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Effects of particle size and toasting of fava beans and forage source on nutrient digestibility, ruminal fermentation, and metabolizable protein supply in dairy cows

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ABSTRACT

The objective of this study was to investigate the effects of heat treatment (toasting) and particle size alterations (grinding; rolling) on nutrient utilization, ruminal fermentation, and supply of metabolizable protein (MP), and to study the interaction between processing conditions of fava beans and forage type. Six Danish Holstein dairy cows fitted with ruminal, duodenal, and ileal cannulas were used in a 6 × 4 incomplete Latin square design with 4 periods of 21-d duration. Cows were fed ad libitum with 6 experimental diets: diets high in either grass-clover silage or corn silage were combined with ground untoasted, ground toasted, or rolled untoasted fava beans. Samples of ruminal fluid, digesta from duodenum and ileum, and feces were collected, and nutrient digestibility was estimated using Cr_2O_3 and TiO_2 as flow markers. Diets high in corn silage resulted in higher ruminal pH and higher proportion of propionate in ruminal volatile fatty acids compared with diets high in grass-clover silage. Diets high in corn silage resulted in higher apparent total-tract digestibility of crude protein and starch but lower apparent ruminal and total-tract digestibility of neutral detergent fiber compared with diets high in grass-clover silage. Rolling of fava beans decreased the in situ small intestinal disappearance of rumen-undegradable protein corrected for particle losses. Compared with grinding, rolling of fava beans reduced apparent ruminal digestibility of starch, true ruminal digestibility of organic matter, crude protein, and AA, and small intestinal digestibility of AA and starch. Grinding of fava beans increased apparent ruminal digestibility of neutral detergent fiber and reduced the proportion of propionate in ruminal volatile fatty acids compared with rolling of fava beans. In addition, rolling of fava beans had no effect on MP supply. Toasting of fava beans had no effect on in vivo nutrient digestibility except for an interaction with forage source on apparent ruminal dry matter and organic matter digestibility. Toasting of fava beans did not affect small intestinal digestion of individual and total AA, and therefore failed to increase MP supply. In conclusion, neither replacing grass-clover silage with corn silage, nor toasting nor rolling of fava beans had an effect on supply of MP.

Key words: amino acid, microbial protein synthesis, methane, field bean

INTRODUCTION

There is increasing public concern about possible negative effects of genetically modified crops and about deforesting of land in tropical regions used for soybean cropping (Tsatsakis et al., 2017). Therefore, the dairy primary industry is seeking locally grown and non-genetically modified alternatives for genetically modified soybean as a protein source for dairy cows (Bertheau and Davison, 2011). Rapeseed is regarded as a proper substitute for soybean meal, and many studies report that replacing soybean meal with rapeseed meal sustains or even increases milk production and N utilization efficiency (Huhtanen et al., 2011; Martineau et al., 2013; Broderick et al., 2015). However, P concentration relative to N concentration in rapeseed is higher than in soybeans (NorFor, 2021), posing a limitation on the use of rapeseed when the risk for eutrophication is taken into account. In recent years, considerable interest has arisen in the use of fava beans as a promising candidate for N and energy supply for dairy production, due to their competitive yield and relative high content of both protein and starch, combined with low P concentration (Crépon et al., 2010).

Some recent studies suggest a decrease in milk yield and milk protein yield when rapeseed meal is replaced by fava beans (Puhakka et al., 2016; Ramin et al., 2017). However, Hansen et al. (2021) found that although feeding toasted fava beans reduced milk protein

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yield compared with feeding soybean meal and rapeseed meal, ECM yield was unaffected. Numerous in situ studies indicate that heat treatment of fava beans increases MP supply (Goelema et al., 1998; Hansen et al., 2021). However, the absence of a positive response in milk yield when untoasted fava beans are replaced by heat-treated fava beans in diets with protein levels below MP requirement indicates that this is not necessarily the case in vivo (Hansen et al., 2021). The lack of response in vivo is probably due to either an overestimation of the positive effect of heat treatment on the supply of RUP or an underestimation of the potential negative effect of heat treatment on ruminal microbial protein synthesis. The mechanisms behind these effects could be either a lower availability of RUP in heat-treated fava beans or a lower ruminal degradability of starch, thereby decreasing intestinal supply of individual EAA from feed and microbes, respectively. Physical processing of feedstuffs, such as milling, would change particle sizes of feeds, which could affect their ruminal digestibility (Larsen et al., 2009). Cherif et al. (2018) reported that feeding ground fava beans increased CP and starch apparent total-tract digestibility but decreased NDF and ADF apparent total-tract digestibility compared with feeding rolled fava beans. However, no differences in production performance and ruminal fermentation were observed. Therefore, further research is required to identify the optimal processing conditions for fava beans to maximize protein and energy efficiency when fed to dairy cows.

A previous study suggested that differences in ruminal and intestinal digestibility of CP, NDF, and starch in corn and grass silages in dairy cows were associated with differences in chemical composition of the silages (Ali et al., 2012). Smith et al. (1993) and Weiss et al. (2009) reported that forage type could also influence the N metabolism and performance of dairy cows. Given the differences in effects of processing condition of fava beans on nutrient digestion and ruminal fermentation reported in previous studies, an interaction might exist between forage sources and processing conditions of fava beans on nutrient digestion, ruminal fermentation, and methane production.

It was hypothesized that (1) rolling of fava beans would decrease ruminal degradation of starch and protein, and ruminal microbial protein synthesis, compared with grinding, due to decreased particle surface; (2) toasting would decrease ruminal degradation of starch and protein as well as ruminal microbial protein synthesis, and would increase small intestinal digestion of AA and starch compared with no toasting, due to thermal protection; and (3) an interaction exists between processing conditions of fava beans and forage source of diets. The study aimed to investigate the effects of physical and thermal processing of fava beans on MP supply, ruminal digestibility, and CH_4 emission in dairy cows fed either grass-clover silage or corn silage-based rations.

MATERIALS AND METHODS

Animals and Experimental Design

The experiment was conducted at Aarhus University (Foulum, Denmark) and complied with the ethical requirements set out in the Danish Ministry of Environment and Food (2014) law no. 474 (May 15, 2014). Six Danish Holstein dairy cows (2 primiparous and 4 multiparous) fitted with rumen cannulas (no. 4C, Bar Diamond Inc.), and duodenal (60 cm caudal to pylorus) and ileal (20 cm cranial to the cecum) simple T-cannulas, were used in an incomplete 6 × 4 Latin square design with 6 experimental diets and 4 periods of 21 d. Three of the diets were high in grass-clover silage, combined with either ground untoasted fava beans (**GGU**), ground toasted fava beans (**GGT**), or rolled untoasted fava beans (**GRU**), and 3 of the diets were high in corn silage combined with either ground untoasted fava beans (**CGU**), ground toasted fava beans (**CGT**), or rolled untoasted fava beans (**CRU**). Due to access to only 4 respiration chambers, 2 cows started the experiment and switched periods 4 d later than the other 4 cows. Cows were housed in tiestalls bedded with rubber mats and sawdust and had free access to water. On average, cows were 187 ± 42 DIM (mean ± SD), with a milk yield of 31.5 ± 9.9 kg at the start of the experiment, and BW of cows at the beginning and end of the experiment were 644 ± 51 and 656 ± 41 kg, respectively. Cows' BCS at the beginning and end of the experiment were 3.08 ± 0.26 and 3.13 ± 0.26, respectively.

Diets and Feeding

The diets were formulated as TMR according to recommendations outlined in NorFor (Volden, 2011) and based on a yearly milk yield potential of 11,500 kg of ECM and a DMI of 24 kg/d. The forage:concentrate ratio was 60:40 on DM basis for all diets. The grass-clover silage to corn silage ratio in the forage part was either 25:75 or 75:25 (DM basis), with urea substituting a minor part of the corn silage in the diets with high corn silage proportion to balance CP content (aimed at 160 g/kg of DM). The concentrate part consisted of 75%

Table 1. Diet composition and nutrient composition of experimental diets (means ± SD; g/kg of DM unless otherwise stated, n = 2)

	Diet[1]					
Item	GGU	GGT	GRU	CGU	CGT	CRU
Ingredient						
Grass-clover silage	450	450	450	150	150	150
Corn silage	150	150	150	444	444	444
Urea, 46% N				6.00	6.00	6.00
Ground untoasted fava beans	300			300		
Ground toasted fava beans		300			300	
Rolled untoasted fava beans			300			300
Sugar beet pulp, dried	80.0	80.0	80.0	80.0	80.0	80.0
Sodium bicarbonate	4.00	4.00	4.00	4.00	4.00	4.00
Minerals[2]	14.0	14.0	14.0	14.0	14.0	14.0
ADE vitamins[3]	2.00	2.00	2.00	2.00	2.00	2.00
Chemical composition						
DM, g/kg of fresh matter	482 ± 3.60	485 ± 7.60	488 ± 15.9	450 ± 1.90	456 ± 3.50	456 ± 4.15
Ash	73.4 ± 0.18	74.1 ± 0.05	74.1 ± 1.28	55.7 ± 0.53	55.7 ± 0.22	56.0 ± 0.88
CP	168 ± 5.31	169 ± 1.72	175 ± 3.75	166 ± 3.75	167 ± 2.34	174 ± 0.16
NDF	274 ± 5.98	273 ± 1.95	276 ± 3.90	287 ± 5.00	288 ± 0.07	286 ± 3.62
Starch	180 ± 7.91	179 ± 3.46	178 ± 5.96	266 ± 5.39	270 ± 0.38	269 ± 0.18

[1]High in grass-clover silage: ground untoasted fava beans = GGU; ground toasted fava beans = GGT; rolled untoasted fava beans = GRU. High in corn silage: ground untoasted fava beans = CGU; ground toasted fava beans = CGT; rolled untoasted fava beans = CRU.

