

Article

Effect of Post-Ruminal Casein Infusion on Milk Yield, Milk Composition, and Efficiency of Nitrogen Use in Dairy Cows [†]

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[†] This article is part of the Ph.D. thesis of Mehrnaz Ardalan.

Abstract: Adequate supply of amino acids can improve the efficiency of nitrogen use. Casein is the predominant milk protein, and its supplementation can improve milk protein synthesis and nitrogen efficiency. We evaluated the effects of post-ruminal supplementation of casein on milk yield and composition and whole-body protein deposition. Two ruminally cannulated Holstein dairy cows (599 kg) were used in a switch-back design, and treatments were an abomasal infusion of 0 or 400 g/day casein. Cows were fed a diet consisting of corn silage, alfalfa hay, wet corn gluten feed, whole cottonseed, and grain mix, and they received 320 g/day dextrose via abomasal infusion to increase energy:metabolizable protein. The experiment used three 8-day periods. Milk, urine, and feces samples were collected to evaluate milk production, milk composition, and nitrogen retention. Abomasal casein infusion increased ($p < 0.01$) milk protein percentage and milk urea nitrogen. Nitrogen retention ($p = 0.03$) and urinary N excretion ($p < 0.001$) were increased and fecal N excretion ($p < 0.001$) was decreased by casein infusion. Results suggest casein stimulated protein deposition and altered nitrogen use in lactating dairy cattle. Adaptation periods of 4 days were appropriate for evaluating responses to casein supplementation. Our data provide elements that can aid the design of future experiments.

Keywords: casein; nitrogen efficiency; milk composition



Citation: Ardalan, M.; Hussein, A.H.; Titgemeyer, E.C. Effect of Post-Ruminal Casein Infusion on Milk Yield, Milk Composition, and Efficiency of Nitrogen Use in Dairy Cows. *Dairy* **2022**, *3*, 163–173. <https://doi.org/10.3390/dairy3010013>

Academic Editor:
Umberto Bernabucci

Received: 19 November 2021

Accepted: 2 February 2022

Published: 24 February 2022

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1. Introduction

To study amino acid use of lactating dairy cattle, it is necessary to have a model where the cow can respond to amino acid supplementation with increases in milk protein production, body protein deposition, or both. A large number of trials have evaluated methionine and lysine supplementation to lactating cows [1,2], but fewer have evaluated other potentially limiting amino acids [3,4]. In part, the study of amino acid use has been hampered by a lack of models that consistently demonstrate responses to amino acid supplementation, particularly in cows fed typical diets and with typical levels of lactational performance.

Post-ruminal casein supplementation serves as an excellent model for determining if lactating cows can demonstrate positive responses to amino acid supplementation [5]. Many casein supplementation studies with dairy cattle have used diets based on grass silages [6–10], which can limit basal supplies of metabolizable protein and therefore exaggerate the responsiveness of cows to supplemental protein. Some of the key studies with casein supplementation to lactating cows have used cows with low levels of milk production [11].

Our objective was to develop a model useful for evaluating amino acid use by dairy cows fed a diet typical of that used by dairy farms in the United States. This pilot study was designed to determine if cows with industry-relevant milk production levels were responsive to abomasal casein supplementation when fed a diet resembling that typical for

the dairy industry in the United States but without added protein sources. Additionally, we determined the appropriate adaptation period for assessing lactational performance and whole-body protein deposition following changes in post-ruminal protein supply.

2. Materials and Methods

2.1. Animals and Experimental Design

The Institutional Animal Care and Use Committee at Kansas State University provided prior approval for all research activities involving the cattle.

Two ruminally cannulated Holstein dairy cows (599 ± 17 kg initial BW) were used in a 38-day experiment, composed of 14 days for adaptation to facilities and diet and three 8-day periods, with samples collected daily. At the start of the adaptation, one cow was 188 days in milk and not pregnant, and the other cow was 242 days in milk and 124 days pregnant. The experiment used a switch-back design. With this design, cows were provided one treatment, then switched to the other treatment, then switched back to the first treatment. The two treatments were provided in opposite sequences to the 2 cows. Cows were housed in tie-stalls with ad libitum access to water and fed twice daily (0500 and 1700 h) a diet consisting of corn silage, alfalfa hay, wet corn gluten feed, whole cottonseed, and grain mix (Table 1) at 27 kg dry matter/day. Cows were observed daily, and the health status of each animal was evaluated and recorded. The basal diet was formulated to provide limiting amounts of protein to create amino acid deficiencies. The diet contained adequate ruminally degradable protein (10.3% of dietary dry matter; Table 1) to ensure that changes in N recycling, which could be affected by treatments, did not affect ruminal microbial growth. To prevent energy from being limiting without increasing microbial protein supply, additional energy was supplied to cows through nearly continuous abomasal infusion of 320 g/day dextrose (providing 291 g/day glucose). The glucose was provided to increase the energy:metabolizable protein ratio to augment amino acid deficiencies and increase the responsiveness of the cows to protein supplementation.

