Effects of toasting and decortication of oat on nutrient digestibility in the rumen and small intestine and on amino acid supply in dairy cows

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ABSTRACT

This study examined the potential for decorticating and toasting of oat (*Avena sativa*) to supply crude protein (CP) and amino acids (AA) in dairy cows. Four lactating Danish Holstein Friesian cows fitted with ruminal, duodenal, and ileal cannulas were assigned to a 4 × 4 Latin square design. Cows were fed experimental diets ad libitum based on grass-clover silage and toasted fava beans, with oat included in different forms arranged in a 2 × 2 factorial: whole oat, decorticated oat, toasted oat, and decorticated toasted oat. In situ rumen degradability of processed oat was also evaluated. Decortication increased starch intake by 0.38 kg/d and reduced NDF intake by 0.91 kg/d. Toasting reduced ruminal AA digestibility and increased duodenal flow of CP by 0.41 kg/d. In situ degradation rate and effective degradability of CP in the rumen were reduced by 0.46 h⁻¹ and 310 g/kg CP due to toasting. Both decortication and toasting increased microbial synthesis of CP by 0.20 and 0.41 kg/d, respectively. Decortication and toasting did not affect small intestinal AA digestibility, but did increase the total digested amount of AA by 154 and 250 g/d, respectively. Milk production was not affected by treatments. Methane production (L/d) decreased with decortication and toasting. In conclusion, unless an interaction exists between decortication and toasting, the results indicate additive effects of toasting and decorticating oat for increasing the supply of digestible AA to the small intestine of dairy cows.

Key words: dehulling, toasting, oat, amino acid, CH₄ emission, microbial synthesis

INTRODUCTION

Within the European Union, organic milk production almost doubled from 2007 to 2016 (Willer et al., 2019), and in Denmark, it reached 11.0% of total milk produc-

tion in 2017 (Danish Agriculture and Food Council, 2019). Organic dairying is associated with low external input, such as restricted use of concentrate (Blanco-Penedo et al., 2012; Scollan et al., 2017) and, based on the European organic farming regulation No. 889/2008 (January 2008), preferably using feedstuffs grown on the farm or obtained from local sources. These factors might limit farmers' options for supplementing their cows with sufficient MP to obtain a high production level. Hence, optimizing the supply of MP via home-grown cereals high in CP or increasing the protein value through processing is needed for more efficient organic milk production.

Oat has fairly high concentrations of CP (Biel et al., 2009) and crude fat (Decker et al., 2014; Qi et al., 2017). The hull of oat (*Avena sativa*) constitutes 28 to 32% of the grain DM, and due to the presence of lignin-carbohydrate/phenolic-carbohydrate complexes (Decker et al., 2014), hulls have very low degradability in the rumen (Garleb et al., 1991). Thus, due to low concentrations of CP and crude fat in the hull (Salo and Kotilainen, 1970), decortication could be a potential processing method to increase the content of CP and crude fat in oat grain.

Synthesized microbial CP is not solely sufficient for fulfilling the protein requirement in high-yielding dairy cows (Savari et al., 2018). Thus, applying heat treatment (e.g., toasting) could be a possible way to reduce ruminal degradation of CP (Kung and Rode, 1996). Heat treating the cereals causes a Maillard reaction between carbonyl groups of reducing sugars and free ε-amino groups of proteins (Oste et al., 1986). McNiven et al. (1995), Lund et al. (2004), and Lashkari et al. (2015) implemented heat treatment to manipulate the site of digestion in the rumen and thereby increase the supply of dietary nutrients in the duodenum.

Methane is a potent greenhouse gas, and organic dairy farms are associated with more CH₄ production per kilogram of fermentable OM than conventional ones. This greater CH₄ production is mainly due to the higher forage:concentrate ratio, at least up to 60% forage, in organic dairying (Fall et al., 2008). Decreasing

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Table 1. Total mixed ration and nutrient composition of experimental diets (g/kg of DM unless otherwise noted)

Item	Diet ¹			
	Oat	D	T	DT
Ingredient				
Whole oat grain	217.1	—	—	—
Decorticated oat grain	—	217.1	—	—
Toasted oat grain	—	—	217.1	—
Decorticated toasted oat grain	—	—	—	217.1
Toasted fava beans	165.2	165.2	165.2	165.2
Grass clover silage	609	609	609	609
Mineral supplements	8.70	8.70	8.70	8.70
Nutrient				
DM, g/kg	540	540	563	561
Ash	77.6	77.4	77.8	77.4
Crude fat	43.9	48.1	40.5	45.1
CP	194	197	193	198
Starch	156	175	151	174
NDF	331	310	331	301

¹D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.

the fiber content and increasing the starch content in the diet are well-known strategies to reduce enteric CH₄ production by cows because CH₄ produced per digested unit is lower for starch than for fiber (Moe and Tyrrell, 1979; Brask et al., 2015).

Our study hypotheses were that decortication would increase protein, fat, and digestible OM intake and decrease the enteric CH₄ production per kilogram of DM intake, and that toasting would increase MP intake. Therefore, the objective of this study was to document the effect of decortication and toasting of oat on nutrient digestibility in rumen and small intestine, protein and AA supply in dairy cows, and enteric CH₄ production.

MATERIALS AND METHODS

Animal and Experimental Design

This experiment complied with the ethical requirements delineated in the Danish Ministry of Justice Law No. 726 (September 9, 1993). Two primiparous and 2 multiparous lactating Danish Holstein dairy cows were fitted with rumen cannulas (#4C; Bar Diamond, Parma, ID) and duodenal and ileal simple polyvinyl chloride T-cannulas (i.d. = 2.5 cm) placed 60 cm caudal to the pylorus (duodenal) and 20 cm cranial to the cecum (ileum). Cows were fed 1 of 4 experimental diets in a 4 × 4 Latin square design. Animals were housed in tie-stalls, and beds were covered with mattresses and sawdust. At the start of the experiment, the average BW was 618 ± 47.9 kg (mean ± SD), the cows were at 61.3 ± 49.9 DIM, and their BCS was 3 ± 0.20. The processing methods, decortication and toasting, were

investigated in a 2 × 2 factorial arrangement of treatments (Table 1). The experimental diets were TMR containing whole-grain oat (oat), decorticated oat (**D**), toasted whole oat (**T**), or decorticated toasted oat (**DT**) as the grain source. All 4 types of oats were rolled weekly before use in the ration. Days 1 to 13 served as the adaptation period, and collection of digesta (i.e., rumen, duodenum, ileum, feces) was carried out from d 13 to 17. On d 17 to 22 the enteric CH₄ emission was measured in respiration chambers.

Feeds, Diet, and Feeding

The oat variety used in this experiment was Dominik, and it was grown in Denmark in 2017. Decortication was conducted using a mobile decorticator with 3 Bühler huller MHSA (Bühler AG, Uzwil, Switzerland) mounted on a truck. Decortication was performed on farm by Gl. Buurholt ApS (Brønderslev, Denmark) with an efficacy of 83%; the remainder was either partially decorticated or intact. Toasting was performed on farm using a Bulldog Toaster (Mecmar S.p.a., Minerbe, Italy) at 121°C. The flow through the toaster was 2,500 kg/h, and the retention time was 35 s. The chemical composition of the ration feedstuffs is shown in Table 2. Samples of all ingredients were taken in each period. The samples taken in periods 1 and 2 were pooled to one sample, and those taken in periods 3 and 4 were pooled to one sample, before the samples were sent for chemical analysis.

The rations were formulated according to NorFor (Volden, 2011). Rations were similar except for the oat portion, which made up 217 g/kg of ration DM (Table 2). The forage:concentrate ratio of the TMR was 60:40

on a DM basis. The TMR consisted of grass-clover silage, toasted fava beans ground on a disc mill, vitamin and mineral supplements, and either oat, decorticated oat, toasted oat, or decorticated toasted oat (Table 2). Cows were fed *ad libitum* with TMR twice a day (0600 and 1630 h). The feed was mixed once daily before the afternoon feeding. Feed offered and the residues were recorded daily, and the DM concentration of feed and residues was determined on d 12 to 21 in each period. Water was provided *ad libitum*, and consumption was recorded using water meters.

