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Enhancing the recovery of human-edible nutrients in milk and nitrogen efficiency throughout the lactation cycle by feeding fatty acid supplements

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Dairy cows convert low-quality feedstuffs unsuitable for human consumption into milk, a high-quality protein source for humans. Even under grazing conditions of well-managed pastures, dietary energy limits the efficiency of animal production, contributing to the excretion of nutrients to the environment. Thus, our study aimed to assess the effect of supplying sources and levels of supplemental fat to grazing dairy cows during early lactation on nutrient excretion and recovery of human-edible (HE) nutrients in milk throughout the lactation cycle. Two experiments were conducted with grazing Jersey × Holstein dairy cows. In Experiment 1, five dairy cows were used in a 5 × 5 Latin Square design. Treatments were a control diet (concentrate without supplemental fat) or diets supplemented with calcium salts of either soybean fatty acids (CSSO) or palm fatty acids (CSPO) fed at two levels of supplementation (400 g/day or 700 g/day) for 24 days. In general, the level of supplemental fat had a minor effect on production parameters and methane emission and intensity. Feeding cows with CSPO increased milk yield by 8.1%, milk energy output by 20%, and milk fat yield by 35.2% compared with CSSO. A tendency was observed for CSPO to decrease methane per unit of milk energy compared with CSSO. In experiment 2, 24 dairy cows were used in a randomized block design. Treatments were a control diet or diets supplemented with calcium salts of either CSSO or CSPO fed at 400 g/day for 13 weeks. The carryover effect of the diets was evaluated until 42 weeks of lactation. During the supplementation period, feeding CSPO improved the recovery of HE energy, protein, and indispensable amino acids compared with control and CSSO. Interestingly, we demonstrated that feeding CSPO improved the recovery of HE energy, protein, and indispensable amino acids compared with control over the lactation cycle. Overall, our results suggested that the dose of supplemental fat used in our study had a minor influence on production and rumen fermentation parameters. We demonstrated that feeding dairy cows with a fatty acid source based on palm oil under tropical grazing conditions reduced nutrient excretion and improved the recovery of HE nutrients throughout the lactation cycle.

KEYWORDS

fat supplementation, nitrogen excretion, rumen fermentation, fatty acid profile, sustainability

1. Introduction

Increasing the production of safe and nutritious food is a growing challenge to satisfy the demands of a global population projected to reach close to 10 billion by 2050. The solution is more complex than enhancing the cultivated area and the number of animals, given the increasingly limited land and resources available and concerns over the environmental impact. Instead, efforts should be directed at enhancing the production efficiency per unit of nutrients and decreasing the environmental footprint of the production systems. An increase in calorie availability to humans by almost 50% could be achieved by changing crop production away from activities, such as bioenergy, livestock feed, and other non-food applications (Foley et al., 2011). Nonetheless, the global concerns related to food security extend beyond caloric needs as the increase in energy consumption from refined grains has been negatively correlated with health outcomes (Vega Mejía et al., 2018). Additionally, in several developing nations, protein quality is a fundamental issue due to the lower digestibility and amino acid profile of grain-based diets compared with animal-based products (Ritchie et al., 2018). In this regard, dairy cows are able to transform feeds unsuitable for human consumption into milk, which is a high-quality protein-rich product, and also provides energy, essential fatty acids, vitamins, and minerals (Górska-Warszewicz et al., 2019).

In tropical areas, dairy milk production relies on the use of grazing systems because forage is an affordable source of nutrients in the diet. A higher potential for forage mass accumulation is observed in tropical forages (Santos et al., 2014), and the development of management strategies based on light interception improved the forages' nutritional value and milk production (Voltolini et al., 2010; Da Silva et al., 2013). Furthermore, using a grazing interval based on light interception resulted in reductions of 40% in nitrous oxide and 20% in methane per unit of milk when compared to the conventional method (Congio et al., 2018, 2019). Nevertheless, improving the grazing management strategy is not enough to match the energy needs of grazing dairy cows, and the use of energy-dense concentrates is required to meet the potential milk production. Concentrates rich in cereal grains are commonly used to increase cows' energy intake in pasture-based systems (Douglas et al., 2021). However, consuming large amounts of starch-based supplements may decrease fiber digestibility, the efficiency of nutrient utilization, and animal performance (Hills et al., 2015; Humer et al., 2018). Supplemental fat could be an alternative to increase dairy cows' energy intake and production efficiency as fat is energetically denser than carbohydrates. We previously demonstrated that feeding a palm oil-based fat supplement to grazing dairy cows enhanced the recovery of milk human-edible (HE) energy and protein as well as indispensable amino acids compared with extensively processed corn grain (Batistel et al., 2021). The use of a palm oil-based supplement reduced the environmental impact of the dairy production system by decreasing nitrogen excretion and methane output per unit of milk (Batistel et al., 2021). Another environmental advantage of incorporating fat supplements into ruminant diets is the reduction in waste generation, as most fat supplements are manufactured from byproducts generated by food and biodiesel industries.

Lately, the impact of the source of fatty acids and fatty acid profile on production responses and efficiency has been demonstrated on cows receiving total mixed rations. For instance, cows receiving a diet with palmitic acid (2% of the diet) improved feed efficiency and the energy was partitioned toward milk synthesis, whereas cows receiving a diet supplemented with soybean oil partitioned more energy toward body tissue gain (Liu et al., 2020). A dose-dependent response was previously reported to feeding increasing levels of palmitic acid replacing soyhulls indicating the need to determine optimal feeding doses that maximize animal performance (Rico et al., 2017). However, there is a lack of information regarding the ideal level of inclusion and fatty acid profile of supplemental fats to dairy cattle grazing tropical pastures. This information is especially important for grazing animals because high levels of fat and unsaturated fatty acids may disrupt rumen function and consequently decrease fiber digestion and animal production (Palmquist and Jenkins, 2017). Furthermore, there is a critical need for long-term studies to determine the potential effect of nutritional strategies implemented during the early lactation phase on the lactation cycle of grazing dairy cows. A carryover effect on the lactation cycle can enhance the efficiency of the production system and farm profitability.

Here, our first objective was to determine the ideal level of inclusion of calcium salts of palm and soybean oil (400 or 700 g/day) on rumen fermentation parameters and animal performance of dairy cows grazing tropical pastures. Subsequently, we investigated the effect of supplying grazing dairy cows with calcium salts of palm or soybean oil (400 g/day) during the early-lactation period on nitrogen excretion and recovery of HE nutrients in milk throughout the lactation cycle. We hypothesized that lower levels of supplemental fat and calcium salts of palm oil would positively influence the production efficiency of grazing dairy cows. Additionally, we postulated that feeding calcium salts of palm oil during early lactation would (a) enhance the recovery of HE nutrients, (b) decrease nitrogen excretion, and (c) enhance the milk production efficiency throughout the lactation cycle.

2. Materials and methods

Experiments were conducted at the University of São Paulo in Piracicaba, São Paulo, Brazil. The Ethical Committee for Animal Research of the University of São Paulo approved all procedures involving the animals.

