

Metabolism of Silage Alcohols in Lactating Dairy Cows

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ABSTRACT

Dairy cows fed silage are subjected to various alcohols and low molecular weight esters. Four lactating Holstein cows fitted with ruminal cannulas and permanent indwelling catheters in the hepatic portal vein, hepatic vein, mesenteric vein, and mesenteric artery were used to study the absorption of alcohols into portal blood and the metabolism of feed alcohols in the rumen and splanchnic tissues. The cows were allocated to 4 experimental treatments in a Latin square design. All treatments were formulated as total mixed rations with the same overall nutrient composition, differing by the source of corn silage. Treatments were a control silage and 3 qualities of problematic corn silage (silage with *Fusarium* toxin, *Penicillium*-infected silage, and silage with a high propanol content). Feeding was followed by a decreasing ruminal pH, as well as decreasing molar proportions of ruminal acetate and isobutyrate. The ruminal concentrations of total VFA, ethanol, propanol, 2-butanol, ethyl acetate, propyl acetate, glucose, and L-lactate, and molar proportions of propionate, butyrate, isovalerate, valerate, and caproate increased after feeding. Treatments affected ruminal concentrations of propanol, propyl acetate, and butyrate and a strong correlation was observed between ruminal propyl acetate and the molar proportion of butyrate ($r = -0.79$). Arterial concentrations of ethanol, propanol, propanal, acetone (sum of acetone and acetoacetate), 3-hydroxybutyrate, L-lactate, glutamate, and glutamine increased, and the arterial concentration of glucose decreased after feeding, but no effects of treatment were observed for arterial variables. The postprandial increase in arterial ethanol was maintained for 5 h. The net portal release of ethanol tended to decrease with the treatment with the lowest ethanol content, and the net splanchnic release of ethanol increased after feeding, but overall, the net splanchnic flux of ethanol was not different from zero, in agreement with the liver being the major organ for alcohol metabolism. The net portal flux and net hepatic flux of propanol were affected by

treatment. All dietary ethanol and propanol were accounted for by absorption of the respective alcohol into the portal blood. The hepatic extraction ratios of ethanol and propanol were, on average, 63 to 66%, and no indications of saturation of hepatic alcohol metabolism were observed at any time. We concluded that typical amounts of alcohols in corn silage do not interfere with splanchnic metabolism of any of the measured variables and do not saturate hepatic pathways for alcohol metabolism. However, even low concentrations of alcohols in feed might affect ruminal metabolism and are followed by hours of elevated peripheral blood concentrations of alcohols.

Key words: dairy cow, alcohol, metabolism

INTRODUCTION

Alcohols are normal constituents of silage, and silage-based rations for dairy cows will contain a variable amount of ethanol, propanol, and 2-butanol as well as a range of esters of these alcohols (Morgan and Pereira, 1962). The presence of alcohols in silage has been linked to problems with the organoleptic quality of milk (Gordon and Morgan, 1972; Randby et al., 1999), changes in ruminal fermentation with increases in ruminal acetate and caproate (Chalupa et al., 1964; Durix et al., 1991), and reduced palatability (Buchanan-Smith, 1990). Danish local extension and veterinary services have repeatedly reported an apparent link between strange silage odors (odor resembling vinegar or acetone) and herd problems primarily related to the productivity and health of fresh cows. Samples of these silages have consistently revealed the presence of propanol and propyl acetate.

Alcohol metabolism related to dairy cow nutrition can be subdivided into at least 4 serially arranged metabolic entities (silage, rumen, ruminal epithelium, and liver). Alcohols can be synthesized in the rumen by fungi (Teunissen et al., 1992) and bacteria (Lauková and Marounek, 1992), and alcohol production is an electron sink in fermentation (Teunissen et al., 1992), in agreement with the observed accumulation of alcohols with high starch intake (Allison et al., 1964). Alcohols are removed from the rumen by microbial metabolism and absorption (Jean-Blain et al., 1992). The multiple in-

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puts and outputs of alcohols indicate that alcohol metabolism in dairy cows represents a complex system, and the quantitative metabolism of alcohols by dairy cows is not well understood and has been sparsely described. The present study was undertaken to study alcohol metabolism in dairy cows given different corn silages, representing those considered problematic in Denmark, with a variable content and composition of alcohols. Corn silage representing different categories of silage problems (with *Fusarium* toxins, *Penicillium*-infected, and with a high propanol content) were collected from dairy farmers across Denmark. The major questions of the study were whether natural variation in the alcohol content of corn silage would affect ruminal fermentation in lactating dairy cows, how much alcohol would be absorbed into the portal blood, and whether alcohol intake affects the overall splanchnic metabolism of alcohols, glucose, lactate, and ketones, as well as the splanchnic metabolism of oxygen.