Table 1. Composition of experimental diet.

Item	% of Dry Matter
Ingredient	
Corn silage	19.9
Alfalfa hay	21.8
Wet corn gluten feed	24.4
Whole cotton seed	5.0
Grain mix	28.9
Nutrient	
Organic matter	92.5
Crude protein	14.7
Rumen-degradable protein	10.3
Neutral detergent fiber	31.7
Acid detergent fiber	21.4

Grain mix composition: 90.5% ground corn, 2.86% sodium bicarbonate, 4.29% calcium carbonate, 1.07% trace mineralized salt (>95.5% NaCl, 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.032% Zn, 0.007% I, and 0.004% Co), 0.71% MgO, 0.039% vitamin A premix (30,000 IU/g), 0.027% vitamin D premix (30,000 IU/g), 0.29% vitamin E premix (44 IU/g), 0.18% Zinpro 4-plex (5.15% zinc from zinc methionine, 2.86% manganese from manganese methionine, 1.80% copper from copper lysine, and 0.36% cobalt from cobalt glucoheptonate), and 0.036% Se premix (600 ppm Se). Nutrient composition: organic matter and crude protein are based on laboratory analyses of diet. Rumen-degradable protein, neutral detergent fiber, and acid detergent fiber calculated using values from NRC [12].

Treatments were abomasal infusions of (1) no protein supplementation (control) and (2) 400 g/day casein. The basal abomasal infusate for each 12 h infusion period was prepared as follows. First, 200 g of sodium caseinate (as is weight) was solubilized in approximately 1600 g of water with a blender. Because the casein solution contained air bubbles, the solution was allowed to sit for 8 h for air bubbles to escape. Then, 160 g dextrose was added and mixed until dissolved. Water was then added to bring the final weight

of abomasal infusate to 2000 g for each 12 h infusion period. For the cow on the control treatment, dextrose was simply dissolved in water.

Treatments were provided as nearly continuous infusions into the abomasum to preclude ruminal degradation. Treatments were infused using a peristaltic pump (Model CP-78002-10; Cole-Parmer Instrument Company, Vernon Hills, IL, USA). The infusions were pumped through Tygon tubing (i.d. = 3.32 mm; Saint-Gobain North America, Valley Forge, PA, USA) into the abomasum with the lines passing through the ruminal cannula, the reticulo-omasal orifice, and the omasum. The infusion line was held in the abomasum with a 10 cm rubber flange. The infusion lines were disconnected from the pump while the cows went to the milking parlor and reconnected when cows returned to their stalls.

2.2. Data and Sample Collection and Analysis

Feed offered and feed refusals were recorded daily to measure feed intake. Samples of the total mixed ration, Orts, and feed ingredients (corn silage, alfalfa hay, wet corn gluten feed, grain mix, and whole cottonseed) were collected daily and frozen (-20°C) for subsequent analysis. Samples were mixed within each period to obtain composite samples, dried in a 55°C forced-air oven for 72 h to determine the partial dry matter, ground to pass through a 1 mm screen (Thomas-Wiley Laboratory Mill Model 4, Thomas Scientific USA, Swedesboro, NJ, USA), and stored for subsequent analysis. Samples of feed, Orts, and feces were analyzed for ash by combustion at 450°C for 8 h.

Cows were milked 3 times daily (0, 1000, and 1700 h). Milk weights were recorded at each milking, and milk samples (25 mL) from individual cows at each milking were collected after mixing all of the milk within the collection vessel, preserved with 2-bromo-2-nitropropane-1,3 diol, and stored at 4°C for analysis of milk components. Milk samples were analyzed for fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments Inc., Chaska, MN, USA), milk urea-N (MUN spectrophotometer; Bentley Instruments Inc.), and somatic cells (SCC 500, Bentley Instruments Inc.) at the Heart of America DHIA, Manhattan, KS, USA.