[2]Concentration of minerals per kilogram of DM: 132 g of chloride, 65 g of magnesium, 50 g of phosphorus, 90 g of sodium, 1.5 g of potassium, 0.5 g of sulfur, 4,500 mg of zinc, 4,000 mg of manganese, 1,500 mg of copper, 225 mg of iodine, 50 mg of selenium, 25 mg of cobalt, 600,000 IU of vitamin A, 190,000 IU of vitamin D, 4,000 IU of vitamin E.

[3]Concentration of vitamins per kilogram of DM: 10 mg of selenium, 5,000,000 IE of vitamin A, 200,000 IE of vitamin D, 10,000 IE of vitamin E.

fava beans (ground untoasted, ground toasted, or rolled untoasted), 20% sugar beet pulp, 1% sodium bicarbonate, and 4% minerals and vitamins (Table 1). Grass-clover silage (first growth; seed weight 60% perennial ryegrass, 23% hybrid ryegrass, 9% white clover, and 8% red clover) and corn silage were ensiled in bunker silos without ensiling additives. All experimental TMR were mixed daily using a stationary auger mixer (Cormall) for 16 min.

Untoasted fava beans were ground using a hammer mill with a 3-mm screen (Skiold A/S). Toasted fava beans were toasted in a flame tumble toaster (Dantoaster, Cimbria) at 125°C for 3 min and subsequently ground as described previously. Rolled untoasted fava beans were rolled in a roller (Skiold A/S) with a roller gap of 2.5 mm. The chemical composition of the diet ingredients is reported in Table 2.

Cows were fed the TMR twice daily (0715 h and 1615 h) for ad libitum intake aiming for 3 to 5 kg residues. Forty percent of the daily feed allowance was fed in the morning and 60% in the afternoon. Orts were collected daily before the afternoon feeding, and feed intake was determined on daily basis. The TMR offered and the collected orts were sampled for DM determination and subsequent nutrient analysis of feed samples. During d 1 to 14 in each period, 10 g of Cr_2O_3 and 13 g of TiO_2 were dosed in the rumen in the morning and afternoon during milking, respectively. Water intake was measured by individual water meters recorded daily at 1030 h during the 5-d sampling period.

Ruminal Fluid, Digesta, Feces, and Urine Sampling

During the 5 d of the sampling period (d 10–14), ruminal fluid, duodenal and ileal digesta content, and fecal samples were collected at 12 different times of the day: at 1000 and 1800 h on d 10; at 0200, 1200, and 2000 h on d 11; at 0400, 1400, and 2200 h on d 12; at 0600 and 1600 h on d 13; and at 0000 and 0800 h on d 14. Ruminal fluid samples were collected by using a 50-mL syringe connected a 90-cm steel rumen sampler (Bar Diamond Inc.) from the ventral rumen. Duodenal (500 mL) and ileal (250 mL) digesta samples were collected by connecting plastic sampling bags to the T-cannulas, and bags were filled by bowel peristalsis. Fecal samples (350 mL) were collected when cows defecated or from the rectum. Urine samples were only collected at 1000 and 1800 h on the first sampling day by collecting urine when the cow was urinating or by mildly stimulating the cow on the area of the perineum. Ruminal fluid samples were stored individually from each of the 12 sampling times, and duodenal and ileal digesta samples and fecal samples from these 12 sampling times were pooled.

Extra ruminal fluid samples were collected for microbial isolation and chemical analysis at 1000 h on d 14. Two liters of ruminal fluid was collected using a vacuum pump, and subsequently filtered through 2 layers of cheesecloth into prewarmed insulated bottles. Microbes were harvested by differential centrifugation, as described by Brask et al. (2015). All samples of

Table 2. Chemical composition of ingredients (means ± SD; g/kg of DM, unless otherwise noted; n = 2)

Item	Ground untoasted fava beans	Ground toasted fava beans	Rolled untoasted fava beans	Sugar beet pulp	Grass-clover silage	Corn silage
DM, g/kg of fresh matter	879 ± 3.55	913 ± 1.90	883 ± 1.75	925 ± 3.68	394 ± 8.55	318 ± 0.70
Ash	32.0 ± 0.20	31.8 ± 0.02	33.0 ± 0.40	72.9 ± 7.23	89.4 ± 0.85	28.5 ± 0.45
CP	277 ± 2.03	277 ± 1.09	299 ± 0.00	96.1 ± 3.62	140 ± 0.16	82.7 ± 0.47
Soluble CP[1]	619 ± 0.32	422 ± 5.30	654 ± 5.25	172 ± 4.35	665 ± 2.83	658 ± 14.4
Crude fat	19.0 ± 1.00	19.0 ± 0.00	18.5 ± 0.50	17.3 ± 9.88	28.5 ± 0.50	33.5 ± 1.50
NDF	194 ± 6.55	173 ± 4.82	188 ± 3.45	334 ± 4.46	326 ± 2.95	399 ± 15.9
Starch	391 ± 0.61	399 ± 3.65	388 ± 5.67	1.88 ± 0.79	4.65 ± 0.23	292 ± 37.8
AAN,[2] % of total N	82.7 ± 0.26	82.9 ± 0.38	83.0 ± 0.07	69.8 ± 0.68	66.7 ± 0.16	67.1 ± 0.88
Total AA	233 ± 1.18	234 ± 1.68	253 ± 0.16	68.3 ± 2.19	94.9 ± 0.12	56.5 ± 0.42
AA, g/kg of AA						
Ala	46.5 ± 0.19	46.7 ± 0.16	46.2 ± 0.19	61.8 ± 0.35	87.1 ± 0.05	106 ± 0.97
Arg	102 ± 0.38	102 ± 0.02	108 ± 0.37	51.4 ± 3.26	25.1 ± 0.39	22.7 ± 0.70
Asp	121 ± 0.03	122 ± 0.09	122 ± 0.15	96.0 ± 0.96	113 ± 0.40	78.2 ± 0.92
Cys	13.6 ± 0.04	13.3 ± 0.03	14.2 ± 0.01	15.7 ± 0.18	8.96 ± 0.09	10.3 ± 0.08
Glu	180 ± 0.10	180 ± 0.12	181 ± 0.19	130 ± 2.67	94.5 ± 0.15	180 ± 0.17
Gly	48.8 ± 0.12	48.7 ± 0.07	47.8 ± 0.01	55.0 ± 1.84	65.0 ± 0.13	52.5 ± 0.12
His	29.3 ± 0.02	29.2 ± 0.06	29.1 ± 0.02	40.8 ± 1.09	20.5 ± 0.02	12.8 ± 0.18
Ile	49.7 ± 0.04	49.7 ± 0.10	48.6 ± 0.21	53.5 ± 1.33	63.0 ± 0.29	51.4 ± 0.15
Leu	83.3 ± 0.08	83.4 ± 0.04	82.5 ± 0.11	82.3 ± 1.55	105 ± 0.23	129 ± 0.81
Lys	74.4 ± 0.01	74.0 ± 0.00	70.9 ± 0.10	78.9 ± 7.56	61.6 ± 0.07	37.7 ± 0.60
Met	8.55 ± 0.02	8.30 ± 0.03	8.23 ± 0.01	22.1 ± 0.51	18.9 ± 0.02	24.4 ± 0.26
Phe	46.6 ± 0.06	46.6 ± 0.12	45.5 ± 0.21	47.4 ± 1.08	60.4 ± 0.13	45.7 ± 0.72
Pro	45.3 ± 0.05	45.3 ± 0.08	45.8 ± 0.29	58.1 ± 0.87	90.4 ± 1.15	90.3 ± 0.21
Ser	56.1 ± 0.03	56.0 ± 0.32	56.3 ± 0.41	66.5 ± 1.02	53.8 ± 0.07	44.1 ± 1.75
Thr	40.4 ± 0.03	40.3 ± 0.05	39.7 ± 0.18	59.9 ± 1.17	52.6 ± 0.17	45.7 ± 0.19
Val	54.8 ± 0.00	55.0 ± 0.16	54.2 ± 0.13	81.0 ± 1.81	80.3 ± 0.20	69.2 ± 0.25
pH	—	—	—	—	4.34 ± 0.01	3.65 ± 0.01
Total VFA, mmol/L	—	—	—	—	12.5 ± 0.07	23.5 ± 0.56
VFA, mmol/L						
Acetate	—	—	—	—	12.4 ± 0.07	19.5 ± 0.58
Propionate	—	—	—	—	ND[3]	2.22 ± 0.02
Butyrate	—	—	—	—	ND	1.23 ± 0.00
Valerate	—	—	—	—	ND	0.44 ± 0.00
Caproate	—	—	—	—	0.12 ± 0.00	0.13 ± 0.00

[1]Grams per kilogram of CP (Crooker et al., 1978).

