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**EFFECTO DE DOS NIVELES DE PROTEÍNA CRUDA EN LA RESPUESTA
PRODUCTIVA Y ECONÓMICA DE VACAS PARDO SUIZO**

**COMO REQUISITO PARCIAL PARA OBTENER EL TITULO DE INGENIERO
AGRÓNOMO ZOOTECNISTA**

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Introducción

La presente investigación se refiere a los problemas de sobrealimentación con proteína cruda (PC) en vacas en lactación, que se puede definir como la precisión de alimentación en la dieta en el porcentaje de PC para reducir el impacto ambiental por contaminación de nitrógeno (N) excretado en heces y orina, así como no ver afectado la producción de leche y una buena relación costo-beneficio (Barros *et al.*, 2017; Broderick y Clayton, 1997).

La característica principal es la falta de conocimiento por productores a pequeña escala en la región sur del Estado de México en la sobrealimentación con PC, pocos trabajos se realizado con este tema como el de Esparza Jiménez *et al.*, (2012), se calcula que los costos por concepto de alimentación para vacas en lactación, representan entre el 50 y 75% del costo de producción de un kg de leche (LACTODATA, 2016) sin embargo muchos de los productores aún siguen sobre alimentando las vacas con PC.

La investigación de esta problemática se realizó por el interés de conocer hasta donde podemos reducir los niveles de PC sin afectar el rendimiento productivo de las vacas en lactación, esto permitió una mejor relación costo-beneficio y reducir el impacto ambiental por la contaminación de N (Barros *et al.*, 2017).

Los tratamientos consistieron en dos dietas con dos niveles de proteína cruda (PC) (14 vs 16%). Estudios anteriores demuestran que hasta un 16% PC en la dieta no afecta el rendimiento productivo (Barros *et al.*, 2017; Broderick y Clayton, 1997), por arriba de estos valores se está sobrealimentando a las vacas y se puede ver reflejado en un análisis de nitrógeno ureico en leche (NUL) (Cerón *et al.*, 2014).

I. Planteamiento del problema

Los niveles altos de proteína cruda en la dieta no tienen una respuesta positiva tanto a la composición de leche y rendimiento productivo, no obstante la proteína en la dieta aumenta los costos de alimentación además de las excreciones de N al medioambiente Albarrán et al., (2018) Reporta que no existen ventajas productivas en usar mayores contenidos de proteína cruda (140 vs 160 g/kg de materia seca)

1.1 Tema

Respuesta a la suplementación con dos niveles de proteína cruda en vacas en lactación sobre el rendimiento productivo y costo-beneficio

1.2 Justificación

Se estima que los gastos de alimentación para vacas en lactación, representan entre el 50 y 75% del costo de producción de un kg de leche (LACTODATA, 2016).

Las vacas lecheras utilizan la proteína cruda (PC) en la alimentación con mayor eficacia que otros rumiantes, pero excretan aun así cerca de 2 a 3 veces más nitrógeno (N) en estiércol que en la leche (Broderick y Clayton, 1997). Esto aumenta los costos de producción de leche más la considerable contaminación ambiental por N. Optimizando la formación de proteínas microbiana en el rumen se obtiene la manera más eficaz para mejorar el estado de la proteína de la vaca en lactación (Cerón Muñoz *et al.*, 2014).

Estudios realizados con anterioridad demuestran que los niveles altos de proteína cruda en la dieta no tienen una respuesta positiva tanto a la composición de leche y rendimiento productivo, no obstante, la proteína en la dieta aumenta los costos de alimentación además de las excreciones de N al medioambiente (Olmos Colmeneros y Broderick, 2006). Trabajo desarrollados en el sur del estado de México, han reportado que bajos

niveles de PC en los suplementos permiten reducir costos de alimentación, así como reducción en los niveles de nitrógeno ureico en leche (NUL) sin afectar los rendimientos productivos (Esparza Jiménez, *et al.*, 2012). Sin embargo, hace falta mayor investigación en México que permitan determinar niveles de eficiencia de utilización de N en los diferentes sistemas de producción de leche.

1.3 Pregunta de investigación

¿Cuál es el efecto de dos niveles de proteína (14% vs 16%) sobre la respuesta productiva de vacas pardo suizo en diferentes etapas de lactación?

¿Cuál es el costo-beneficio de la suplementación con dos niveles de proteína (14% vs 16%) en la dieta de vacas pardo suizo en diferentes etapas en lactación?

1.4 Objetivo general

Evaluar la respuesta productiva y el costo-beneficio en dos diferentes niveles de proteína cruda (14% vs 16%) en la dieta de vacas en lactación.