Rumen, Digesta, and Feces Sampling

Chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) were used as digestion markers. Ten grams of Cr_2O_3 and 13 g of TiO_2 in disintegrable bags were placed into the rumen twice a day at milking. Rumen liquid, intestinal chyme, and feces were sampled 12 times over 5 d (i.e., d 13–17) at 1000 and 1800 h (d 13); 0200, 1200, and 2000 h (d 14); 0400, 1400, and 2200 h (d 15); 0600, 1600, and 2400 h (d 16); and 0800 h (d 17). Rumen liquid was

sampled from the ventral rumen using a 90-cm steel rumen sampler (Bar Diamond Inc.) with an attached 50-mL syringe.

Duodenal (0.5 L) and ileal (0.2 L) samples were collected from the T-cannula. Feces (approximately 50 g) was sampled during defecation or by grab sampling from the rectum. The 12 samples for duodenal and ileal contents and feces were pooled.

Rumen liquid samples were analyzed for pH directly after sampling using a digital pH meter (Meterlab PHM 220, Radiometer, Brønshøj, Denmark). Rumen microbes were harvested on d 17 at 1200 h. Two liters of rumen liquid were collected with a cup and filtered through 2 layers of cheesecloth into prewarmed thermos bottles and immediately transferred to the laboratory. Feed particles and protozoa were removed by centrifuging twice at $500 \times g$ for 5 min at 3°C . Next, the supernatant was centrifuged at $17,300 \times g$ for 20 min at 3°C . The pellet was resuspended in 200 mL of saline solution (0.9% NaCl) and centrifuged at $17,300 \times g$ for 20 min at 3°C , after which the pellet (microbial matter) was harvested.

Table 2. Chemical composition of feedstuffs (means $\pm$ SD; g/kg of DM unless otherwise noted)

	Feedstuff						
	Grass-clover silage	Toasted fava beans	Oat	Decorticated oat	Toasted oat	Decorticated toasted oat	Oat hull ¹
DM, g/kg of fresh matter	302 $\pm$ 0.84	946 $\pm$ 0.95	882 $\pm$ 0.74	881 $\pm$ 1.39	986 $\pm$ 1.14	975 $\pm$ 1.43	946
Ash	95.2 $\pm$ 0.20	38.0 $\pm$ 0.24	21.6 $\pm$ 0.04	20.5 $\pm$ 0.04	22.1 $\pm$ 0.08	20.5 $\pm$ 0.10	25.6
CP	192 $\pm$ 1.44	294 $\pm$ 1.33	91.0 $\pm$ 0.13	107 $\pm$ 0.38	87.9 $\pm$ 0.46	109 $\pm$ 0.07	58.1
Buffer solubility, g/kg of CP	627 $\pm$ 8.06	198 $\pm$ 3.01	206 $\pm$ 4.86	256 $\pm$ 47.5	105 $\pm$ 12.6	94.9 $\pm$ 0.41	247
Crude fat	29.4 $\pm$ 0.92	19.0 $\pm$ 0.0	63.5 $\pm$ 4.95	83.0 $\pm$ 1.41	48.0 $\pm$ 2.83	69.0 $\pm$ 2.83	NA ²
NDF	377 $\pm$ 1.24	139 $\pm$ 0.51	319 $\pm$ 1.74	223 $\pm$ 3.37	319 $\pm$ 2.80	184 $\pm$ 0.5	597
OMD ³	789 $\pm$ 8.10	865 $\pm$ 0.0	690 $\pm$ 7.05	830 $\pm$ 9.50	695 $\pm$ 2.45	823 $\pm$ 0.31	NA
Starch	NA	304 $\pm$ 0.21	449 $\pm$ 1.75	535 $\pm$ 1.36	422 $\pm$ 1.53	530 $\pm$ 3.51	221
Total AA	134 $\pm$ 3.48	250 $\pm$ 2.55	74.0 $\pm$ 2.22	88.5 $\pm$ 1.55	68.4 $\pm$ 0.96	86.9 $\pm$ 0.65	NA
Amino acids, g/kg of AA							
Ala	91.3 $\pm$ 6.71	47.6 $\pm$ 0.07	53.3 $\pm$ 0.43	52.7 $\pm$ 0.09	55.1 $\pm$ 0.22	54.7 $\pm$ 0.18	NA
Arg	23.6 $\pm$ 0.32	98.9 $\pm$ 0.42	72.3 $\pm$ 0.45	74.2 $\pm$ 0.31	69.0 $\pm$ 0.05	67.3 $\pm$ 0.19	NA
Asp	135 $\pm$ 4.83	121 $\pm$ 0.02	86.5 $\pm$ 0.40	85.4 $\pm$ 0.01	88.5 $\pm$ 0.28	87.9 $\pm$ 0.24	NA
Cys	6.81 $\pm$ 0.29	13.6 $\pm$ 0.10	36.6 $\pm$ 0.09	36.7 $\pm$ 0.09	35.5 $\pm$ 0.12	35.7 $\pm$ 0.08	NA
Glu	107 $\pm$ 0.004	185 $\pm$ 0.02	211 $\pm$ 1.64	215 $\pm$ 0.04	219 $\pm$ 0.07	224 $\pm$ 0.52	NA
Gly	59.3 $\pm$ 0.00	48.3 $\pm$ 0.22	58.3 $\pm$ 0.33	58.2 $\pm$ 0.21	60.0 $\pm$ 0.31	60.2 $\pm$ 0.23	NA
His	20.3 $\pm$ 0.60	29.3 $\pm$ 0.07	24.5 $\pm$ 0.33	24.9 $\pm$ 0.05	22.5 $\pm$ 0.03	21.9 $\pm$ 0.04	NA
Ile	59.8 $\pm$ 0.41	47.8 $\pm$ 0.36	42.6 $\pm$ 1.78	41.9 $\pm$ 0.22	43.6 $\pm$ 0.39	43.8 $\pm$ 0.98	NA
Leu	93.3 $\pm$ 1.11	84.1 $\pm$ 0.30	81.1 $\pm$ 0.11	80.7 $\pm$ 0.53	82.0 $\pm$ 0.04	82.6 $\pm$ 0.92	NA
Lys	48.1 $\pm$ 2.87	70.4 $\pm$ 0.09	47.8 $\pm$ 1.88	46.2 $\pm$ 0.03	36.6 $\pm$ 0.01	32.9 $\pm$ 0.22	NA
Met	19.6 $\pm$ 0.30	8.37 $\pm$ 0.05	20.3 $\pm$ 0.02	20.4 $\pm$ 0.04	20.0 $\pm$ 0.03	19.6 $\pm$ 0.02	NA
Phe	52.5 $\pm$ 0.71	47.7 $\pm$ 0.16	53.3 $\pm$ 0.14	53.5 $\pm$ 0.27	54.5 $\pm$ 0.004	55.0 $\pm$ 0.34	NA
Pro	98.0 $\pm$ 4.58	46.1 $\pm$ 0.16	57.3 $\pm$ 0.19	57.0 $\pm$ 1.02	57.6 $\pm$ 0.87	57.1 $\pm$ 0.53	NA
Ser	53.3 $\pm$ 1.95	56.7 $\pm$ 0.20	57.1 $\pm$ 0.85	57.0 $\pm$ 0.18	57.6 $\pm$ 0.47	57.4 $\pm$ 0.45	NA
Thr	54.4 $\pm$ 0.77	40.6 $\pm$ 0.11	38.9 $\pm$ 0.38	38.6 $\pm$ 0.13	39.6 $\pm$ 0.27	39.1 $\pm$ 0.12	NA
Val	77.5 $\pm$ 0.75	54.4 $\pm$ 0.94	58.8 $\pm$ 1.09	58.1 $\pm$ 0.12	59.4 $\pm$ 0.12	60.4 $\pm$ 1.56	NA

¹Samples of obtained hulls are for comparison and hulls were not used in diets.

²NA = not analyzed.

³In vivo OM digestibility (OMD) based on in vitro OM digestibility of silage and concentrate.