Fecal and urine samples were collected 3 times daily (0730, 1030, and 1330 h). Fecal samples (200 g) were collected either when cows spontaneously defecated or directly from the rectum, if necessary, and then were mixed to obtain a single composite for each day. Urine samples (20 mL, mixed to obtain a composite for each day) were collected either when cows spontaneously urinated or following stimulation of the vulva to induce urination. Samples of urine (60 mL) were diluted with 15 mL of 1.5 M H_2SO_4 and frozen (-20°C) for analysis of creatinine, and N. Urinary creatinine concentrations were measured using HPLC as described by Brake et al. [13]. Urinary creatinine excretion was estimated as body weight (kg) $\times$ 29 mg/kg of BW, and daily urine volumes were estimated from urinary creatinine concentrations [14]. Samples of the feed, Ort, and feces were analyzed for indigestible acid detergent fiber (internal marker for determining diet digestibility and fecal output). An *in situ* experiment was performed to measure indigestible acid detergent fiber. The samples (feed, Ort, and feces pooled across each period) were weighed (approximately 0.5 g) into F57 filter bags (Ankom Technology, Macedon, NY, USA) to produce 6 replicates of each sample, placed in duplicate into mesh bags along with duplicate blank F57 filter bags for each mesh bag ($n = 32$ for each mesh bag), and inserted into the rumen of 3 heifers (one mesh bag per heifer) for 192 h of incubation. After 192 h, the Ankom filter bags were removed from the rumen, rinsed under running cold water by hand, and allowed to dry at room temperature. Next, the dried bags were analyzed for acid detergent fiber [15] using the batch procedure of Ankom Technology. For calculating digestibilities, abomasal infusates were not included as part of the intake. Fecal output was calculated as indigestible acid detergent fiber intake divided by fecal indigestible acid detergent fiber concentration. Nitrogen concentrations of casein, feed, Ort, wet feces, and urine samples were determined through combustion (Nitrogen Analyzer Model FP-2000, Leco Corporation, St. Joseph, MI, USA). Milk N was calculated by dividing milk protein yield by 6.38. Nitrogen retention was determined as

the difference between N intake (feed refusals + casein infused) and N lost as feces, urine, and milk.

2.3. Statistical Analyses

Treatments were arranged in a switch-back design and included 0 and 400 g/day casein. This design allowed us to control for differences between cows and experimental periods in a manner similar to that for a Latin square. Data were analyzed using the MIXED procedure of SAS System 9.3 for Windows (SAS Inst. Inc., Cary, NC, USA). Data were analyzed with a repeated-measures analysis with a model including fixed effects of period (3 periods during which treatments were applied), treatment (0 or 400 g/day casein), day within period (8 days within each of the 3 periods), and the interaction between treatment and day. Cow was included as a random effect. Significance was declared at $p \leq 0.10$ due to the preliminary nature of this study. Treatment means were calculated using the LSMEANS option. Because data demonstrated that adaptation required 4 days (see Results and Discussion), data from days 5 through 8 were used to analyze the overall effects of treatment. These analyses were completed as described above, but with only the data from days 5 through 8 included.

3. Results and Discussion

3.1. Adaptation Time

Our work was conducted as a pilot study to provide information about casein use by dairy cattle and to develop a model for evaluating amino acid use by high-producing dairy cows. The time course data that was used to determine the necessary adaptation periods for responses to abomasal casein infusion are presented for milk urea-N (Figure 1A), N intake (Figure 1B), milk N secretion (Figure 1C), urinary N excretion (Figure 1D), fecal N excretion (Figure 1E), retained N (Figure 1F), productive N (Figure 1G), N efficiency (Figure 1H), total dry matter intake (Figure 2A), milk yield (Figure 2B), milk protein yield (Figure 2C), milk protein content (Figure 2D), milk fat yield (Figure 2E), milk fat content (Figure 2F), milk lactose yield (Figure 2G), and milk lactose content (Figure 2H). We observed interactions between treatment and day for urinary N excretion (Figure 1D; $p = 0.06$), N retention (Figure 1F; $p = 0.06$), and N efficiency (Figure 1H; $p = 0.09$) for the data from days 1 through 8. For the 400 g/day casein infusion, urinary N excretion increased over the initial 4 days of the period, whereas retained N and N efficiency decreased over these initial 4 days, with these effects demonstrating the time required for equilibrium to be reached. For the control infusions, urinary N excretion decreased over the initial 4 days, with N efficiency increasing over the initial 4 days. Retained N demonstrated some variation for the control treatment on days 3 and 4 but nonetheless appeared to reach equilibrium before day 5. Although other response criteria did not demonstrate significant treatment by day interactions, most criteria followed similar patterns (or inverse for responses moving in opposite directions), suggesting that a 4-day adaptation period would be appropriate for evaluating responses to post-ruminal protein supplementation. Notably, data indicated that a 4-day adaptation period was appropriate to allow cows to reach a stable response for milk protein percentage (Figure 2D) and milk fat percentage (Figure 2F). The appropriateness of a 4-day adaptation is further supported by a lack of interaction between treatment and day for any of the N output responses when data from days 5 through 8 were analyzed ($p \geq 0.66$; Table 2). The appropriateness of short adaptation periods is not surprising because ruminants adapt rapidly to changes in the post-ruminal supply of nutrients where there is no need for ruminal adaptation to take place [16,17]. This rapid adaptation is in agreement with the findings by Whitelaw et al. [11] that examined the effects of casein supplementation on cows using short adaptation periods (5 days) between treatments. The results [11] indicated that milk yield and composition responded rapidly to abomasal infusion of casein, and the major changes in milk yield and composition were observed within 24 h. These authors suggested that short adaptation periods, considering no change in basal diet and intake