MATERIALS AND METHODS

The present experiment complied with Danish Ministry of Justice Law no. 382 (June 10, 1987), Act no. 726 (September 9, 1993), concerning experiments with animals and the care of experimental animals.

Animals and Feeding

Four primiparous Danish Holstein cows (568 ± 40 kg of BW) fitted with ruminal cannulas (#1C; Bar Diamond, Parma, ID) and permanent indwelling catheters in the mesenteric artery, mesenteric vein, hepatic portal vein, and hepatic vein were used in the study. Surgery was done according to the procedures described by Huntington et al. (1989), with slight modifications of the catheters. Catheters made of Tygon (S-54-HL, 1.02 mm i.d. \times 1.78 mm o.d.; Cole-Parmer, Vernon Hills, IL) were inserted 45 cm into the mesenteric artery and 15 cm into 2 separate branches of the mesenteric vein. Catheters implanted in the hepatic portal vein and the hepatic vein were made of Teflon (PTFE 16 TW, 1.32 mm i.d. \times 1.93 mm o.d.; Cole-Parmer) sheathed in silicone tubing (Silastic, 1.57 mm i.d. \times 3.18 mm o.d.; Cole-Parmer). The portal catheter had a 13-cm silicone free tip that was inserted into the portal vein. The hepatic catheter had a 10-cm silicone free tip, and the tip was placed at least 5 cm from the caudal vena cava under ultrasound guidance. Cows underwent surgery at 236 ± 23 d in milk, with first sampling 21 ± 1 d after surgery. Cows were randomly allocated to 4 experimental treatments in a Latin square design with 14-d periods.

Treatments were a control silage and 3 different corn silages representing common corn silage problems [*Fu-*

Table 1. Ingredient and nutrient composition of experimental diets

Item	Treatment ¹			
	Control	<i>Fusarium</i>	<i>Penicillium</i>	Propanol
Ingredient, % of DM				
Corn silage	54.0	52.5	53.1	53.4
Grass silage	11.4	11.7	11.6	11.5
Rapeseed cake	13.8	14.3	14.1	14.0
Rolled barley	13.3	13.8	13.6	13.5
Molasses, sugar beet	5.4	5.6	5.6	5.5
Mineral mix ²	1.1	1.1	1.1	1.1
Urea	0.5	0.5	0.5	0.5
NaCl	0.3	0.3	0.3	0.3
Vitamin mix ³	0.2	0.2	0.2	0.2
Nutrient				
DM, %	41.4	41.9	42.7	40.7
	(g/kg of DM)			
OM	942	937	940	938
NDF	315	293	297	302
CP	155	153	153	150
Crude fat	39	42	42	41
Starch	277	262	263	276
Glucose	33.3	31.0	33.4	28.8
L-Lactate	23.5	25.9	20.9	20.7
Acetate	19.7	14.2	14.8	26.6
Ethanol	14.2	10.0	6.7	4.4
Propanol	3.4	0.6	0.6	5.6
2-Butanol	ND ⁴	ND	ND	0.03
Ethyl acetate	0.76	0.44	0.33	0.36
Propyl acetate	0.15	0.02	0.03	0.42

¹All diets were mixed with the intention of having the same overall nutrient composition except for the different alcohol contents and had the same overall energetic value, calculated to be 0.94 Scandinavian feed units/kg of DM (13.6 MJ of digestible energy/kg of DM).

²Mineral mix (Optimin 1; Vitfoss, Gråsten, Denmark) contained (per kg): 180 g of Ca (from limestone), 10 g of P, 65 g of Mg (from magnesium sulfate and magnesium oxide), 80 g of Na (from NaCl), 30 g of S (from magnesium sulfate), 693 kIU of vitamin A, 146 kIU of vitamin D, 4,204 mg of α -tocopherol, 3,080 mg of Mn [manganese(II)oxide], 693 mg of Cu (from copper sulfate), 3,460 mg of Zn (from zinc oxide), 173 mg of I (calcium iodate), 19 mg of Co [from cobalt(II)sulfate], and 38.5 mg of Se (from sodium selenite).

³Vitamin mix (Rød Suplex; Vitfoss) contained (per kg): 5,000 kIU of vitamin A, 200 kIU of vitamin D, 9,100 mg of α -tocopherol, and 10 mg of Se (from sodium selenite).