In Situ Study

Rumen in situ CP degradation of the 4 tested oats and a hull sample was determined according to the standard NorFor procedure (Åkerlind et al., 2011). Dacron bags (7 × 6 cm, 38-μm pore size) were filled with approximately 1 ± 0.01 g of ground (1.5-mm) sample and 12 mg/cm² sample-to-surface area ratio (Nocek, 1988). The bags were incubated in the rumen of 3 dry cows fed at maintenance level for 0, 2, 4, 8, 24, 48, and 96 h. After rumen incubation, all bags were rinsed in running cold water and subsequently washed using a domestic washing machine for 10 min using 2 × 22 L of water (25°C). Residues were then transferred to N-free filter paper (retention value 2, Whatman AGF 607-90 mm) for DM (103°C) and N analysis (Kjeldahl). Water solubility was estimated over filter paper as described by Hvelplund and Weisbjerg (2000). Particle loss was determined as the difference between the loss from the nylon bags when they were only washed and the water solubility measured on filter paper (Hvelplund and Weisbjerg, 2000).

Total-tract disappearance (**TTD**) was determined using the mobile bag technique according to Hvelplund et al. (1992). The Dacron bags (6 × 6 cm, 12-μm pore size) were sealed after loading 1.0 ± 0.01 g of the samples. Bags were ruminally pre-incubated using 3 dry cows for 16 h, as described above. In each cow, 2 replicates of each sample were incubated (6 observations for each treatment). Bags were incubated in HCl (pH 2.4) for 1 h and subsequently incubated for 2 h in pepsin-HCl solution (200 mg of pepsin dissolved in 2 M HCl) at 40°C in a Daisy incubator with rotation (Ankom, Macedon, NY) to simulate abomasal digestion. Thereafter, the bags were inserted into the small intestine of 2 lactating cows through the duodenal cannula (open T-piece placed 60 cm caudal to the pylorus). After fecal recovery, bags were machine-washed as mentioned above, and residues were transferred to N-free filter paper using the same procedure as for rumen bags to determine the total-tract CP disappearance.

Gas Exchange Measurement

Gas exchange was measured (d 17–22) by means of open-circuit indirect calorimetry using 4 respiration chambers according to Hellwing et al. (2012). The chambers were positioned in a square in the same barn where the collection of digesta took place. To balance out any possible differences in inlet air composition, the cows were switched between the chambers diagonally, after 3 of the 5 d of measurements. Gas concentrations of inlet and outlet air and flow in each of the 4 chambers (CH₄, CO₂, O₂, and H₂) were measured every 12.5

min as described by Hellwing et al. (2012). Calculations for gas exchange were based on standard conditions for temperature and pressure (0°C and 101.325 kPa). The recovery (% ± SD) of CH₄ and CO₂ gases measured at chamber level was 99.5 ± 0.4% and 99.9 ± 0.3%. For other gases, the average recovery of CH₄ and CO₂ was assumed (99.7%).

Chemical Analysis

All feed and digesta samples were stored frozen at –20°C and subsequently freeze-dried before laboratory analysis. Dry matter was determined by oven-drying at 60°C for 48 h (Åkerlind et al., 2011). The ash content was determined by combustion at 525°C for 6 h. The Dumas method (Hansen, 1989) was used to determine the nitrogen content of feed, digesta, and microbial matter using a Vario MAX CN (Elementar Analysensysteme GmbH, Hanau, Germany). Nitrogen in bag residues from rumen degradation and from mobile bags was analyzed using the Kjeldahl method, and CP was calculated as N times 6.25. Soluble N in samples was analyzed by extraction in a borate-phosphate buffer (pH 6.75) at 39°C for 1 h according to Åkerlind et al. (2011). Crude fat was determined using a Soxhlet apparatus with petroleum ether extraction after 3 M HCl hydrolysis (Stoldt, 1952). Starch concentration of feedstuffs was determined by an enzymatic colorimetric method (Bach Knudsen et al., 1987). In this method, 150 mg of the ball-milled grains (approximately 100 mg starch) was weighed into 50-mL centrifuge tubes with screw caps. Heat-stable α-amylase and acetate buffer (0.1 M, pH 5.0, 40 mL) were added, and the mixture was then incubated for 1 h at 100°C. To extend the breakdown of released oligosaccharides to glucose monomers a further incubation of the samples with an amyloglucosidase from *Aspergillus oryzae* for 2 h at 60°C was done. The tubes were centrifuged (3,000 × g, 10 min), and after dilution (500 μL to 100 mL), the released glucose monomers in the supernatant were measured with a glucose oxidase reagent. The factor 0.9 was then used to convert the values for glucose to the equivalent starch polysaccharide. The NDF concentration was analyzed according to Mertens (2002) method in a Fibertec M6 System (Foss Analytical, Hillerød, Denmark) using sodium sulfite and heat-stable amylase treatments and corrected for residual ash.

In vitro OM digestibility (**IVOS**) of silage in the basal diet was determined according to Tilley and Terry (1963) by incubating samples in rumen fluid for 48 h, followed by a 48-h digestion by pepsin and HCl. Incubation residues were then combusted to determine OM digestibility.

In vitro enzyme digestible OM (**EDOM**) for concentrate was measured by treating the samples with a pepsin-HCl solution for 24 h, after which the samples were heated at 80°C for 45 min. After washing, the residues were treated with an enzyme mixture for 24 h at 40°C and then incubated for 19 h at 60°C. The enzyme mixture consisted of 20 mL of Celluclast 1.5 L (EC 232-734-4; Novozymes Inc., Copenhagen, Denmark), 10 mL of Viscozym L from Novozymes Inc., 2.175 g of amyloglycosidase (EC 3.2.1.3, 3,200 U/mL), 17.0 mL Novozym 51054 from Novozymes Inc., and 0.1 g of chloramphenicol (EC 200-287-4) with 800 mL of acetate buffer (pH 4.8 ± 0.1). The acetate buffer consisted of 8.16 g of sodium acetate (EC 204-823-8) dissolved in 0.5 L of water and 7.5 mL of acetic acid (30% wt/wt 5.8 M, EC 200-580-7) and water added to 1 L. Residues were washed and acetone extracted before determination of undigested residues (Weisbjerg and Hvelplund, 1993).

Ammonia in rumen liquid was alkalized with potassium hydroxide followed by distillation and titration using a Kjeltac 2400 (Foss Analytical; Brask et al., 2013a). Volatile fatty acids of rumen 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 2900 Biochemistry Analyzer (YSI Inc., Yellow Springs, OH). Chromic oxide in digesta and feces was oxidized to chromate and determined colorimetrically (Schürch et al., 1950). Titanium dioxide was digested with sulfuric acid, hydrogen peroxide was added, and absorbance was measured spectrophotometrically according to Myers et al. (2004), with the modification that 15 mL of 30% hydrogen peroxide was added instead of 10 mL and an additional 5 drops were added before the absorbance was measured. Microbial matter and duodenal chyme were analyzed for total purine content by the method of Zinn and Owens (1986) as modified by Thode (1999). Briefly, the nucleotides were hydrolyzed by the addition of perchloric acid, after which purines precipitated into complexes with silver nitrate and were measured spectrophotometrically.

Amino acids were determined using the EEC (98/64/EC) method (European Commission, 1998). Samples were oxidized with performic acid followed by hydrolysis with HCl, 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).

Fat, protein, and lactose (monohydrate) concentrations in milk were determined by an infrared analyzer (Milkoscan Msc4000, Foss Analytical) at Eurofins Steins Laboratories (Vejen, Denmark).

Calculations and Statistical Analysis

Data on gas exchange, DMI, water intake, and milk yield and composition were averaged per cow per period. Digesta DM flows were calculated as average of flows for each marker. Microbial net synthesis (flow to the duodenum) was calculated according to Lund et al. (2003), using the concentration of nitrogen and purines in rumen-isolated bacteria, the concentration of purines in duodenal contents, and duodenal DM flow. The calculation of microbial efficiency was expressed as the amount of microbial CP synthesized relative to the true rumen digested OM (g CP/kg true rumen digested OM; Lund et al., 2003). The amount of true rumen digested OM was calculated by subtracting duodenal OM flow (corrected for duodenal microbial OM flow) from OM intake.