[2]Amino acid nitrogen.

[3]ND = not detected.

feed, feed residues, ruminal fluid, duodenum and ileum digesta, feces, and isolated microbes were stored at −20°C until laboratory analysis.

Gas Exchange

Gas exchange was measured by using 4 transparent polycarbonate respiration chambers based on the open-circuit indirect calorimetry system, as described by Hellwing et al. (2012). Chambers were positioned in a square in the same barn where the collection of digesta samples took place, to decrease stress. Gas exchange was measured over two 48-h periods, where cows swapped chambers diagonally after the first 48 h to balance out any possible difference in background air. The gas exchange was measured d 18 to 22 in each period for all cows, but due to only 4 chambers being available, the cows started the experiment in a staggered way with a 4-d delay for 2 cows, as explained earlier. The first 4 of the 6 cows were moved to the respiration chambers after the afternoon milking on d 18 in each period, swapped chambers on the afternoon of d 20, and exited from the chambers on the afternoon of d 22, after which the cows were fed the diet for the next period, equally to d 1 in the period.

The flow and concentration of inlet and outlet gases (CH_4, CO_2, O_2, and H_2) in a given chamber were measured every 12.5 min. Recovery tests for CH_4 (99.6 ± 2.03%) and CO_2 (99.8 ± 0.98%) were conducted before and after the experiment. Gas exchange was calculated based on standard conditions for temperature (0°C) and pressure (101.325 kPa). The measurements from when the chambers were opening for feeding and milking were deleted (around 60 min per day). The gas exchange data during this period was assumed equal to the mean of the rest of the day. Gas exchange data were finally corrected based on the respective recovery test, and H_2 and O_2 were corrected with the average CH_4 and CO_2 recovery.

Milk Yield and Composition

Cows were milked twice daily at 0600 and 1630 h. Milk yield was recorded daily, and milk samples were collected from 6 consecutive milkings from afternoon milking on d 1 to morning milking on d 4 during the sampling period. Milk crude protein, fat, and lactose monohydrate concentrations were analyzed using a Milkoscan 4000 infrared analyzer (Foss Electric; ISO, 2013) at Eurofins Steins Laboratorium (Vejen, Denmark).

In Situ Analyses

Ruminal CP degradation of the 3 tested fava bean samples was estimated by using the standard NorFor Dacron bag method (Åkerlind et al., 2011). Samples were ground at 1.5 mm on a cutter mill (Pulverisette 15, Fritsch GmbH). A total of 1.0 g of sample was weighed out in polyester bags (11 × 8.5 cm, 38-μm pore size). Thereafter, the bags were placed in the rumen of 3 dry cows fed at maintenance level (69:31 forage-to-concentrate ratio; primarily hay as forage and barley and oat grain as concentrate) for 0, 2, 4, 8, 16, 24, 48, and 96 h. After ruminal incubation, all bags were rinsed with cold tap water and frozen. After recovery of all bags, bags were thawed and subsequently washed in a domestic washing machine for 10 min with 2 cycles of 22 L of water (25°C). Residues left in the bags were then transferred to nitrogen-free filter paper (retention value 2, Whatman AGF 607–90 mm) and analyzed for DM (103°C) and N (Kjeldahl method; Hansen, 1989). Water solubility was estimated over filter paper as described by Hvelplund and Weisbjerg (2000). Particle loss was estimated as the difference between the loss from the polyester bags when they were only washed and the water solubility measured on filter paper (Hvelplund and Weisbjerg, 2000).

Total-tract disappearance of CP (**TPD**) was determined using the mobile bag technique according to Hvelplund et al. (1992). Sealed polyester bags (6 × 6 cm, 12-μm pore size) including 1.0 g of feed sample were pre-incubated for 16 h in the rumen of 3 dry cows fed at maintenance level; 6 bags were used for each sample (6 replicates; 2 bags per cow). Bags were subsequently incubated for 2 h in pepsin-hydrochloride solution to simulate abomasal digestion. Afterward, the bags were inserted into the duodenum of 2 lactating cows, with 3 bags for each cow, through the T-shaped duodenal cannula, recovered from feces, and washed in a washing machine. The cows used (160 DIM on average) were fed twice daily for ad libitum intake of a TMR with a forage-to-concentrate ratio of 54:46, consisting of mainly grass-clover silage, corn silage, and barley, with the following chemical composition (g/kg of DM): 167 CP, 323 NDF, and 160 starch. Residues were analyzed for DM (60°C) and N (Kjeldahl method).

Chemical Analysis

The DM was determined in feed ingredients, TMR, and orts using a forced-air oven at 60°C for 48 h. Duodenum and ileum digesta, isolated ruminal microbes, and feces samples were freeze-dried before chemical analysis. Ash content was measured by combustion at 525°C for 6 h. The N content of feed, ruminal microbial matter, and duodenal, ileal, and fecal samples was analyzed by the Dumas method (Hansen, 1989) and calculated as 6.25 × total N, using a Vario MAX CN (Elementar Analysesysteme GmbH). Total N concentration in urine and residues from in situ fermentation was measured by the Kjeldahl method using a Kjeltec 2400 distillation unit (Foss Analytical). Crude fat was measured by using Soxhlet extraction with petroleum ether (Soxtec2050, Foss Analytical) after hydrolysis with HCl (Stoldt, 1952). Starch content in feed, digesta, and feces samples was measured enzymatically (Kristensen et al., 2007). Neutral detergent fiber was determined by using a Fibertec M6 System (Foss Analytical), using heat-stable α-amylase and sodium sulfite (Mertens et al., 2002), and reported as ash-free NDF.

The concentration of Cr_2O_3 in digesta and feces samples was measured via spectrophotometry after oxidation to chromate (Schürch et al., 1950). The concentration of TiO_2 in digesta and feces samples was determined by digesting TiO_2 with sulfuric acid and hydrogen peroxide, and absorbance was measured spectrophotometrically. This method was modified from the method of Myers et al. (2004). The modification included the addition of 15 mL of 30% hydrogen peroxide instead of 10 mL, and an additional 0.25 mL were added before the absorbance was measured. The total contents of purine in microbial matter and duodenal digesta content samples were analyzed according to the method of Zinn and Owens (1986), as modified by Thode (1999). In brief, perchloric acid was used to hydrolyze nucleotides, after which purines precipitated into complexes with silver nitrate and were measured spectrophotometrically.

The pH of urine and ruminal samples was measured immediately after sampling using a pH meter (Meterlab PHM 220, Radiometer). Concentrations of VFA in ruminal fluid were analyzed by gas chromatography as described by Kristensen et al. (1996). Glucose and L-lactate were determined with membrane-immobilized substrate-specific oxidases using an YSI 2900D Biochemistry Analyzer (YSI Inc.). Concentration of NH_3 in ruminal fluid was measured using Randox Ammonia

Kit-AM1015 and Cobas Mira Plus (Roche); samples were diluted by phosphate buffer (100 mmol/L).

Amino acids in feedstuffs, TMR, and duodenal and ileal digesta samples were analyzed using the EEC (European Economic Community) method (98/64/EC; European Commission, 1998). Samples were oxidized with performic acid, followed by hydrolysis with HCl at 0°C for 23 h, and individual AA were subsequently determined on a Biochrom B20 automated AA analyzer. Isoleucine, Ser, and Val were corrected to account for incomplete recovery, using a factor of 1.06 (Rudemo et al., 1980). The particle size distributions of ground untoasted, ground toasted, and rolled untoasted fava beans were determined according to Razzaghi et al. (2016).

Calculations and Statistical Analysis

The geometric mean diameter and geometric standard deviation by mass of particle size distribution were calculated according to ASABE (2013). Crude protein was calculated as total N × 6.25. Data on DMI was averaged per cow over the last 7 d within each period, and data on gas exchange was averaged per cow over the last 4 d within each period. Digesta DM flow was calculated as the average of the DM flows estimated by the 2 markers. One observation of duodenum DM flow was detected as outlier for small intestine digestibility, probably due to nonrepresentative sampling with too low DM content; therefore digestibility data from the corresponding cow was removed. Microbial net synthesis (microbial mass flow from rumen to duodenum) was calculated according to Lund et al. (2003), using the concentration of N and purines in rumen-isolated bacteria, the content of purines in duodenal content, and duodenal DM flow. Microbial N efficiency was expressed as the amount of microbial N synthesized relative to the amount of true ruminal digested OM (Lund et al., 2003). The amount of true ruminal digested OM was calculated by subtracting duodenal OM flow (corrected for duodenal microbial OM flow) from OM intake.

Milk yield and fat, protein, and lactose concentrations were calculated as the average over last 72 h in each sampling period. Energy-corrected milk yield (kg/d) was calculated using the following formula: ECM = milk yield × (383 × milk fat + 242 × milk protein + 157.1 × milk lactose + 20.7)/3,140 (Sjaunja et al., 1991), where lactose is lactose monohydrate; milk fat, protein, and lactose are expressed as the daily yield (kg/d); and assuming 3.14 MJ/kg of ECM.

