Dairy Science*, [online] 97(5), pp. 3073–3080. https://doi.org/10.3168/jds.2013-7622

Cabrita, A.R.J., Dewhurst, R.J., Melo, D.S.P., Moorby, J.M. and Fonseca, A.J.M., 2011. Effects of dietary protein concentration and balance of absorbable amino acids on productive responses of dairy cows fed corn silage-based diets. *Journal of Dairy Science*, [online] 94(9), pp. 4647–4656. https://doi.org/10.3168/jds.2010-4097.

FAO, 2002. *World Agriculture: Towards 2015/2030. Summary Report. FAO*. [online] Available at: <https://www.fao.org/3/y3557e/y3557e.pdf>.

Hanigan, M.D., Reynolds, C.K., Humphries, D.J., Lupoli, B. and Sutton, J.D., 2004. A model of net amino acid absorption and utilization by the portal-drained viscera of the lactating dairy cow. *Journal of Dairy Science*, [online] 87(12), pp. 4247–4268. https://doi.org/10.3168/jds.S0022-0302(04)73570-5.

Hristov, A.N., Price, W.J. and Shafii, B., 2005. A meta-analysis on the relationship between intake of nutrients and body weight with milk volume and milk protein yield in dairy cows. *Journal of Dairy Science*, [online] 88(8), pp. 2860–2869. https://doi.org/10.3168/jds.S0022-0302(05)72967-2.

Huhtanen, P., Cabezas-Garcia, E.H., Krizsan, S.J. and Shingfield, K.J., 2015. Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. *Journal of Dairy Science*, [online] 98(5), pp. 3182–3196. https://doi.org/10.3168/jds.2014-8215.

IDF, 2015. *A Common Carbon Footprint Approach for the Dairy Sector. The International Dairy Federation Guide to Standard Life Cycle Assessment Methodology*. IDF, Brussels, Belgium.

Ipharraguerre, I.R. and Clark, J.H., 2005. Varying protein and starch in the diet of dairy cows. II. Effects on performance and nitrogen utilization for milk production. *Journal of Dairy Science*, [online] 88(7), pp. 2556–2570. https://doi.org/10.3168/jds.S0022-0302(05)72932-5.

Law, R.A., Young, F.J., Patterson, D.C., Kilpatrick, D.J., Wylie, A.R.G. and Mayne, C.S., 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. *Journal of Dairy Science*, [online] 92(3), pp. 1001–1012. https://doi.org/10.3168/jds.2008-1155.

Letelier, P., Zanton, G.I. and Wattiaux, M.A., 2022. Production performance of Holstein cows at 4 stages of lactation fed 4 dietary crude protein concentrations. *Journal of Dairy Science*, 105(12), pp. 9581–9596. https://doi.org/10.3168/jds.2022-22146.

Liu, E. and VandeHaar, M.J., 2020. Low dietary protein resilience is an indicator of the relative protein efficiency of individual dairy cows. *Journal of Dairy Science*, [online] 103(12), pp. 11401–11412. https://doi.org/10.3168/jds.2020-18143.

Mutsvangwa, T., Davies, K.L., McKinnon, J.J. and Christensen, D.A., 2016. Effects of dietary crude protein and rumen-degradable protein concentrations on urea recycling, nitrogen balance, omasal nutrient flow, and milk production in dairy cows. *Journal of Dairy Science*, [online] 99(8), pp. 6298–6310. https://doi.org/10.3168/jds.2016-10917.

NASEM Dairy-8, 2021. *The National Academies of Sciences, Engineering and Medicine. Nutrition Requirements of Dairy Cattle. 8th rev. ed. The National Academies Press, 2021*. Available at: <https://www.nap.edu/catalog/25806/nutrient-requirements-of-dairy-cattle-eighth-revised-edition>.

NRC, 2001. *Requirements of Dairy Cattle. 7th ed.* Washington, DC.: Natl. Acad. Press,.

Olmos Colmenero, J.J. and Broderick, G.A., 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *Journal of Dairy Science*, 89(5), pp. 1704–1712. hhttps://doi.org/10.3168/jds.s0022-0302(06)72238-x

Prestegaard-Wilson, J.M., Daley, V.L., Drape, T.A. and Hanigan, M.D., 2021. A survey of United States dairy cattle nutritionists' practices and perceptions of reducing crude protein in lactating dairy cow diets. *Applied Animal Science*, [online] 37(6), pp. 697–709. https://doi.org/10.15232/aas.2021-02179.

SAS OnDemand, 2021. SAS® OnDemand for Academics. https://welcome.oda.sas.com/login. [online] Available at: <https://welcome.oda.sas.com/login>.

Stanton, T.L., Jones, L.R., Everett, R.W. and Kachman, S.D., 1992. Estimating Milk, Fat, and Protein Lactation Curves with a Test Day Model. *Journal of Dairy Science*, [online] 75(6), pp. 1691–1700. https://doi.org/10.3168/jds.S0022-0302(92)77926-0.

Tamminga, S., 1992. Nutrition Management of Dairy Cows as a Contribution to Pollution Control. *Journal of Dairy Science*, [online] 75(1), pp. 345–357. https://doi.org/10.3168/jds.S0022-0302(92)77770-4.

Uddin, M.E., Larson, R. a. and Wattiaux, M.A., 2020. Effects of dairy cow breed and dietary forage on greenhouse gas emissions from manure during storage and after field application. *Journal of Cleaner Production*, 270, p.122461. https://doi.org/10.1016/j.jclepro.2020.122461.

Velarde-Guillén, J., Arndt, C. and Gómez, C.A., 2022. Carbon footprint in Latin American dairy systems. *Tropical Animal Health and Production*, 54(1), pp. 1–7. https://doi.org/10.1007/s11250-021-03021-6.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt, H.F. and Lesch, T.N., 1982. A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. *Journal of Dairy Science*, 65(3), pp. 495–501. https://doi.org/10.3168/jds.S0022-0302(82)82223-6.

Wolf, C.A., 2010. Understanding the milk-to-feed price ratio as a proxy for dairy farm profitability. *Journal of Dairy Science*, [online] 93(10), pp. 4942–4948. https://doi.org/10.3168/jds.2009-2998.

Wu, Z. and Satter, L.D., 2000. Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein 1. *Journal of Dairy Science*, [online] 83(5), pp. 1042–1051. https://doi.org/10.3168/jds.S0022-0302(00)74968-X.

Yang, C.T., Ferris, C.P. and Yan, T., 2022. Effects of dietary crude protein concentration on animal performance and nitrogen utilisation efficiency at different stages of lactation in Holstein-Friesian dairy cows. *Animal*, [online] 16(7), p. 100562. https://doi.org/10.1016/j.animal.2022.100562.