The predicted in vivo OMD of grass-clover silage was calculated based on Møller et al. (1989), according to the following equation:

$$\text{OMD} = 41.0 + 0.959 \times \text{IVOS},$$

where OMD is the calculated in vivo digestibility of OM (g/kg of OM). In vivo OMD for concentrate was calculated by the equation suggested by Weisbjerg and Hvelplund (1993):

$$\text{OMD} = 53.8 + 0.867 \times \text{EDOM},$$

where OMD is the calculated in vivo digestibility of OM (g/kg of OM).

Energy-corrected milk yield (3.14 MJ/kg) 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., 1990), where lactose is lactose monohydrate, and milk fat, protein, and lactose are expressed as the daily yield (kg/d).

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). Here, $\text{Deg}(t)$ 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 depending on time, and c (h^{-1}) is the degradation rate of the fraction b . Effective degradability (**ED**) in the rumen was calculated according to Ørskov and McDonald (1979) using 0.08 h^{-1} rumen fractional passage rate (k) in the following equation: $\text{ED} = a + b[c/(c + k)]$.

The small intestinal disappearance (**SID**, g/kg) of CP escaping ruminal degradation was calculated based on ED and TTD (Hvelplund et al., 1992): $\text{SID} = (\text{TTD} - \text{ED})/(1,000 - \text{ED})$.

Data were analyzed in R (R Core Team, 2018), using the Fit Linear Mixed-Effects Models (LMM) procedure through the “lmer” function in “lme4” package (Bates et al., 2014). The following model was used for in vivo data:

$$Y_{ijkl} = \mu + D_i + T_j + DT_{ij} + P_k + C_l + E_{ijkl},$$

where Y is the dependent variable, μ is the overall mean, and the model includes the fixed effects of decortication (D_i), toasting (T_j), the interaction (DT_{ij}), the k th period (P_k), and the random effect of l th cow (C_l). E_{ijkl} is the random error.

The main effect of decortication and toasting is reported in full terms; that is, decortication refers to the main effect of decortication in D and DT diets, and toasting refers to the main effect of toasting in T and DT diets. Further, diurnal variations in rumen parameters were assessed by a mixed model as above, including time as an independent repeated measurement.

The parameters for the in situ study were analyzed using the LMM procedure in R:

$$Y_{ijk} = \mu + D_i + T_j + DT_{ij} + C_k + E_{ijk},$$

where Y is the dependent variable; μ is the overall mean; decortication (D_i), toasting (T_j), and the interaction (DT_{ij}) are fixed effects; the effect of k th cow (C_k) is random; and E_{ijk} is the random error.

Values presented in Tables 3–9 and Supplemental Tables S1–S4 (<https://doi.org/10.3168/jds.2019-17142>) are least squares means with corresponding standard error of means. Significance was declared at $P < 0.05$ and a tendency at $0.05 < P < 0.10$. When significant interactions were found, least squares means were compared using Tukey-Kramer test in $\alpha = 0.05$. Unless there is interaction between decortication and toasting, the results indicate that main effects are additive.

RESULTS

Composition of Experimental Feeds

Decortication (the difference between decorticated oats and undecorticated oats) increased the concentration of CP, starch, and crude fat by 18.6, 97.0, and 20.3 g/kg of DM, and it decreased the NDF concentration by 115 g/kg of DM compared with the oat (Table 2). Digestibility (OMD) estimated by an in vitro enzymatic method was considerable higher for the decorticated oat. In addition, total AA concentration was highest in decorticated oat, and the Lys proportion of total AA was lower for decorticated oat than for oat. Toasted oat

had lower solubility of CP and lower crude fat concentration. The composition of the removed hull mirrored the difference between oat and decorticated hull, with lower CP and starch and higher NDF concentration in hulls than in oat (Table 2).

Intake, Digested Nutrients, and Digestion

Compared with untreated oat, decortication increased ($P = 0.01$) starch intake by 0.38 kg/d and reduced ($P = 0.01$) NDF intake by 0.91 kg/d. Dry matter and OM intake tended ($P = 0.08$) to be increased by toasting (Table 3). Decortication increased ($P = 0.02$) the ruminal digested amount of starch by 0.29 kg/d and increased the digested amount of starch in the small intestine ($P < 0.01$) and in the total tract ($P < 0.01$). Decortication lowered ($P = 0.05$) the digested amount of NDF in the rumen by 0.79 kg/d. Toasting decreased ($P = 0.05$) the digested amount of CP in the rumen by 0.28 kg/d and increased ($P = 0.04$) the amount digested in the small intestine by 0.35 kg/d. Toasting also decreased ($P = 0.02$) the digested amount of AA in the rumen and decortication tended ($P = 0.09$) to decrease it. The amount of digested AA in the small intestine increased by 0.15 and 0.26 kg/d due to decortication ($P = 0.02$) and toasting ($P < 0.01$), respectively. Decortication decreased ($P < 0.01$) the proportion of Lys and it increased ($P < 0.01$) the proportion of Met in AA intake (Supplemental Table S1; <https://doi.org/10.3168/jds.2019-17142>). Moreover, toasting reduced ($P < 0.01$) the proportion of Lys, His, and Met in AA intake. Both decortication and toasting increased the digested amount (g/d) of Lys and Met in small intestine (Supplemental Table S2; <https://doi.org/10.3168/jds.2019-17142>). Ruminal digestibility of Arg, Asp, Cys, Glu, Lys, Phe, Pro, Ser, and Val was reduced ($P \leq 0.05$) by toasting (Supplemental Table S3; <https://doi.org/10.3168/jds.2019-17142>).

Toasting decreased ($P = 0.03$) the concentration of CP and AA in microbial DM (Table 4). Toasting also decreased ($P \leq 0.05$) the proportion of Arg and Glu, whereas it increased ($P = 0.01$) Gly in microbial AA (g/kg AA). Decortication tended to decrease ($P = 0.07$) the proportion of Ile, and it increased ($P = 0.05$) Ser. Both decortication and toasting increased ($P < 0.01$) the daily microbial OM flow (kg/d) by 0.29 kg/d. Decortication and toasting also increased ($P = 0.01$) the duodenal flow of microbial CP by 0.17 and 0.15 kg/d and microbial AA flow at the duodenum by 0.12 and 0.13 kg/d on average, respectively. Decortication increased ($P = 0.03$) microbial efficiency by 22.7 g of microbial CP per kilogram of true rumen digested OM.

Ruminal digestibility of OM and starch was not affected by treatments (Table 5). Decortication, how-

Table 3. Intake and digested amount of nutrients in rumen, small intestine, hindgut, and total tract (kg/d unless otherwise noted)