Ruminal degradability of CP was fitted in a nonlinear least square model to the equation $\text{Deg (t)} = a + b(1 - e^{-ct})$ (Ørskov and McDonald, 1979) and corrected for particle loss. In the equation, Deg (t) is the fraction of CP degraded at time t of incubation (h); a is the immediately degradable (soluble) fraction at 0 h of incubation; b is the fraction not soluble, but potentially degradable over time; and c (h^{-1}) is the degradation rate of fraction b. Effective CP degradability (**EPD**, g/kg) in the rumen was calculated using a fractional rate of passage of 5%/h. Corrected EPD (**EPD*corr***) was calculated by correcting EPD for particle loss, estimated as the difference between solubility at 0 h and solubility over nitrogen-free filter paper (Hvelplund and Weisbjerg, 2000).

The small intestine disappearance (**SID**, g/kg) of RUP was calculated based on EPD*corr* and TPD of CP: SID = (TPD − EPD*corr*)/(1,000 − EPD*corr*) × 1,000 (Hvelplund and Weisbjerg, 2000).

Data were analyzed in R (version 3.6.3; R Foundation for Statistical Computing) using the Fit Linear Mixed-Effects Models (LMM) through the lmer function in the lme4 package (Bates et al., 2015). The following model was used for in vivo data:

$$Y_{ijk} = \mu + T_i + P_j + C_k + E_{ijk},$$

where Y is the dependent variable (n = 24), μ is the overall mean, T_i is the fixed effects of diet (i = GGU, GGT, GRU, CGU, CGT, or CRU), P_j is the fixed effect of period (j = 1 to 4), C_k is the random effect of cows (k = 1 to 6), and E_{ijk} is the random error, assumed to be independent and normally distributed.

The effects of (1) forage source, (2) particle size, (3) heat treatment, (4) interaction between forage source and particle size, and (5) interaction between forage source and heat treatment were estimated by contrasts. The effect of (1) forage source was tested by contrasting GGU, GGT, and GRU versus CGU, CGT, and CRU; the effect of (2) particle size by contrasting GGU and CGU versus GRU and CRU; the effect of (3) heat treatment by contrasting GGU and CGU versus GGT and CGT; the interaction between (4) forage source and particle size by contrasting GGU and CRU versus GRU and CGU; and the interaction between (5) forage source and heat treatment by contrasting GGU and CGT versus GGT and CGU. Least squares of means were presented in tables, and significance or trends were declared at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

The parameters for the in situ study were analyzed using the lm function in R:

$$Y_{ij} = \mu + T_i + E_{ij},$$

where Y is the dependent variable (n = 6), μ is the overall mean, T_i is the fixed effect of treatments (i =

Table 3. Particle size distribution (μm) of processed fava beans (g/kg unless otherwise noted)

Item	Ground untoasted	Ground toasted	Rolled untoasted
>4,000	0.30	0.18	331
2,000–4,000	11.0	2.92	557
1,000–2,000	477	188	64.3
500–1,000	313	363	25.4
250–500	87.9	183	10.0
125–250	36.5	83.7	4.57
63–125	27.8	74.4	3.75
45–63	17.7	36.2	2.17
0–45	28.8	68.6	1.81
d_{gw},[1] μm	743	407	2,827
S_{gw},[1] μm	776	529	1,728

[1]Geometric mean diameter (d_{gw}) and geometric standard deviation (S_{gw}) by mass calculated according to ASABE (2013).

ground untoasted, ground toasted, rolled untoasted), and E_{ij} is the random error.

RESULTS

Experimental Diets and Feeds and Particle Size Distribution of Fava Beans

Table 1 shows ingredient proportion and chemical composition of the 6 diets, and chemical composition of the 6 main ingredients (ground untoasted fava beans, ground toasted fava beans, rolled untoasted fava beans, sugar beet pulp, grass-clover silage, corn silage) are reported in Table 2. The DM contents for ground untoasted, ground toasted, and rolled untoasted fava beans were 879, 913, and 883 g/kg, respectively (Table 2). Grass-clover silage had DM, CP, NDF, starch, and total AA contents of 394 g/kg, and 140, 326, 4.65, and 94.9 g/kg of DM, respectively, compared with contents of 318 g/kg, and 82.7, 399, 292, and 56.5 g/kg of DM, respectively, for corn silage. Geometric mean diameters and geometric standard deviations by mass of the 3 fava beans used in the current study are shown in Table 3.

Intake and Digestibility in Different Parts of the Gastrointestinal Tract

Significant interactions occurred between forage source and heat treatment on DM, OM, and NDF intake (Table 4). When feeding diets high in grass-clover silage, inclusion of ground untoasted fava beans showed higher DM, OM, and NDF intakes than inclusion of ground toasted fava beans. In contrast, inclusion of ground toasted fava beans showed higher DM, OM, and NDF intakes than inclusion of ground untoasted when diets high in corn silage were fed. Compared with feeding the diets high in corn silage, feeding the diets high in grass-clover silage resulted in greater water and AA intake but less intake of CP and starch. Inclusion of rolled untoasted fava beans resulted in 3% greater CP intake and 4% greater AA intake than inclusion of ground untoasted fava beans (Table 4). We detected no effect of toasting of fava beans on nutrient intake.

Interactions between forage source and heat treatment on DM and OM apparent ruminal digestibility were observed (Table 4). When feeding diets high in grass-clover silage, inclusion of ground untoasted fava beans resulted in higher DM and OM apparent ruminal digestibility than ground toasted fava beans; conversely, inclusion of ground toasted fava beans resulted in higher DM and OM apparent ruminal digestibility than ground untoasted when feeding diets high in corn silage. Moreover, a significant interaction between forage source and particle size on hindgut NDF digestibility was noted. When feeding diets high in grass-clover silage, inclusion of ground untoasted fava beans resulted in a lower hindgut NDF digestibility than rolled untoasted fava beans, whereas when feeding diets high in corn silage, inclusion of ground untoasted fava beans instead resulted in greater hindgut NDF digestibility. Feeding diets high in grass-clover silage resulted in higher apparent ruminal NDF digestibility, small intestine DM and OM digestibility, and total-tract NDF digestibility, but lower total-tract CP digestibility and apparent ruminal and total-tract starch digestibility than when diets high in corn silage were fed. Rolling of fava beans resulted in lower true ruminal OM, CP, and AA digestibility, total-tract CP digestibility, feed-ileum AA digestibility, and apparent ruminal, small intestine, hindgut, and total-tract starch digestibility compared with ground untoasted fava beans (Table 4). In contrast, rolling of fava beans resulted in higher apparent ruminal and total-tract NDF digestibility compared with ground untoasted fava beans.

Microbial Protein Synthesis and Small Intestine AA Digestion

Inclusion of ground toasted fava beans resulted in higher proportion of Ala in microbial AA (Table 5), whereas lower proportion of Arg was found when compared with ground untoasted fava beans. Feeding diets high in grass-clover silage resulted in higher Ala and Met proportions of total microbial AA, but lower Arg and Ser proportions and lower microbial OM flow compared with feeding diets high in corn silage. Rolled untoasted fava beans resulted in a higher Asp proportion of total microbial AA but lower His proportion and microbial OM, CP, and AA flow compared with ground untoasted fava beans.

Table 4. Intake and digestibility of nutrients in rumen, small intestine, hindgut, and total tract (g/kg unless otherwise noted) of dairy cows fed diets varying in forage source and particle size and heat treatment of fava beans[1]