⁴ND = not detected.

sarium toxins, *Penicillium*-infected, and propanol (strong vinegar smell)] obtained from farmers across Denmark. The silages were fed in a TMR with the same overall composition (Table 1). Except for the control treatment, 2 silages were used within treatment to avoid confounding the silage source and growth conditions, corn variety, maturity of the crop, and so on. Silages used in the *Fusarium* treatment were 2 silages with the highest level of deoxynivalenol, identified based on reports by farmers who had had their silage analyzed by commercial labs (4.5 ppm, Blgg Oosterbeek, Oosterbeek, the Netherlands; 1.5 ppm, Steins Laboratorium A/S, Brørup, Denmark). *Penicillium*-infected silages were selected based on the presence of visible growth of blue-green molds in the silage. On one

farm where silage with *Penicillium* molds was found, grass meal cubes had been added at ensiling, and to avoid adding grass meal to the experimental ration, moldy silage was separated by hand and mixed into silage without grass meal at 9% of corn silage. Corn silage used for the propanol treatment was identified by a strong vinegar smell, and the presence of propanol was confirmed by headspace GC-MS (see below). Corn silage was transported to the research center in the early morning and mixed into the TMR before 1000 h (one silage sample was picked up at around 2200 h the evening before feed mixing and stored on the truck overnight). Within 2 to 3 h after mixing, the TMR was bagged in portions of 10 to 15 kg, evacuated using a chamber vacuum-packaging machine (Webomatic I22; Webo A/S, Årup, Denmark), and frozen at -20 to -25°C . During experimental samplings, feed was maintained in a freezer at -6°C . Before feeding, vacuum bags were allowed to thaw overnight on the barn floor. Feed intake of the cows was restricted to 17 kg of DM/d to ensure comparable feeding levels among treatments. Within each experimental period, cows were fed the control ration on d 1 to 3, 50% control ration and 50% experimental ration on d 4 to 7, and only the experimental ration on d 8 to 14, with sampling on d 14. Cows were fed equally sized portions of the TMR at 0700 and 1900 h; orts, if present, were removed before feeding in the morning. Cows were milked at 0600 and 1600 h. Two randomly selected vacuum bags from each TMR were used for feed analysis.

Experimental Samplings

On sampling days, continuous infusion of *p*-aminohippuric acid (**pAH**; 30.9 ± 0.7 mmol/h) into the mesenteric vein was initiated at 0530 h. The pAH infusate was a 250-mM solution of pAH (99% 4-aminohippuric acid; Acros, Geel, Belgium) adjusted to pH 7.4, filtered (Vacu Cap 0.8/0.2 μm ; Pall Corp., Ann Arbor, MI), and autoclaved. Ten sets of ruminal and blood samples were obtained at 0.5 h before feeding, and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding. Blood was sampled by simultaneously drawing blood from the artery, hepatic portal vein, and hepatic vein into 20-mL syringes. A total of 40 mL of blood was obtained from each catheter at every sampling time, and blood was immediately transferred to heparin vacuettes (#455051; Greiner BioOne GmbH, Kremsmuenster, Austria). Plasma was harvested by centrifugation at $3,000 \times g$ for 20 min and stored at -20°C until analysis. Separate blood samples were obtained in 1-mL heparinized syringes for blood gas measurements immediately before collecting the main blood samples. Ruminal fluid was sampled using a suction strainer (#RT; Bar Diamond) and a 50-mL

syringe. Ruminal fluid pH was measured immediately after sampling (IQ 150 pH meter; IQ Scientific Instruments, Inc., Carlsbad, CA). Immediately after reading the pH, a subsample of ruminal fluid was stabilized with 5% metaphosphoric acid and frozen at -20°C .