1.4.1 Objetivos específicos

Determinar los rendimientos de producción:

- Leche kg/vaca/día
- Grasa g/kg
- Proteína g/kg
- Lactosa g/kg
- Nitrógeno ureico en leche mg/dL
- Peso (kg/vaca)
- Condición corporal

Realizar análisis costo-beneficio en vacas en lactación con dos niveles de proteína cruda en la dieta.

- Costo de producción por litro de leche por concepto de alimentación
- Costo de alimentación vaca/día
- Ingresos por venta de leche
- Relación costo-beneficio

1.5 Hipótesis

El suministro de los dos niveles de proteína cruda en la dieta no afecta la respuesta productiva de vacas de la raza Pardo Suizo en diferentes etapas de lactación.

La suplementación con un nivel de 14% PC tiene una mejor relación costo-beneficio que un nivel de PC de 16%.

1.6 Variables

- Costo de producción por litro de leche por concepto de alimentación.
- Costo de alimentación vaca/día
- Relación costo-beneficio
- Niveles de proteína: 14% PC vs 16% PC
- Leche kg/vaca/día
- Grasa g/kg
- Proteína g/kg
- Lactosa g/kg
- Nitrógeno ureico en leche mg/dL
- Peso (kg/vaca)
- Condición corporal

II. Antecedentes del tema y problema de investigación

2.1 Marco teórico

2.1.1 Alimentación con proteína cruda

En los reportes de Zanton y Heinrichs, (2009) concluyen que el aumento de la ingesta de nitrógeno (N) aumenta la digestibilidad de la Materia orgánica (MO), cuya magnitud depende del nivel de forraje del alimento proporcionado.

La cantidad de proteína que llega al intestino delgado para ser absorbida es la suma de la PBM y de la proteína del pienso que escapa a la digestión ruminal o sale ilesa de la misma. Con tasas elevadas de producción, la PBM sola puede ser ineficiente para cubrir las demandas de proteína de animal productivo (Church, 1988).

2.1.2 Metabolismo de la proteína en rumiantes

Los rumiantes tienen de la capacidad única de subsistir y producir sin disponer de una fuente de proteína dietética debido a la síntesis de proteína microbiana en el interior del rumen, no obstante, los microbios del rumen son aprovechados por el animal y, junto con la proteína que escapa de la degradación en el rumen, proporciona al intestino delgado proteína para ser digerida y absorbida (Church, 1988).

Las proteínas llevan a cabo diferentes funciones en el cuerpo de los animales, la mayor parte de las proteínas corporales están presentes en forma de componentes de las membranas celulares, en el músculo y en otras estructuras de soporte, como la piel, pelo y las pezuñas (Pond *et al.*, 2003). Asimismo, las proteínas del plasma sanguíneo, las enzimas, las hormonas y los anticuerpos realizan funciones especializadas en el cuerpo aun cuando no contribuyen de manera significativa al contenido proteico total.

2.1.3 Importancia de los microorganismos del rumen como fuente de proteína

Los microorganismos del rumen contienen generalmente entre 20 y el 60% de su sustancia seca en forma de proteína bruta. La fuente de N que emplean los microbios para la síntesis de proteína consiste tanto en proteína de la dieta como en N no proteico (NNP) así como también N reciclado hacia el rumen para su reutilización los microbios ruminales se adaptan generalmente en unos pocos días al empleo de nuevas fuentes de proteína o energía aunque precisan períodos de adaptación más prolongados para ciertos compuestos como el biuret, que es una fuente de NNP (Church, 1988).

2.1.4 Reciclado de nitrógeno hacia el rumen

El N es reciclado continuamente hacia el rumen desde la corriente sanguínea para ser utilizado (Figura 1). Este mecanismo de conservación permite sobrevivir a los rumiantes con dietas muy pobres en N (Church, 1988).