level among treatments and high digestibility of casein infused into the abomasum can be adequate.

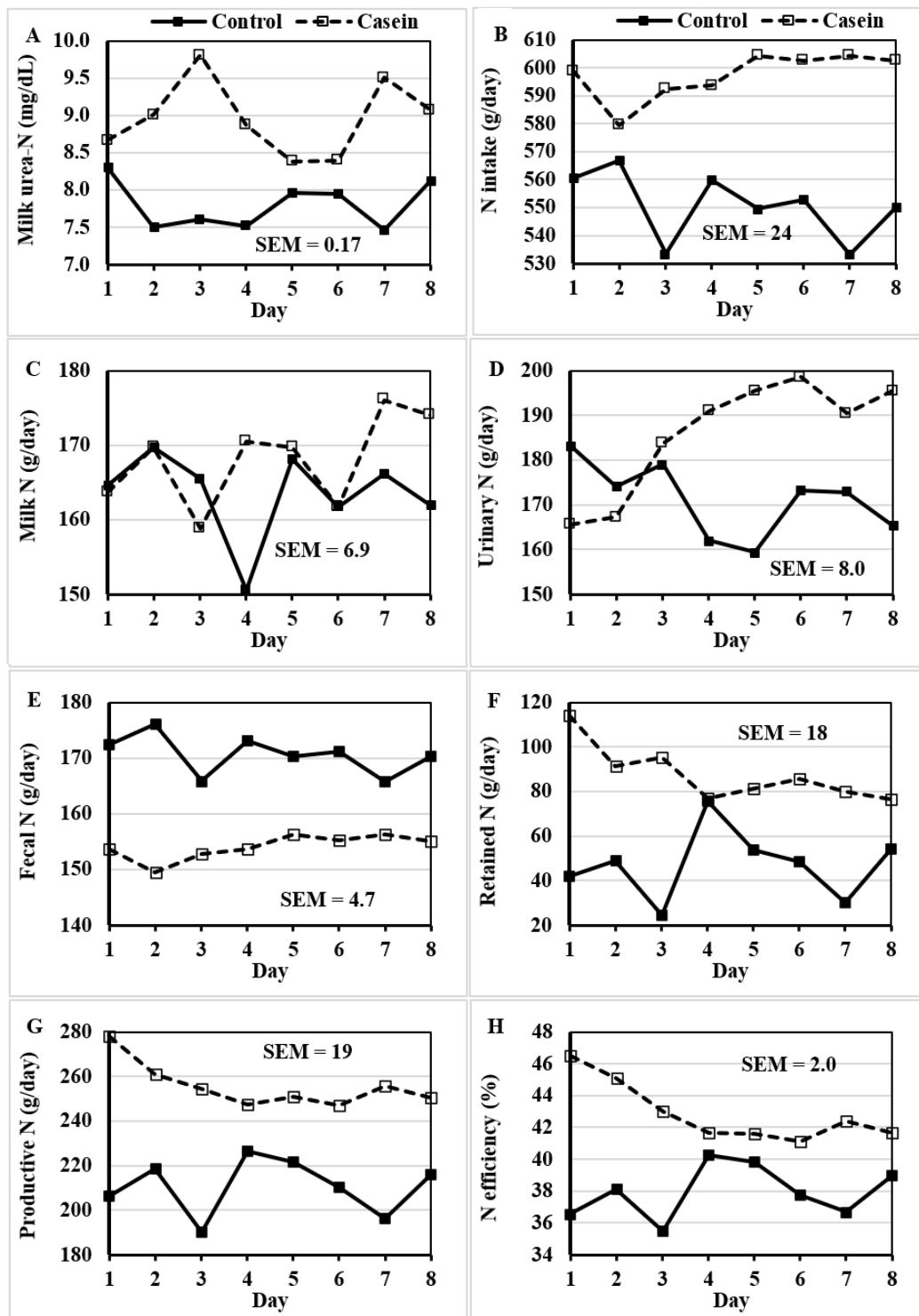


Figure 1. Effect of casein supplementation on milk urea-N and measures of N use from days 1 through 8 of each period. (A) milk urea-N: casein, $p < 0.001$; casein $\times$ day, $p = 0.61$. (B) N intake: casein, $p < 0.001$; casein $\times$ day, $p = 0.33$. (C) milk N secretion: casein, $p = 0.08$; casein $\times$ day, $p = 0.75$. (D) urinary N excretion: casein, $p < 0.001$; casein $\times$ day, $p = 0.06$. (E) fecal N excretion: casein, $p = 0.01$; casein $\times$ day, $p = 0.31$. (F) retained N: casein, $p < 0.001$; casein $\times$ day, $p = 0.06$. (G) productive N: casein, $p < 0.001$; casein $\times$ day, $p = 0.21$. (H) N efficiency: casein, $p = 0.001$; casein $\times$ day, $p = 0.09$.

