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RESEARCH ARTICLE

Effect of partial or total replacement of soybean meal by ground peas in the diet on milk production responses, rumen metabolism and nitrogen excretions of dairy cows

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ABSTRACT

Soybean (*Glycine max* (L.) Merr.) meal is the predominant protein supplement in dairy production, but its high cost and considerable C footprint have encouraged the search for alternative protein sources. This study evaluated the effects of replacing soybean meal (S) with ground pea (*Pisum sativum* L.) grain (P) on productive and metabolic parameters in the diet of dairy cows. Twelve multiparous Holstein Friesian cows (152 ± 18.6 d in milk; milk yield 26.0 ± 1.9 kg d^{-1}) were used; following a 3 × 3 Latin square design, replicated in four balanced squares to account for residual effects. Animals were randomly assigned to one of three dietary treatments: 55% grass silage, 9% alfalfa hay, 17.5% concentrate, 10% ground corn, and 8.5% S (100S-0P); 55.5% grass silage, 9% alfalfa hay, 11.5% concentrate, 4.5% ground corn, 5% S, and 14.5% P (50S-50P); 56% grass silage, 9% alfalfa hay, 7% concentrate, 2.5% ground corn, and 25.5% P (0S-100P). Replacing S with P did not affect DM intake, which averaged 22.3 kg DM d^{-1}. However, milk yield decreased linearly from 23.96 to 22.61 kg d^{-1} with total replacement of soybean meal ($p < 0.05$), whereas milk fat concentration increased from 4.99% to 5.17% ($p = 0.04$). Feed costs decreased from 6.55 to 5.90 USD cow^{-1} d^{-1} ($p < 0.0001$) as the inclusion of peas increased. In the rumen, butyric acid concentration increased from 13.6 to 14.0 mol 100 mol^{-1}, while acetate proportion decreased ($p < 0.05$). Urinary N excretion tended to decrease from 279.3 to 256.6 g d^{-1}, and N use efficiency showed a quadratic trend, being greatest in cows fed 0S-100P. Partial replacement (50%) of S with P maintained milk production while reducing feed costs and potentially improving the environmental sustainability of dairy systems.

Key words: Dairy cows, *Glycine max*, milk production, nitrogen use efficiency, pea, *Pisum sativum*, soybean meal.

INTRODUCTION

In humid temperate regions (such as Chile, Ireland, and New Zealand), the main feed resources for dairy cattle are permanent pastures, which are characterized by comparatively low production costs that ensure the competitiveness and economic sustainability of the region's dairy farms (Pulido et al., 2024). However, climatic variation between seasons results in marked differences in pasture production throughout the year, with lower pasture growth rates occurring during winter and a decline in nutritional quality during summer as physiological maturity progresses. This leads to an increase in fiber content and a decrease in digestible protein, resulting in lower DM intake (DMI) (Keim et al., 2015). These seasonal fluctuations represent a challenge for dairy farmers who produce milk year-round (Wilkinson et al., 2020). Therefore, it is essential to provide supplementary feeds to meet the nutritional requirements of lactating cows. Traditional strategies for supplementing grazing cows involve concentrates fed in the milking parlor, complemented with forages such as silages, forage crops, and by-products offered as a partial mixed ration (PMR) before or after milking (Pulido et al., 2024).

Over the past six decades, the steadily increasing demand for vegetable oil for food, industrial, and energy uses has led to an expansion of oil crop cultivation areas (Phalan et al., 2013) and the intensification of production practices (Pretty, 2018). Since 2014, oil crops have occupied over 300 million hectares globally approximately 19% of total cropped land (Phalan et al., 2013). Remarkably, more than 85% of the world's vegetable oil is produced by only four crops: Oil palm, soybean, rapeseed, and sunflower (FAO, 2021), which are cultivated across diverse climatic zones. The clearing of native vegetation to meet the growing demand for these crops represents a considerable source of greenhouse gas (GHG) emissions, further exacerbated by intensive cultivation and post-harvest processing (Chataut et al., 2023).

Soybean (*Glycine max* (L.) Merr.) meal is the most used protein source in livestock nutrition and is also among the most expensive (Alcock et al., 2022). The search for alternative protein sources that can replace soybean meal without compromising animal performance is crucial to improving profitability by reducing feed costs. In addition, the use of alternative plant protein sources aims to decrease soybean imports into the European Union and countries such as Chile, while partially reducing the inclusion of genetically modified organisms in the food chain (Osmane et al., 2017). This approach may also help lower the C footprint of dairy production, since soybean has a relatively high C footprint (4.25 kg CO_2-eq kg^{-1} product) (Alcock et al., 2022) compared with other protein sources such as peas (0.49 kg CO_2-eq kg^{-1} product) (Nette et al., 2016).

Peas (*Pisum sativum* L.) are an increasingly important protein crop in Chile, offering significant benefits for animal production and environmental sustainability when compared to soybean meal. Cultivating peas generally results in a lower C footprint than soybean production, as peas require less synthetic N fertilizer due to their ability to fix atmospheric N, thereby reducing GHG emissions and soil degradation. Additionally, peas can improve soil structure and fertility, further enhancing the sustainability of agricultural systems in Chile (Rioja et al., 2024).