Analytical Procedures

Blood sampled in 1-mL syringes was immediately taken for blood gas and oximetry analysis (ABL 520; Radiometer A/S, Copenhagen, Denmark). The hematocrit was determined on heparin-stabilized arterial samples by centrifugation of capillary tubes at $13,000 \times g$ for 6 min. Plasma was analyzed for glucose (D-glucose oxidase), L-lactate (L-lactate oxidase), L-glutamate (L-glutamate oxidase), and L-glutamine (glutaminase plus L-glutamate oxidase; YSI 7100; YSI Inc., Yellow Springs, OH). Plasma concentrations of propanal, acetone, ethanol, propanol, and 2-butanol were determined by headspace GC-MS using a modification of the method described by Felby and Nielsen (1994). Five hundred microliters of sample was transferred to a 10-mL headspace vial with 500 μL of a saturated NaCl solution, 100 μL of deionized water (or standard solution for calibration), and 100 μL of 5 mM pentanol (internal standard). The samples were capped and incubated for 60 min at 100°C for full decarboxylation of acetoacetate to acetone and for denaturing protein in the sample. The values obtained for acetone therefore represent the total amount of acetone and acetoacetate in the samples. Samples were analyzed using a Trace DSQ GC-MS instrument (Thermo Electron, Austin, TX) equipped with a CTC Combipal autosampler with the static headspace option (CTC Analytics, Zwingen, Switzerland). Samples were equilibrated in the agitator for 5 min at 70°C . Five hundred microliters of gas was transferred, using a 2.5-mL syringe maintained at 80°C to the injector, which was maintained at 120°C and a split of 1:18. The column (Stabilwax 30 m, 0.25 mm i.d., 0.25 μm df; Restek, Bellefonte, PA) was maintained at 37°C for 3 min and then programmed to increase at $5^{\circ}\text{C}/\text{min}$ to 80°C . The column flow was constant at 1.1 mL/min. The mass spectrometer was operated in selected ion monitoring mode with a dwell time of 100 ms. The ion source was maintained at 200°C and the emission current was 100 μA , with electron energy of 70 eV. The method was calibrated daily by spiking blood plasma with 100 μL (see above) of a serial dilution (1, 2, 5, and 10%) of a standard containing (mM) the following: ethanol, 15; propanol, 15; butanol, 5; 2-butanol, 5; propanal, 0.2; acetone, 8. The following retention times and masses were used for the individual compounds (min, *m/z*): propanal, 1.61, 58; acetone, 1.72, 58; ethanol, 2.73, 45; 2-butanol, 4.30, 45; propanol, 4.60,

59; butanol, 7.24, 56; pentanol, 10.08, 55. The recovery of propanal, acetone, and alcohols in the plasma fraction of whole blood was investigated by spiking 5 mL of plasma and 5 mL of whole blood from 3 cows with 125 and 250 μ L of the standard described above and using deionized water as the balance. The relative responses in plasma from spiked whole blood relative to directly spiked plasma for the individual compounds were (%) as follows: propanal, 92 ± 7 ; acetone, 113 ± 6 ; ethanol, 106 ± 3 ; propanol, 106 ± 1 ; 2-butanol, 108 ± 2 ; butanol, 104 ± 1 . Plasma concentrations of pAH were determined using the method described by Harvey and Brothers (1962), modified to run on a Cobas Mira autoanalyzer (Triolab A/S, Brøndby, Denmark). D-3-Hydroxybutyrate was determined in plasma using a Cobas Mira autoanalyzer and a kit based on D-3-hydroxybutyrate dehydrogenase (Ranbut; Randox Laboratories Ltd., Crumlin, UK).

Ruminal samples were analyzed for VFA by gas chromatography (Kristensen et al., 1996b). Alcohols and esters were determined in ruminal fluid using the head-space GC-MS method described above except that the mass spectrometer was operated in full scan mode and the column was programmed to increase at $5^\circ\text{C}/\text{min}$ to 90°C , from an initial temperature of 40°C with a 1-min hold. The method was calibrated daily in a water matrix using a serial dilution of a standard containing the following components (mM): methanol, 32; ethanol, 32; propanol, 32; butanol, 16; 2-butanol, 4; ethyl acetate, 0.5; propyl acetate, 0.15. The following retention times and masses were used for the individual compounds (min, m/z): ethyl acetate, 2.04, 70; methanol, 2.08, 31; ethanol, 2.39, 31; propyl acetate, 2.83, 43; 2-butanol, 3.45, 45; propanol, 3.65, 31; butanol, 5.57, 31; pentanol, 7.97, 55. Glucose and L-lactate were determined in ruminal fluid using the YSI analyzer as described above.