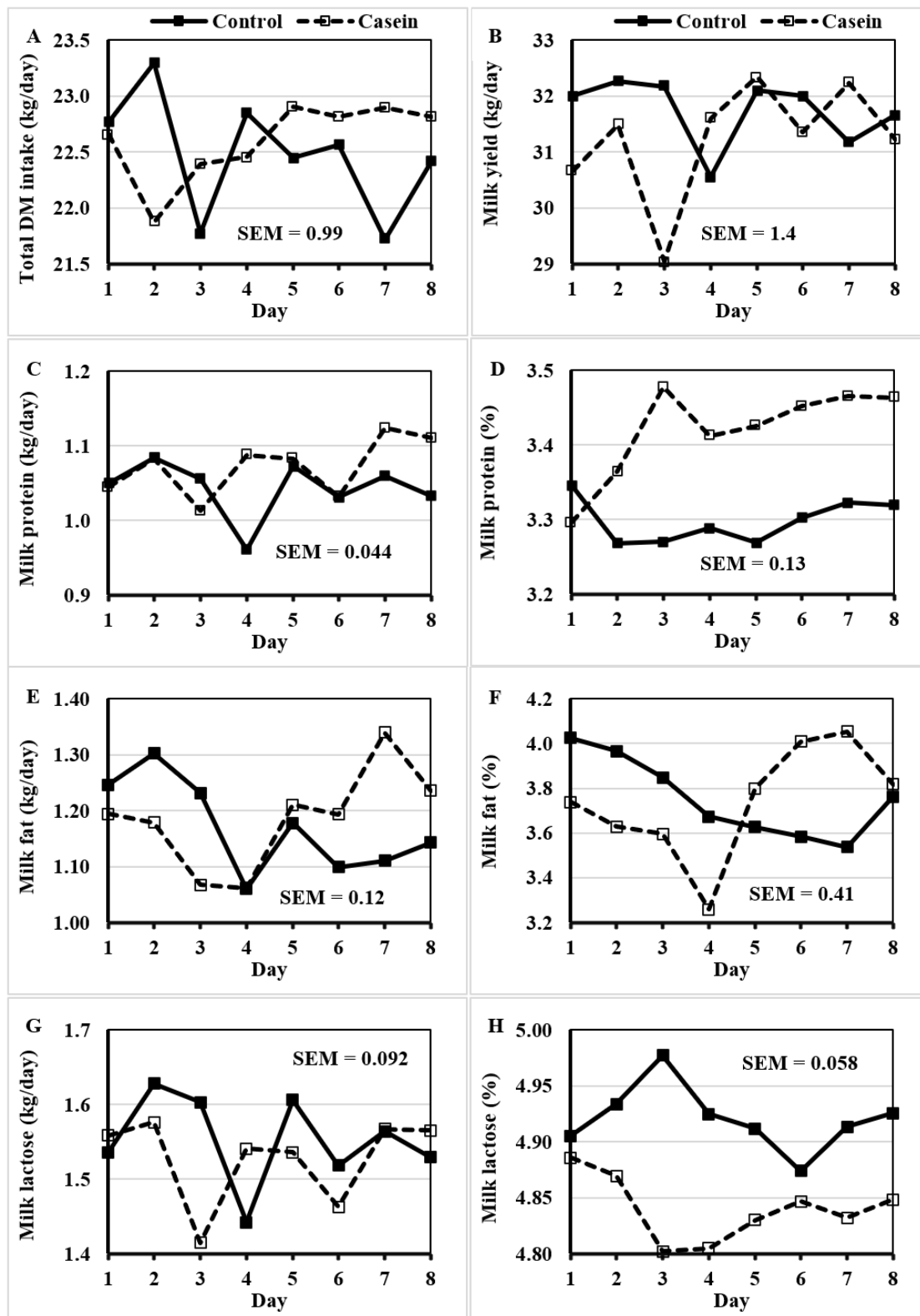


Figure 2. Effect of casein supplementation on dry matter intake, milk production, milk composition, and milk component yields from days 1 through 8 of each period. (A) total dry matter (DM) intake: casein, $p = 0.77$; casein $\times$ day, $p = 0.33$. (B) milk yield: casein, $p = 0.32$; casein $\times$ day, $p = 0.73$. (C) milk protein yield: casein, $p = 0.08$; casein $\times$ day, $p = 0.75$. (D) milk protein content: casein, $p < 0.001$; casein $\times$ day, $p = 0.32$. (E) milk fat yield: casein, $p = 0.90$; casein $\times$ day, $p = 0.71$. (F) milk fat content: casein, $p = 0.97$; casein $\times$ day, $p = 0.92$. (G) milk lactose yield: casein, $p = 0.49$; casein $\times$ day, $p = 0.76$. (H) milk lactose content: casein, $p < 0.001$; casein $\times$ day, $p = 0.37$.

Table 2. Effect of casein supplementation on nitrogen (N) intake, excretion, balance, efficiency, and diet digestibilities from days 5 through 8 of each period.

Item	Treatment			p-Value	
	Control	Casein	SEM	Treatment	Treatment × Day
Total N intake, g/day	548.3	601.5	20.8	0.008	0.82
Dietary N intake, g/day	548.3	543.6	20.8	0.78	0.82
Casein N infused, g/day	0.0	57.9	-	-	-
Milk N, g/day	164.5	170.4	5.1	0.42	0.93
Fecal N, g/day	169.3	155.7	2.5	0.002	0.79
Urinary N, g/day	167.0	195.7	4.8	<0.001	0.84
Retained N, g/day	48.4	78.9	13.3	0.03	0.83
Productive N, g/day	215.0	247.2	16.6	0.17	0.66