Peas are available in several forms, including raw, split, ground, and extruded. They are one of the few feed ingredients that provide relatively high concentrations of crude protein (CP), together with significant energy content in the form of starch, all at a comparatively lower cost (NASEM, 2021). Pea protein is composed primarily (85%-100%) of albumins and globulins, making it highly soluble and degradable in the rumen (Kudlinskienė et al., 2016). This characteristic can potentially reduce milk production and N use efficiency in lactating dairy cows (Mendowski et al., 2019). In terms of amino acid profile, peas have higher lysine and lower methionine and tryptophan contents compared with soybean meal; pea protein typically contains about 7.4% lysine and 0.8% methionine (NASEM, 2021).

In general, studies evaluating ground peas as a protein source for dairy cows are limited and mostly dated, despite significant advances in pea breeding over the past three decades. Therefore, it is necessary to reassess the inclusion of ground peas in dairy cow diets and their effects on DMI, milk yield and composition, blood metabolites, and ruminal parameters (Naeiny et al., 2019). Tufarelli et al. (2012) reported that total replacement did not affect milk yield or composition. More recently, Pulido et al. (2024) replaced up to 60% of corn grain and soybean meal with peas in the diets of grazing lactating dairy cows without affecting milk yield or composition. However, they observed a linear increase in milk urea N, suggesting reduced N utilization and increased urinary N excretion, which may lead to greater ammonia (NH_3) and nitrous oxide (N_2O) emissions (Beltran et al., 2022). Thus, there remains uncertainty regarding the effect of total replacement of soybean meal with ground peas on productive responses, rumen metabolism, and N excretion in lactating dairy cows.

Therefore, the aim of this study was to evaluate the effect of a partial (50%) or total (100%) replacement of soybean meal by ground peas in a grass-silage based diet on production performance, rumen metabolism and N balance of lactating dairy cows.

MATERIALS AND METHODS

The study was conducted at the Experimental Research Station of Universidad Austral de Chile, Valdivia, Chile, between January and March 2023. All experimental procedures were approved by the Universidad Austral Institutional Animal Care and Use Committee (CICUA, Approval Number: 444/2022).

Animals, housing and experimental design

Cows were subjected to a 15 d uniformity period during which all cows were offered the control diet. The animals were selected according to milk production and body weight (BW) prior to the experiment (26 kg milk d^{-1}, 560 kg BW, and 152 d in milk). Twelve multiparous lactating Holstein Friesian dairy cows were randomly allocated to the three dietary treatments based on milk production measured during the uniformity period. The experimental design was a replicated (n = 4) 3 × 3 Latin square design with three 21 d periods. Each experimental period consisted of 14 d of adaptation to the diets and 7 d of experimental measurements. Cows were grouped into four squares according to milk production and BW. The sequences of dietary treatments were balanced for residual effects of previous treatments; thus, all combinations of treatments (100S-0P to 50S-50P, 100S-0P to 0S-100P, 50S-50P to 100S-0P, 50S-50P to 0S-100P, 0S-100P to 100S-0P, and 0S-100P to 50S-50P) occurred when changing cows from one experimental period to another.

All animals were housed in the same tie-stall barn, equipped with rubber bedding and individual feeders. Animals were milked twice daily at 07:00 and 16:00 h in a milking parlor adjacent to the barn and had ad libitum access to water. The control diet (100S-0P) consisted of 12.7 kg DM grass silage, 2 kg DM alfalfa hay, 4 kg DM commercial concentrate, 2.3 kg DM ground corn, and 2 kg DM soybean (*Glycine max* (L.) Merr.) meal (S). The other two treatments replaced 50% (50S-50P) and 100% (0S-100P) of the soybean meal with ground peas (*Pisum sativum* L.) (P). To maintain isoenergetic and isonitrogenous diets, the amounts of commercial concentrate and ground corn were adjusted accordingly. Thus, 50S-50P consisted of 12.7 kg DM grass silage, 2 kg DM alfalfa hay, 2.6 kg DM commercial concentrate, 1.1 kg DM ground corn, 1.2 kg DM soybean meal, and 3.4 kg DM ground peas, whereas 0S-100P included 12.7 kg DM grass silage, 2 kg DM alfalfa hay, 1.7 kg DM commercial concentrate, 0.7 kg DM ground corn, and 5.9 kg DM ground peas. The commercial concentrate was based on small grain cereals and by-products, but did not contain soybean meal or peas. A 250 g mineral mixture was offered along with the diets.

Chemical composition and *in situ* rumen degradation parameters

Prior to the beginning of the experiment, all feed ingredients were analyzed for their chemical composition and *in situ* degradation parameters (Table 1). Samples for the chemical analyses of commercial concentrate, soybean meal, ground corn and ground peas were taken once per experimental period. For each sample, ash and ether extract (EE) were analyzed according to AOAC (1996; methods 942.05 and 920.39 for ash and EE, respectively); N content was determined by combustion (FP-428 Nitrogen Determinator, Leco Corporation, St. Joseph, Minnesota, USA) and was used to calculate crude protein (CP) content (N × 6.25). Neutral detergent fiber (NDF) was determined as aNDFom according to Van Soest et al. (1991) using sodium sulfite (Sigma-Aldrich, St. Louis, Missouri, USA) heat-stable amylase (Ankom Technology Corporation, Macedon, New York, USA) and expressed exclusive of residual ash and acid detergent fiber (ADF) ADFom according to AOAC (1996; method 973.18) expressed exclusive of residual ash.

