

# Protein supplement source and processing for cattle fed forage-based diets: Effects on nitrogen and energy use and methane emissions

Pauliane Pucetti<sup>1</sup>, Christy A. Finck<sup>1</sup>, Zachary E. Carlson<sup>2</sup>, Julia T. da Silva<sup>3</sup> and Kendall C. Swanson<sup>1</sup>

*Protein supplementation improves nitrogen retention, reduces methane losses and enhances energy use efficiency regardless of supplement source or processing. Supplementation with distillers grains led to greater nitrogen retention due to their higher protein content. Pelleting altered nitrogen and energy metabolism, which warrants further investigation.*

## Summary

This study tested how protein source (DDGS vs. soybean-based) and supplement form (meal vs. pellet) affect nitrogen and energy use in steers. Protein supplementation increased nitrogen intake and retention, reduced methane emissions and improved overall energy efficiency. Supplementing DDGS led to greater nitrogen retention than a soybean-based supplement, mainly due to its higher protein content. Pelleting changed how nitrogen and energy were used, including increased urinary nitrogen losses and reduced fecal energy losses. These results show that protein supplementation improves nutrient utilization, reduces methane output and supports more efficient energy use in forage-fed cattle. Pelleting may further influence these responses and warrants more investigation.

## Introduction

Forage-based diets often fail to meet the protein requirements of beef cattle, especially during winter or drought conditions. When crude protein (CP) levels drop below 9-10%, fiber digestion is impaired, feed intake declines and nutrient imbalances can lead to increased nitrogen excretion and greater methane emissions (NASEM, 2016). Protein supplementation is a well-established strategy to improve nutrient utilization and support animal performance, but its impact extends beyond digestion; it also influences nitrogen and energy partitioning and contributes to the environmental footprint of cattle production.

The form and composition of protein supplements can influence digestion dynamics and affect energy and nitrogen utilization. Pelleted supplements, or “range cubes,” are widely adopted in extensive grazing systems due to their ease of handling and distribution, but how pelleting affects nitrogen use efficiency, methane production and energy metabolism remains unclear. The use

of DDGS has traditionally dominated supplementation programs in the northern Great Plains (Mueller, 2024). However, since 2023, North Dakota has expanded its soybean processing capacity, increasing the interest in soybean-based protein supplements as alternatives to DDGS sources. This study aimed to evaluate how protein supplementation, source (DDGS vs. soybean-based) and processing method (meal vs. pellet) affect methane emissions and how nitrogen and energy use in cattle fed a forage-based diet.

## Experimental Procedures

All procedures involving animals were approved by the North Dakota State University Institutional Animal Care and Use Committee. Five Jersey steers (initial body weight [BW] =  $994 \pm 20$  lb), fitted with ruminal, duodenal and ileal cannulas, were used in a  $5 \times 5$  Latin square design with a  $2 \times 2 + 1$  factorial arrangement of treatments. Factors included two protein sources — distillers dried grains with solubles (DDGS) or a soybean-based supplement (52.5% soybean meal, 47.5% soyhulls) — each in meal or pelleted form and a negative control with no supplementation. Supplements were offered daily at 0.5% of BW. All animals received free-choice access to grass hay, mineral supplement and water. Supplements were fed at 7:30 a.m. and hay at 8:00 a.m., adjusted daily to maintain ~5% refusals.

<sup>1</sup>NDSU Department of Animal Sciences

<sup>2</sup>Danisco Animal Nutrition and Health, IFF Inc.

<sup>3</sup>Colorado State University

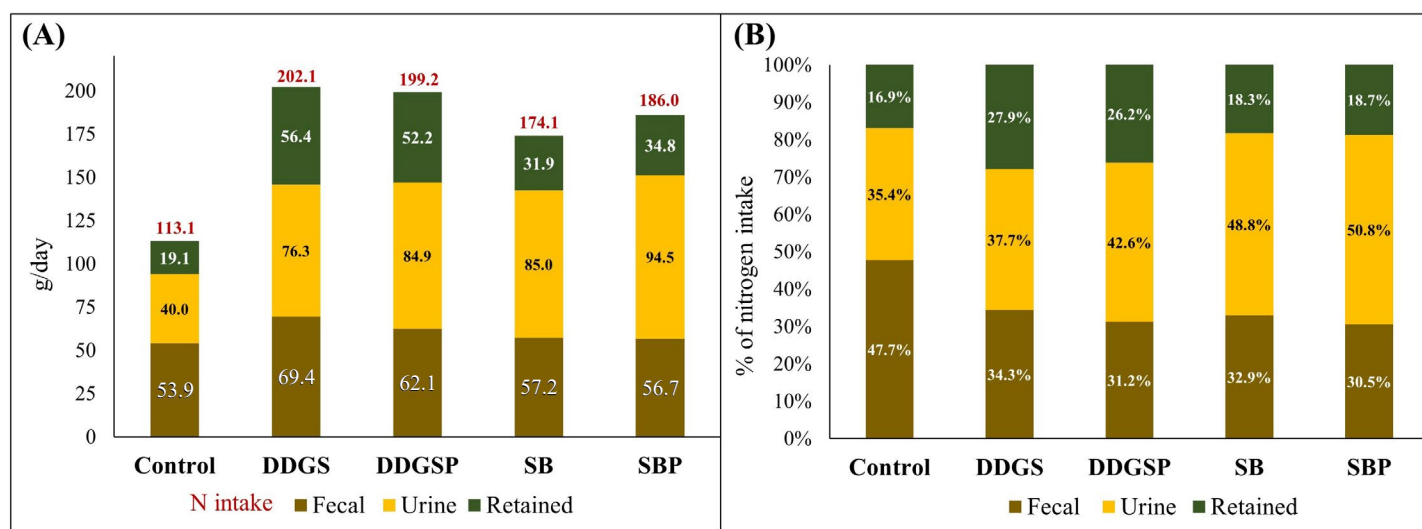
Each period lasted 14 days, with seven days of adaptation and seven days of data collection. Offered feed (hay and supplements) and refusals were weighed and sampled daily during the collection phase. Total feces and urine were collected over five consecutive days (days 8 to 12). Feces were collected using fecal bags, weighed and sampled twice daily. Feed, refusals and fecal samples were composited by steer and period, dried, ground and analyzed for nutrient and energy content. Urine was collected using funnels and vacuum systems, preserved in HCl and subsampled daily. Total volume was recorded, and aliquots were frozen for nitrogen and energy analysis.