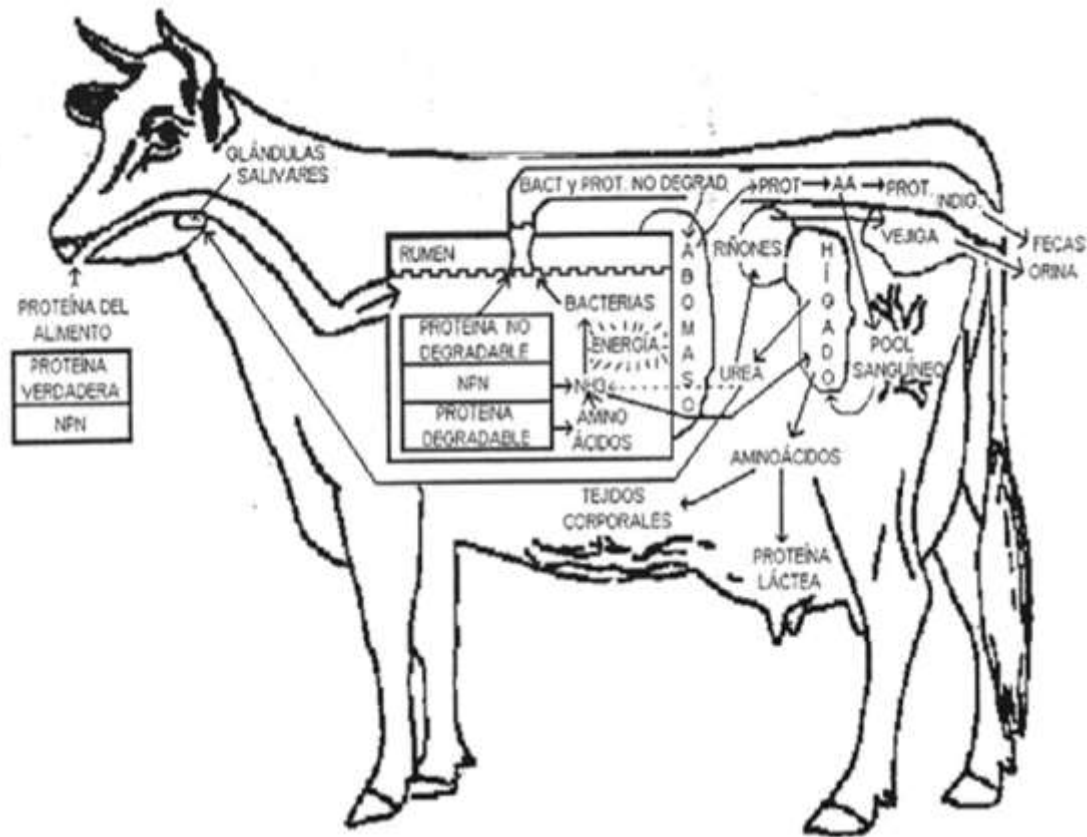


Figura 1. Esquema del metabolismo de la proteína en la vaca lactante. Modificado por Stern y Satter (Church, 1988)

2.1.5 Calidad nutritiva de la proteína microbiana

La composición en aminoácidos de la digesta duodenal es más constante que la composición de aminoácidos de los alimentos debido a la dilución con proteína bruta microbiana (PBM). El valor nutritivo de la proteína microbiana y la complementariedad con la proteína de la dieta no fermentada son hechos que no han sido valorados totalmente. Los estudios iniciales indicaban que la proteína que la PBM posee una calidad alta aunque no la ideal cuando se determina su valor biológico (VB) (Church, 1988)

La cantidad de proteína que llega al intestino delgado para ser absorbida es la suma de la PBM y de la proteína del pienso que escapa a la digestión ruminal o sale ilesa de la misma. Con tasas elevadas de producción, la PBM sola puede ser ineficiente para cubrir las demandas de proteína de animal productivo (Church, 1988).

2.2 Marco referencial

2.2.1 Nitrogeno ureico en leche

La determinación del nitrógeno ureico en leche (NUL) es una herramienta efectiva y práctica para los productores y técnicos a la hora de tomar decisiones relacionadas con el plan de alimentación en diversos tipos de rumiantes, el resultado obtenido tiene relación con el exceso o déficit de proteína y carbohidratos solubles en la dieta de los animales (Cerón *et al.*, 2014).

De acuerdo a Cerón Muñoz, *et al.*, (2014) si el NUL es superior a 18 mg/dL y la proteína de la leche es menor que 3.0%, la dieta tiene exceso de proteína soluble o degradable en relación con la disponibilidad de carbohidratos fermentables; si está entre 3.0 y 3.2% hay exceso de proteína y de energía en la dieta; y si es superior a 3.2%, hay un exceso de proteína pero baja disponibilidad de carbohidratos fermentables.

El nitrógeno ureico de la leche (NUL) se correlaciona con el equilibrio de nitrógeno (N), la ingesta de N y el contenido de N en la dieta, y por lo tanto es un buen indicador del manejo adecuado de la alimentación con respecto a las proteínas. De acuerdo con Aguilar, *et al.*, (2012) Se usa comúnmente para monitorear programas de alimentación para alcanzar objetivos ambientales; sin embargo, la diversidad genética también existe entre las vacas. La diversidad fenotípica entre las vacas podría sesgar las decisiones de

gestión de los alimentos cuando las herramientas de monitoreo no consideran la diversidad genética asociada con el (NUL).