Glucose, L-lactate, VFA, alcohols, and esters in feed were determined as described for ruminal fluid using water extracts of feed. Two hundred fifty grams of thawed feed was combined with 1,000 mL of deionized water, blended for 2×30 s (Waring 24CB10; Waring Commercial, New Hartford, CT), centrifuged ($2,300 \times g$ at 10°C for 20 min), stabilized with 5% metaphosphoric acid, and stored at -20°C until analysis. Feed DM was determined after drying at 60°C for 48 h in a forced-air oven. Organic matter was determined as DM—crude ash, where crude ash was determined after combustion at 525°C for 6 h. Crude fat was determined as petroleum ether extract following acid hydrolysis (Stoldt, 1952). Neutral detergent fiber was determined as described by Mertens (2002). Starch was determined according to a modification of the procedure described by Salomonsson et al. (1984) as follows: Thirty milligrams of dry sample (equivalent to 7 to 8 mg of starch) ground

through a 0.5-mm screen or 5 to 30 mg of pure starch for calibration was weighed into 13-mL culture tubes, suspended in 10 mL of acetate buffer (0.1 M; pH 5.0), and incubated for 70 min at 100°C with 50 μ L of thermo-stable α -amylase (3,000 U/mL; Megazyme Int., Bray, Ireland). Samples were cooled, combined with 50 μ L of amyloglucosidase (3,260 U/mL; Megazyme Int.), and incubated overnight at 60°C . Samples were incubated for 30 min at 100°C , cooled to room temperature, and centrifuged at $3,000 \times g$ for 30 min at 20°C , and the supernatant was assayed for glucose using a YSI 7100 analyzer (YSI Inc., Yellow Springs, OH). Samples incubated without enzymes were used to correct for non-starch glucose in the samples. Milk was analyzed for fat, protein, and lactose by infrared spectrometry using a MilkoScan 4000 (Foss Electric, Hillerød, Denmark).

Calculations and Statistical Procedures

The 12-h sampling window was defined from 0600 to 1800 h. Data presented as means are weighted by time. Each sample represents a sampling window starting at the time halfway between the previous and the actual sample and ending at the time halfway between the actual and the following sample. The net portal flux_{ijk} was calculated as follows: portal blood or plasma flow_{ijk} \times (portal concentration_{ijk} – arterial concentration_{ijk}), using either whole blood or plasma values as indicated, where *i* is cow 1 to 4, *j* is treatment 1 to 4, and *k* is sample 1 to 10. The net hepatic flux_{ijk} was calculated as follows: hepatic blood or plasma flow_{ijk} \times hepatic concentration_{ijk} – [(portal blood or plasma flow_{ijk} \times portal concentration_{ijk}) + [(hepatic blood or plasma flow_{ijk} – portal blood or plasma flow_{ijk}) \times arterial concentration_{ijk}]]. The hepatic extraction ratio_{ijk} was calculated as follows: (–) net hepatic flux_{ijk}/[(portal blood or plasma flow_{ijk} \times portal concentration_{ijk}) + [(hepatic blood or plasma flow_{ijk} – portal blood or plasma flow_{ijk}) \times arterial concentration_{ijk}]]. The net splanchnic flux_{ijk} was calculated as follows: hepatic blood or plasma flow_{ijk} \times (hepatic concentration_{ijk} – arterial concentration_{ijk}).

Whole blood flows were calculated as follows: plasma flow/[1 – (hematocrit/100)]. Whole blood fluxes for alcohols were calculated after correction for plasma recovery of alcohols added to whole blood as described above. The ME intake of alcohols was calculated, assuming that the heat of combustion was 1,367 and 2,020 kJ/mol for ethanol and propanol, respectively. Energy corrected milk was calculated according to Sjaunja et al. (1990).

Data for ruminal variables, arterial variables, and blood and plasma fluxes were analyzed as a split-plot design over time with cows as blocks, treatment as the whole-plot factor, and time as the split-plot factor using

Table 2. Feed intake and milk production

Item	Treatment ¹				SEM ²	P ³
	Control	<i>Fusarium</i>	<i>Penicillium</i>	Propanol		
Alcohol ME intake, MJ/d	8.7 ^a	5.1 ^b	3.8 ^b	5.2 ^b	1.0	0.04
Alcohol intake, % of total ME ⁴	4.9	2.9	2.0	3.0	0.6	0.06
DMI, kg/d	15.8	16.2	16.5	16.0	0.4	0.64
Yield, kg/d						
Milk	20.5	22.3	23.0	23.1	1.3	0.50
ECM ⁵	20.6	21.3	22.6	22.7	1.0	0.40
Fat	0.86	0.82	0.89	0.92	0.04	0.51
Protein	0.65	0.74	0.78	0.75	0.04	0.16

^{a,b}Means within row without a common superscript differ ($P < 0.05$).

¹Diets contained corn silages selected to represent the control and 3 qualities of problematic corn silage.

² $n = 4$.

³Probability for the effect of treatment.

⁴Total ME calculated as digestible energy $\times 0.82$.