	Diet[2]							Contrast, *P*-value[4]				
Item	GGU	GGT	GRU	CGU	CGT	CRU	SEM[3]	S	Pa	H	S × Pa	S × H
Intake, kg/d												
Drinking water, L/d	84.4	88.2	80.2	72.8	75.1	71.3	7.33	<0.001	0.18	0.16	0.54	0.74
DM	22.9	22.3	22.3	23.1	23.7	23.1	1.52	<0.01	0.13	0.88	0.26	0.03
OM	21.2	20.6	20.6	21.8	22.3	21.8	1.42	<0.001	0.10	0.80	0.25	0.03
CP	3.84	3.75	3.92	3.85	3.96	4.00	0.25	0.03	0.05	0.80	0.52	0.09
AA	2.91	2.84	2.99	2.67	2.74	2.80	0.18	<0.001	<0.01	0.86	0.54	0.07
NDF	6.29	6.10	6.14	6.65	6.80	6.59	0.44	<0.001	0.11	0.75	0.49	0.03
Starch	4.16	3.93	3.87	6.17	6.35	6.12	0.37	<0.001	0.21	0.85	0.42	0.18
Digestibility, g/kg												
DM												
Apparent rumen	370	337	345	347	395	349	25.2	0.38	0.51	0.66	0.48	0.05
True rumen	496	478	449	480	541	486	44.5	0.15	0.38	0.30	0.26	0.11
Small intestine	534	544	538	509	488	492	19.4	<0.01	0.68	0.71	0.53	0.35
Hindgut	101	73.7	121	125	102	112	34.8	0.60	0.90	0.44	0.64	0.95
Total-tract	733	722	723	723	725	704	10.5	0.23	0.11	0.58	0.64	0.52
OM												
Apparent rumen	466	438	443	436	475	431	22.1	0.91	0.36	0.73	0.57	0.05
True rumen	637	610	582	613	649	583	21.8	0.66	0.02	0.73	0.42	0.07
Small intestine	502	507	501	475	454	459	20.6	0.01	0.63	0.64	0.66	0.46
Hindgut	59.1	31.0	90.4	88.6	64.0	82.1	36.4	0.53	0.72	0.45	0.61	0.96
Total-tract	746	733	735	734	736	715	10.6	0.17	0.09	0.51	0.64	0.44
CP												
Apparent rumen	−149	−198	−168	−160	−93.5	−137	42.2	0.16	0.95	0.78	0.56	0.11
True rumen	463	395	320	456	459	373	32.4	0.07	<0.001	0.15	0.21	0.15
Small intestine	701	709	692	728	717	695	11.7	0.16	0.06	0.88	0.25	0.33
Hindgut	−9.68	−27.2	5.47	−22.0	−19.4	−12.3	33.0	0.79	0.71	0.82	0.94	0.78
Total-tract	648	645	625	682	687	646	10.0	<0.01	0.01	0.92	0.55	0.71
AA												
Apparent rumen	−113	−183	−141	−203	−172	−202	45.6	0.14	0.72	0.58	0.71	0.20
True rumen	490	408	319	414	411	345	48.2	0.63	0.01	0.28	0.24	0.36
Small intestine	728	743	712	748	747	708	12.9	0.46	0.05	0.59	0.36	0.51
Feed-ileum[5]	686	698	671	695	703	648	10.2	0.71	<0.01	0.26	0.10	0.82
NDF												
Apparent rumen	660	674	708	595	630	621	27.2	<0.001	0.03	0.10	0.47	0.46
Small intestine	−89.9	−165	−196	−235	−191	−47.6	96.0	0.89	0.55	0.81	0.06	0.39
Hindgut	−18.9	−0.36	124	101	24.1	11.1	53.1	0.76	0.51	0.47	0.02	0.28
Total-tract	631	626	693	573	573	610	20.2	<0.001	<0.01	0.87	0.45	0.89
Starch												
Apparent rumen	789	712	661	821	833	756	29.5	<0.01	<0.01	0.15	0.20	0.08
Small intestine	762	753	505	768	693	484	44.0	0.44	<0.001	0.24	0.72	0.39
Hindgut	591	552	307	616	667	282	57.2	0.35	<0.001	0.91	0.62	0.39
Total-tract	982	968	882	985	987	911	8.92	<0.01	<0.001	0.32	0.08	0.28

[1]Intake, hindgut digestibility, and total-tract digestibility were based on 24 observations in total (4 observations for each diet), but rumen, true rumen, and small intestine digestibility were based on 23 observations in total (3 observations for rolled untoasted fava beans in diet high in grass-clover silage, 4 observations for the other 5 diets).

[2]High in grass-clover silage: ground untoasted fava beans = GGU; ground toasted fava beans = GGT; rolled untoasted fava beans = GRU. High in corn silage: ground untoasted fava beans = CGU; ground toasted fava beans = CGT; rolled untoasted fava beans = CRU.

[3]Largest value was reported, due to missing observation.

[4]Silage (S) = forage source; particle (Pa) = particle size; heat (H) = heat treatment (toasting). Probability of contrasts: S = GGU, GGT, and GRU vs. CGU, CGT, and CRU; Pa = GGU and CGU vs. GRU and CRU; H = GGU and CGU vs. GGT and CGT; S × Pa = GGU and CRU vs. GRU and CGU; S × H = GGU and CGT vs. GGT and CGU.

[5]Refers to the total AA digestibility based on the intake and the ileal outflow.

Forage source, toasting, and rolling of fava beans had no significant effect on small intestine total AA digestion (Table 6). However, diets high in grass-clover silage and rolling of fava beans both resulted in a smaller amount of Gly digested in the small intestine compared with diets high in corn silage and ground untoasted fava beans, respectively. Toasting of fava beans had no effect on small intestine individual AA digestion but resulted in higher proportions of Arg, Cys, Glu, His, Phe, and Pro and a lower proportion of Met in total AA digested in the small intestine compared with ground untoasted fava beans. Feeding diets high in grass-clover

Table 5. Chemical composition of ruminal microbes (g/kg of microbial DM unless otherwise noted), microbial flow to the duodenum (kg/d), and microbial efficiency (g/kg of OM truly digested in the rumen) of dairy cows fed diets varying in forage source and particle size and heat treatment of fava beans[1]

	Diet[2]							Contrast, *P*-value[4]				
Item	GGU	GGT	GRU	CGU	CGT	CRU	SEM[3]	S	Pa	H	S × Pa	S × H
AA composition, g/kg of AA												
Ala	79.8	81.5	79.2	79.1	80.0	79.5	0.75	0.05	0.75	<0.01	0.20	0.27
Arg	51.0	50.4	50.6	52.1	51.0	51.4	0.51	0.02	0.18	0.05	0.59	0.55
Asp	128	128	128	127	129	129	0.30	0.18	<0.01	0.02	0.40	0.03
Cys	11.1	11.2	11.2	10.6	10.9	11.0	0.23	0.05	0.17	0.36	0.31	0.48
Glu	135	135	135	135	134	135	0.60	0.56	0.78	0.15	0.43	0.21
Gly	59.1	59.3	59.0	59.0	59.3	59.1	0.32	0.88	0.95	0.31	0.79	0.87
His	18.2	18.0	17.9	18.6	18.1	18.1	0.27	0.21	0.05	0.08	0.72	0.51
Ile	63.3	63.0	63.1	62.5	62.6	62.9	0.44	0.09	0.65	0.82	0.39	0.61
Leu	78.3	78.4	78.8	79.4	79.4	79.0	0.76	0.12	0.88	0.92	0.41	0.87
Lys	88.5	88.2	88.5	88.1	87.6	87.9	1.23	0.54	0.90	0.67	0.98	0.96
Met	24.5	23.8	23.9	23.3	23.1	23.1	0.54	0.03	0.33	0.31	0.70	0.69
Orn	1.82	1.95	1.72	1.51	1.77	1.56	0.17	0.08	0.85	0.17	0.61	0.67
Phe	51.4	51.6	51.8	51.4	52.0	51.6	0.67	0.89	0.52	0.37	0.80	0.67
Pro	35.9	35.5	36.0	36.2	36.4	35.8	0.39	0.13	0.63	0.74	0.43	0.35
Ser	48.0	48.2	48.4	49.0	49.0	49.0	0.35	0.01	0.56	0.73	0.48	0.80
Thr	58.1	58.4	58.4	58.4	58.7	58.6	0.23	0.09	0.19	0.14	0.79	0.90
Val	68.6	68.5	68.3	68.4	68.0	68.4	0.35	0.47	0.64	0.45	0.69	0.50
Microbial flow, kg/d												
OM	3.60	3.49	2.89	3.91	4.11	3.32	0.28	<0.01	<0.001	0.68	0.63	0.24
CP	2.31	2.21	1.89	2.44	2.33	2.02	0.17	0.09	<0.001	0.20	0.99	0.99
AA	1.75	1.65	1.40	1.67	1.69	1.53	0.13	0.55	<0.01	0.50	0.13	0.40
Microbial efficiency												
g of N/kg of OM truly digested in the rumen	27.8	28.3	25.8	28.8	25.6	25.8	1.81	0.63	0.11	0.35	0.70	0.22
g of AA/kg of OM truly digested in the rumen	131	132	118	123	116	122	7.27	0.16	0.25	0.59	0.35	0.48

[1]Chemical composition of microbes and AA composition were based on 24 observations in total (4 observations for each diet); microbial flow and microbial efficiency were based on 23 observations in total (3 observations for rolled untoasted fava beans in diet high in grass-clover silage, 4 observations for the other 5 diets).

[2]High in grass-clover silage: ground untoasted fava beans = GGU; ground toasted fava beans = GGT; rolled untoasted fava beans = GRU. High in corn silage: ground untoasted fava beans = CGU; ground toasted fava beans = CGT; rolled untoasted fava beans = CRU.

[3]Largest value was reported, due to missing observations.

[4]Silage (S) = forage source; particle (Pa) = particle size; heat (H) = heat treatment (toasting). Probability of contrasts: S = GGU, GGT, and GRU vs. CGU, CGT, and CRU; Pa = GGU and CGU vs. GRU and CRU; H = GGU and CGU vs. GGT and CGT; S × Pa = GGU and CRU vs. GRU and CGU; S × H = GGU and CGT vs. GGT and CGU.

silage resulted in higher proportions of Arg, His, and Phe in AA digested in the small intestine compared with feeding diets high in corn silage. Inclusion of rolled untoasted fava beans resulted in higher proportions of Arg, Glu, His, and Orn but lower proportion of Met in AA digested in the small intestine compared with inclusion of ground untoasted fava beans.