El uso actual de pastos de rye grass y trébol con alto contenido de nitrógeno (N) finalmente resulta en proporcionar un exceso de N a las vacas lecheras y, posteriormente, grandes pérdidas de N en la orina como costo metabólico y desperdicio. Al proporcionar un pasto diverso, se puede lograr una reducción significativa en las pérdidas de N en la orina, lo que proporciona un beneficio ambiental (Totty *et al.*, 2013).

Olmos Colmeneros & Broderick (2006) Reportan que la producción de leche y proteínas mostró tendencias para las respuestas cuadráticas a la proteína cruda (PC) dietética y fue, respectivamente, de 38.3 y 1.18 kg / día con un 16.5% de PC. Como proporción de la ingesta de nitrógeno (N), la excreción urinaria de N aumentó de 23.8 a 36.2%, mientras que la N-secretada aumentó de 36.5 a 25.4%, ya que la proteína de la dieta aumentó de 13.5 a 19.4%. Bajo las condiciones de este estudio, el rendimiento de la leche y las proteínas no aumentaron al alimentar a más de 16.5% de PC.

III. Marco contextual

Alrededor de 150 millones de hogares en todo el mundo se dedican a la producción de leche. En los tres últimos decenios, la producción lechera mundial ha aumentado en más del 58 por ciento, pasando de 522 millones de toneladas en 1987 a 828 millones de toneladas en 2017. La leche produce ganancias relativamente rápidas para los pequeños productores y es una fuente importante de ingresos en efectivo (FAO, 2019).

La población del número de cabezas de ganado lechero a nivel nacional en el 2015 de acuerdo a LACTODATA, (2014) fue de 118 millones de animales con un aumento de 1.7% anual, para el 2010 el Estado de México contaba con 674,861 cabezas de ganado, teniendo Temascaltepec 2,993 cabezas de ganado expresando en porcentajes 0.44% del número de cabezas que tenía en ese entonces el Estado de México.

La producción nacional de leche por año para el 2012 fue de 10,946,015 litros, el cual el Estado de México produjo 468,733 litros de leche, esto representaba el 4.2% de la producción nacional (LACTODATA, 2014).

3.1 Generalidades de la región sur del estado, importancia en producción pecuaria, población bovina.

Texcoco es el primer municipio del estado con mayor población de ganado bovino (mayoritariamente ganado lechero especializado), seguido por cuatro municipios que se ubican en la región sur del Estado (Tlatlaya, Amatepec, Luvianos y Tejupilco). Un gran número de ésta población de bovinos, corresponde a vacas de doble propósito que producen leche principalmente en la época de lluvias. Por lo tanto, existe un número importante de ganado bovino no especializado que contribuye de manera importante a la

producción de leche, la cual es transformada en su mayoría a queso, por lo que no existe un dato confiable sobre los niveles de producción de leche de la región (SAGARPA, 2015).

México registro una producción de leche de 12.3 millones de toneladas de leche en 2018, con una población de 2.5 millones de vacas lecheras. El estado de México ocupa el octavo lugar a nivel nacional en producción de leche, con una población de 110,817 vacas lecheras especializadas (Holstein); éste dato, no considera vacas de doble propósito de la región sur del Estado que producen leche al menos durante la época de lluvias, y en menor grado en la época de secas (SAGARPA, 2015).

IV. Marco metodológico

4.1 Tipo de investigación, alcance y diseño de

4.1.1 Materiales y métodos

El trabajo experimental se llevó a cabo en el rancho "El Potrero" ubicado en la localidad de Telpintla, municipio de Temascaltepec, Estado de México. Ubicando en las coordenadas geográficas GPS: Longitud: -100.048333 y una Latitud: 19.063611. La localidad se encuentra a una mediana altura de 1880 metros sobre el nivel del mar, el clima predominante es templado subhúmedo, presenta una temperatura media anual que oscila entre los 18°C y los 22°C (INEGI, 2018).

Se utilizaron 23 vacas de la raza Pardo Suizo (600 ± 58 ; 2.0 ± 0.4 , peso vivo y condición corporal, respectivamente), en estabulación, estratificadas por etapa de lactación (42 ± 30 , 161 ± 33 y 321 ± 28 días, lactación temprana, media y tardía, respectivamente). Se ordeñaron dos veces al día (07:00 y 15:00 h). Posterior a la ordeña, las vacas recibieron las dietas experimentales mezcladas (concentrado y forrajes, cuadro 1) a libre acceso.