N efficiency, %	38.7	41.3	1.7	0.28	0.76
Dry matter digestibility, %	63.1	63.1	2.6	0.96	-
Organic matter digestibility, %	64.7	65.4	2.6	0.55	-

Productive N = Milk N + Retained N; N efficiency = Productive N/Total N intake.

3.2. Dry Matter Intake and Lactation Responses

Due to the necessity of 4-day adaptation periods, as discussed above, the data presented in Tables 2 and 3 are from days 5 through 8 of each period. Total dry matter intake and milk production and composition are presented in Table 3.

Table 3. Effect of casein supplementation on dry matter intake (DMI) and milk production and composition from days 5 through 8 of each period.

Item	Treatment			p-Value	
	Control	Casein	SEM	Treatment	Treatment × Day
Total DMI, kg/day	22.4	22.8	0.86	0.64	0.81
Dietary DMI, kg/day	22.1	22.1	0.86	1.00	0.81
Glucose DMI, kg/day	0.291	0.291	-	-	-
Casein DMI, kg/day	0	0.385	-	-	-
Milk yield, kg/day	31.5	32.0	0.97	0.67	0.93
Milk protein yield, kg/day	1.05	1.09	0.03	0.42	0.93
Milk protein, %	3.31	3.44	0.11	0.01	0.90
Milk fat yield, kg/day	1.13	1.24	0.10	0.45	0.83
Milk fat, %	3.63	3.92	0.42	0.63	0.85
Milk lactose yield, kg/day	1.54	1.55	0.07	0.94	0.96
Milk lactose, %	4.90	4.85	0.05	0.28	0.81
Milk solids-not-fat yield, kg/day	2.86	2.92	0.10	0.68	0.95
Milk solids-not-fat, %	9.11	9.15	0.07	0.68	0.69
Milk urea-N, mg/dL	7.82	8.89	0.25	0.002	0.26
Milk somatic cell count, per μ L	12.9	25.9	4.70	0.02	0.74