2.1. Experiment 1

2.1.1. Experiment description

Five multiparous Jersey × Holstein dairy cows (rumen-cannulated) were used in a 5 × 5 Latin Square design. Cows at the start of the experiment had 428 ± 14 kg of body weight (mean ± standard deviation), milk yield of 16.5 ± 3.1 kg, and 115 ± 8.1 days in milk. Each experimental period lasted 24 days: 14 days for treatment adjustment and 10 days for sampling. The dietary treatments were as follows: (1) control (concentrate with no supplemental fat); (2) concentrate with a low dose of calcium salts of soybean fatty acids (CSSO; 400 g/day; Megalac-E, Química

Geral do Nordeste, Rio de Janeiro, Brazil); (3) concentrate with a high dose of CSSO (700 g/day; Megalac-E); (4) concentrate with a low dose of calcium salts of palm fatty acids (CSPO; 400 g/day, Magnapac, Tectron Inc., Toledo, Parana, Brazil); and (5) concentrate with a high dose of CSPO (700 g/day, Magnapac). For the fat supplement dose, assuming an intake of 17.5 kg/day, we aimed to provide 2.0 and 3.5% of fatty acids of diet dry matter for low and high doses, respectively (NRC, 2001). We provided the treatments as a concentrate supplement (8 kg dry matter/day) fed twice a day individually before milking at 0400 and 1600 h. Grazing and cow management are described in detail elsewhere (de Souza et al., 2017). In summary, all cows were allocated daily to a new paddock (2,000 m²) grazing elephant grass (*Pennisetum purpureum* cv. Cameroon) with free access to natural shade and fresh water. The pasture was managed in a rotational grazing system based on canopy height. The entry height was 103 cm (Voltolini et al., 2010), and cows grazed the canopy down to 62 ± 2.2 cm. This procedure was adopted to avoid a reduction in short-term forage intake by sward structure, and therefore, animals should graze the canopy down to a maximum of 40% of the entry height (Fonseca et al., 2013). The ingredients and nutritional constituents of the concentrates and forage are presented in Supplementary Table 1.

2.1.2. Sample collection and analysis

During the sampling period, we recorded milk yield as a sum of both milkings. We collected milk samples from both milkings every 2 days and preserved them with a bronopol preservative pill. The contents of fat, protein, lactose, and milk urea nitrogen were determined in the milk samples using infrared procedures (Foss 4000; Foss North America, Eden Prairie, MN).

Composite forage, concentrate, and fecal samples were analyzed for dry matter, ash (AOAC, 2005), total nitrogen content by the Dumas combustion method using an N analyzer (Leco FP-2000 N Analyzer; Leco Instruments Inc., St. Joseph, MI), neutral detergent fiber (NDF; Van Soest et al., 1991) with sodium sulfite and heat-stable α -amylase for concentrate samples, acid detergent fiber (ADF) and lignin (AOAC, 2005), and gross energy by bomb calorimeter (Parr Instrument Inc., Moline, IL).

Forage intake and total dry matter intake were estimated from feed indigestibility values and total fecal excretion. To determine fecal output, we dosed cows twice daily with titanium dioxide (20 g/cow per day) during the last 15 days of each experimental period (de Souza et al., 2015). Fecal samples were collected during the last 5 days after milkings and then stored at -20°C. Subsequently, samples were thawed, dried at 55°C in a forced-air oven, ground through a 1-mm screen (Wiley mill, Thomas Scientific), and composited by the cow. Fecal samples were analyzed for titanium concentration according to Myers et al. (2004). We determined the indigestible NDF (iNDF) content of forage, concentrate, and fecal samples according to Huhtanen et al. (1994). The iNDF contents of forage and concentrate were divided by the iNDF content in the feces of each cow to calculate the indigestibility of feeds for each animal. Total fecal excretion, fecal excretion coming from the concentrate, forage intake, and total dry matter intake were calculated as previously described (de Souza et al., 2015).

On day 20 of each period, we collected samples of ruminal fluid from three locations in the ventral rumen sac. Samples were taken at 0 (relative to morning concentrate feeding), 2, 4, 6, 8, 12, 18, and 24 h. Samples of ruminal fluid were then preserved by adding 1 mL of 50% sulfuric acid and stored at -20°C. The ruminal fluid samples were thawed and processed by centrifugation (15,000 × g, 4°C, 30 min); the supernatant was analyzed for short-chain fatty acids (SCFA) by gas chromatography (Erwin et al., 1961) and ammonia nitrogen by the phenol-hypochlorite procedure (Chaney and Marbach, 1962). We calculated methane using SCFA stoichiometry using the equations generated by Williams et al. (2019).

2.1.3. Statistical analyses

We analyzed the data using the Mixed procedure of SAS 9.4 (SAS Institute) in a linear model that included the fixed effect of treatment and the random effect of cow and period. The data were checked for normality of the residuals using normal probability and box plots and for homogeneity of variances with plots of residuals vs. predicted values. For rumen variables that were collected through the day of sampling, a repeated procedure was used. We used the variance components matrix as the covariate structure for repeated-measures analysis based on the lowest Bayesian information criterion. Three preplanned orthogonal contrasts were tested to assess the overall effect of supplemental fat (CON vs. FAT), the effect of source of fatty acids (CSSO vs. CSPO), and the effect of supplement dose (low vs. high). Treatment differences were determined at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

2.2. Experiment 2

2.2.1. Experiment description

For this study, we used a subset of cows from a previously published study (de Souza et al., 2017). In a previous publication, we presented results for nutrient digestibility production responses and milk fatty acid profile. A subset of 24 multiparous Jersey × Holstein dairy cows, with 490 ± 40 kg of body weight and 18 ± 2.0 days in milk, were used in a randomized complete block design. The experiment lasted 39 weeks, with supplementation and carryover periods lasting 13 and 26 weeks, respectively. Cows were blocked by previous lactation milk production, and blocks were balanced by body condition score and parity. Treatment diets were offered for 13 weeks during the supplementation period (from 3 to 16 weeks postpartum). The dietary treatments were as follows: (1) control (concentrate with no supplemental fat); (2) concentrate with CSSO (400 g/day; Megalac-E); and (3) concentrate with CSPO (400 g/day, Magnapac). All diets were formulated to meet predicted requirements (NRC, 2001). During the treatment period, all cows rotationally grazed elephant grass (*Pennisetum purpureum* cv. Cameroon) and the treatments were added to a concentrate supplement. The treatments were fed twice a day at 0400 and 1600 h individually before milking. Grazing management practices were the same as described in Experiment 1. From 17 to 42 weeks postpartum (carryover period), all cows received a common

diet (without fat supplement) fed as a total mixed ration once a day at 0900 h.

2.2.2. Sample collection and analysis

We collected a weekly sample of concentrate and forage throughout the study. These samples were analyzed for dry matter, crude protein, NDF, ADF, lignin, ash, and fatty acids using the same methods described in Experiment 1. Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide, glucose concentration was measured with glucose oxidase, and absorbance was determined with a spectrophotometer. Cows were milked twice a day, and milk yield was recorded daily. Milk samples from both milkings were collected once a week and analyzed using the same methods described in Experiment 1.

We determined forage and total dry matter intakes on weeks 4, 8, 12, 24, and 36 from fecal excretion and feed indigestibility. We dosed titanium dioxide during 15 days of each period and collected fecal samples in the last 5 days. The methods and procedures to determine feed intake were the same as described in Experiment 1.