Methane emission was measured using headbox respiration chambers over 24 hours on day 13 of each period. Heat production was estimated by indirect calorimetry. Steers were tethered comfortably and had ad libitum access to hay and

water. Air samples were collected from the inlet and outlet of the chambers and analyzed with a Siemens Ultramat 23 gas analyzer for oxygen, carbon dioxide and methane. Total air flow was recorded using a wet gas meter and corrected for temperature and humidity. Methane energy loss was calculated by multiplying CH<sub>4</sub> volume by 9.45 kcal/L. Energy balance was calculated from gross energy intake and energy losses via feces, urine, methane and heat. Data were analyzed using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC). The model included treatment as a fixed effect and steer and period as random effects. Preplanned orthogonal contrasts were used to test effects of supplementation (control vs. supplemented), source (DDGS vs. soybean), processing (meal vs. pellet) and source × form interaction. Significance was declared at  $P < 0.05$  and tendencies at  $0.05 \leq P \leq 0.10$ .

## Results and Discussion

Protein supplementation increased nitrogen (N) intake ( $P < 0.001$ ; Figure 1), with greater values for the DDGS-based compared to the soybean-based supplement ( $P = 0.001$ ). Fecal N excretion followed a similar pattern, increasing with supplementation ( $P = 0.005$ ) and being greater for DDGS-based supplements ( $P < 0.001$ ); a tendency for lower fecal N was observed with pelleting ( $P = 0.071$ ). Urinary N excretion also increased with supplementation ( $P < 0.001$ ) and was greater for soybean-based supplements ( $P = 0.041$ ), likely due to greater ruminal ammonia production from the more degradable protein. Without sufficient fermentable energy from the hay to support microbial uptake, excess ammonia was likely absorbed and excreted as urea in the urine. Pelleting increased urinary N excretion ( $P = 0.043$ ), demonstrating that processing affects N metabolism. Nitrogen retention was increased by



**Figure 1. (A) Daily nitrogen (N) intake and partitioning (g/day) and (B) N partitioning as percentage of intake in steers fed grass hay and supplemented or not at 0.5 % of body weight with a soybean-based or DDGS-based protein supplement, offered in meal or pelleted form. Control = hay only; DDGS = hay supplemented with DDGS in meal form; DDGSP = hay supplemented with DDGS in pellet form; SB = hay supplemented with soybean-based supplement (52.5% soybean meal and 47.5% soyhulls) in meal form; SBP = hay supplemented with soybean-based supplement in pellet form. Standard error mean (g/day) for panel A variables were: intake = 11.4, fecal = 4.20, urine = 6.86, digested = 7.99, retained = 7.09.  $P$ -values (supplementation, source, and processing, respectively) were  $< 0.001$ ,  $0.001$ , and  $0.409$  for N intake;  $0.005$ ,  $< 0.001$ , and  $0.071$  for fecal N;  $< 0.001$ ,  $0.041$ , and  $0.043$  for urinary N; and  $< 0.001$ ,  $0.001$ , and  $0.892$  for retained N. No interaction effect was detected ( $P > 0.10$ ).**

supplementation ( $P < 0.001$ ) and was greater with DDGS supplementation ( $P = 0.001$ ), reflecting their higher crude protein content. Nitrogen retention as a percentage of intake also increased with supplementation ( $P = 0.016$ ) and was greater for DDGS ( $P < 0.001$ ), with no effects of pelleting or the interaction ( $P > 0.10$ ).