For the *in situ* procedure, feed samples were lyophilized and ground through a 5 mm sieve. Polyester bags of 10 × 20 cm (40-60 Zm porosity) contained 4 g sample achieving a ratio of 16 mg sample cm^{-2}. Two replicates (bags) by incubation period were introduced in a fistulated cow (Holstein breed in a state of maintenance) in reverse order to re-move all the bags from the rumen at the same time. Incubation times corresponded to 0, 2, 4, 8, 10, 14, 24 and 48 and 72 h. The cannulated cows were fed a ration formulated according to its nutritional requirements and managed in a housing yard. Prior to ruminal incubation, the bags were placed in 20 × 30 cm porous laundry bags and soaked in warm water (30 °C) for 20 min and then introduced into the cows' rumen. Once removed from the rumen, the bags were washed with running water until water was clear and then frozen at -20 °C. Thereafter samples were thawed and washed in a conventional washing machine for 10 min and dried in a forced air oven at 60 °C for 48 h. The time 0 h was not incubated in the rumen and was used to determine the soluble fraction of the DM and CP. Residues after ruminal incubation were weighed to determine the amount of feed degraded at a certain incubation time.

Degradation dynamics of the DM and CP were determined using the model proposed by Ørskov and McDonald (1979), through the nonlinear procedure of the GraphPad Prism v6.0 software (GraphPad Software, San Diego, California, USA), using the exponential model without a lag phase: $PD = A + B\ (1 - e^{-kt})$, where: PD is the potential degradability, A is the soluble fraction (g kg^{-1} fraction of bags washed at time 0 h), B is the insoluble but potentially degradable fraction (g kg^{-1}), k is the degradation rate (% h^{-1}), and t is the incubation time (h).

Effective degradability (ED) was calculated from the aforementioned parameters assuming fractional passage rates (kp) of 5% h^{-1} according to McDonald (1981):

$$ED\ (g\ kg^{-1}) = A + B[k/(k + kp)]e^{-kp}$$

where A (g kg^{-1}) is the soluble fraction; B (g kg^{-1}) is the insoluble but potentially degradable fraction; k and kp (h^{-1}), are the degradation rate of the feed and the passage rate recommended (5%), respectively.

A correction for small particle losses was made according to Hvelplund and Weisbjerg (2000). Thus, samples of each feed (1.5 g) were placed in containers to which 40 mL water was added and stored at room temperature (20 °C) for 1 h and then filtered through a paper filter and washed 8 times with 20 mL water, the residue was dried in a forced air oven at 60 °C for 48 h and then weighed, calculating the soluble (SOL) fraction. Assuming that the losses of small particles are degraded similarly to the particles left in the bag, corrections can be made for the loss:

$$PDcor\ (ti) = PD\ (ti) - P\ [1 - (PD\ (ti) - (P + SOL))/(1 - (P + SOL)))], \quad (1)$$

$$EDcor = SOL + [((1 - SOL)/(1 - (P + SOL))) \times (ED - (P + SOL))], \quad (2)$$

$$acor = A - P, \quad (3)$$

$$bcor = B + P\ [B / (1 - (P + SOL))], \quad (4)$$

$$ccor = c \quad (5)$$

where PDcor (ti) is the degradability corrected at the time of incubation ti, EDcor is the corrected effective degradation, P are the losses of small particles and SOL is the water solubility.

Table 1. The nutrient concentrations of the feed ingredients and diets (% on DM basis if not otherwise stated). 100S-0P: 100% Soybean meal; 50S-50P: 50% soybean meal and 50% ground pea; 0S-100P: 100% ground pea; GS: grass silage; AH: alfalfa hay; CC: commercial concentrate; GC: ground corn; CP: crude protein; ADF: acid detergent fiber; NDF: neutral detergent fiber; EE: ether extract; ME: metabolizable energy; NFC: non-fibrous carbohydrates; aDM: soluble fraction of DM; bDM: insoluble but potentially degradable fraction of DM; cDM: rate of degradation of bDM (h^{-1}); DMPD: DM potential degradability; DMED: DM effective degradability assuming a passage rate of 5% h^{-1}; aCP: soluble fraction of CP; bCP: insoluble but potentially degradable fraction of CP; cCP: rate of degradation of bCP (h^{-1}); CPPD: CP potential degradability; CPED: CP effective degradability assuming a passage rate of 5% h^{-1}.

	Diets			Ingredients					
Item	100S-0P	50S-50P	0S-100P	GS	AH	CC	GC	SM	GP
Chemical composition									
DM	58.3	58.0	57.5	33.2	90.4	89.8	87.5	88.7	87.1
Ash	9.3	9.1	8.8	11.9	11.7	5.5	1.4	6.9	2.7
CP	19.2	19.4	18.7	17.8	16.9	15.1	8.2	52.0	23.3
ADF	24.6	24.9	25.0	33.9	39.1	9.8	2.7	5.6	7.6
NDF	39.6	40.4	41.0	51.3	48.3	28.5	13.2	8.6	22.6
EE	2.9	2.7	2.5	3.2	1.5	2.7	4.5	1.4	0.9
ME, Mcal kg DM^{-1}	2.68	2.72	2.74	2.49	2.19	2.80	3.43	3.28	3.41
NFC	28.9	28.5	28.9	17.8	21.6	48.2	72.7	31.1	50.6
Ca	0.58	0.54	0.50	0.43	1.82	1.00	0.00	0.07	0.10
P	0.35	0.33	0.31	0.34	0.18	0.44	0.25	0.51	0.25
In situ degradation parameters									
aDM	49.6	47.8	46.2	43.9	35.4	74.2	55.4	39.6	43.2
bDM	36.0	38.4	40.4	38.4	33.1	16.5	41.3	60.4	56.7
cDM	0.092	0.092	0.093	0.074	0.098	0.042	0.17	0.234	0.144
DMPD	85.6	86.2	96.6	82.3	68.6	90.6	96.9	97.8	99.9
DMED	71.4	71.2	70.9	65.1	56.0	82.3	86.5	87.7	83.3
aCP	67.8	67.9	68.1	75.7	58.0	80.6	47.7	17.0	51.2
bCP	26.5	26.6	26.6	17.1	30.1	16.4	50.0	81.3	48.7
cCP	0.199	0.193	0.187	0.192	0.156	0.21	0.181	0.176	0.176
CPPD	94.2	94.5	94.8	92.8	88.0	97.0	97.6	98.3	99.9
CPED	88.2	88.2	88.1	88.7	79.7	93.4	85.2	85.4	87.6

Dry matter and nutrient intake

Prior to feeding, all feeds were weighed and mixed according to each cow's dietary treatment and offered after a.m. and p.m. milkings.