Item	Diet ¹				SEM	P-value		
	Oat	D	T	DT		D	T	DT
Intake								
Water, L/d	86.6	85.1	86.5	87.5	10.8	0.87	0.49	0.44
DM	21.7	20.7	22.0	21.9	2.07	0.25	0.08	0.25
OM	20.4	19.5	20.7	20.7	2.05	0.25	0.08	0.25
Starch	3.35	3.60	3.28	3.79	0.34	0.01	0.53	0.19
NDF	6.95	5.76	7.04	6.40	0.62	0.01	0.22	0.35
CP	4.13	4.00	4.14	4.26	0.40	0.92	0.12	0.13
AA	3.00	2.95	3.03	3.11	0.30	0.79	0.10	0.24
Digested nutrients								
DM								
Rumen	8.16	7.47	8.19	7.21	0.75	0.13	0.81	0.77
Small intestine	7.00	7.71	7.50	8.74	0.83	0.01	0.03	0.37
Hindgut	1.11	0.88	0.84	0.94	0.20	0.73	0.57	0.39
Total tract	16.3	16.1	16.5	16.9	1.52	0.81	0.13	0.39
OM								
Rumen	9.36	8.86	9.54	8.75	0.85	0.19	0.93	0.74
True rumen	11.4	11.2	11.8	11.4	1.12	0.45	0.49	0.80
Small intestine	5.27	5.74	5.48	6.56	0.65	0.02	0.08	0.27
Hindgut	0.54	0.40	0.36	0.43	0.15	0.82	0.62	0.50
Total tract	15.2	15.0	15.4	15.7	1.41	0.74	0.14	0.41
Starch								
Rumen	2.85	3.08	2.81	3.15	0.32	0.02	0.85	0.55
Small intestine	0.34 ^a	0.39 ^a	0.34 ^a	0.49 ^b	0.03	<0.01	0.06	0.03
Hindgut	0.11	0.09	0.09	0.09	0.01	0.13	0.29	0.49
Total tract	3.30	3.56	3.24	3.73	0.33	<0.01	0.53	0.22
NDF								
Rumen	4.32	3.64	4.77	3.87	0.43	0.05	0.34	0.76
Small intestine ²	−0.36	−0.21	−0.61	−0.01	0.18	0.07	0.88	0.24
Hindgut	0.39	0.38	0.31	0.37	0.13	0.85	0.69	0.78
Total tract	4.36	3.80	4.46	4.23	0.37	0.15	0.30	0.52
CP								
Rumen	0.25	−0.02	−0.09	−0.23	0.12	0.12	0.05	0.63
True rumen	1.67	1.56	1.47	1.51	0.19	0.76	0.32	0.50
Small intestine	2.69	2.91	3.04	3.26	0.33	0.15	0.04	0.98
Hindgut	0.11	0.05	0.05	0.06	0.05	0.49	0.50	0.47
Total tract	3.05	2.95	3.01	3.08	0.29	0.80	0.44	0.17
AA								
Rumen	0.07	−0.03	−0.12	−0.30	0.08	0.09	0.02	0.57
True rumen	1.17	1.18	1.10	1.06	0.13	0.78	0.16	0.64
Small intestine	2.20	2.29	2.39	2.61	0.26	0.02	<0.01	0.20
Feed-ileum	2.27	2.25	2.27	2.31	0.23	0.74	0.53	0.55

^{a,b}Means in the same row with different superscripts differ ($P < 0.05$).

¹D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.

²Negative small intestine NDF digestibilities are mentioned in the discussion.

ever, increased ($P < 0.01$) small intestinal digestibility of DM, OM, and starch with a tendency ($P = 0.07$) for increasing small intestinal digestibility of AA. As shown in Table 6, decortication increased ($P \leq 0.05$) the digestibility of several individual AA (e.g., Ala, Glu, Met, and Pro). Toasting increased ($P = 0.01$) small intestinal digestibility of starch. Although toasting decreased ($P = 0.03$) ruminal digestibility of total AA, it did not influence small intestinal digestibility of individual AA. However, total-tract digestibility of AA, as the AA disappearance between intake and ileal flow (feed-ileum), was decreased ($P = 0.03$) by toasting by 13.5 g/kg of intake AA, calculated as the difference between the main effects (Table 5).

Composition of AA (g/kg of AA) in duodenal and ileal digesta is presented in Supplemental Table S4 (<https://doi.org/10.3168/jds.2019-17142>). Decortication increased ($P = 0.03$) His and slightly increased ($P = 0.06$) Met proportion in duodenal AA. Toasting did not change His and Met proportions, but it decreased that of Lys ($P = 0.04$). Methionine in ileal AA composition tended ($P = 0.07$) to decrease by decortication and it increased ($P = 0.01$) by toasting.

In Situ Study

Decortication did not change rumen degradation parameters. However, toasting decreased water solubility

and water-soluble fraction (*a*) ($P < 0.01$), with a consequent increase ($P < 0.01$) in the potentially degradable fraction (*b*) for CP (Table 7). Toasting reduced ($P < 0.01$) degradation rate (*c*) and ED of CP in the rumen by 0.46 h^{-1} and 310 g/kg of CP, respectively. Total-tract disappearance of CP increased ($P < 0.01$) due to both decortication and toasting, and it increased more for the decorticated and toasted oat than expected from the sole effects of decortication and toasting (interaction, $P = 0.01$). Decortication reduced ($P < 0.01$) SID of RUP, but toasting increased it ($P < 0.01$). The decorticated and toasted oat resulted in higher SID than the sole effects (interaction $P < 0.001$).

Rumen Fluid

No differences related to the rumen pH and total short-chain fatty acid (SCFA) concentration were observed among treatments (Table 7). However, decortication decreased acetate ($P = 0.03$) and increased propionate ($P = 0.05$) proportion. Toasting tended to

reduce acetate ($P = 0.06$) and isobutyrate ($P = 0.07$), and it reduced ($P = 0.01$) the isovalerate proportion in total SCFA. The diurnal pattern of rumen SCFA (results are not shown) was in accordance with the mean results presented in Table 8.

Milk Production

Milk production and composition were not affected by treatments (results are not shown). On average, the following results were recorded: milk yield ($32.56 \pm 6.96 \text{ kg/d}$), ECM yield ($33.1 \pm 7.45 \text{ kg/d}$), fat concentration ($41.0 \pm 7.96 \text{ g/kg}$ of milk), protein concentration ($34.7 \pm 3.35 \text{ g/kg}$ of milk), and lactose concentration ($49.1 \pm 1.68 \text{ g/kg}$ of milk).

Gas Exchange

The net production of CH_4 decreased with both decortication ($P = 0.03$) and toasting ($P = 0.02$) by 12.0 and 12.4 L/d , respectively (Table 9).

Table 4. Chemical composition of microbes and microbial flow to the duodenum (g/kg of microbial DM unless otherwise noted)

Item	Diet ¹				SEM	P-value		
	Oat	D	T	DT		D	T	DT
Chemical composition								
Ash	186	189	206	192	9.34	0.56	0.20	0.32
CP	568	559	539	545	8.53	0.79	0.03	0.40
AA	440	429	423	422	4.56	0.22	0.03	0.27
Purine	113	110	106	107	2.94	0.69	0.12	0.38
AA composition, g/kg of AA								
Ala	80.2	79.6	80.6	80.8	0.79	0.80	0.32	0.63
Arg	50.6	51.3	50.1	50.4	0.45	0.16	0.05	0.57
Asp	128	128	129	129	0.66	0.92	0.20	0.89
Cys	11.6	11.5	11.5	11.4	0.09	0.19	0.19	0.95
Glu	137	137	135	135	0.49	0.63	<0.01	0.35
Gly	59.1	59.0	59.3	59.5	0.16	0.55	0.01	0.22
His	17.3	17.2	16.8	17.0	0.27	0.83	0.18	0.51
Ile	66.2	66.1	66.9	66.0	0.34	0.07	0.26	0.13
Leu	80.2	80.7	81.3	81.0	0.66	0.83	0.22	0.46
Lys	82.1	81.8	81.7	82.2	0.69	0.75	0.96	0.37
Met	24.3	24.0	24.0	24.4	0.34	0.79	0.97	0.20
Orn	1.91	1.68	1.85	1.93	0.08	0.29	0.21	0.06
Phe	52.6	53.4	53.7	52.9	0.62	0.99	0.57	0.14
Pro	36.4	36.7	36.8	36.0	0.39	0.50	0.70	0.22
Ser	47.3 ^a	48.4 ^b	47.9 ^{ab}	47.7 ^{ab}	0.20	0.05	0.88	0.01
Thr	58.4	58.4	59.0	58.7	0.39	0.77	0.24	0.77
Val	66.3	65.6	65.6	66.6	0.48	0.76	0.77	0.11
Microbial flow, kg/d								
OM	2.05	2.30	2.30	2.62	0.29	<0.01	<0.01	0.56
CP	1.43	1.57	1.56	1.75	0.19	0.01	0.01	0.61
AA	1.10	1.21	1.22	1.35	0.15	0.01	0.01	0.74
Microbial efficiency								
g of CP/kg of OM true digested in the rumen	132	150	139	166	8.50	0.03	0.18	0.58
g of AA/kg of OM true digested in the rumen	102	115	109	129	6.87	0.03	0.14	0.61

^{a,b}Means in the same row with different superscripts differ ($P < 0.05$).