Total dry matter intake was not affected by casein supplementation ($p = 0.64$). A meta-analysis [18] investigated the effects of supplemental casein in dairy cows, and they observed that dry matter intake tended to increase by 0.18 kg/day with casein supplementation across 48 studies in the literature. With our limited number of observations, we would not have been able to detect a difference of 0.18 kg/day.

No differences in milk production were observed in response to casein administration (Table 3). Milk fat yield (1.24 vs. 1.13 kg/day) and percentage (3.92% vs. 3.63%) were numerically greater when cows received casein with the responses being potentially important, but these effects lacked significance ($p \geq 0.45$) due to large variation and lack of adequate sample size. In terms of model development, we conclude that substantial increases in the number of observations would be required for the evaluation of effects on milk fat concentration and yield.

Hanigan et al. [19] demonstrated that dairy cows in late lactation, which received abomasal infusions of four levels of casein (200, 400, or 600 g/day) for 10 days, did not show any significant differences in fat percentage, but fat yield increased quadratically for cows receiving casein supplementation. Cohick et al. [20] investigated the effect of post-ruminal infusion of 395 g/day casein compared with water in four Holstein cows; results indicated that casein supplementation decreased milk fat percentage and increased milk yield.

When casein was infused, milk protein percentage and milk urea-N were greater ($p < 0.01$) than for the control. Milk protein yield was not statistically different ($p = 0.42$) from control when casein was infused, but infusion of casein improved milk protein yield by about 4%, indicating that casein supplementation may have stimulated protein synthesis [21], although our experiment was not designed to detect differences of this size. Supplementing deficient essential amino acids into the intestinal digesta can similarly improve milk production [22]. Somatic cell count was greater ($p = 0.02$) for cows receiving post-ruminal casein infusions, although this effect was largely driven by high somatic cell counts for one cow in one period when it received the casein treatment.

Hurtaud et al. [23], using ruminally and duodenally fistulated Holstein cows fed a diet consisting of 70% forage and 30% concentrate and receiving ruminally infused volatile fatty acids, demonstrated a tendency for elevated milk protein yields in response to duodenal infusion of sodium caseinate. This suggests that casein supplementation increased supplies of amino acids that were limiting for milk protein synthesis. Broderick et al. [21] used cows averaging 31 kg/day milk to evaluate the effects of abomasal infusions of 800 g/day of methionine-supplemented sodium caseinate. Casein supplementation significantly enhanced milk protein concentration and milk protein yield, and these authors suggested that the increases in milk protein were due to the provision of amino acids that limited milk protein synthesis. Griinari et al. [24] observed increases in milk yield (11%) and milk protein yield (10%) following abomasal casein infusion in rumen-fistulated Holstein cows (184 days postpartum). They also suggested that the increased milk protein yield could be a consequence of improved amino acid supply. Clark [25] suggested that enhanced milk protein after treatment with casein might be associated with: (1) supplementation of amino acids that are limiting for milk protein synthesis, (2) improvement in the supply of glucogenic amino acids [26], and (3) alteration in hormonal status, such as growth hormone, prolactin, and insulin [27]. Consistent with our observations, other authors have reported that post-ruminal casein infusions in cows fed grass silage-based diets increased milk urea-N [7–9]. The increases in milk urea-N might result from the catabolism of amino acids, which can significantly affect blood urea-N [28] and a positive correlation between blood urea and milk urea [29].

Martineau et al. [5] conducted a meta-analysis based on 23 experiments that post-ruminally supplemented casein to lactating dairy cows. They found that casein infusion increased milk true protein concentration and milk and component yields; however, supplemental casein decreased milk concentrations of fat and lactose. Results also illustrated a positive effect of casein on N retention and an increase in milk and blood urea concentrations. Elevations of urea suggest that some of the amino acids, following administration of casein, were catabolized, resulting in elevated urea synthesis.