⁵Calculated according to Sjaunja et al. (1990).

the MIXED procedure of SAS (SAS Institute, 2001). Cow, treatment, and sampling time were included in the model. Effect of treatment was tested against the random cow \times treatment factor. Treatment means were separated using the PDIF option of the LSMEANS procedure in MIXED, protected by the overall F -test. Data for ME intake of alcohols, daily DMI, and milk yield were analyzed by one-way ANOVA using the GLM procedure of SAS. The difference between the total intake and total net portal flux of ethanol and propanol was evaluated by a paired t -test using the MEANS procedure of SAS. The data set was complete except for the loss of a hepatic catheter from one cow in her last sampling (propanol treatment). Significance was declared at $P < 0.05$, with a tendency at $0.05 \leq P < 0.10$. Data are presented as means \pm standard error.

RESULTS

Feed Intake and Milk Yield

The total DM offered was limited to 17 kg/d for all treatments, and actual intakes did not differ ($P = 0.64$) between treatments. The ME intake of alcohols was higher for the control treatment compared with the other treatments; however, alcohol intake accounted for a relatively small fraction (approximately 2 to 5%) of the total ME intake (Table 2). Milk yield, ECM, and fat and protein yields did not differ ($P = 0.16$ to $P = 0.50$) between treatments (Table 2).

Ruminal Variables

All ruminal variables were affected by the time of sampling ($P = 0.04$ to $P < 0.01$; Table 3) and generally showed the largest relative changes between the first

and second sampling times, as illustrated by the ruminal profile of ethanol and ethyl acetate shown in Figure 1. Ruminal pH and ruminal concentrations of ethanol, 2-butanol, ethyl acetate, glucose, L-lactate, and total VFA did not differ ($P = 0.10$ to $P = 0.57$) between treatments. The ruminal concentrations of propanol and propyl acetate increased ($P < 0.01$) with the control and propanol treatments compared with the *Fusarium* and *Penicillium* treatments (Table 3). Ruminal propanol increased ($P < 0.01$) with the propanol treatment. The ethanol-ethyl acetate and the propanol-propyl acetate ratios were higher ($P < 0.01$) in feed (35 ± 1 and 39 ± 3 , respectively) compared with ruminal fluid (8 ± 2 and 12 ± 3 , respectively). The molar proportion of butyrate in the rumen increased ($P = 0.03$) with *Fusarium* and *Penicillium* compared with propanol, and the molar proportion of butyrate increased with *Fusarium* compared with the control. The molar proportion of acetate and isobutyrate tended ($P = 0.08$ and $P = 0.05$, respectively) to decrease with the *Fusarium* and *Penicillium* treatments.

Arterial Variables

Arterial oxygen, hematocrit, and pAH were not affected ($P = 0.50$ to $P = 0.73$) by sampling time; however, all other arterial variables tested were affected ($P \leq 0.01$) by sampling time. The pattern of ethanol in arterial and portal blood is shown in Figure 2. None of the measured variables in arterial blood were affected ($P = 0.18$ to $P = 0.95$) by treatment (Table 4). The postprandial increase in arterial alcohol concentrations varied considerably between individual cows, and values as high as 1.5 mM ethanol were recorded in one cow, whereas in other cows, ethanol concentrations in the

Table 3. Ruminal variables

Item	Treatment ¹				SEM ²	P ³	
	Control	<i>Fusarium</i>	<i>Penicillium</i>	Propanol		Trt	Time
Ruminal pH	6.62	6.65	6.64	6.54	0.05	0.44	<0.01
	(mmol/L)						
Ethanol	2.86	2.81	2.47	1.12	0.45	0.14	<0.01
Propanol	0.63 ^a	0.30 ^b	0.35 ^b	0.95 ^c	0.05	<0.01	<0.01
2-Butanol	0.015	0.020	0.010	0.037	0.005	0.86	<0.01
Propanal	0.005 ^a	0.004 ^a	0.005 ^a	0.006 ^b	0.0003	<0.01	<0.01
Ethyl acetate	0.38	0.28	0.34	0.12	0.07	0.10	<0.01
Propyl acetate	0.058 ^a	0.021 ^b	0.032 ^b	0.065 ^a	0.008	<0.01	<0.01
Glucose	0.04	0.09	0.06	0.05	0.02	0.52	0.04
L-Lactate	1.24	2.03	2.27	1.07	0.57	0.35	<0.01
Total VFA	95	95	94	107	4	0.10	<0.01
	(mol/100 mol)						
Acetate	63.4	59.6	61.1	62.1	0.8	0.08	<0.01
Propionate	20.4	22.0	21.3	22.9	0.8	0.46	<0.01
Isobutyrate	0.7	0.6	0.6	0.7	0.02	0.05	<0.01
Butyrate	11.7 ^{ac}	14.3 ^b	13.3 ^{bc}	10.9 ^a	0.8	0.03	<0.01
Isovalerate	1.3	0.9	0.9	1.2	0.1	0.12	<0.01
Valerate	1.6	1.7	2.0	1.5	0.1	0.37	<0.01
Caproate	1.0	0.8	0.9	0.6	0.1	0.17	<0.01

^{a-c}Means within row without a common superscript differ ($P < 0.05$).