¹D = decorticated; T = toasted; DT = decorticated and toasted.

Table 5. Rumen, intestine, hindgut, and total-tract digestibilities¹ of different nutrients

Item	Diet ²				SEM	P-value		
	Oat	D	T	DT		D	T	DT
DM, g/kg of DM								
Rumen	380	360	376	330	17.5	0.09	0.33	0.45
Small intestine	519	582	544	593	8.95	<0.01	0.08	0.44
Hindgut	165	164	134	152	25.0	0.73	0.42	0.72
Total tract	751	777	754	770	4.83	<0.01	0.64	0.32
OM, g/kg of OM								
Rumen	471	463	474	434	14.7	0.14	0.37	0.30
True rumen	543	545	550	520	12.5	0.31	0.50	0.23
Small intestine	496	561	511	569	10.7	<0.01	0.29	0.76
Hindgut	96.1	95.8	70.1	84.0	28.5	0.82	0.52	0.81
Total tract	764	790	766	782	4.31	<0.01	0.50	0.27
Starch, g/kg starch								
Rumen	845	851	855	831	11.1	0.35	0.62	0.15
Small intestine	677	741	704	777	9.59	<0.01	0.01	0.66
Hindgut	661	659	667	613	25.5	0.24	0.39	0.27
Total tract	984	987	986	985	1.15	0.20	0.79	0.12
NDF, g/kg of NDF								
Rumen	632	626	677	602	28.1	0.20	0.73	0.26
Small intestine	-144	-89.2	-278	-8.52	74.7	0.06	0.72	0.17
Hindgut	103	155	109	137	44.6	0.39	0.90	0.79
Total tract	631	660	637	662	22.6	0.12	0.80	0.89
CP, g/kg of CP								
Rumen	47.9	-10.8	-15.1	-43.4	29.4	0.17	0.14	0.62
True rumen	395	380	357	365	22.2	0.88	0.26	0.64
Small intestine	695	727	716	721	12.0	0.16	0.53	0.28
Hindgut	82.3	52.9	52.0	45.1	37.8	0.57	0.56	0.72
Total tract	735	739	729	724	5.71	0.95	0.10	0.44
Total AA, g/kg of AA								
Rumen	25.4	-34.8	-38.1	-87.1	24.8	0.11	0.03	0.88
True rumen	391	392	361	346	16.7	0.67	0.06	0.64
Small intestine	747	766	756	763	7.20	0.07	0.62	0.35
Feed-ileum ³	754	763	747	743	5.73	0.66	0.03	0.24

¹Digestibility of nutrients in rumen, small intestine, hindgut, and total tract is reported in grams of digested nutrient per kilogram of nutrient intake, duodenal flow, ileal flow, and intake, respectively.

²D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.

³Total AA digestibility based on the intake and the ileal outflow.

Table 6. Small intestinal digestibility of AA (g/kg of duodenal AA flow)

AA	Diet ¹				SEM	P-value		
	Oat	D	T	DT		D	T	DT
Ala	686	711	703	714	8.91	0.05	0.25	0.39
Arg	820	836	825	833	6.45	0.07	0.88	0.43
Asp	740	759	749	757	6.88	0.09	0.64	0.44
Cys	584	609	601	604	10.6	0.21	0.58	0.32
Glu	733	755	745	753	6.99	0.05	0.47	0.32
Gly	857	863	859	856	8.97	0.54	0.37	0.11
His	732	754	740	751	8.10	0.08	0.78	0.51
Ile	713	733	723	735	8.29	0.06	0.44	0.59
Leu	745	763	754	760	7.68	0.12	0.66	0.41
Lys	809	824	811	820	7.05	0.10	0.87	0.65
Met	723	747	726	744	9.20	0.04	0.95	0.73
Orn	481	543	527	533	27.7	0.23	0.52	0.31
Phe	706	729	717	729	9.85	0.08	0.56	0.58
Pro	677	707	691	701	8.62	0.05	0.65	0.27
Ser	726	740	734	737	7.24	0.27	0.73	0.42
Thr	694	715	710	717	7.55	0.11	0.28	0.37
Val	734	756	747	758	7.49	0.06	0.36	0.48

¹D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.

Table 7. In situ degradability and small intestinal and total-tract digestibility of CP (g/kg of CP)

Item ¹	Treatment				SEM	P-value			Oat hull ²
	Oat	Decorticated oat (D)	Toasted oat (T)	Decorticated toasted oat (DT)		D	T	DT	
BS	216	198	80.6	100					247
SOL	98.5	94.5	0.0 ³	40.3					69.6
<i>p</i>	668	655	280	308					615
<i>a</i>	769	751	381	394	0.84	0.83	<0.01	0.10	685 ± 5.63
<i>b</i>	154	198	478	514	2.51	0.15	<0.01	0.89	132 ± 6.00
<i>c</i>	0.44	0.58	0.08	0.04	0.09	0.54	<0.01	0.32	0.69 ± 0.19
ED	899	922	595	576	2.54	0.92	<0.01	0.41	800 ± 5.21
SID	323 ^b	209 ^a	836 ^c	872 ^c	7.81	<0.01	<0.01	<0.01	486 ± 1.86
TTD	932 ^a	938 ^b	934 ^a	946 ^c	0.80	<0.01	<0.01	0.01	897 ± 0.37

^{a-c}Means in the same row with different superscripts differ ($P < 0.05$).

¹BS = buffer solubility; SOL = water solubility; *p* = particle loss calculated as 0 h loss from the bag minus SOL; *a* = soluble fraction; *b* = potentially degradable fraction; *c* = rate of degradation of *b* fraction (h^{-1}); ED = effective degradability of CP; SID = small intestinal disappearance of rumen undegraded protein (g/kg RUP); TTD = total-tract disappearance (g/kg CP).

²Mean ± SD.

³Measured slightly negative (−4.31 g/kg), reported as zero.

DISCUSSION

Processing of Oat

Decorticating of grains is a practical tool for increasing the digestible energy content of dairy cow diets without altering the forage-to-concentrate ratios (Yang et al., 1997; Wang et al., 2017). Decortication resulted in the expected changes in composition and in vitro digestibility (Panah et al., 2019). The composition of the removed oat hull showed that hulls are low in CP and high in NDF. Starch content was considerably higher (221 g/kg of DM) than the feed table value (130 g/kg of DM) for oat hull meal from the cereal industry (NorFor, 2019). The considerable starch concentration in the hulls indicated that the obtained hulls should

be used as feed, for example, as substitute for straw in rations for dry cows and heifers. Earlier studies also showed that oat hull is mainly made up of low digestible cell wall (Decker et al., 2014), and fat and protein in the grain are mostly stored with the starch mass throughout the endosperm (Peterson and Wood, 1997). Therefore, decorticating exposes the digestible groat to ruminal fermentation.

On-farm toasting is used in practice in Denmark, and the oat used in this study was toasted on farm at 121°C. Toasting increased DM concentration by approximately 100 g/kg and reduced buffer solubility of CP by 117 g/kg of CP and in situ rumen protein degradability by 357 g/kg of CP without decreasing total-tract mobile bag digestibility, showing that the toasting process performed as planned. This finding is in agreement with

Table 8. Rumen fermentation parameters

Item	Diet ¹				SEM	P-value		
	Oat	D	T	DT		D	T	DT
pH rumen	6.32	6.33	6.37	6.32	0.12	0.50	0.60	0.32
Total SCFA, ² mmol/L	130	132	127	130	5.82	0.36	0.24	0.79
SCFA, mol/100 mol of total SCFA								
L-Lactate ³	1.61	1.49	1.79	2.44	0.74	0.62	0.31	0.48
Acetate	59.9	58.9	59.2	57.5	1.10	0.03	0.06	0.55
Propionate	21.0	22.6	22.2	23.1	0.53	0.05	0.13	0.51
Isobutyrate	0.83	0.85	0.79	0.77	0.09	0.98	0.07	0.60
Butyrate	13.1	12.6	12.7	12.8	0.43	0.30	0.73	0.15
Isovalerate	1.32	1.44	1.15	1.07	0.14	0.77	0.01	0.25
Valerate	1.63	1.69	1.64	1.68	0.04	0.27	0.96	0.78
Caproate	0.56	0.51	0.52	0.55	0.05	0.82	0.95	0.28
NH ₃ -N, mmol/L	9.28	9.53	8.84	9.22	0.62	0.53	0.45	0.89
Glucose, mmol/L	0.88	1.06	0.90	1.02	0.10	0.10	0.91	0.78

¹D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.