4.2 Universo de estudio, unidad de análisis y muestra

4.2.1 Tratamientos y diseño experimental

Los tratamientos consistieron en dos dietas con dos niveles de proteína cruda (PC) (14 vs 16%) (Cuadro 1). Las raciones fueron balanceadas utilizando el programa Dairy NRC (2012). Las vacas se dividieron al azar en dos grupos (corrales), asignando al azar los tratamientos.

El experimento se dividió en dos periodos experimentales (PE) de tres semanas cada uno, dos semanas de acostumbramiento a la dieta y la tercera semana para la toma de

registros productivos (rendimiento de leche, composición de leche, peso, condición corporal, y toma de muestras de las dietas experimentales). Posterior el primer periodo, se cambiaron las dietas experimentales de forma que los dos grupos de vacas recibieron ambas dietas.

4.2.2 Análisis estadístico

Los datos fueron analizados con un procedimiento MIXED del programa SAS 9.0 (2002), con un diseño de bloques completos al azar, en el que la vaca estuvo definida como factor aleatorio, en el que se tomaron las medidas repetidas a lo largo del experimento, con base en el siguiente modelo:

$$y_{ijk} = \mu + T_i + PE_j + EL_k + (T*EL)_{ik} + Vaca_l + \varepsilon_{ijkl}$$

En donde: y_{ijk} = variable de respuesta, μ = media general, τ_i = efecto fijo del tratamiento (i = tratamiento 14 y 16 % PC), PE_j = efecto fijo del periodo experimental ($j = 1$ y 2), EL_k = efecto fijo de la etapa de lactación ($k =$ temprana, media y tardía), efecto fijo de la interacción tratamiento por etapa de lactación $(\tau*EL)_{ik}$ + vaca (efecto aleatorio dentro de cada tratamiento) ε_{ijk} = error aleatorio.

Cuadro 1. Ingredientes de las dietas experimentales (14 vs 16% de proteína cruda, base seca (%))

Alimento	14% PC	16% PC
Sorgo	32.2	28.3
Soya	3.7	7.4
Alfalfa	34.7	40.6
Ensilado de avena	29.0	23.3
Minerales	0.4	0.4
Total (%)	100	100

Fuente. Elaboración propia con datos de campo.

4.2.3 Análisis económico

Para realizar este análisis solo fueron tomados en cuenta los costos por concepto de alimentación.

Cuadro 2. Costos por concepto de alimentación por tipo de dieta (14% PC) y (16% PC) durante el estudio.

Tratamientos	14% PC	16% PC
Costo por kg de alimento	\$2.5	\$2.9
Costo total de alimentación	\$37,982	\$41,864
Precio de venta/kg de leche	\$8.0	\$8.0
Total de gastos en efectivo	\$37,982	\$41,864

Fuente. Elaboración propia con datos de campo.

El tratamiento que registró mayor producción de leche fue el de 16% PC, con un total de 9,122 kg de leche, por otro lado, fue el que presentó un mayor gasto total de alimentación, con una diferencia total de \$3,882 con respecto al tratamiento de 14% PC.

El tratamiento al 14% PC registró un menor costo de producción (\$4.3) respecto al tratamiento al 16% PC (\$4.6). Por lo que el primero presenta un margen/kg de leche mayor (\$3.7) comparado con (\$3.4) del tratamiento dos.

Carta de confirmación de envío

Benito Albarran Portillo

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Resultados

EFFECT OF TWO DIETARY CRUDE PROTEIN CONCENTRATIONS ON BROWN SWISS COWS PRODUCTIVE AND ECONOMIC PERFORMANCE

EFFECTO DE DOS NIVELES DE PROTEÍNA CRUDA EN LA RESPUESTA PRODUCTIVA Y ECONÓMICA DE VACAS PARDO SUIZO

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ABSTRACT

Background. Dairy cows convert between 24 to 32% of dietary nitrogen (N) into milk protein, the rest of the dietary N is excreted in urine and feces that contributes to environmental N pollution. Besides the N excretions, dietary crude protein (DCP) represents up to 69% of the diet total cost. Therefore, the best way to reduce environmental pollution and to increase dairy profits is the reduction of crude protein in the diet of cows. **Objective.** To determine the effect of two dietary crude protein (DCP) levels 14 vs 16% on the productive and economic performance of Brown Swiss under confined management. **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 dietary treatments 14 and 16% of DCP on a crossover design with two experimental periods of three weeks each. Cows on experimental DCP 14% in period 1 switched to 16% in the second period, whereas cows that received a 16% diet in period 1 switched to 14% in period 2. Milk-to-feed price ratio and income over feeding cost were estimated as indicators of profitability. **Results.** There 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% DCP had higher yields (0.57) than 14% DCP (0.55, kg/day). Cows in early and mid-lactation stage had higher performance than on late lactation ($P < 0.05$), on most of the response variables. Income over feeding costs were 0.24 and 0.21 (\$ USD/kg) for DCP 14 and 16%, respectively. Total income over feeding cost per treatment was \$1,020 for DCP 14%, and 917 for DCP 16%. **Implications.** The reduction of dietary crude protein in the diet of lactating cows will allow the reduction of N excretions to the environment and for farmers this strategy allows reductions in feeding cost. **Conclusions.** There were no differences in Brown Swiss cows performance to a