¹Diets contained corn silages selected to represent control and 3 qualities of problematic corn silage.

²Standard error of the mean ($n = 4$).

³Probability for the effect of treatment (Trt) and sampling time (Time).

arterial blood barely changed from the baseline following feeding (data not shown).

Blood Flows

Portal and hepatic blood flows were affected ($P \leq 0.01$) by sampling time; however, no effect ($P = 0.50$ to $P =$

0.68) of treatment was observed for hepatic arterial, portal, or hepatic blood flows (Table 5). The postprandial increase in portal and hepatic blood flows was approximately 12 and 20%, respectively (Figure 3).

Net Portal Fluxes

The net portal fluxes of oxygen and carbon dioxide were not affected ($P = 0.22$ to $P = 0.70$) by treatment

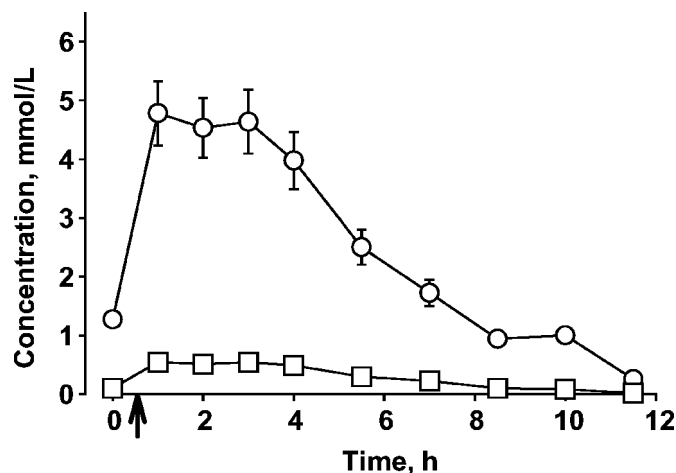


Figure 1. Ruminal concentration of ethanol (○) and ethyl acetate (□) in dairy cows sampled 0.5 h before as well as 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding. The arrow indicates the time of feeding. No effects ($P = 0.14$ and $P = 0.10$, respectively) of treatment were observed for ruminal ethanol and ethyl acetate. Each data point is the mean of 16 observations \pm SE.

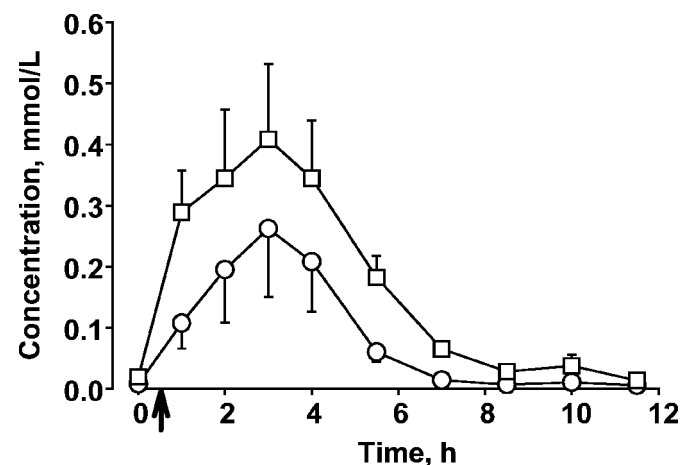


Figure 2. Arterial (○) and portal (□) plasma concentrations of ethanol in dairy cows sampled 0.5 h before as well as 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding. The arrow indicates the time of feeding. No effects ($P = 0.18$ and $P = 0.11$, respectively) of treatment were observed for arterial and portal ethanol and each data point is the mean of 16 observations \pm SE.