3.3. Nitrogen Retention

Nitrogen retention was measured as an estimate of lean tissue deposition and was used in conjunction with milk protein yield to calculate the efficiency of N use. Table 2 shows the N retention responses of dairy cows to supplementation with casein. By design, the casein treatment increased ($p = 0.008$) total N intake. Abomasal infusion of casein increased N excreted ($p < 0.001$) in urine by 29 g/day and reduced fecal N output by 14 g/day compared to control. There were no differences between the control and casein groups for organic matter or dry matter digestibility (Table 2).

In general agreement with our observations, Cohick et al. [20], working with rumen-fistulated Holstein cows that received 0 or 395 g/day casein abomasally, showed total N intake (55 g/day), absorbed N (54 g/day), urinary N excretion (28 g/day), and milk N (13 g/day) were increased through abomasal casein infusion. These authors also indicated that casein tended to increase N retention. Elevated urinary N excretion might suggest that amino acids supplied by casein were more than the cow's requirement for protein synthesis [30], and therefore increased deamination and greater urinary losses of dietary protein were taking place [31]. Huhtanen et al. [9] also indicated a significant increase in urinary N excretion in lactating Ayrshire cows in response to duodenal infusion of 400 g/day of casein.

A potential explanation for the reduction in fecal N in response to casein supplementation is that casein may have improved small intestinal starch digestion by increasing pancreatic amylase secretion, activity of small intestinal carbohydrases, or both [32]; this would reduce the amount of fermentable substrate entering the large intestine, which could reduce large intestinal microbial protein synthesis and, ultimately, microbial N excretion in the feces. Some experimental observations that support this hypothesis include Bruckental et al. [33], who fed a high-starch diet based on cracked corn and observed that abomasal casein infusion decreased fecal N excretion, as well as Whitelaw et al. [11], who fed a very low starch diet and observed that abomasal casein infusion led to a small but significant increase in fecal N excretion.

Nitrogen retention was significantly augmented ($p = 0.03$) with casein supplementation compared with control. Consistent with our observations, other authors have reported that casein infusion into the abomasum increased N retention in steers [33–35].

Productive N and N efficiency were not affected by treatment, although the effect of casein supplementation on productive N was larger than the significant effect of casein on N retention. Increases in N retention, instead of alterations in milk N secretion, in response to supplemental casein in our experiment, demonstrate that dairy cattle do not use supplemental protein only for milk synthesis. Given the lack of change in milk protein secretion, we would conclude that the basal diet had already met the amino acid requirement for maximal production of milk protein. However, cows also can partition some protein toward body reserves even when milk production is not maximal. Whitelaw et al. [11] illustrated concurrent use of supplemental casein for milk protein synthesis and body protein deposition. They observed progressive increases in milk N for casein supplementation levels of 200, 400, or 600 g/day with 42%, 23%, and 15%, respectively, of the supplemental casein N being used for milk protein synthesis, whereas the use of infused casein N for productive N (milk N plus retained N) was consistently near 64%. These observations demonstrate partitioning of the supplemental casein N between milk N and retained N across all levels of casein supplementation. Because supplemental protein can be used for protein deposition, instead of just milk synthesis, it is important to consider N retention as part of the response by the lactating cow.

4. Conclusions

The results of this experiment demonstrate that post-ruminal casein supplementation is a useful model for evaluating the efficiency of protein use in lactating dairy cows. Supplementation of casein led to increased urine N output, but at the same time, it increased productive N. Based on increases in N retention, casein supplementation improved whole-body protein deposition, presumably by providing an increased supply of amino acids. Changes over time for key response variables indicated that a 4-day adaptation period was appropriate. Data from this experiment can be used in power analyses during the development of future experiments to ensure adequate replication is available to evaluate treatments.

Author Contributions: Conceptualization, E.C.T.; methodology, E.C.T.; validation, M.A., A.H.H., and E.C.T.; formal analysis, E.C.T.; investigation, M.A. and A.H.H.; resources, E.C.T.; data curation, M.A.; writing—original draft preparation, M.A.; writing—review and editing, A.H.H. and E.C.T.;

visualization, M.A., A.H.H., and E.C.T.; supervision, E.C.T.; project administration, E.C.T.; funding acquisition, E.C.T. All authors have read and agreed to the published version of the manuscript.

Funding: Contribution no. 21-235-J from the Kansas Agricultural Experiment Station. This work was supported by the USDA National Institute of Food and Agriculture, Hatch project 1001435.

Institutional Review Board Statement: The Institutional Animal Care and Use Committee at Kansas State University provided prior approval for all research activities involving the cattle (#3319).

Informed Consent Statement: Not applicable.

Data Availability Statement: Dataset is available from corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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