Feeds offered and orts were recorded all days of week 3 of each period. Subsamples of each feed ingredient and orts were collected and their DM content determined in a forced-air oven at 105 °C for 12 h. The nutrient intake of each feed ingredient was calculated by multiplying the DM intake (DMI) of each ingredient and its nutrient concentration. Samples of grass silage and alfalfa hay were collected once a week, freeze-dried, ground through a 1 mm screen (Wiley Mill, Philadelphia, Pennsylvania, USA) and stored for chemical analyses.

Milk production and composition

Cows were milked at 07:00 and 16:00 h, and milk yield was recorded daily with a flow sensor (MPC580 DeLaval, Tumba, Sweden) throughout the experiment. The daily average for the final week of each period was used in the statistical analysis and reported in the manuscript. Milk samples (350 mL) were collected with milk meters (Waikato, Hamilton, New Zealand) at morning and afternoon milking times on three non-consecutive days of each experimental period for fat, crude protein and urea analyses by mid-infrared spectrophotometry (Foss 4300 Milko-scan, Foss Electric, Hillerød, Denmark).

Energy corrected milk (ECM) was calculated as:

$$ECM = (12.82 \times kg\ fat) + (7.13 \times kg\ protein) + (0.323 \times kg\ milk).$$

Fat-protein corrected milk (FPCM) was calculated as:

$$FPCM = kg\ milk \times (0.1226 \times \%\ fat + 0.0776 \times \%\ protein + 0.2534)$$

Rumen fermentation

Rumen fluid was collected using an intraesophageal scoop (Flora Rumen Scoop; Prof-Products, Guelph, Ontario, Canada) at 09:00 and 15:00 h on 1 d of each data collection period. Samples were strained through four layers of cheesecloth. A 10 mL sample was drawn off, mixed with 0.2 mL 50% (w/v) sulfuric acid, and stored at -20 °C pending determination of volatile fatty acids (VFA) and NH_3 concentrations. Rumen fluid was allowed to thaw for 16 h at 4 °C and then centrifuged at 10 000 × g for 10 min at 4 °C. Six milliliters of supernatant were drawn off and then centrifuged at 10 000 × g for 10 min at 4 °C. Thawed supernatant of rumen fluid samples was analyzed for VFA by gas chromatography (GC). Briefly, the GC was equipped with a flame ionization detector (FID) and the conditions were as follows: A 0.50 µL sample volume was injected using a split/splitless injector set at 230 °C. Helium was used as the carrier gas at a constant flow rate of 0.5 mL min^{-1}. Separation was achieved on an ELITE-23 free fatty acid phase (FFAP) capillary column (high polarity; Perkin Elmer, Waltham, Massachusetts, USA). The oven temperature program started at 105 °C held for 3 min, followed by a temperature ramp at 8 °C min^{-1} up to 230 °C, which was then maintained for 8 min. The detector temperature was maintained at 230 °C throughout the analysis. Total VFA was considered as the sum of acetate, butyrate, propionate, isobutyrate, valerate, and isovalerate. Ammonia was determined by the phenol-hypochlorite reaction method.

Urine collection microbial protein synthesis and N use efficiency

Rumen microbial N flow was estimated based on purine derivatives (PD). Spot urine samples (20 mL) were collected by subvulvar stimulation every 3 h during 1 d in each experimental period. Samples were acidified with 2 mL sulfuric acid (10% v/v) and stored at -20 °C. A composite sample per cow was made for each period and analyzed for allantoin, uric acid and creatinine by HPLC. A Waters Alliance 2996 sensitive module HPLC (Waters, Milford, Massachusetts, USA) equipped with a UV spectrophotometric detector set at 220 nm was used for these analyses. The stock standard solutions (1 mg mL^{-1}) of allantoin and creatinine were freshly prepared in water. The uric acid standard was dissolved in water (1 mg mL^{-1}) by adding 0.01 N sodium hydroxide solution (5 mL 100 mL^{-1} stock standard solution) to make the pH 7. The quantitative HPLC separations were performed at a temperature of 30 °C on a C18 reversed-phase column (250 × 4.60 mm ID, 5 µm particle size). The mobile phase was 10 mM potassium dihydrogen phosphate solution (pH 7.0). The flow rate was 1 mL min^{-1}, and the absorbance detection was set at 220 nm. Compound peaks were identified by the retention times and quantified by comparison of the peak areas of the samples with those of authentic standards on a 20 mL

injection. The equations used in calculating the estimated microbial N supply outlined below have been described previously according to Chen and Gomes (1992).