Urine samples were collected from each cow on weeks 4, 8, 12, 24, and 36. The samples were acidified with sulfuric acid (pH of urine < 5) and stored at -20°C until further analysis. We determined creatinine, allantoin, and uric acid using an HPLC instrument (Pimpa et al., 2001). Total nitrogen was measured by the micro-Kjeldahl procedure (AOAC, 1990). We estimated the total urine yield using the creatinine content of each sample, accounting for a constant excretion of creatinine at 0.213 mmol/kg of body weight (Chizzotti et al., 2008). We considered the intake of concentrate and pasture and their respective nitrogen contents to calculate N intake. The excretion of urinary nitrogen was calculated from total urine yield multiplied by its nitrogen content. Milk nitrogen efficiency (assuming no retention or mobilization of body nitrogen) calculated by dividing milk nitrogen output (milk crude protein/6.34) by total nitrogen intake. Fecal nitrogen excretion was calculated from fecal nitrogen \times fecal output.

2.2.3. Recovery of HE nutrients

We estimated HE nutrient recovery using the approach proposed by Takiya et al. (2019). Based on sugar, starch, true protein, and fat concentrations in corn grain, soybean meal products, and fat supplements, we estimated HE metabolizable energy and protein contents of diets. During the supplementation period, we postulated two situations to calculate HE nutrient recovery in milk: First, we considered corn, soybean meal, and fat supplements as HE feeds and the pasture as unedible (Pasture Scenario); and in the second scenario, we considered that the land used for pasture production could be used to grow soybeans and corn (Land Scenario). For the land scenario, we estimated the HE metabolizable energy and protein using a blend of corn and dehulled soybeans (82 and 18% on a dry matter basis, respectively) in place of forage (elephant grass). Thus, we could grow 0.70 ha of corn and 0.30 ha of soybeans for every hectare used for elephant grass production. The annual yields (on a dry matter basis) of corn (5,319 kg/ha) and soybeans (3,032 kg/ha), retrieved from CONAB 2021/2022 Annual Crop Brazilian Production Summary

(CONAB, 2022), were used to determine the potential HE energy and protein displaced by pasture production. We discounted the yield of soybean by 7.3% (Perkins, 1995) as soyhulls is not HE. Based on the yield of pasture leaves for elephant grass we calculated the total forage yield as previously reported (de Souza et al., 2017). To calculate the recovery of HE nutrients during the whole lactation, we used the average input and output of the pasture scenario plus the calculated input and output during the carryover period.

To account for amino acid recovery, we utilized the standardized ileal digestibility (SID) library and estimated the HE digestible amino acid inputs and outputs using the feed's total amino acid content. Recoveries of HE nutrients in milk were calculated as the ratio between nutrients secreted in milk and the corresponding HE nutrient intake by cows. To calculate the recovery of HE digestible AA during the whole lactation, we used the average input and output of the pasture scenario plus the calculated input and output during the carryover period.

2.2.4. Statistical analyses

We analyzed the data using the Mixed procedure of SAS 9.4 (SAS Institute) in a linear model including the fixed effect of treatment, time, and its interaction, and the random effect of the block. For the data accounting for the recovery of nutrients for the whole lactation, a reduced model without the time effect was used. The data were checked for normality of the residuals using normal probability and box plots and for homogeneity of variances with plots of residuals vs. predicted values. For rumen variables that were collected over the day of sampling, a repeated procedure was used. We used the variance components matrix as the covariate structure for repeated-measures analysis based on the lowest Bayesian information criterion. Treatment differences were determined at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

3. Results

3.1. Experiment 1

Animal performance was affected by the fatty acid source of the supplemental fat (Figures 1A–I). Feeding cows with CSPO increased milk yield by 8.1% compared with CSSO ($P = 0.02$). Although intake was not affected by treatments, CSPO increased milk energy output by 20% compared with CSSO ($P < 0.01$). This result was associated with the CSPO treatment increasing milk fat content by 25.6% ($P < 0.01$) and milk fat yield by 35.2% ($P < 0.01$) when compared with feeding CSSO. Similarly, CSPO increased the yield of milk protein by 8.2% ($P = 0.03$) and milk lactose by 8.4% ($P = 0.03$) compared with CSSO. In general, the level of supplemental fat had a minor effect on production parameters. We observed a tendency for feeding the high dose of supplemental fat to decrease milk lactose content ($P = 0.07$) and milk fat yield ($P = 0.10$) compared with the low dose.

Compared with the control, feeding supplemental fat decreased the rumen concentration of isovalerate ($P = 0.02$; Table 1). There was no effect of fat supplementation on methane emission and