Methane ( $\text{CH}_4$ ) production in liters per day was not affected by treatment ( $P > 0.10$ ). However, when expressed per kg of DM intake,  $\text{CH}_4$  tended to decrease with supplementation ( $P = 0.052$ ), and was reduced when expressed per kg of digested DM ( $P = 0.016$ ), suggesting improved fermentation efficiency. No effects of source, pelleting or interaction were observed for  $\text{CH}_4$  production ( $P > 0.10$ ). These results indicate that protein supplementation reduces  $\text{CH}_4$  emissions per unit of digested feed.

Gross energy (GE) intake was not affected by treatment ( $P > 0.10$ ), but supplementation reduced fecal energy losses ( $P = 0.041$ ) and increased urinary energy losses ( $P < 0.001$ ), consistent with greater N intake and excretion. Methane energy losses were reduced with supplementation ( $P = 0.012$ ), while heat production increased ( $P = 0.003$ ). Retained energy increased with supplementation ( $P = 0.025$ ), indicating improved energy utilization. When expressed as a percentage of GE intake, supplementation reduced fecal ( $P < 0.001$ , Figure 2) and methane ( $P < 0.001$ ) losses, and it increased urinary losses ( $P < 0.001$ ) and energy retention ( $P = 0.016$ ). Pelleting reduced fecal energy losses ( $P = 0.048$ ), and methane energy losses tended to be greater with soybean-based supplements than with DDGS ( $P = 0.096$ ). Retained energy was not affected by protein source, pelleting or their interaction ( $P > 0.10$ ).

In conclusion, protein supplementation improved nitrogen retention and reduced methane

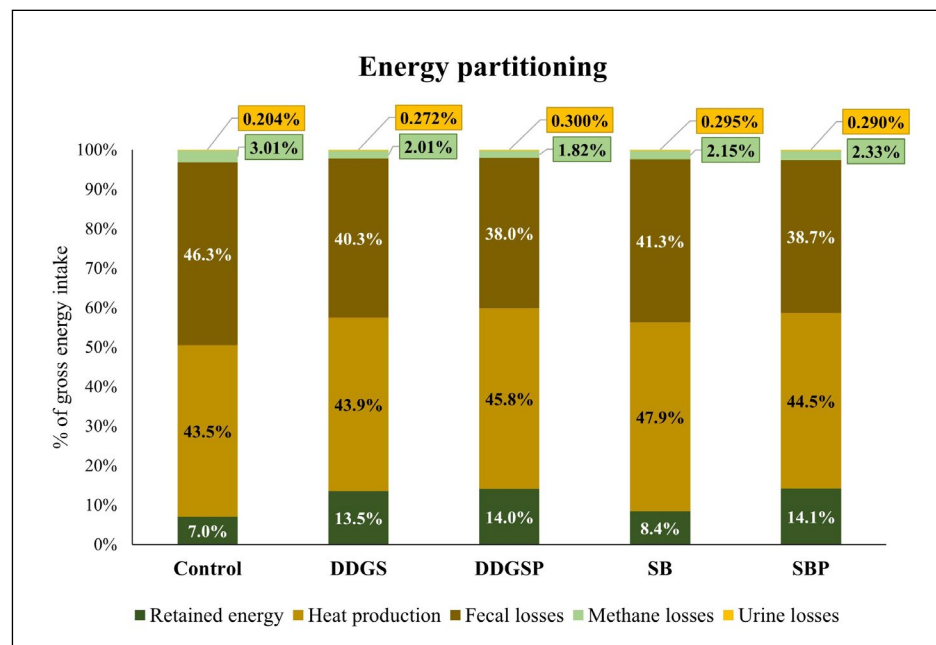
emissions per unit of digested dry matter, indicating more efficient nutrient use. Supplement with DDGS led to greater nitrogen retention than soybean-based supplementation, likely due to higher protein content and a greater proportion of rumen-undegradable protein. In contrast, the soybean-based supplement increased urinary nitrogen losses, likely due to excess rumen ammonia being converted to urea and excreted. Pelleting reduced fecal energy losses but increased urinary nitrogen, showing that supplement processing affects nutrient partitioning. Properly formulated soybean-based pellets may offer a practical alternative to DDGS for forage-based systems.

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## References

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**Figure 2. Energy partitioning as percentage of gross energy intake in steers fed grass hay and supplemented or not at 0.5 % of body weight with a soybean-based or DDGS-based protein supplement, offered in meal or pelleted form. Control = hay only; DDGS = hay supplemented with DDGS in meal form; DDGSP = hay supplemented with DDGS in pellet form; SB = hay supplemented with soybean-based supplement (52.5% soybean meal and 47.5% soyhulls) in meal form; SBP = hay supplemented with soybean-based supplement in pellet form. Standard error mean values (%) were: fecal = 1.03, urine = 0.0190, methane = 0.404, heat = 2.23, retained = 2.955.  $P$ -values (supplementation, source, and processing, respectively) were  $<0.001$ , 0.251, and 0.005 for fecal losses;  $<0.001$ , 0.614, and 0.410 for urinary losses;  $<0.001$ , 0.096, and 0.980 for methane losses; 0.248, 0.396, and 0.647 for heat production; and 0.016, 0.178, and 0.101 for retained energy. No interaction effect was detected ( $P > 0.10$ ).**