The PD index was calculated based on total PD [Allantoin (mmol L^{-1}) + Uric acid (mmol L^{-1})] as:

$$\text{PD index} = (\text{Total PD (mmol L}^{-1}\text{)}/\text{Creatinine (mmol L}^{-1}\text{)}) \times BW^{0.75}$$

Urine volume was estimated using creatinine concentration as a marker and assuming a daily creatinine excretion of 26 mg kg^{-1} body weight (BW). The estimated urinary creatinine excretion (0.9 mmol kg^{-1} $BW^{0.75}$) was included in the following equation to estimate the daily excretion of PD (mmol kg^{-1} $BW^{0.75}$):

$$\text{Daily excretion of PD (dPD)} = \text{mmol/kg } BW^{0.75} = \text{PD index} \times 0.9$$

From this, the amount of purines absorbed daily was estimated:

$$\text{Daily absorbed purines (daP)} = [\text{dPD (mmol/kg } BW^{0.75}) - 0.385 \times BW^{0.75}] + 0.85$$

Microbial N (g N d^{-1}) supply was estimated using the following equation:

$$\text{Microbial N (g N d}^{-1}\text{)} = (\text{daP} \times 70)/(0.116 \times 0.83 \times 1000)$$

Nitrogen balance was calculated as follows:

$$\text{N intake} = \text{MN} + \text{UN} + \text{FN}$$

Total N intake (g d^{-1}) was determined by multiplying DMI and dietary N concentration. Milk N (MN) was calculated and reported as described as:

$$\text{MN (g N d}^{-1}\text{)} = \text{Milk yield} \times (\%\text{ CP in milk}/6.38)/100$$

Urinary N (UN) was estimated from the N concentration in urine and the daily urine volume (based on the urinary creatinine concentration, considering a daily creatinine excretion of 0.212 mmol kg^{-1} BW), while fecal N (FN) was estimated as the difference between N intake and N excreted in milk and urine.

Economic analyses

The selling price per liter of milk was determined according to the Nestlé Chile S.A. payment scheme, which considers a base price, fat and protein contents, colony-forming unit count, somatic cell count, and applicable bonuses. This calculation yielded the net income from milk sales. The cost per kilogram of DM of each feed ingredient used in the different treatments was also determined. The commercial costs of feed ingredients at the date of the trial were used for commercial concentrate (USD 0.36 kg^{-1} DM), ground corn (USD 0.46 kg^{-1} DM), soybean meal (USD 0.79 kg^{-1} DM), ground peas (USD 0.41 kg^{-1} DM), alfalfa hay (USD 0.41 kg^{-1} DM), whereas for grass silage (USD 0.18 kg^{-1} DM) the costs of production and transportation on farm were considered. The margin over feed cost (MOFC) was calculated as the difference between net income and total feed costs.

Statistical analyses

Prior to statistical analyses, the assumptions of normality and homogeneity of variances were verified. Data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, North Carolina, USA) to account for carryover effects according to the following model:

$$Y_{ijklm} = \mu + S_i + A_{i(j)} + P_k + T_l + C_m + e_{ijklm}$$

where Y_{ijklm} is an observation for each dependent variable; μ is the general mean; S_i is the fixed effect of the i^{th} treatment sequence (i = 1 to 6); $A_{i(j)}$ is the random effect of the j^{th} cow in the i^{th} sequence; P_k is the fixed effect of the k^{th} period (k = 1 to 3); T_l is the fixed effect of the l^{th} treatment (l = 1 to 3); C_m is the fixed carryover effect from the previous period (C = 0, if period = 1); and e_{ijklm} is the random error.

As carryover effects were not detected, a simplified model for a replicated Latin square was applied:

$$Y_{ijkl} = \mu + S_i + A_{i(j)} + P_k + T_l + e_{ijkl}$$

where Y_{ijkl} is the observation for dependent variables; μ is the general mean; S_i is the random effect of the i^{th} square (i = 1 to 4); $A_{i(j)}$ is the random effect of cow nested within square; P_k is the fixed effect of the k^{th} period (k = 1 to 3); T_l is the fixed effect of the lth treatment (l = 1 to 3); and e_{ijkl} is the random error.

Data for DM and nutrient intake, milk yield, milk composition, production efficiency (PE), and milk N (MN) below the defined threshold were summarized by day. Data for volatile fatty acids (VFA) and ammonia N (NH_3-N) were analyzed using the same model but included sampling time as a repeated measure, with cow as the subject, and incorporated the interaction between treatment and sampling time.

The estimation method was restricted maximum likelihood (REML), and degrees of freedom were adjusted using the Kenward-Roger method. The variance-covariance structure that produced the lowest corrected

Akaike information criterion (AICc) was compound symmetry, which was selected for the final model. Orthogonal polynomial contrasts were used to assess linear and quadratic effects of ground pea inclusion.

All data are presented as least squares means (LSM) ± standard error of the mean (SEM). Statistical significance was declared at $p \leq 0.05$, and trends were discussed at $0.05 < p \leq 0.10$.

RESULTS

Dry matter and nutrient intake

No effect of diet was observed on DM intake (DMI), which averaged 22.3 kg DM cow^{-1} d^{-1}, or on non-fibrous carbohydrate (NFC) intake ($p > 0.05$; Table 2). A quadratic effect ($p < 0.0001$) was observed for metabolizable energy (ME) intake, which increased from 64.6 to 64.9 Mcal d^{-1} in cows fed 100S-0P and 50S-50P, respectively, but decreased to 62.3 Mcal d^{-1} in cows fed 0S-100P.