reduction of DCP from 16 to 14%. Dietary crude protein treatment had lower milk production cost and generated higher incomes over feeding cost.

Keywords: crude protein; economic and productive performance; dairy cows.

RESUMEN

Antecedentes. Las vacas lecheras convierten entre el 24 y el 32% del nitrógeno (N) de la dieta en proteína en la leche, el resto del N de la dieta se excreta en la orina y las heces, lo que contribuye a la contaminación ambiental por N. Además de las excreciones de N, la proteína cruda representa hasta el 69% del costo total de la dieta. Por lo tanto, lo mejor 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 (PCD) de 14 vs 16% sobre el desempeño productivo y económico de vacas Pardo Suizo en estabulación. **Metodología.** Se utilizaron 23 vacas Pardo Suizo multíparas estratificadas por etapa de lactación en temprana, media y tardía y sometidas a dos tratamientos dietéticos 14 y 16% de PCD en un diseño cruzado con dos periodos experimentales de tres semanas cada uno. Las vacas con nivel de proteína cruda de 14% en el período 1 cambiaron al 16% en el segundo período, mientras que las vacas que recibieron una dieta del 16% en el período 1 cambiaron al 14 % en el período 2. La relación 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 rendimiento de proteína de la leche (kg/día) ($P = 0,03$), donde el 16 % de PCD tuvo mayores rendimientos (0.57) que el 14% de PCD (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 PCD 14 y 16%, respectivamente. El ingreso total sobre el costo de alimentación por tratamiento fue de \$1,020 para PCD 14% y 917 para PCD 16%.

Implicaciones. La reducción de proteína cruda en la dieta permitirá reducir las excreciones de nitrógeno al medio ambiente, pero más importante permitirá a los productores reducir costos de producción por concepto de alimentación. **Conclusiones.**

No hubo diferencias en el rendimiento de las vacas Pardo Suizo a una reducción de PCD de 16 a 14%. El tratamiento con 14% de PCD tuvo un menor costo de producción de leche y generó mejores ingresos sobre costo de alimentación.

Palabras clave: proteína cruda; respuesta económica y productiva; vacas lecheras.

INTRODUCTION

Dairy farms contribute to environmental nitrogen (N) pollution from forage production (fertilizer) and excretions from cows in manure and urine. Of the total N farm input, only about 15% is transformed into animal protein (meat and milk) (Tamminga, 1992). Over the last two decades there has been pressure on dairy and beef cattle production to reduce nitrogen excretions to the environment (Bequette and Nelson, 2006). Cattle manure contributes to nitrogen (N) pollution as ammonia and nitrous oxide volatilization into the atmosphere, nitrate leakage in groundwater, and N runoff in surface water (Tamminga, 1992). The conversion of dietary N 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 into milk protein (Huhtanen et al., 2015).

The concentration of dietary N and N intake are the main factors that determine N excretions in dairy cows 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 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 (Prestegard-Wilson et al., 2021).

Wu and Satter (2000) 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.

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 well balance between CP and energy in the diet to maximize milk yield without overfeeding CP.

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 pool that cows rely upon temporarily to sustain steady productivity.

Model predictions indicated that factors like dietary energy concentration and dietary protein degradability influenced urine nitrogen excretions of dairy cows, however, the best way to increase milk nitrogen efficiency is the reduction of crude protein in the diet of lactating cows (Kebreab et al., 2002).

To the best of our knowledge, in Mexico, little attention has been put on the dietary crude protein of lactating cows or the effects of overfeeding with crude protein. Therefore, the study aimed to determine the effect of two dietary crude protein (DCP) levels 14 vs 16% on the productive and economic performance of Brown Swiss under confined management.

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, 19° 03' 48.5 north latitude, at an altitude of 1,798 meters above sea level.

The farm is 50 ha of extension of which 10 were destined for maize crop during the spring-summer, and during autumn and winter oat was established and preserved as silage. The experimental cows were housed in two separate freestall barns.