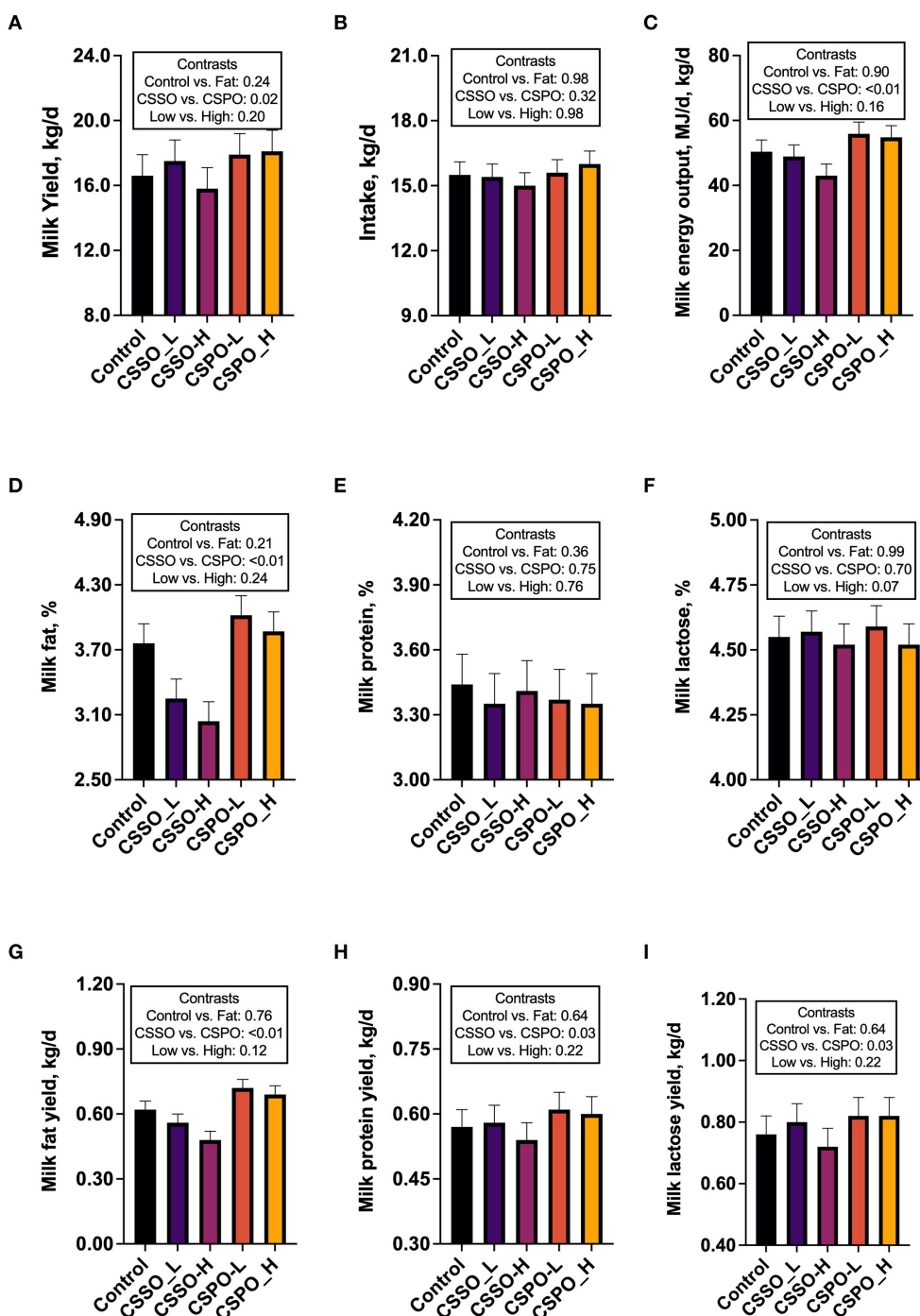


FIGURE 1 Milk production parameters (A–I) in cows that grazed elephant grass and received treatments as a concentrate supplement (Experiment 1). Treatments were a control diet (concentrate without supplemental fat) or diets supplemented with calcium salts of either soybean fatty acids (CSSO) or palm fatty acids (CSPO) fed at two levels of supplementation (L: 400 g/day; H: 700 g/day).

intensity. CSPO increased rumen total SCFA production ($P = 0.02$) and acetate ($P = 0.02$) compared to CSSO. Furthermore, CSPO tended to increase butyrate by 5.0% ($P = 0.09$) and isovalerate by 4.3% ($P = 0.10$) and decrease methane per unit of milk energy by 16.3% ($P = 0.07$) compared with CSSO. Feeding a high dose of supplemental fat tended to reduce total SCFA production ($P = 0.10$) and acetate ($P = 0.06$) compared to a low dose.

3.2. Experiment 2

In the pasture scenario during the supplementation period, feeding both CSSO and CSPO increased HE energy intake compared with control ($P = 0.01$; Table 2). Regarding the land scenario, supplemental fat did not impact intakes of energy and protein ($P > 0.10$). Cows fed CSPO had greater milk energy ($P <$

TABLE 1 Rumen fermentation parameters and methane estimates of dairy cows receiving the treatment diets^a (Experiment 1).

	Control	CSSO		CSPO		SEM	Contrast ^b		
		Low	High	Low	High		Control vs. FAT	CSSO vs. CSPO	Low vs. High
Short-chain fatty acids, mmol									
Acetate	68.3	67.5	65.9	70.2	67.9	1.45	0.71	0.02	0.06
Propionate	20.0	19.7	19.2	20.4	19.8	0.81	0.71	0.24	0.31
Butyrate	12.8	12.0	12.6	13.1	12.8	0.53	0.63	0.09	0.57
Isobutyrate	1.03	0.98	0.98	1.05	0.99	0.07	0.29	0.20	0.29
Isovalerate	1.99	1.81	1.79	1.94	1.82	0.12	0.02	0.10	0.28
Valerate	1.12	1.04	1.06	1.10	1.05	0.06	0.23	0.56	0.82
Total	105.3	103.1	101.6	108.0	104.6	2.73	0.61	0.02	0.10
Acetate: Propionate	3.48	3.45	3.47	3.49	3.45	0.10	0.85	0.95	0.89
pH	6.34	6.38	6.32	6.30	6.32	0.12	0.83	0.38	0.71
N-NH ₃ , mg/dL	21.2	20.0	19.5	19.6	20.1	1.74	0.38	0.93	0.98
Methane, g/d	329	321	317	329	335	15.4	0.81	0.35	0.91
Methane, g/kg intake	21.1	20.8	21.1	21.1	21.0	0.45	0.73	0.87	0.87
Methane, g/kg milk	19.9	18.6	21.5	18.5	19.0	1.92	0.73	0.33	0.21
Methane, g/kg protein yield	587	559	627	549	569	53.6	0.81	0.39	0.27
Methane, g/kg milk energy	6.68	6.61	7.88	5.90	6.22	0.70	0.97	0.07	0.20

^aCows grazed elephant grass and received treatments as a concentrate supplement. Treatments were a control diet (concentrate without supplemental fat); or diets supplemented with calcium salts of either soybean fatty acids (CSSO) or palm fatty acids (CSPO) fed at two levels of supplementation (low: 400 g/d; high: 700 g/d). ^bP-values are associated with preplanned orthogonal contrasts. The test evaluated the overall effect of supplemental fat (control vs. FAT), the effect of source of fatty acids (CSSO vs. CSPO), and the effect of supplement dose (low vs. high).

0.01) and protein output ($P = 0.04$) compared with control and CSSO. For total lactation, in both scenarios, we did not observe a treatment effect for HE intakes of energy and protein. In contrast, CSPO increased total lactation energy ($P < 0.01$) and protein output ($P = 0.02$) compared with CSSO and control.

Regarding the pasture scenario during the supplementation period, feeding CSPO improved the recovery of HE energy by 10 and 25% compared with control and CSSO, respectively ($P < 0.01$; Figure 2A). Similarly, CSPO enhanced the recovery of HE protein by 14 and 12% compared with control and CSSO, respectively ($P < 0.01$; Figure 2B). Feeding cows with CSSO reduced the recovery of HE energy ($P < 0.01$) but did not affect the recovery of protein compared to the control. The HE energy ($P < 0.01$) and protein ($P < 0.01$) recoveries decreased in the land scenario when compared to the pasture scenario. In the land scenario during the supplementation period, CSPO improved the recovery of HE energy ($P < 0.01$; Figure 2C) and protein ($P < 0.01$; Figure 2D) compared with other treatments. For total lactation, in both scenarios feeding CSPO increased ($P < 0.01$) the recovery of HE energy and tended to increase ($P \leq 0.10$) the recovery of HE protein compared with other treatments.

We did not observe treatment differences for nitrogen excretion in the feces in both the supplementation period and total lactation (Figure 3A; $P > 0.10$). During the supplementation period, feeding CSPO reduced the excretion of nitrogen in urine by 4% compared with CSSO and control (Figure 3B; $P = 0.01$). Feeding CSPO tended to increase milk nitrogen efficiency by 7 and 13% compared with CSSO and control, respectively, in both the supplementation period and total lactation (Figure 3C; $P = 0.09$). We also calculated the

recovery of amino acids considering the pasture scenario. Feeding cows with CSPO increased the recovery of all indispensable amino acids evaluated by 27 and 14% compared with control and CSSO, respectively ($P < 0.05$; Figure 4). Also, cows fed with CSSO had greater recovery of all amino acids evaluated (between 9 and 11%) compared with the control ($P < 0.05$). Overall, CSPO tended to increase the recovery of all indispensable amino acids evaluated by 23% over control during total lactation ($P < 0.10$).