Table 2. Effect of replacing soybean meal by ground pea on DM and nutrient intake in the diet of mid-lactating dairy cows. 100S-0P: 100% Soybean meal; 50S-50P: 50% soybean meal and 50% ground pea; 0S-100P: 100% ground pea; SEM: standard error of the mean; ME: metabolizable energy; CP: crude protein; NDF: neutral detergent fiber; EE: ether extract; NFC: non-fibrous carbohydrates.

					p-Value	
Intake	100S-0P	50S-50P	0S-100P	SEM	Linear	Quad.
DM, kg DM d^{-1}	22.35	22.45	22.25	0.144	0.512	0.265
ME, Mcal d^{-1}	64.64	64.87	62.34	0.155	< 0.0001	< 0.0001
CP, kg DM d^{-1}	4.56	4.61	4.19	0.011	< 0.0001	< 0.0001
NDF, kg DM d^{-1}	9.48	9.75	9.18	0.019	< 0.0001	< 0.0001
EE, kg DM d^{-1}	0.71	0.64	0.57	0.001	< 0.0001	0.3928
Ash, kg DM d^{-1}	2.21	2.16	2.01	0.001	< 0.0001	< 0.0001
NFC, kg DM d^{-1}	6.92	7.02	6.82	0.145	0.512	0.265

A quadratic effect ($p < 0.0001$) was also detected for crude protein (CP) intake, which increased from 4.56 to 4.61 kg d^{-1} with partial replacement of soybean meal by pea grain, and then decreased to 4.19 kg d^{-1} with total replacement. Similarly, a quadratic effect ($p < 0.0001$) was observed for neutral detergent fiber (NDF) intake, which increased from 9.48 to 9.75 kg d^{-1} in cows fed 100S-0P and 50S-50P, respectively, before decreasing to 9.18 kg d^{-1} in cows fed 0S-100P.

Finally, a linear decrease ($p < 0.0001$) was observed for ether extract (EE) and ash intakes, from 0.71 to 0.57 kg DM d^{-1} and from 2.21 to 2.01 kg DM d^{-1}, respectively, between treatments 100S-0P and 0S-100P.

Milk production and composition

No differences were observed among treatments for feed conversion, milk fat yield, crude protein concentration, milk urea N, milk price per liter, net income, or margin over feed cost ($p > 0.05$) (Table 3). However, a linear decrease ($p < 0.05$) in milk yield was detected, from 23.96 to 22.61 kg d^{-1}, with the total replacement of soybean meal by pea grain. Conversely, a linear increase ($p < 0.05$) in milk fat concentration was observed, by 0.18 percentage units, with total replacement of soybean meal by pea grain in the diet.

A linear reduction ($p < 0.05$) was also observed in CP yield, from 0.89 to 0.81 kg d^{-1}, with total replacement of soybean meal by pea grain (Table 3). Similarly, linear effects ($p < 0.05$) were observed for energy corrected milk (ECM) and fat-protein corrected milk (FPCM) with the total replacement of soybean meal by ground pea. Finally, a linear decrease ($p < 0.001$) in feeding cost was detected, from 6.55 to 5.90 USD cow^{-1} d^{-1}, when soybean meal was fully replaced by ground pea in the diet.

Table 3. Effect of replacing soybean meal by ground pea on milk production and composition in the diet of mid-lactating dairy cows. 100S-0P: 100% Soybean meal; 50S-50P: 50% soybean meal and 50% ground pea; 0S-100P: 100% ground pea; SEM: standard error of the mean; MY/DMI: milk yield to DM intake ratio; CP: crude protein; ECM: energy corrected milk; FCM 4%: 4% fat corrected milk; FPCM: fat and protein corrected milk; MOFC: margin over feed costs.

					p-Value	
	100S-0A	50S-50P	0S-100P	SEM	Linear	Quad
Milk yield, kg d^{-1}	23.96	23.61	22.61	0.776	0.002	0.295
MY/DMI, kg	1.03	1.05	1.01	0.038	0.339	0.212
Fat, %	4.99	5.02	5.17	0.146	0.044	0.403
Fat, kg d^{-1}	1.17	1.15	1.13	0.040	0.166	0.836
CP, %	3.79	3.76	3.72	0.063	0.078	0.897
CP, kg d^{-1}	0.89	0.87	0.81	0.022	0.0004	0.239
ECM, kg d^{-1}	28.86	28.36	27.27	0.790	0.017	0.546
FPCM, kg d^{-1}	27.13	26.65	25.63	0.738	0.016	0.547
Urea, mg L^{-1}	444.3	460.3	425.9	15.37	0.222	0.074
Feed cost, USD cow^{-1} d^{-1}	6.55	6.31	5.9	36.46	< 0.0001	0.047
Milk price, USD kg^{-1} d^{-1}	0.54	0.53	0.53	8.38	0.344	0.108
Net income, USD cow^{-1} d^{-1}	12.41	12.3	11.88	292.53	0.142	0.622
MOFC, USD cow^{-1} d^{-1}	5.86	5.98	5.98	283.47	0.713	0.833

Rumen fermentation

There was no effect of diet on total volatile fatty acids (VFA) concentrations; relative proportions of propionic, isobutyric, and valeric acids; acetate-to-propionate ratio; or ammonia concentrations ($p > 0.05$; Table 4). A significant Diet × Time of day interaction ($p < 0.05$) was observed for the relative proportion of acetic acid, showing a linear decrease from 100S-0P to 0S-100P in the morning, with no differences detected in the afternoon. The relative proportion of butyric acid increased linearly ($p < 0.05$) from 13.6 to 14.0 mol 100 mol^{-1} with increasing inclusion of ground pea. For valeric and isovaleric acids, significant Diet × Time interactions ($p < 0.001$) were also observed, showing a linear increase with the inclusion of ground pea in the morning, while no differences were detected among dietary treatments in the afternoon.