In Situ CP Degradation

Toasting of fava beans resulted in lower water solubility and corrected soluble fraction (a*corr*) of CP and higher corrected potentially degradable fractions (b*corr*) of CP and TPD compared with ground untoasted fava beans (Table 7). Rolling of fava beans resulted in a higher water solubility and corrected potentially degradable fraction (b*corr*) of CP, as well as higher corrected effective degradability of CP (EPD*corr*) and TPD, and lower corrected soluble fraction (a*corr*) of CP and SID of RUP compared with ground untoasted fava beans. In situ data should be interpreted with care due to the uniform milling before incubation and analysis.

Ruminal Fermentation

Toasting of fava beans resulted in lower isobutyrate proportion compared with ground untoasted fava beans. Inclusion of rolled untoasted fava beans resulted in greater acetate proportion and acetate:propionate ratio compared with inclusion of ground untoasted fava beans, whereas the proportions of propionate and valerate were lower for rolled untoasted fava beans compared with ground untoasted fava beans (Table 8). Diets high in grass-clover silage resulted in lower average ruminal pH compared with diets high in corn silage (Table 8). The concentrations of ruminal NH_3 and glucose were respectively 33% and 34% higher for diets high in corn silage than for diets high in grass-clover silage. Feeding diets high in grass-clover silage resulted in higher butyrate proportion and acetate:propionate ratio but lower propionate proportion and isovalerate proportion compared with feeding diets high in corn silage. Rolling and toasting of fava beans both resulted in a higher urine pH compared with ground untoasted fava beans.

Gas Exchange

Interactions between forage source and heat treatment on daily CH_4 production and daily CO_2 production were noted (Table 9). In diets high in grass-clover silage, ground untoasted fava beans showed greater CH_4 and CO_2 production compared with ground toasted fava beans, whereas ground untoasted fava beans resulted in lower CH_4 and CO_2 production in diets high in corn silage.

Rolled untoasted fava beans resulted in greater daily CH_4 production, CH_4 liters per kilogram of DMI, CH_4 in percentage of gross energy intake, and CH_4:CO_2 ratio compared with ground untoasted fava beans. No effect of toasting of fava beans on gas exchange was observed (Table 9).

Feeding diets high in grass-clover silage resulted in greater daily CH_4 production, CH_4 in percentage of gross energy intake, daily H_2 production, H_2 in liters per kilogram of DMI, and CH_4:CO_2 ratio (Table 9) compared with feeding diets high in corn silage.

Milk Production

Toasting of fava beans lowered milk protein and urea concentration and milk protein yield compared with ground untoasted fava beans (Table 9). Rolling of fava beans resulted in lower milk protein and urea concentration and ECM, milk protein, and lactose yield compared with ground untoasted fava beans. Feeding diets high in corn silage resulted in greater milk yield, ECM, milk protein and lactose yield, and milk lactose concentration, and lower milk fat concentration compared with feeding diets high in grass-clover silage.

Supplemental Data

Amounts of nutrients digested in the rumen, small intestine, hindgut, and total tract; microbial chemical composition; duodenal flow of individual AA; and small intestine individual AA digestibility are reported in Supplemental Tables S1 to S3 (https://doi.org/10.5281/zenodo.6655348; Wang et al., 2022).

DISCUSSION

Effects of Forage Source

The grass-clover silage and corn silage used in the current study had typical contents of DM, CP, NDF, and starch (Khan et al., 2015; Alstrup et al., 2016). In agreement with several previous grass-clover silage and corn silage comparison studies, diets high in corn silage resulted in higher DMI (Hart et al., 2015; Khan et al., 2015; Tayyab et al., 2019). The high rumen-digestible starch and starch content in corn silage may enhance ruminal fermentation, thereby increasing rumen passage rate and resulting in higher DMI (Jensen et al., 2005; Brask et al., 2013). The higher DMI further resulted in higher CP and starch intake but lower AA intake in diets high in corn silage. The reason for higher

Table 6. Small intestinal individual AA digestion[1] (g/d) and individual AA proportion in dairy cows fed diets varying in forage source and particle size and heat treatment of fava beans[2]

	Diet[3]							Contrast, *P*-value[5]				
Item	GGU	GGT	GRU	CGU	CGT	CRU	SEM[4]	S	Pa	H	S × Pa	S × H
Amount digested, g/d												
Ala	142	153	145	150	144	144	12.4	0.87	0.86	0.67	0.58	0.26
Arg	135	153	153	142	142	144	9.80	0.49	0.21	0.21	0.33	0.27
Asp	262	290	279	276	274	270	10.9	0.78	0.71	0.34	0.43	0.29
Cys	26.9	31.8	29.2	29.2	29.4	28.3	2.17	0.84	0.69	0.15	0.39	0.20
Glu	295	332	323	315	315	317	10.9	0.96	0.38	0.26	0.45	0.29
Gly	288	297	263	321	299	285	5.74	0.04	0.01	0.48	0.59	0.15
His	48.1	54.7	52.5	50.6	50.8	50.5	10.4	0.60	0.43	0.20	0.42	0.25
Ile	127	139	132	132	131	128	10.9	0.75	0.94	0.37	0.50	0.34
Leu	185	205	196	194	192	190	10.9	0.71	0.77	0.36	0.50	0.32
Lys	180	195	186	191	188	185	9.48	0.83	0.99	0.47	0.49	0.29
Met	37.9	39.1	37.1	39.9	37.5	36.7	9.25	0.98	0.30	0.76	0.54	0.36
Orn	2.55	2.82	2.91	2.93	2.53	2.89	11.9	0.91	0.57	0.81	0.43	0.18
Phe	105	118	111	107	108	106	10.2	0.38	0.63	0.20	0.53	0.35
Pro	84.9	98.2	93.9	91.6	91.4	90.2	12.0	0.76	0.48	0.20	0.35	0.22
Ser	116	132	127	125	124	122	10.2	0.79	0.54	0.26	0.30	0.23
The	115	125	120	122	118	118	11.7	0.86	0.91	0.54	0.43	0.24
Val	137	151	143	144	141	138	12.6	0.67	1.00	0.46	0.50	0.31
Total	2,287	2,515	2,399	2,430	2,387	2,359	10.2	0.93	0.86	0.38	0.43	0.24
Individual proportion, g/kg of total AA digested												
Ala	62.0	61.0	60.3	61.6	60.2	61.1	0.99	0.77	0.13	0.08	0.37	0.75
Arg	58.7	60.5	63.1	58.0	59.2	60.6	1.74	0.01	<0.001	0.02	0.16	0.64
Asp	115	115	116	114	115	115	1.81	0.11	0.08	0.24	0.47	0.81
Cys	11.8	12.8	12.2	12.3	12.4	12.2	0.59	0.80	0.69	0.05	0.34	0.13
Glu	129	131	135	130	132	135	3.38	0.54	<0.001	0.04	0.89	0.97
Gly	127	119	114	131	126	123	14.8	0.28	0.13	0.31	0.69	0.81
His	21.0	21.7	21.8	20.8	21.2	21.4	0.39	0.02	<0.01	<0.01	0.57	0.41
Ile	55.3	55.2	54.8	54.5	55.0	54.2	0.87	0.22	0.45	0.78	0.84	0.58
Leu	80.7	81.3	81.2	79.8	80.3	80.1	1.82	0.22	0.74	0.56	0.91	0.94
Lys	78.3	77.4	77.4	78.8	78.5	78.3	1.10	0.09	0.21	0.25	0.71	0.59
Met	16.5	15.5	15.5	16.3	15.6	15.4	0.34	0.84	<0.001	<0.001	0.79	0.61
Orn	0.84	1.12	1.19	0.89	1.13	1.25	0.20	0.72	0.03	0.09	0.98	0.91
Phe	45.6	46.6	46.1	43.8	45.2	44.6	1.02	<0.01	0.28	0.05	0.83	0.70
Pro	37.1	39.0	38.8	37.8	38.4	38.2	0.88	0.72	0.08	0.04	0.28	0.31
Ser	50.8	52.4	52.9	51.8	52.1	51.9	0.93	0.77	0.06	0.08	0.08	0.22
Thr	50.2	49.9	50.2	50.4	49.5	50.1	0.94	0.80	0.76	0.20	0.73	0.52
Val	60.1	59.8	59.3	59.2	58.9	58.5	0.92	0.10	0.23	0.63	0.96	0.99

[1]Small intestine AA digestion is regarded as indicator for MP supply.

[2]Three observations for rolled untoasted fava beans in diet high in grass-clover silage, 4 observations for the other 5 diets.

[3]High in grass-clover silage: ground untoasted fava beans = GGU; ground toasted fava beans = GGT; rolled untoasted fava beans = GRU. High in corn silage: ground untoasted fava beans = CGU; ground toasted fava beans = CGT; rolled untoasted fava beans = CRU.

[4]Largest value was reported, due to missing observations.

[5]Silage (S) = forage source; particle (Pa) = particle size; heat (H) = heat treatment (toasting). Probability of contrasts: S = GGU, GGT, and GRU vs. CGU, CGT, and CRU; Pa = GGU and CGU vs. GRU and CRU; H = GGU and CGU vs. GGT and CGT; S × Pa = GGU and CRU vs. GRU and CGU; S × H = GGU and CGT vs. GGT and CGU.