4. Discussion

Recently, considerable advances in forage management practices have been made to improve the nutritional value of tropical grasses (Da Silva et al., 2013). Nevertheless, energy is the major constraint to improving dairy cattle production efficiency in tropical pasture-based systems. Currently, concentrates rich in cereal grains are commonly used to increase cows' energy intake in pasture-based systems (Douglas et al., 2021). However, feeding large amounts of starch-based supplements may decrease fiber digestibility, the efficiency of nutrient utilization, and animal performance (Hills et al., 2015; Humer et al., 2018). Additionally, the use of this type of concentrate represents a direct competition of food ingredients for humans. We previously demonstrated that adding a supplemental fat of palm oil was more efficient than cereal grains to improve the efficiency of recovery of nutrients in milk and to reduce methane emissions and nitrogen excretion in tropical grazing conditions during the supplementation period (Batistel et al., 2021). However, there is a lack of information

TABLE 2 Human-edible (HE) nutrient inputs and outputs of dairy cows receiving the treatment diets during the supplementation period and total lactation^a (Experiment 2).

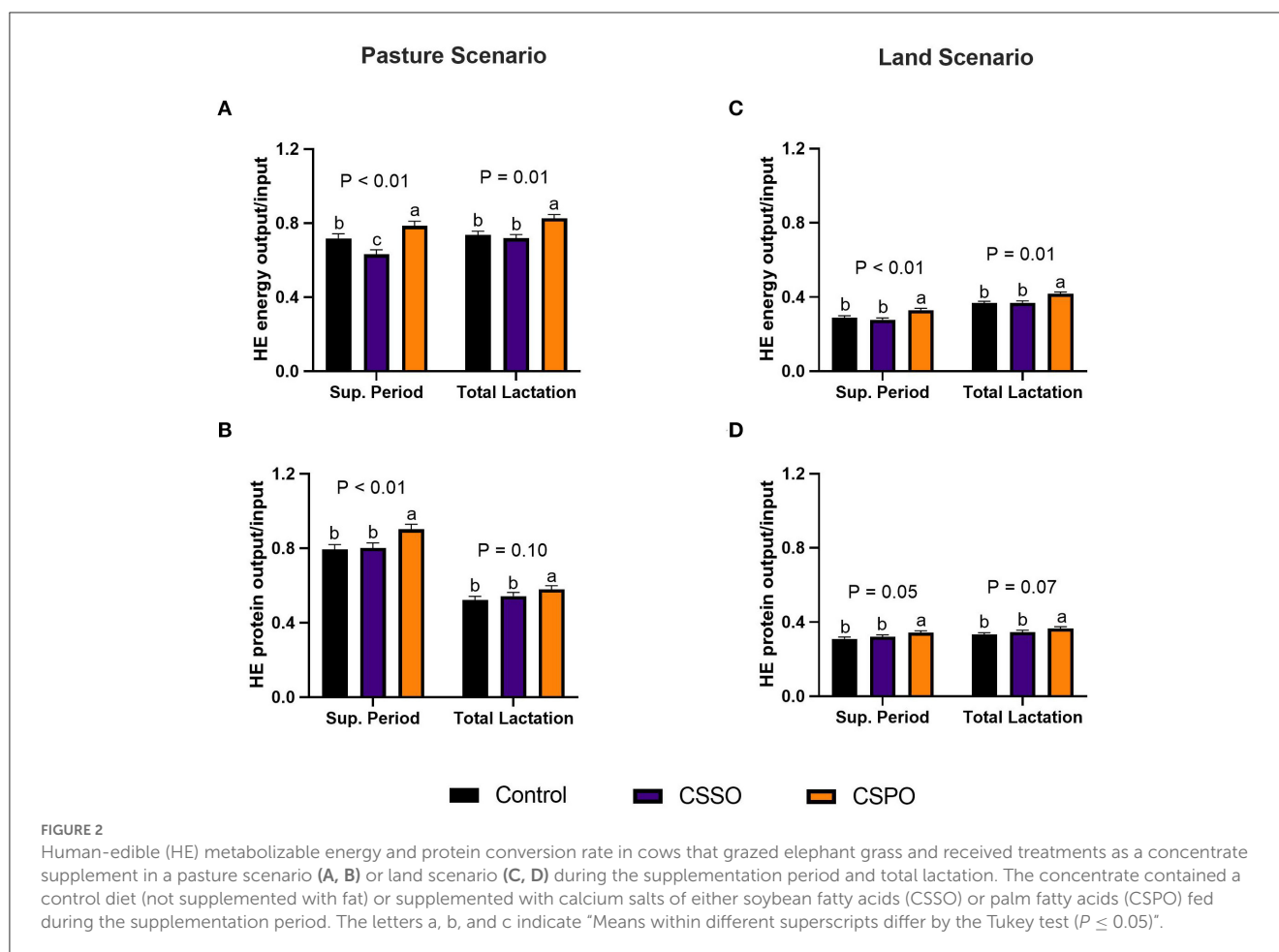
	Treatment				P-value ^b		
	Control	CSSO	CSPO	SEM	Treatment	Time	Treatment × Time
Supplementation period							
Pasture scenario^c							
HE metabolizable energy intake, MJ/d	100 ^y	110 ^z	106 ^z	2.20	0.01	<0.01	0.39
HE crude protein intake, kg/d	1.04	1.03	1.00	0.02	0.33	0.01	0.43
Land scenario^d							
HE metabolizable energy intake, MJ/d	248	251	254	5.25	0.67	0.01	0.43
HE crude protein intake, kg/d	2.66	2.58	2.62	0.05	0.58	0.01	0.45
Output							
Milk energy, MJ/d	71.0 ^y	68.3 ^y	82.8 ^z	2.09	<0.01	0.01	0.90
Milk protein, kg/d	0.82 ^y	0.82 ^y	0.89 ^z	0.02	0.04	0.03	0.67
Total lactation							
Pasture scenario^c							
HE metabolizable energy intake, MJ	23257	23709	23812	728.8	0.88	NA	NA
HE crude protein intake, kg	374.1	364.2	369.7	13.70	0.84	NA	NA
Land scenario^d							
HE metabolizable energy intake, MJ	46529	46076	46763	1316	0.93	NA	NA
HE crude protein intake, kg	586.8	568.3	586.8	17.8	0.74	NA	NA
Output							
Milk energy, MJ	17034 ^y	16935 ^y	19410 ^z	508.3	<0.01	NA	NA
Milk protein, kg	194.3 ^y	195.4 ^y	209.8 ^z	3.81	0.02	NA	NA

^aCows grazed elephant grass and received treatments as a concentrate supplement. Treatments were a control diet (concentrate without supplemental fat) or diets supplemented with calcium salts of either soybean fatty acids (CSSO) or palm fatty acids (CSPO). ^bP-values are associated with the effect of treatment, time, and its interaction. ^cCorn, soybean meal, and soybean and palm oil were considered human-edible foods. ^dLand used for pasture production was considered to grow corn and soybean for direct human consumption. ^{y,z}Means within a row with different superscripts differ by the Tukey test ($P \leq 0.05$).

regarding the ideal level of inclusion and fatty acid profile of supplemental fats to enhance dairy cattle production efficiency and decrease nitrogen excretion in tropical grazing systems. Furthermore, the long-term impacts of feeding strategies during early lactation on production efficiency are poorly understood. Thus, our first objective was to determine the ideal level of inclusion of calcium salts of palm and soybean oil (400 or 700 g/day) on rumen fermentation and animal performance of grazing dairy cows. Our follow-up objective was to investigate the effect of supplying calcium salts of palm or soybean oil to dairy cows grazing tropical pastures during the early-lactation period on nitrogen excretion and recovery of HE nutrients in milk throughout the lactation cycle.