Cows and experimental diets

Twenty-three multiparous Brown Swiss cows of 600 ± 58 body weight (BW) (mean \pm 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) (n = 7), mid (ML) (161 ± 33) (n = 6)

and late (LL) lactation (321 ± 28 ; days in milk) ($n = 10$). Half of the cows in all strata were randomly assigned to one of two groups to have even numbers of cows according to the lactation stage on each experimental diet, except early lactation strata. Once the groups were formed the experimental diets containing 14 or 16% of dietary crude protein (DCP) were randomly assigned to each group. The two groups of cows were housed in separated free stall barns where experimental diets were fed as a total mixed ratio (TMR) offered in two meals at 9:00 am and 4:00 pm.

Experimental design

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. Cows on experimental DCP 14% in period 1 switched to 16% in the second period, whereas cows that received a 16% diet in period 1 switched to 14% in period 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 diets according to average cows characteristics by group (parity, milk yield and composition, body weight, body condition score, days in milk etc.). Afterward, during the third week of the first and second EP diet 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 ashes were obtained by incineration in a muffle furnace at 550°C for 6 h. Crude

protein (CP) was estimated by the Kjeldahl method, and neutral detergent fibre (NDF) and acid detergent fibre (ADF) by the Ankom micro-bag technique (AOAC, 1995).

The dry matter intake (DMI) (kg/cow/day) was derived from the NRC (2001) estimates using inputs according to individual cow characteristics. The amount of feed assigned to every group was estimated according to the average dry matter intake per cow multiplied by the number of cows in each group, plus an extra 10% feed.

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, during 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).

Body weight and body condition score

Cows were weighed after morning milking for two consecutive days on the last week of the 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 (Wildman et al., 1982).

Income over feeding cost

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

Statistical analysis

Data 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} = dependent variable, μ = overall mean, T_i = effect of treatment (dietary CP; $i = 1$ to 2), P_j = effect of the period ($j = 1$ to 2), LS_k = effect of lactation stage ($k = 1, 2$ and 3), $T*P_{ij}$ = 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.

RESULTS

Nutritional composition of the diets

The nutritional characteristics of the experimental diets according to the NRC (2001) estimates are presented in Table 1. Diets contained ~64% forage (oat silage and alfalfa hay) and ~36% concentrate mix (sorghum, soybean meal and minerals). 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 very similar.

Cow performance and feed efficiency

There 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 (0.57) than 14% DCP (0.55, kg/day). There was a trend towards higher lactose yield ($P = 0.08$), for cows that received 16% compared with 14% DCP. Fat, protein and lactose mean concentrations were 44.2, 29.9 and 42.1 (g/kg), respectively. Mean BW and BSC were 613 (kg) and 2.2, respectively. Mean Body weight change (BWc) and body condition score change (BCSc) were 0.6 and 0.2, respectively. Mean 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

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

Lactation stages

There were significant differences in most performance variables (Table 2), except for DMI (kg/day), fat concentration (g/kg) and yield (kg/day), protein and lactose yield (kg/day), BWc (kg/day) and BCSc ($P > 0.05$). The Mil yield of EL cows (21.2) was significantly higher than ML (18.1) and LL (16.6 kg/day) cows ($P < 0.01$). Energy-corrected milk and FPCM (kg/day) tended to be higher ($P = 0.06$) for EL decreasing as lactation progressed. Fat content and yield were not affected by the lactation stage ($P > 0.05$), whereas protein and lactose contents (g/kg) were significantly lower in EL lactation than in ML and LL lactation. Protein and lactose yield (kg/day) were not significant to the lactation stage ($P > 0.05$). Body weight was higher for 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 FPCM/DMI) were significantly higher for EL than ML and LL ($P < 0.019$).

Feeding cost and returns

Dietary crude protein supplement of 16% CP was 11% more expensive than 14% CP. The amount of milk produced by treatment 16% was 3% higher than treatment 14%. Milk-to-feed price ratios were 2.5 and 2.2 for DCP 14 and 16%. Income over feeding costs

were 0.24 and 0.21 (\$/kg) for DCP 14 and 16%, respectively. Total income over feeding cost per treatment was \$1,020 for DCP 14%, and 917 for DCP 16%.

DISCUSSION

The DCP levels tested in the present study did not affect DMI (kg/day) of cows at any lactation stage, 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 compared with 17.3% of DCP. Olmos Colmenero and Broderick, (2006) evaluated several levels of DCP ranging from low 13.5 to high 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) when reducing DCP below 13.1% in late lactation cows.