CP intake but lower AA intake in diets high in corn silage was inclusion of urea in the corn silage diets. The N from urea was accounted as the N from CP, whereas the N from urea will not provide AA to cows. Diets high in corn silage also resulted in higher total-tract starch digestibility, which is probably due to high digestibility of starch from corn silage in the rumen (Brask et al., 2013; Moharrery et al., 2014). However, in accordance with previous studies, diets high in corn silage resulted in lower total-tract NDF digestibility (Juniper et al., 2008; Brask et al., 2013), which could be attributed to lower ruminal pH, less favorable for fibrolytic bacteria. However, ruminal pH was higher in the diets high in corn silage compared with diets high in grass-clover silage in the present study. Huhtanen et al. (2006) reported that the effect of ruminal pH on fiber digestion is relatively small when the ruminal pH is above 6.2, which was the case in all 6 diets. In addition, the lower total-tract NDF digestibility for corn silage-based diets can also be partly attributed to the faster passage rate in the rumen caused by higher DMI for these diets (Kuoppala et al., 2009). The lower apparent ruminal and total-tract NDF digestibility for diets high in corn silage could therefore be due to intrinsic differences between corn and grass-clover in the physiochemical characteristics of the NDF fraction. In the current study, lower total-tract NDF digestibility complied with the lower ruminal acetate proportion and higher propionate proportion in ruminal fluid, and lower CH_4 production, in cows fed diets high in corn silage, as also found by van Gastelen et al. (2015). This is despite DMI being higher in diets high in corn silage and DMI being considered as the most critical factor associated with CH_4 production (Niu et al., 2018). In addition, it has been reported that starch-rich diets could reduce the protozoa numbers in the rumen, and, because protozoa are important for transferring hydrogen to methanogens and eventually contribute to methanogenesis, higher starch intake in diets high in corn silage could thereby contribute to mitigating CH_4 production by reducing hydrogen supply (Hegarty, 1999). The higher H_2 production in diets high in corn silage also confirmed that hydrogen was not efficiently utilized for CH_4 production, possibly due to a suboptimal transfer by protozoa. However, it is surprising to see significant effects on nutrient intake and digestibility, ruminal fermentation, and CH_4 production without an effect on microbial protein synthesis. Diets high in corn silage were associated with higher ruminal ammonia concentration, probably due to the supplementation of urea in these diets. However, we found no difference between diets high in grass-clover silage and diets high in corn silage on milk urea concentration.

A significant interaction between forage source and heat treatment was found for several parameters related to ruminal fermentation. We detected a positive effect of toasting of fava beans on DM and OM intake, apparent ruminal DM and OM digestibility, and emission of CH_4 and CO_2 when cows were fed diets high in corn silage, and a negative effect on DM and OM intake, apparent ruminal DM and OM digestibility, and emission of CH_4 and CO_2 when cows were fed diets high in grass-clover silage. This indicates that toasting of fava beans increases ruminal fermentation when cows were fed diets high in corn silage and decreases ruminal fermentation when cows were fed diets high in grass-clover. However, this was not reflected in similar responses in ruminal pH, NDF apparent ruminal di-

Table 7. In situ degradability and small intestinal and total-tract digestibility of CP (g/kg of CP, unless otherwise noted) of ground untoasted, ground toasted, and rolled untoasted fava beans[1]

	Fava bean				*P*-value[3]	
Item[2]	Ground untoasted	Ground toasted	Rolled untoasted	SEM	H	Pa
SOL	360	272	445	6.01	0.002	0.002
P	241	203	236	16.8	0.21	0.83
a*corr*	414	248	322	2.90	<0.001	<0.001
b*corr*	537	753	770	6.69	<0.001	<0.001
c	0.13	0.13	0.12	0.01	0.72	0.33
EPD*corr*[4]	802	789	858	11.9	0.51	0.04
SID, g/kg of RUP[5]	561	609	435	27.2	0.30	0.05
TPD[5]	913	918	920	1.01	0.03	0.01

[1]Two observations for each feedstuff.

[2]SOL = water solubility; P = particle loss, calculated as 0-h loss from the bags minus SOL; a*corr* = soluble fraction corrected for particle loss; b*corr* = potentially degradable fraction corrected for particle loss; c = rate of degradation of b fraction (h^{-1}); EPD = effective degradability of CP; EPD*corr* = EPD corrected for particle loss; SID = small intestine disappearance of RUP (g/kg of RUP); TPD = total-tract disappearance of CP.

[3]H = heat treatment (toasting); Pa = particle size.

[4]A fractional rate of passage of 5%/h was used to calculate EPD.

[5]Hvelplund and Weisbjerg (2000).

Table 8. Ruminal fermentation characteristics and urine pH in dairy cows fed diets varying in forage source and particle size and heat treatment of fava beans[1]

Item	Diet[2]						Contrast, *P*-value[3]				
	GGU	GGT	GRU	CGU	CGT	CRU	SEM	S	Pa	H	S × Pa	S × H
Rumen pH	6.30	6.23	6.29	6.38	6.45	6.41	0.08	0.02	0.84	0.95	0.77	0.28
L-Lactate, mmol/L	0.68	1.08	0.97	0.54	0.51	0.95	0.25	0.26	0.19	0.48	0.81	0.44
NH_3, mmol/L	5.49	5.14	5.78	9.19	7.00	8.15	0.78	<0.01	0.64	0.13	0.43	0.28
Glucose, mmol/L	0.64	0.59	0.55	0.98	0.87	0.86	0.08	<0.001	0.14	0.23	0.82	0.70
Total VFA, mmol/L	132	128	128	127	126	128	4.20	0.28	0.49	0.31	0.33	0.56
VFA, mol/100 mol of total VFA												
Acetate	60.4	61.5	62.2	59.5	60.3	61.6	0.94	0.07	<0.01	0.10	0.83	0.83
Propionate	21.8	20.8	19.9	23.8	22.2	21.8	1.23	0.02	0.02	0.12	0.97	0.72
Isobutyrate	0.75	0.70	0.72	0.76	0.70	0.76	0.04	0.35	0.56	0.04	0.49	0.97
Butyrate	13.2	13.5	13.5	12.3	13.1	11.9	0.55	0.04	0.93	0.31	0.51	0.64
Isovalerate	1.26	1.21	1.26	1.50	1.29	1.71	0.10	<0.001	0.13	0.08	0.17	0.30
Valerate	1.80	1.68	1.59	1.63	1.64	1.51	0.06	0.06	0.01	0.33	0.49	0.30
Caproate	0.74	0.67	0.70	0.65	0.76	0.66	0.09	0.84	0.78	0.79	0.73	0.24
Acetate:propionate	2.81	3.00	3.14	2.55	2.82	2.91	0.20	0.03	<0.01	0.06	0.92	0.73
Urine pH	8.04	8.15	8.03	7.84	8.08	8.08	0.05	0.09	0.04	0.004	0.03	0.22

[1]A total of 24 observations for all 6 diets in each parameter (4 observations for each diet in each parameter).

[2]High in grass-clover silage: ground untoasted fava beans = GGU; ground toasted fava beans = GGT; rolled untoasted fava beans = GRU. High in corn silage: ground untoasted fava beans = CGU; ground toasted fava beans = CGT; rolled untoasted fava beans = CRU.

[3]Silage (S) = forage source; particle (Pa) = particle size; heat (H) = heat treatment (toasting). Probability of contrasts: S = GGU, GGT, and GRU vs. CGU, CGT, and CRU; Pa = GGU and CGU vs. GRU and CRU; H = GGU and CGU vs. GGT and CGT; S × Pa = GGU and CRU vs. GRU and CGU; S × H = GGU and CGT vs. GGT and CGU.

gestibility, and rumen microbial protein synthesis. The biological mechanism behind the interactions between forage source and toasting of fava beans on DM and OM intake and digestibility and gas exchange remains unclear, and further research is required in this regard.

Effects of Toasting of Fava Beans

Based on in situ data, toasting has been widely used as an effective approach to improve the protein utilization of different protein feeds, especially for fava beans (Goelema et al., 1998; Mogensen et al., 2010). In accordance with previous studies (Yu et al., 1998, 2000; Hansen et al., 2021), toasting provided additional drying for fava beans, whereby the DM content was increased. Yu et al. (2000) observed an increase in CP content on fava beans by pressure toasting, although Yu et al. (2002) also reported a decrease in CP content of fava beans by dry toasting, which is probably due to the higher toasting temperature used during dry toasting. The decrease of CP content was not observed when fava beans were toasted at 100, 118, and 136°C for 7, 15, and 30 min (Yu et al., 2000), whereas a decrease in CP content was seen when fava beans were toasted at 150°C for 45 min, but an increase was also seen when toasted at 150°C for 15 and 30 min. Therefore, the effect of toasting on CP content of fava beans is not consistent, as it depends not only on the temperature of toasting but also on the duration and type of toasting. Moreover, the moisture content of fava beans could also influence the effects of toasting on nutrient composition, as Cleale IV et al. (1987) suggested that hydrating of fava beans before toasting could increase the rate of the Maillard reaction.

In the present study, no in vivo effect of toasting of fava beans on CP and AA digestibility in rumen and small intestine was observed, whereas a lower CP content in microbial DM was observed, but without affecting the microbial protein synthesis. Lund et al. (2004) reported a numerical but not significant decrease in microbial synthesis in diets containing heat-treated fava beans. In addition, given that microbial synthesis was estimated only based on a single ruminal fluid collection, the effect on microbial synthesis should be interpreted with care, as an unaccounted-for diurnal variation on rumen microflora might exist (Salfer et al., 2021).