Interestingly, animal production, rumen fermentation, and methane emissions were primarily influenced by the fatty acid source in the diet but not by the feeding level. It has been previously established that the fatty acid profile of a fat supplement is most likely the primary factor affecting production responses to it (Lock and de Souza, 2016). Additionally, determining the response to different sources of fat is essential, considering the sustainability aspects of different agricultural activities. Lately, sustainability and deforestation concerns have been raised over

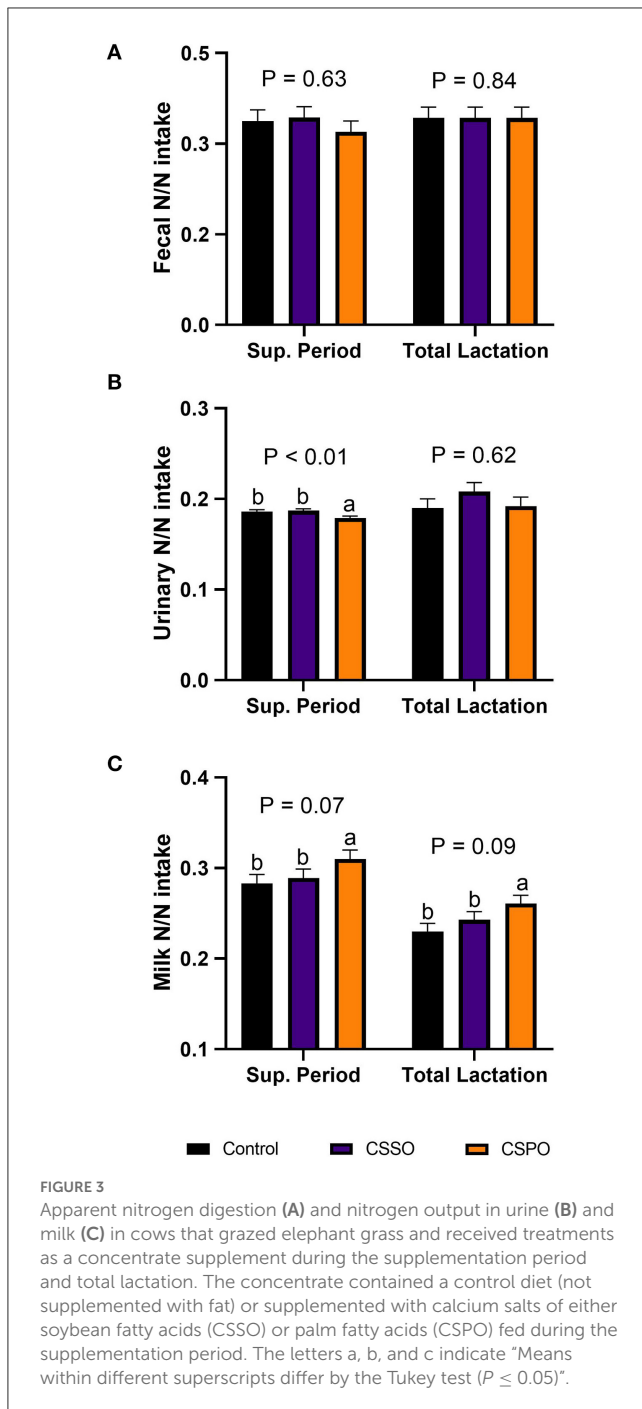
the utilization of palm oil (Teng et al., 2020; Murphy et al., 2021). We observed that feeding CSPO increased milk yield by 8.1% compared with CSSO indicating a major influence of the fatty acid profile on milk yield responses. Feeding fat supplements not only increases the dietary energy density but also increases energetic efficiency in lactating cows due to increased ATP efficiency compared to SCFA and by direct utilization of long-chain fatty acids for milk fat synthesis (Palmquist and Jenkins, 2017). Additionally, fatty acids may spare glucose utilization as the downregulation of *de novo* fatty acids synthesis may reduce the need for reducing equivalents for milk fat synthesis (Storry et al., 1973). In our prior study, both fat supplements decreased *de novo* milk fatty acids (<16 carbon) (de Souza et al., 2017), so the mechanism mentioned above may be at least in part responsible for these results. Feeding CSPO increased the yield of milk fat and energy output by 35 and 20%, respectively, when compared to CSSO. Improved milk yield and yield of milk components when fat supplements contain more saturated fatty acids have been previously reported (de Souza et al., 2018). A recent study indicated increased milk fat output (22%) and milk energy output (8%) partially due to changes in insulin metabolism when feeding a palmitic acid



supplement rather than soybean oil in dairy cows (Liu et al., 2020). In our study, we fed the supplements as calcium salts to reduce the negative effect of feeding fat to the rumen microbial population. However, ruminal pH and the degree of unsaturation of the fat source majorly influence the stability of calcium salts (Sukhija and Palmquist, 1990). In general, diet-induced milk fat depression is commonly detected in cows receiving plant or fish oil supplements and/or fed with rapidly degradable carbohydrates (Dewanckele et al., 2020). Under specific dietary conditions, altered ruminal biohydrogenation of unsaturated fatty acids may lead to the formation of biohydrogenation intermediates that inhibit fat synthesis in the mammary gland (Bauman et al., 2011). Furthermore, CSSO supplementation tended to reduce acetate, butyrate, and total SCFA in the rumen. Acetate and butyrate are major sources of energy generated by rumen fermentation and building blocks for de novo milk fat synthesis (Palmquist and Harvatine, 2020). Similar to our results, a previous study feeding CSSO to grazing cows also observed a negative effect on several isoacids and parameters of rumen fermentation (Macedo et al., 2016). A decrease in microbial growth can be postulated as the reduced concentration of these isoacids in the rumen fluid of CSSO-supplemented cows may result in a reduction in the population of acetate-producing cellulolytic bacteria. Altogether, our results indicated that fat supplements based on palm oil

improved production outcomes compared to supplements based on soybean oil.