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

Cows productive performance was not different between DCP content which was in line with other reports at similar CP levels (Olmos Colmenero and Broderick, 2006; Barros et al., 2017; Yang, Ferris and Yan, 2022). Mean 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 Holstein cows in late lactation, with 30.4 kg/day of MY and 34.9 and 47 (g/kg) of protein and lactose, respectively. Fat concentration in the present study (44 g/kg) was higher than the one 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 was adequate to meet the metabolizable protein requirements of the cows under the study conditions.

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 international literature, aimed to increase dietary N utilization as well as reductions of N excretions to the environment. However, it has been pointed out by Yang et al. (2022), the results of short term studies like the present report should be interpreted with caution before putting them into practice on commercial farms and for complete lactation.

In this regard, Barros et al. (2017) reported that when reducing DCP from 16.2 to 13.1% a MY reduction was observed after the fourth week, and with a more pronounced reduction of DCP (from 16.2 to 11.8%) MY dropped one week after. So, the ability of cows to temporarily sustain milk yields with deficient DCP is related to the ability to increase urea N recycling to the gastrointestinal tract (Mutsvangwa et al., 2016).

A recent report showed that there are cows that can sustain milk production when fed low protein diets, due to a more metabolic efficiency than their contemporaries. 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) (Liu and VandeHaar, 2020). 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 or more desirable.

There were small numerical but not significant differences in milk protein among (Table 2). It could be 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, more than an effect due to DCP. This same situation could have happened as well with lactose yield which showed a trend for higher yields at 16% DCP.

The lack of interaction between DCP and lactation stages indicates that all the significant differences in the response variables were due to the advance of lactation. As the lactation stage advances a shift in nutrients partitioning occurs, then pregnancy becomes a priority over milk production (NRC 2001). Protein and lactose content was lower at the early lactation stage than during mid and late lactation, which was expected as MY decreased towards the end of lactation, and milk components tend to increase (Stanton et al., 1992). However, this trend was not significant for fat content, despite the numerical differences that showed increasing fat content as the stage of lactation increased.

The economic analysis shows that the 14% DCP allowed a lower production cost of ~11% (\$/kg), with better indicators like milk to feed price ratio (2.5 vs 2.2), and income over feeding cost (IOFC) (0.24 vs 0.21; \$/kg) than the 16% DCP. Total income over feeding

cost was 10% higher for the 14% DCP than 16% DCP. Buza et al. (2014) reported an average IOFC (\$/cow/day) of 7.71 ranging from -0.33 to 16.60, from USA dairy farms. In the present study IOFC (\$/cow/day) were 4.02 and 3.74, for 14 and 16% DCP, respectively, which were lower than those mentioned above.

With the current high volatility of commodities like animal feeds and fertilizers on the one hand and the small profit margins for dairy farmers the dairy industry is focusing on reductions of DCP in the diets of lactating cows, rather than environmental concerns (Prestegaard-Wilson et al., 2021).

In the present study, alfalfa hay and soybean meal were the main sources of crude protein in the experimental diets which accounted for 58% of the supplement cost of the diet at 14%, while for the diet at 16% crude protein accounted for 68%. Those main sources of protein are imported to most of the dairy farms in México and Latin America, a situation that increased its cost apart from the carbon footprint involve in transportation (Velarde-Guillén, Arndt and Gómez, 2022). So, the reduction of protein in the diets of cows to reduce milk production costs should be as important as the efficient use of external inputs like soybean meal and to produce food with a minimum environmental impact.

CONCLUSION

There were no differences in Brown Swiss cows performance to a reduction of DCP from 16 to 14%. The significant differences found were due to the advancement of lactation rather than a diet effect. A dietary CP of 14% allowed reductions of ~11% in milk production cost (\$/kg). Income over feeding costs were 4.02 and 3.74 \$/cow/day for 14 and 16% DCP, respectively.

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Statements and Declarations

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Conflicts of interest/competing interests

The authors declare that they have no conflict of interest.

Compliance with ethical standards

The research did not involve direct work with animals or persons, and followed guidelines accepted by the support 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.

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Table 1. Ingredient and chemical treatments according to NRC (2001)

I. Item	Dietary crude protein, % of DM	
	14	16
Ingredient, % of DM		
Oat silage	29.1	23.4
Alfalfa hay	34.8	40.8
Sorghum grain ground	32.4	28.4
Soybean meal	3.7	7.4
Nutrient		
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
NEL, 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

Table 2. 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 performance

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.

Table 3. 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

S14 = 50% ground maize ears with husks: 50% 18% CP commercial dairy concentrate; S16 = 43% ground maize ears with husks: 50% 18% CP commercial dairy concentrate: 7% soya bean meal; SC16 = 16% commercial dairy concentrate

V. Bibliografía Protocolo

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