Table 4. Effect of replacing soybean meal by ground pea on rumen volatile fatty acid (VFA) and ammonia (NH_3) in the diet of mid-lactating dairy cows. 100S-0P: 100% Soybean meal; 50S-50P: 50% soybean meal and 50% ground pea; 0S-100P: 100% ground pea; SEM: standard error of the mean; D×t: Diet × Time interaction.

								p-Value	
	100S-0A	50S-50P	0S-100P	SEM	Diet	Time	D×t	Linear	Quad
Total VFA, mM	135.7	132.8	131.7	3.89	0.587	< 0.001	0.542	0.359	0.652
VFA, mol 100 mol^{-1}									
Acetate	63.4	63.4	62.9	0.26	0.082	< 0.001	0.005	0.046	0.289
Propionate	16.5	16.4	16.3	0.12	0.396	0.035	0.475	0.188	0.712
Isobutyrate	2.92	2.96	3.06	0.07	0.19	< 0.001	0.033	0.069	0.971
Butyrate	13.6	13.5	14.0	0.12	0.004	0.955	0.082	0.005	0.067
Isovalerate	1.83	1.84	1.91	0.04	0.071	< 0.001	0.003	0.025	0.557
Valerate	1.80	1.79	1.85	0.04	0.282	< 0.001	0.019	0.173	0.395
Ammonia, mmol L^{-1}	8.17	8.5	8.37	0.42	0.869	0.01	0.442	0.811	0.635

Urinary purine derivatives and N use efficiency

There were no effects of dietary treatment on purine derivatives (PD) (allantoin, uric acid, creatinine), microbial N production, or urinary N excretion ($p > 0.05$; Table 5). A quadratic effect ($p < 0.05$) was observed for N intake, which increased from 729.4 to 737.5 g d^{-1} in cows fed 100S-0P and 50S-50P, respectively, and then decreased to 671.9 g d^{-1} in cows fed 0S-100P. A linear decrease ($p < 0.05$) in milk nitrogen output was observed, from 138.9 to 131.1 g d^{-1} for 100S-0P and 0S-100P, respectively. There was a trend ($p < 0.10$) toward a linear decrease in urinary N excretion with increasing inclusion of ground pea, whereas fecal N output tended to increase linearly ($p < 0.10$) with the total re-placement of soybean meal by ground pea. A quadratic trend ($p < 0.10$) was observed for fecal N concentration, which was highest in cows fed 50S-50P. Nitrogen use efficiency also showed a trend toward a quadratic response, decreasing from 100S-0P to 50S-50P, and then increasing in cows fed 0S-100P.

Table 5. Effect of replacing soybean meal by ground pea on purine derivatives (PD) and predicted microbial N in the diet of mid-lactating dairy cows. 100S-0P: 100% soybean meal; 50S-50P: 50% soybean meal and 50% ground pea; 0S-100P: 100% ground pea; SEM: standard error of the mean; NUE: N use efficiency.

					p-Value	
	100S-0P	50S-50P	0S-100P	SEM	Linear	Quad
Allantoin, mmol d^{-1}	656.7	613.1	619.2	25.6	0.209	0.341
Uric acid, mmol d^{-1}	24.9	24.5	22.5	3.48	0.608	0.846
Creatinine, mmol d^{-1}	138.1	137.4	137.6	2.75	0.611	0.565
Total PD, mmol d^{-1}	681.6	638.5	641.2	24.4	0.146	0.342
PD excretion, mmol d^{-1}	598.7	562.4	563.4	21.8	0.143	0.369
N intake, g d^{-1}	729.4	737.5	671.9	2.15	< 0.0001	< 0.0001
Microbial N, g d^{-1}	455.7	425.9	426.7	18.1	0.143	0.375
Milk N, g d^{-1}	138.9	136.1	131.1	2.86	0.047	0.734
Urinary N, %	12.6	12.7	12.7	0.36	0.828	0.836
Urinary N, g d^{-1}	279.3	275.8	256.6	14.5	0.066	0.445
Fecal N, %	2.82	2.97	2.82	0.04	0.111	0.081
Fecal N, g d^{-1}	201.6	212.2	213.9	4.25	0.097	0.195
NUE, %	19.1	18.5	19.5	0.40	0.381	0.081

DISCUSSION

Dry matter and nutrient intake and productive performance

When analyzing the effect of supplementing lactating cows by replacing soybean meal with ground peas, no negative effects on DM intake (DMI) were observed. This is consistent with previous findings by Naeiny et al. (2019), who replaced up to 66% of soybean meal with ground peas in the diet of dairy cows, as well as Volpelli et al. (2009), who included up to 15% pea grain to replace soybean meal and corn grain in diets of primiparous and multiparous lactating cows. Similarly, Pulido et al. (2024) reported no effects on DMI when evaluating the inclusion of up to 60% pea grain in a commercial concentrate for dairy cows under restricted grazing.

Although there were no differences in DMI, differences were observed in the intake of some nutrients, which were likely associated with the nutritional composition of the forages. It should be noted that in this study, the ingredients comprising the experimental diets were mixed manually in individual cubicles. Feed refusals consisted mainly of grass silage and alfalfa hay. This is relevant since DMI is known to be a key determinant of milk production in both grazing systems (Ruiz-Albarran et al., 2016) and in confinement systems with high concentrate inclusion (NASEM, 2021).