Knowledge about the optimal dietary inclusion level of fat supplements with specific fatty acid profiles brings the advantage of optimizing animal performance and farm profitability. In our study, feeding increasing levels of supplemental fat did not influence feed intake, milk yield, and the yield of milk components. Replacing a corn-based concentrate with 400 g/day of CSPO increased the yields of milk and energy-corrected milk and did not affect feed intake in the first 16 weeks postpartum in grazing cows (Batistel et al., 2017). However, the production response to different doses of fat inclusion in the diet has been variable. A previous study reported a dose-dependent response to feeding increasing levels of palmitic acid replacing soyhulls in the diet of dairy cows with animal performance peaking when the supplement was fed at 420 g/day (Rico et al., 2017). Increasing the level of a CSPO supplement in the diet (from 200 to 600 g/d) of early lactating dairy cows grazing a tropical pasture linearly increased milk yield and the yield of milk components (dos Santos Neto et al., 2022). One reason for the lack of results in our study is the possibility that the high feeding level was over the optimal level. Additionally, the inconsistency in production responses due to different levels of supplemental fat may be related to the total dietary fat, nutrient replaced (fiber vs. starch), and stage of lactation. In our study,



we also did not observe major effects of feeding levels on rumen fermentation parameters as only acetate concentration was reduced as the feeding level increased. Acetate is the major SCFA produced in rumen, and it is strongly related to fiber digestion. In general, based on our results, feeding supplemental fat levels over 400 g/day did not influence rumen fermentation and animal performance of grazing cows. As fat supplements are generally more expensive than cereal grains, the lack of production responses precludes feeding higher doses of supplemental fat.

Several targets of mitigation practices for the dairy industry have been discussed lately, and the reduction of enteric methane

production is one of the main goals (Hristov et al., 2013). Therefore, to improve the sustainability and profitability of dairy production systems, nutritional strategies to mitigate enteric methane emissions are warranted (Shetty et al., 2017). In our study, we utilize SCFA stoichiometry to estimate methane output rather than directly measuring it. This technique has been used previously due to the strong correlation between rumen fermentation profile and methane output (Cabezas-Garcia et al., 2017; de Souza et al., 2020). Importantly, different methods of measuring methane have different degrees of uncertainty and variability in methane measurements (Hristov et al., 2018). In this study, we observed a tendency for CSPO to decrease methane per unit of milk energy output by 16.3% compared with CSSO with no differences in feed intake between treatments. Similarly, our previous study with grazing dairy cows indicated a reduction in methane per unit of milk (-31%) and per unit of milk energy output (-29%) when CSPO was fed compared with non-fat control diet (Batistel et al., 2021). Feeding supplemental fat has been shown to decrease methane yield in cattle and sheep (Grainger and Beauchemin, 2011). The effect of supplemental fat on methane yield may depend on the fatty acid profile and form of the additional fat. For example, feeding a diet supplemented with calcium salts of palm plus linseed oil decreased methane yield and methane per unit of milk compared with linseed oil and a non-fat control diet (Kliem et al., 2019). Also, the recent publication of NASEM (2021) suggested that intake, fiber digestibility, and dietary fat levels as factors that influence enteric methane production in dairy cows. It also proposes a linear decrease in methane production as dietary fat level increases (NASEM, 2021). Reduction in methane yield may be achieved by strategies that reduce feed intake. Nonetheless, reducing feed intake will also decrease animal performance, increasing methane intensity by a unit of milk, which is not desired. Our data suggest that feeding CSPO can decrease methane intensity mostly due to the increased performance of cows receiving CSPO without influencing intake.

Increasing the efficiency of livestock production has been proposed to reduce the environmental impact of the livestock sector (Patel et al., 2017). Recently, Wilkinson (2011) proposed a new method of measuring efficiency considering edible feeds to relate HE nutrient inputs and outputs in animal products. A decrease in the utilization of pesticides, soil erosion, and greenhouse emissions was suggested in a food system model assuming minimal HE feeds used for feeding animals (feeding animals with a large amount of grass and by-products) (Schader et al., 2015). As a result, supplying animals with ingredients inedible for human consumption, such as by-products and grasses, will have a positive impact on global food production and environmental impact. However, there is a role for strategically using HE feeds to increase the efficiency of milk production because forage-only systems are associated with relatively low output per head (Wilkinson and Lee, 2018). In our second experiment, the recovery of HE nutrients was calculated under two scenarios. First, we assumed soybean meal, corn, and fat supplement as HE feeds and the pasture as inedible (Pasture scenario); and then, we assumed that the land used for pasture production could be used to grow soybeans and corn (Land Scenario). In the pasture scenario, during the supplementation period feeding CSPO improved the recovery of HE energy by 10 and 25% compared with control and CSSO,

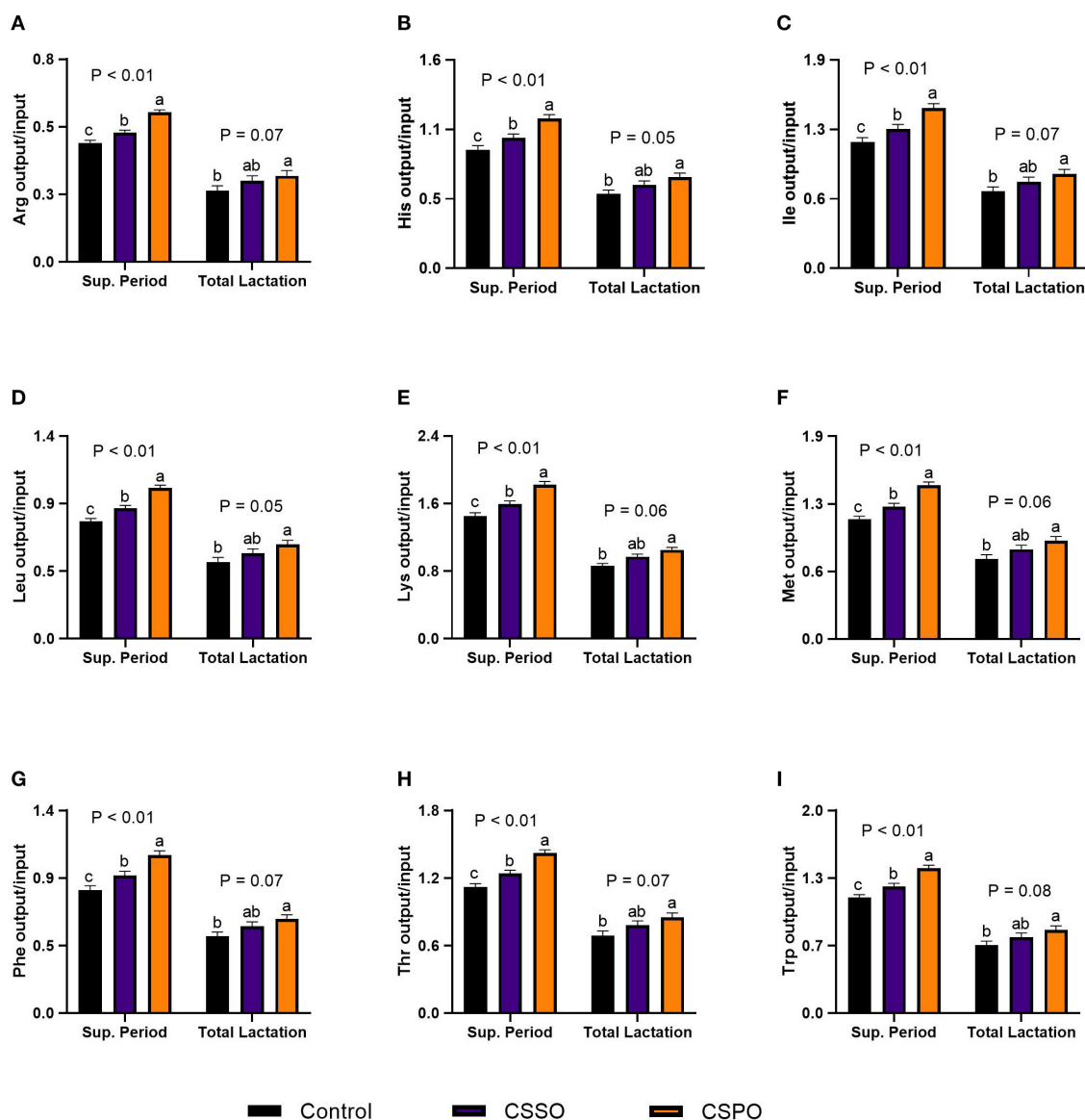


FIGURE 4

Milk recovery of indispensable amino acids (A–I) in cows that grazed elephant grass and received treatments as a concentrate supplement during the supplementation period and total lactation. The concentrate contained a control diet (not supplemented with fat) or supplemented with calcium salts of either soybean fatty acids (CSSO) or palm fatty acids (CSPO) fed during the supplementation period. The letters a, b, and c indicate “Means within different superscripts differ by the Tukey test ($P \leq 0.05$)”.