²Short-chain fatty acids.

³Total lactate approximately 2 times L-lactate.

Table 9. Dry matter intake during gas measurement and gas exchanges (d 17–22)

Item ¹	Diet ²				SEM	P-value		
	Oat	D	T	DT		D	T	DT
DMI, kg/d	21.7	21.3	22.0	21.6	1.90	0.45	0.50	0.98
CH ₄ , L/d	617	598	597	593	41.8	0.03	0.02	0.12
CH ₄ , L/kg of DMI	28.6	28.2	27.3	27.6	0.90	0.97	0.12	0.53
CH ₄ , L/kg of TOMD	63.4	59.8	59.0	63.5	2.66	0.86	0.90	0.16
CH ₄ , L/kg of ECM	20.7	17.9	18.3	17.8	1.34	0.19	0.30	0.36
CO ₂ production, L/d	7,298	7,292	7,264	7,423	547	0.45	0.63	0.42
H ₂ , L/d	6.51	6.13	5.53	5.57	0.83	0.80	0.26	0.75
CH ₄ :CO ₂ ratio	0.0846	0.0822	0.0824	0.0803	0.002	0.09	0.12	0.89
O ₂ consumption, L/d	6,550	6,540	6,527	6,680	508	0.48	0.56	0.43

¹TOMD = true rumen OM digested; CH₄:CO₂ = the ratio of produced CH₄ (L/d) to produced CO₂ (L/d).

²D = decorticated oat; T = toasted oat; DT = decorticated and toasted oat.

McNiven et al. (1995), who showed that heat treatment reduced ruminal degradation of CP in barley. Profiles of rumen protein degradation for toasted oat fit more poorly with the exponential function used to describe the degradation than profiles for oat due to a very high increase in degradability from 0 to 2 h incubation time. This finding indicates that toasting rendered some water soluble protein insoluble but still very quickly degradable.

Digestion of CP and AA in the Small Intestine

As decortication removes the physical barriers associated with the hull, we expected to find higher DMI with decortication. To the contrary, numerically lower DMI was observed, probably due to increased energy content of the diet (Yang et al., 1997). The numerically lower intake may have counterbalanced the increased CP content in oats caused by decortication, resulting in similar intake of CP and AA for the 4 diets. Similar milk production also reflected the similar intake of CP and AA.

In the present study, with similar intake of CP and AA among treatments, both toasting and decortication increased the microbial synthesis of CP and AA. The digested amount of AA in the small intestine was increased by both decortication and toasting. The availability of rapid fermentable carbohydrates along with an adequate degradable protein (Schwab and Broderick, 2017) increased microbial synthesis or microbial efficiency. Therefore, the higher microbial efficiency of protein synthesis due to decortication might be explained by higher availability of starch as an energy supply for rumen microbes. In agreement with our findings, Archimède et al. (1997) found an average of 23.6 ± 9.3 g of N/kg of OM truly digested in the rumen for the microbial efficiency based on a database review involving 157 publications. In their review, higher inclusion of starch was associated with higher microbial

efficiency until the point at which cellulolytic activity of rumen microbes started to be hampered. Moreover, it was previously reported that synchronization for rapid fermentation through provision of fast degradable starch and nitrogen increases the efficiency of microbial AA synthesis (NRC, 2001) and thereby leads to higher duodenal flow of microbial CP (Herrera-Saldana et al., 1990). Therefore, in the present study the increased starch intake upon decortication may have improved the synchronization between the digestible carbohydrate and CP and thereby the efficiency of microbial synthesis. Decortication did not change the rumen pH to the point at which cellulolytic activity could have been impaired because pH averaged 6.34 ± 0.02 .

The slightly higher DMI due to toasting may partly explain the increasing effect of toasting on microbial synthesis of AA and CP. However, microbial efficiency was also numerically increased. Regardless of the increasing effect of toasting on total microbial CP and AA synthesis (kg/d), toasting reduced the concentration of CP and AA in microbial DM. In accordance, Hvelplund and Madsen (1993) reported that variations in the chemical composition of rumen microbes could be related to the diet to some extent; for example, energy, some nutrients, and the supply of rumen degradable protein and its quality contribute to this variation.

Methionine, Lys, and His are typically considered as the limiting AA for high-yielding dairy cattle (Schwab et al., 2005; Schwab and Broderick, 2017). The replacement of oat with decorticated, toasted, or decorticated and toasted oat did not change the Lys, Met, and His proportion in total microbial AA. However, the proportions of Arg, Glu, Gly, Ile, Orn, Phe, and Ser in total microbial AA changed due to the treatments. The microbial AA profile found in the present study was comparable to the values reported in the literature (Larsen et al., 2000; Johansen et al., 2018), with only minor variations in microbial AA composition due to the treatments. This difference in microbial AA compo-

sition was possibly related to the variations in nutrient supply to the microbes, such as starch and rumen degradable AA (Hvelplund and Madsen, 1993; Johansen et al., 2017), because decortication increased starch intake and toasting reduced ruminal AA digestibility and ruminal *in situ* degradability of CP.

Both decortication and toasting decreased the Lys proportion in total AA intake. The similar Lys proportion in the duodenal flow, which was likely due to the high proportion of Lys in microbial AA, can offset the reducing effect of decortication on Lys intake. Toasting decreased His proportion, whereas Met proportion increased due to decortication and decreased upon toasting. However, regardless of these differences in the proportion of AA intake in the total AA among treatments, the trend of changes in AA composition of duodenal flow was to some extent different from the AA composition of the feed. Unaffected ruminal digestibility of Met (g/kg of AA intake) due to decortication along with its increased proportion in AA intake could explain the tendency of decortication to increase Met in duodenal AA. The increased Cys proportion in duodenal AA by toasting, despite its decrease in AA intake, reflects reduced ruminal digestibility of Cys due to toasting. For Lys, regardless of the fact that toasting significantly reduced its ruminal digestibility, its proportion in duodenal flow was reduced due to toasting. This outcome reflected the reduced proportion of Lys in AA intake due to toasting, possibly as a result of thermal damage. Decortication had only minor effects on ruminal digestibility of some AA (Ala, Asp, Lys, Phe, Ser, and Val), but toasting was shown to be a protective shield against ruminal digestion of AA, based on the results for Lys, Arg, Asp, Cys, Glu, Phe, Pro, Ser, and Val.

Additionally, the endogenous AA-N originating from glycoproteins in mucus, shed epithelial cells, and bile and digestive enzymes secreted in the abomasum and duodenum constitutes approximately 25% of total AA-N flow at the duodenum with a small intestinal digestibility of 62% (Larsen et al., 2000, 2001). In the present study, because the duodenal cannula was located after the bile duct entrance, a high proportion of Gly in the duodenal protein shows the presence of endogenous AA in the duodenal samples originating from bile. This finding is in agreement with Larsen et al. (2000), who reported that Gly is the most abundant AA originating from cow's bile.

Previous nylon bag studies showed that despite minor deviations, the proportion of AA in RUP is similar to that of the protein intake (Skórko-Sajko et al., 1994; Skiba et al., 1996; Hvelplund and Hesselholt, 1987); therefore, variation in the composition of AA intake is also expected to cause variation in the duodenal flow

of AA. As a result, differences in the AA composition in the duodenal flow could mainly originate from AA composition of feed RUP and partially from variable contributions of microbial and endogenous AA. However, postruminal digestibility of AA in RUP may vary within the same protein source, depending on the treatment applied on the feedstuff because lower ruminal degradability of AA was reported to be associated with higher digestibility of RUP (Hvelplund and Madsen, 1993). Nevertheless, neither decortication nor toasting resulted in any decline in small intestinal digestibility of AA, which is in line with the reduced or tendency to be reduced ruminal digestibility of individual AA due to our treatments.