The inclusion of ground peas linearly reduced milk yield from 23.96 to 22.61 kg d^{-1}, in agreement with Pereira et al. (2017), who reported decreased milk yield with high levels of split pea inclusion (≥ 24% of dietary DM). This reduction is probably related to deficiencies in metabolizable protein supply, particularly of the essential amino acids lysine and methionine, which limit milk production (Carder and Weiss, 2017). In our study,

no differences were observed in microbial N synthesis or rumen-undegradable protein, suggesting that the lower methionine concentration in ground peas compared with soybean meal (NASEM, 2021) may have limited milk yield. This hypothesis is supported by Pereira et al. (2017), who found that supplementing peas with rumen-protected lysine and methionine restored milk yield without affecting milk composition.

The linear increase in milk fat concentration in cows supplemented with ground peas could be attributed to the higher ruminal butyric acid concentration, as butyric acid is one of the main precursors for milk fatty acid synthesis in the mammary gland (Cheng et al., 2020). Additionally, variations in milk fat content are often associated with changes in milk yield, with higher milk fat concentrations observed when milk yield declines (Daley et al., 2022).

The quadratic trend observed for milk urea (higher in cows fed the diet with 50% of soybean meal (S) and 50% of peas (P) "50S-50P", followed by the diet with 100% of soybean meal as protein source "100S-0P" and the diet with 100% of peas as protein source "0S-100P") may be explained by the higher N intake, which is a key factor influencing milk urea concentration (Guliński et al., 2016). Previous studies have shown a linear relationship between N intake and milk urea content (Zhao et al., 2025), which is consistent with the results of this study.

Although the inclusion of ground peas reduced feeding costs, it did not improve the margin over feed costs due to the lower milk yield, particularly in cows fed 0S-100P. Thus, the main advantage of replacing soybean meal with ground peas may be associated with the lower C footprint of peas compared with soybean meal (Nette et al., 2016; Alcock et al., 2022).

Rumen fermentation

According to Dieho et al. (2016), production increases with greater DMI and fermentable organic matter. In the present study, the inclusion of ground peas did not affect total VFA concentration, likely because the diets had similar digestibility, as shown by the *in situ* rumen degradation parameters and estimated effective degradabilities, which did not differ among treatments. Dhakal et al. (2024) reported that total volatile fatty acids (VFA) concentration in the rumen is closely associated with dietary digestibility.

The inclusion of ground peas caused a linear increase in the concentrations of butyric and isovaleric acids at the expense of acetic acid in the rumen. Similar changes in the VFA profile have been reported by Oba et al. (2015) when high-starch diets are fed to dairy cows. In this regard, peas contain approximately 42% starch (NASEM, 2021), and in the 50S-50P and 0S-100P diets, soybean meal and commercial concentrate were replaced with ground peas and ground corn. Ruminal ammonia concentration was not affected by the inclusion of peas, remaining within the optimal range of 5 to 11 mmol L^{-1} recommended by Schwab and Broderick (2017) to maximize microbial growth.

Urinary purine derivatives and N metabolism

Nitrogen intake did not affect microbial protein synthesis, although a quadratic trend was observed in N use efficiency, which was higher in cows fed 0S-100P. Microbial protein synthesis levels were consistent with the moderate milk yields observed (Dineen et al., 2021). Despite differences in N intake among treatments, microbial N production was not affected by the inclusion of ground peas, suggesting that the additional N was not utilized for microbial protein synthesis. This is reflected by the higher milk urea concentrations observed in cows fed the 100S-0P and 50S-50P diets. Excess N intake is generally excreted into the environment via urine (Beltran et al., 2021). Increased urinary N excretion is partly a consequence of elevated ruminal ammonia concentrations. Excess ammonia is metabolized in the liver to urea, which can be recycled through saliva or excreted in urine (Keim and Anrique, 2011).

Nitrogen excreted in feces and urine represents an indirect source of greenhouse gases, such as N_2O, and non-CO_2 gases, such as ammonia NH_3. The environmental significance of N_2O is related to its high global warming potential, which is 265 times greater than that of CO_2, while ammonia is considered an atmospheric pollutant due to volatilization losses and as an indirect source of N_2O emissions (Beltran et al., 2021).

Because potential N_2O emissions from urine are five times greater than those from feces, nutritional strategies should aim to shift N excretion from urine to feces or to reduce urinary N excretion. In this study, the inclusion of peas in the diet tended to reduce urinary N excretion and increase fecal N excretion, which may contribute to lowering nitrous oxide emissions (Ribeiro-Filho et al., 2020).

CONCLUSIONS

Partial replacement (50%) of soybean meal with ground peas maintained milk yield while reducing feed costs in mid-lactation dairy cows. In contrast, total replacement reduced milk production despite increasing milk fat concentration. The inclusion of ground peas also tended to decrease urinary N excretion and improve N use efficiency, suggesting that peas may represent a more environmentally sustainable alternative protein source for dairy cow diets.

Author contribution

Conceptualization: J.P.K. Methodology: J.P.K., M.G. Software: J.P.K. Validation: J.P.K., M.G. Formal analysis: J.P.K. Investigation: J.P.K., H.U., V.H. Resources: J.P.K. Data curation: J.P.K., H.U., V.H. Writing-original draft preparation: J.P.K., H.U., V.H. Writing-review and editing: J.P.K., M.G. Visualization: J.P.K., H.U. Supervision: J.P.K. Project administration: J.P.K. Funding acquisition: J.P.K. All authors have read and agreed to the published version of the manuscript.

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