respectively. Similarly, CSPO enhanced the recovery of HE protein by 14 and 12% compared with control and CSSO, respectively. Corroborating our results, a previous study with grazing cows indicated enhanced recovery of HE energy (+20%) and protein (+17%) when the fat supplement was provided in diets (Batistel et al., 2021). We observed a decrease in the recovery of HE energy and protein between the pasture and land scenarios (-40 and -51%, respectively); however, feeding CSPO also improved the recovery of HE energy and protein compared with other treatments regardless of the scenario. It is essential to point out that non-arable land can be used to produce forage, but our model considered that all land would be used for crop production rather than forage production. Altogether, our results suggest that partial replacement of cereal

grains (corn) by supplemental fatty acids improved HE energy and protein recoveries, but the magnitude of response varies by the fatty acid profile of the supplemental fat.

Although some studies suggested a positive effect on food supply and climate impact of reducing consumption of animal protein (Foley et al., 2011; González et al., 2011), it is important to consider that ruminants can convert poor quality nitrogen that is inedible to human consumption into a high-quality protein. In many developing nations, accounting for protein quality is essential when comparing protein sources as protein consumption is generally limited (Ritchie et al., 2018). When considering HE feed conversion efficiency, the nutritional value of animal protein is twice greater than plant protein (Patel et al., 2017). In our

study, we accounted for protein quality based on the value of milk-digestible amino acids. On average, we observed that the recovery of HE indispensable amino acids varied from 47 to 187%. Recovery rates over 100% were observed for lysine (162%), methionine (127%), isoleucine (129%), and tryptophan (127%). Recovery of over 100% of the supply of indispensable amino acids contributes to food security as these are essential nutrients to human diets. Enhancing the recovery of lysine and methionine is important as these amino acids are considered limited and are presented in suboptimal proportions for human needs in grain-based diets (Mariotti and Gardner, 2019). In addition, our results indicated that during the supplementation period feeding cows with CSPO increased the recovery of all amino acids evaluated by 27 and 14% compared to control and CSSO, respectively. Also, cows fed with CSSO had higher recovery of all amino acids (between 9 and 11%) compared with the control. Thus, our data suggest that it is possible to improve the efficiency of recovery of HE nutrients, especially indispensable amino acids, by manipulating fatty acid sources in the dairy cows' diet.

In this study, we first demonstrate improving the recovery of HE nutrients over the course of a whole lactation cycle by strategically altering animal nutrition strategies during a specific period (early lactation). The use of supplementation with HE feeds strategically to increase the efficiency of milk production in forage-based systems is important (Wilkinson and Lee, 2018). We target supplemented grazing cows with CSSO and CSPO during early lactation (over 13 weeks) but assess its impact over the lactation (42 weeks). Interestingly, we demonstrated that feeding CSPO improved the recovery of HE energy (+13%), protein (+11%), and indispensable amino acids (+23%) compared with control over the lactation period. Previous studies have demonstrated that early lactation feeding programs may have a carryover effect (Jørgensen et al., 2016). Usually, each extra kilogram of milk secreted at peak production results in approximately 200 kg of milk subsequent during lactation (Roche et al., 2013), suggesting that supplementation during early lactation that increases milk production at peak could have positive effects even after supplementation ceased. Therefore, increased response beyond the supplementation period not only improved the efficiency of nutrients recovered in milk but also may have a positive impact on the economic viability of this strategy. We also indicated an improvement in milk nitrogen efficiency by 13% over the lactation period when feeding CSPO compared with the control. Improving nitrogen efficiency is a central goal of livestock production as excess nitrogen can contribute to air, water, and soil pollution. Although several nutrition strategies have been proposed to improve nitrogen efficiency, their utilization may negatively influence production and reproduction performance (Hristov et al., 2013; Dijkstra et al., 2018). In agreement with our results, previous studies reported that feeding fatty acids supplements enhanced milk nitrogen efficiency by 8 to 17% in dairy cows under confinement or grazing systems (Nichols et al., 2018; Batistel et al., 2021). Fatty acids do not directly contribute to glucose synthesis. However, providing dietary fatty acids may spare glucose as preformed fatty acids may be incorporated into milk fat, reducing the need for glucose associated with *de novo* synthesis (Rico et al., 2017). Therefore, providing energy precursors and preformed fatty acids may reduce the use of amino acids for gluconeogenesis or energy-yielding oxidative

processes favoring the utilization of indispensable amino acids for milk protein synthesis (Rius et al., 2010). Our results indicated that supplemental fat based on palm oil can improve nitrogen efficiency not only during the supplementation period suggesting a key role of energy in nitrogen metabolism. Importantly, in our study, the improvement in the recovery of HE nutrients is associated with a greater nutrient output instead of a reduction in input. Altogether, our results suggest that target supplementation of fat supplements can improve the recovery of HE nutrients and reduce nitrogen excretion throughout lactation in dairy cows.

5. Conclusion

In this study, we demonstrated that changing the source of supplemental fat in the diet of grazing dairy cows reduced nitrogen excretion and methane emissions in dairy systems. Production, rumen fermentation, and methane emissions were largely influenced by the source of fatty acid in the diet but not by the feeding level. Furthermore, grazing dairy cows are efficient at using human-edible foods yielding a net increase in digestible indispensable amino acids. Our results suggest that partial replacement of cereal grains (corn) by supplemental fatty acids improves the recovery of human-edible energy and protein, but the magnitude of response varies by the fatty acid profile of supplemental fat. Furthermore, we first demonstrate improving the recovery of human-edible nutrients over the course of a whole lactation cycle by strategically altering nutrition during early lactation. We also demonstrated that feeding dairy cows with a fatty acid source based on palm oil under tropical grazing conditions reduced nutrient excretion and improved the recovery of HE nutrients over the lactation cycle.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Ethical Committee for Animal Research of the University of São Paulo, Piracicaba.

Author contributions

FB, JS, and FS contributed to the overall design of the study. FB and JS carried out the experiments, acquisition of data, analyses, and drafted and revised the first version of the manuscript. All authors reviewed and approved the final manuscript.

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Conflict of interest

JS is employed by the company Perdue Agribusiness.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2023.1186454/full#supplementary-material>

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