The effects of the applied treatments on changing the postruminal flow of CP and AA was highlighted by the different quantities of CP and AA digested in the small intestine. Along with increased supply of microbial AA at the duodenum, decortication also increased the daily digested amount of AA in the small intestine with a slight increase of the small intestinal digestibility of AA. The apparent small intestinal digestibility of the individual AA shows that decortication generally increased or tended to increase the digestibility of most AA. For instance, although decortication did not change the proportion of Ala, Glu, and Met in microbial AA, it increased both the intake of these AA and their small intestinal digestibility. This outcome could be due to the removal of the low digestible hull and its negative effect on intestinal digestibility (Welch et al., 1983). On the other hand, the lower digestibility of whole oat grain was attributed to the extensive ester cross-link bonds between polysaccharides and lignin in oat hull and its high fiber content (Yu et al., 2005; Kirkkari, 2008). The results of the mobile bag study showed that lower TTD of CP in the intact oat could be related to the low digestibility of hull protein in addition to its negligible CP concentration. Hvelplund (1985) also reported a lower small intestinal digestibility of RUP in some feedstuffs due to higher proportion of cell wall bound nitrogen content. Another study likewise suggested a negative correlation between the fiber content and nutrient digestibility (Wang et al., 2017).

The increased amount of digested AA (kg/d) due to toasting could be explained by increased duodenal flow of AA with unaffected small intestinal digestibility. Increased duodenal flow of CP and AA, caused by toasting, derived from the reduced ruminal digestibility and the digested amount of AA and the increased microbial AA synthesis. In accordance, heat treatment has been used to reduce ruminal degradation of feed proteins and increasing the supply of dietary protein at the duodenum (McNiven et al., 1995; Schwab, 1995). This effect is induced by denaturation of proteins and

formation of protein-carbohydrate (Maillard reactions) cross-links via a reaction between ϵ -amino groups of AA and carbonyl group of reducing sugars, which reduces protein degradability in the rumen (Kung and Rode, 1996; NRC, 2001; Lund et al., 2004). McNiven et al. (1994) showed a decreased ruminal and total-tract digestibility of CP for oat when it was heat treated at 168°C. In the present study, the feed-ileum digestibility of AA was decreased on average by 13.5 g/kg of AA intake due to toasting, indicating minor heat damage to AA in the feed. However, toasting at 121°C did not influence the small intestinal digestibility of CP and individual AA, and it increased the amount of digested AA in the small intestine. Therefore, the applied heat in the present study did not overprotect CP and AA for small intestinal digestibility, and toasting of oat caused a shift in the site of AA digestion from the rumen to the small intestine. Chowdhury et al. (2018) showed that Lys is one of the most heat-sensitive AA. Data on the Lys digestibility for diets with toasted oat were similar to the other treatments, which indicates that toasting oat at 121°C did not overprotect the CP.

Toasting reduced in situ degradability, and in situ results quantitatively confirmed the flow of CP at the duodenum for the in vivo study. Mobile bag results for total-tract CP digestibility showed a slight increase, whereas feed-ileum digestibility of AA showed a slight decrease. The increased SID due to toasting was due to the reduced ED and equivalent increased RUP. Hvelplund et al. (1992) reported that lower ruminal degradability of CP was associated with higher small intestinal digestibility of RUP. This finding might also explain the lower small intestinal digestibility of CP due to decortication because decortication numerically increased ED. Total-tract disappearance (mobile bags) of CP was statistically increased by both decortication and toasting, but the difference was numerically small.

Digestibility of Other Nutrients

Based on a meta-analysis of in vivo studies in dairy cows, Moharrery et al. (2014) showed a negative correlation between starch intake and its ruminal digestibility in corn, wheat, barley, and sorghum, mainly due to its positive correlation with DMI resulting in an increased passage rate. In agreement with that study, the constant DMI and digested amount of DM in the rumen among our treatments indicate a rather constant passage rate due to treatments. Therefore, the increased starch intake due to decortication and increased starch concentration in feed DM did not influence ruminal starch digestibility. In our study, higher small intestinal and total-tract digestibility of DM and OM and higher small intestinal digestibility

of starch in decorticated oat are presumably related to the lower fiber concentration. The increased intake of starch due to decortication, along with the unaffected ruminal digestibility of starch, could also explain the increased digested starch in the small intestine as well as its increased small intestinal digestibility. This possibility is in line with Moharrery et al. (2014), who reported a higher small intestinal digestion of starch due to increased flow of starch at the duodenum. Toasting slightly increased DMI without influencing the starch intake and ruminal digestibility of starch in the present study. It was previously reported that toasting at 130°C decreased ruminal degradability of starch in fava beans, peas, and lupins (Goelema et al., 1998). However, the insignificant influence of toasting on ruminal digestibility of starch in this study could be due to the different applied temperature (i.e., 121 vs. 130°C), starch structures in different grains, and the variable influence of heat treatment on different grains (Theurer, 1986). Although the in situ digestibility of lost particles was previously assumed to be similar to the particles remaining in the bag (Hvelplund and Weisbjerg, 2000), the interpretation of in situ observations in the case of oat grains must be done with caution. Particle loss for DM and CP is substantial in oat (Panah et al., 2019). Therefore, due to the methodology of in situ evaluation, the in vivo ruminal starch digestibility cannot be explained by reduced DM and CP particle loss in situ due to toasting. Regardless of unaffected starch intake and ruminal digestibility of starch due to toasting, toasting increased small intestinal digestibility of starch in oat and it was within the range reported by Moharrery et al. (2014). The increased small intestinal digestibility of starch by toasting might be due to the structural changes induced to the starch granules, making them more susceptible to small intestinal digestion (Theurer, 1986).

Consistent with other studies (Lund et al., 2007; Brask et al., 2013b; Olijhoek et al., 2016; Johansen et al., 2017), the small intestinal digestibility of NDF was negative for all diets, possibly due to problems with representative sampling from intestinal cannulas with respect to the fiber fraction (Olijhoek et al., 2016).

Rumen Fluid

Ruminal pH and total VFA concentration were not affected by treatments, which fits with almost the same amount of fermented OM being found. Although ruminal pH was not affected, the results of the present study showed slightly lower acetate production along with a slightly higher propionate proportion due to decortication. This result reflected the lower intake of NDF and the higher intake of starch due to decortication. It was

previously reported that a higher intake of NDF can be expected to enhance acetic acid production, and higher starch intake enhances propionate production in the rumen (Brask et al., 2013a). The reducing effect of toasting on ruminal degradability of AA was in accordance with the reduced proportions of isovalerate and isobutyrate in rumen SCFA because rumen branched-chain SCFA originates from the degradation of branched-chain AA (Johansen et al., 2017). This is in agreement with McNiven et al. (1995), who reported a lower isovaleric acid in the ruminal fluid of the cows fed heat-treated barley due to recorded lower ruminal degraded AA. The $\text{NH}_3\text{-N}$ concentration was unaffected by treatments, although we had expected a lower $\text{NH}_3\text{-N}$ due to toasting because toasting decreased the ruminal digestibility of AA and increased microbial synthesis.

Methane Production

As expected, decortication reduced CH_4 production (L/d) due to an increased proportion of easily digestible carbohydrates, reduced NDF, and increased proportion of propionate in total SCFA (Boadi et al., 2004; Brask et al., 2013a); however, opposite to our expectation, decortication did not reduce CH_4 production per kilogram of DM intake. Toasting also reduced daily CH_4 production (L/d), possibly due to the tendency for decreased acetate proportion and numerically increased propionate proportion due to toasting. However, the results are difficult to interpret because OM digested in the rumen was similar.

CONCLUSIONS

Decortication increased the daily intake of starch. Small intestinal digestibility of starch increased by both decortication and toasting. Toasting increased small intestinal digested CP. Both decortication and toasting increased the amount of AA digested in the small intestine, decortication mainly due to increased efficiency of microbial protein synthesis and toasting due to decreased rumen degradation of feed protein. Both decortication and toasting reduced CH_4 production per day but not per kilogram of DM intake.

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