Toasting of fava beans had no effect on in situ EPD and SID and increased TPD. However, the EPD was numerically lower and SID was numerically higher for toasted fava beans compared with untoasted ground fava beans. This indicated that CP digestion of fava beans partly shifted from the rumen to the small intestine and increased total-tract CP digestion. The in situ

Table 9. Gas exchange, milk yield, and milk composition in dairy cows fed diets varying in forage source and particle size and heat treatment of fava beans[1]

	Diet[3]							Contrast, *P*-value[4]				
Item[2]	GGU	GGT	GRU	CGU	CGT	CRU	SEM	S	Pa	H	S × Pa	S × H
Gas exchange												
CH_4, L/d	632	612	682	569	631	630	27.1	0.01	<0.01	0.13	0.70	0.02
CH_4/DMI, L/kg	27.1	26.7	28.9	25.6	27.6	28.1	1.25	0.32	<0.01	0.20	0.60	0.09
CH_4/ECM,[5] L/kg	21.2	20.1	20.7	22.6	20.5	20.0	2.35	0.81	0.46	0.44	0.62	0.81
CH_4, % of GEI[6]	5.93	5.74	6.51	5.32	5.67	5.87	0.26	<0.01	<0.01	0.57	0.95	0.09
H_2, L/d	13.0	14.0	13.1	6.99	9.55	8.29	1.04	<0.001	0.46	0.08	0.55	0.43
H_2/DMI, L/kg	0.59	0.62	0.56	0.32	0.41	0.37	0.07	<0.001	0.88	0.24	0.41	0.51
CO_2, L/d	7,754	7,625	7,643	7,515	7,746	7,622	388	0.47	0.98	0.51	0.20	0.05
CH_4:CO_2 ratio	0.082	0.081	0.089	0.077	0.082	0.083	0.00	0.04	<0.01	0.18	0.91	0.08
O_2, L/d	6,672	6,639	6,573	6,518	6,644	6,574	357	0.34	0.73	0.45	0.25	0.24
Milk production												
Milk, kg/d	30.2	31.2	29.0	32.4	31.2	31.4	4.34	0.01	0.09	0.89	0.91	0.12
ECM, kg/d	31.4	31.3	29.8	32.5	31.7	31.6	2.87	0.01	0.01	0.28	0.42	0.48
Fat, g/d	1,274	1,271	1,237	1,259	1,263	1,263	85.5	0.99	0.42	0.99	0.44	0.88
Protein,[7] g/d	1,111	1,080	1,016	1,173	1,115	1,110	110	<0.01	<0.01	0.03	0.42	0.51
Lactose, g/d	1,422	1,456	1,342	1,571	1,495	1,485	197	<0.01	0.02	0.52	0.95	0.14
Milk composition												
Fat, %	4.46	4.35	4.45	4.17	4.30	4.23	0.39	0.02	0.74	0.93	0.69	0.18
Protein,[7] %	3.81	3.63	3.62	3.72	3.66	3.65	0.19	0.60	<0.01	<0.01	0.16	0.17
Lactose, %	4.71	4.72	4.65	4.78	4.76	4.75	0.08	<0.01	0.08	0.86	0.55	0.58
Urea,[8] mmol/L	3.82	3.65	3.62	3.71	3.65	3.64	0.19	0.36	<0.01	<0.01	0.07	0.13

[1]A total of 24 observations for all 6 diets in each parameter (4 observations for each diet in each parameter).

[2]When referring to gas exchange data, DMI and ECM were only from gas exchange period.

[3]High in grass-clover silage: ground untoasted fava beans = GGU; ground toasted fava beans = GGT; rolled untoasted fava beans = GRU. High in corn silage: ground untoasted fava beans = CGU; ground toasted fava beans = CGT; rolled untoasted fava beans = CRU.

[4]Silage (S) = forage source; particle (Pa) = particle size; heat (H) = heat treatment (toasting). Probability of contrasts: S = GGU, GGT, and GRU vs. CGU, CGT, and CRU; Pa = GGU and CGU vs. GRU and CRU; H = GGU and CGU vs. GGT and CGT; S × Pa = GGU and CRU vs. GRU and CGU; S × H = GGU and CGT vs. GGT and CGU.

[5]Energy-corrected milk yield calculated according to Sjaunja et al. (1991).

[6]GEI = gross energy intake (MJ/d).

[7]Milk crude protein.

[8]Milk urea concentration.

studies were performed using standardized methods in different types of cows. Ruminal CP degradation was conducted in dry cows, and intestinal degradation was conducted in lactating cows, which might have influenced the obtained results. The toasting of fava beans had no effect on nutrient digestibility, and small intestine individual AA digestion indicated that toasting of fava beans failed to improve MP supply and nutrient digestion in the present study, and that in situ methods therefore probably overestimate the effect of heat treatment on MP supply. Lund et al. (2007) suggested that the optimal toasting temperature might be between 120°C and 150°C to increase small intestine starch digestion. In addition, Lund et al. (2004) observed an increase in duodenal flow of undegraded feed amino acid nitrogen in ground toasted fava beans, thereby increasing MP supply by toasting fava beans at 140°C for 90 to 120 s. Therefore, the absence of effect of toasting on MP supply in the current study might be due to a lower-than-optimal temperature of toasting. Yu (2005) also found that pressure toasting at 136°C for 15 min might be within the optimal heat treatment range for fava beans to prevent N loss in the rumen, thereby improving MP supply.

The absence of effect of toasting of fava beans on ruminal fermentation and gas production was supported by the unaffected DM and OM intake and digestion, in accordance with results on toasted oats reported by Panah et al. (2020). However, toasting of fava beans gave a lower milk protein percentage and yield in the current study, as also observed by Hansen et al. (2021). Hansen et al. (2021) speculated that the negative effect of toasting of fava beans on milk protein could be due to a lower Met proportion in MP. The current observation of a lower Met proportion in total digested AA in the small intestine supports the hypothesis. Patton (2010) reported that rumen protected Met could increase milk protein percentage and yield. Further, the negative effect on milk protein could also be partly attributed to a potential negative effect of heat treatment on the biological availability of digested Lys (Dakowski et al., 1996), which would not be detected in analyzed Lys.

Effects of Particle Size of Fava Beans

The smaller particle size of ground untoasted fava beans was associated with a higher true ruminal OM, CP, and AA digestibility, apparent ruminal starch digestibility, and total-tract CP, AA, and starch digestibility compared with rolled untoasted fava beans. This was expected, as smaller feed particles have a larger surface area, thereby increasing the accessibility of nutrients to microbial enzymes (Wondra et al., 1993; Naves et al., 2016). Byars et al. (2021) and Luhovyy et al. (2017) suggested that both starch and protein digestibility in navy beans increased as the particle size decreased in an in vitro study. A significant increase in total-tract digestibility of CP and starch was also observed by Rémond et al. (2004) as the particle size of corn decreased. In addition, higher small intestinal Gly digestion and digestibility was observed in diets containing ground untoasted fava beans than in diets containing rolled untoasted fava beans. Although Benchaar et al. (1994) found greater intestinal availability of Gly in extruded fava beans than raw fava beans, the apparent digestion in the small intestine was lower in extruded fava beans. The duodenal flows of Gly are high in the current study compared with some other studies (Schwab et al., 1992; Erasmus et al., 1994). The reason for this discrepancy in Gly flows is the supply of Gly originating from bile glycocholic acid; Gly makes up 30% of endogenous AA in duodenal digesta. The physical placement of the duodenal cannula in relation to the bile duct therefore affects the amount of bile and thereby Gly concentration in duodenal digesta (Weisbjerg et al., 1992; Larsen et al., 2000). Inclusion of ground untoasted fava beans with smaller particle size in diets resulted in a lower apparent ruminal and total-tract apparent NDF digestibility compared with diets containing rolled untoasted fava beans in the current study. Goelema (1999) suggested that smaller particle size could cause higher ruminal passage rate, resulting in lower total-tract fiber digestibility. In addition, the smaller particle size may result in more rapid starch fermentation, which could reduce ruminal pH. However, we detected no difference in ruminal pH between diets containing ground untoasted and diets containing rolled untoasted fava beans, probably because the amount of fava beans included in the diets was not sufficient to induce such differences. Corresponding with high apparent ruminal starch digestion and lower apparent ruminal NDF digestion and digestibility, diets containing ground untoasted fava beans also resulted in a lower acetate:propionate ratio, CH_4, CH_4 per unit of ECM, and CH_4 proportion of gross energy intake compared with diets containing rolled untoasted fava beans.

CONCLUSIONS

Forage source and rolling and toasting of fava beans had no effect on MP supply. Rolling of fava beans reduced ruminal fermentation and apparent ruminal and total-tract digestibility of nutrients, except for NDF, where digestibility was increased. Rolling of fava beans also reduced microbial protein synthesis compared with ground untoasted fava beans. Toasting of fava beans had no effect on digestibility, except for an interaction

with forage source on DM and OM apparent ruminal digestibility.

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