Table 4. Arterial variables

Item	Treatment ¹				SEM ²	P ³	
	Control	<i>Fusarium</i>	<i>Penicillium</i>	Propanol		Trt	Time
Whole blood							
pH	7.44	7.44	7.44	7.43	0.01	0.95	<0.01
Hematocrit, %	24.9	25.6	25.8	24.4	0.6	0.37	0.71
Oxygen, mmol/L	4.89	5.06	4.96	4.97	0.12	0.87	0.50
Carbon dioxide, mmol/L	25.1	24.6	25.2	25.3	0.7	0.85	<0.01
Blood plasma							
p-Aminohippuric acid	0.065	0.064	0.056	0.060	0.004	0.48	0.73
Ethanol	0.165	0.104	0.041	0.015	0.048	0.18	<0.01
Propanol	0.004	0.002	0.001	0.003	0.001	0.35	0.01
2-Butanol	ND ⁴	ND	ND	ND			
Propanal	0.0013	0.0017	0.0017	0.0014	0.0003	0.78	<0.01
Acetone ⁵	0.104	0.159	0.110	0.103	0.018	0.18	<0.01
3-Hydroxybutyrate	0.717	0.832	0.773	0.616	0.052	0.18	<0.01
Glucose	3.831	3.961	3.964	4.042	0.066	0.27	<0.01
L-Lactate	0.756	0.578	0.514	0.723	0.087	0.30	<0.01
Glutamate	0.071	0.062	0.071	0.050	0.016	0.71	<0.01
Glutamine	0.242	0.283	0.245	0.273	0.020	0.46	<0.01

¹Diets contained corn silages selected to represent control and 3 qualities of problematic corn silage.

²n = 4.

³Probability for the effect of treatment (Trt) and sampling time (Time).

⁴ND = not detected.

⁵Total concentration of acetone + acetoacetate in the samples determined after decarboxylation of acetoacetate by incubation at 100°C for 60 min.

or sampling time. The net portal fluxes of ethanol and propanol were affected ($P < 0.01$) by sampling time, and ethanol tended ($P = 0.08$) to decrease with the propanol treatment. The net portal flux of propanol was smallest with the *Fusarium* and *Penicillium* treatments, increased ($P < 0.01$) with the control, and was highest with the propanol treatment (Table 5). The net portal flux of propanal was not affected by treatment or sampling time ($P = 0.51$ and $P = 0.36$, respectively). The net portal fluxes of acetone, 3-hydroxybutyrate, glucose, glutamate, and glutamine were not affected ($P = 0.30$ to $P = 0.84$) by treatment; however, for acetone, 3-hydroxybutyrate, and glucose, an effect ($P < 0.01$) of sampling time was observed. The net portal flux of glucose peaked approximately 5 h after feeding (Figure 4), and the net portal flux of glucose differed from the net portal flux of alcohols, which peaked immediately after feeding (data not shown). A tendency ($P = 0.06$) for a time effect on the net portal flux of glutamate had no obvious relationship with time after feeding (data not shown). The net portal flux of L-lactate tended ($P = 0.09$) to increase with the *Penicillium* and propanol treatments compared with the control. Net portal fluxes of glutamate and glutamine were not affected ($P = 0.84$ and $P = 0.30$, respectively) by treatment (Table 5).

Net Hepatic Fluxes

The net hepatic flux of oxygen became more negative after feeding ($P < 0.01$), and the pattern of oxygen up-

take in the liver was different from the relatively stable oxygen uptake by the portal-drained viscera (PDV; Figure 5). The hepatic vein-arterial concentration difference of oxygen increased ($P = 0.02$) after feeding (data not shown), and the increased hepatic uptake of oxygen after feeding was therefore not only an arithmetic effect of an increased blood flow. There was no effect ($P = 0.67$) of sampling time on the hepatic flux of carbon dioxide, and there were no effects ($P = 0.86$ and $P = 0.66$, respectively) of treatment on the net hepatic fluxes of oxygen and carbon dioxide (Table 5). The net hepatic fluxes of ethanol and propanol were affected ($P < 0.01$) by sampling time and followed a pattern similar to the net portal flux of the same alcohols (data not shown). The net hepatic flux of ethanol was not affected ($P = 0.19$) by treatment; however, propanol was affected ($P = 0.01$) by treatment, with the same ranking of treatments as observed for the net portal flux: *Fusarium* = *Penicillium* < control < propanol. The hepatic extraction of ethanol (0.63 ± 0.05) and propanol (0.66 ± 0.04) was not affected ($P = 0.86$ and $P = 0.16$, respectively) by treatment but did increase ($P < 0.01$) after feeding; the highest mean values observed were 0.78 ± 0.05 and 0.93 ± 0.02 for ethanol and propanol, respectively. A numerically small net uptake of propanal was observed across the liver; however, no effect of treatment ($P = 0.70$) or time of sampling ($P = 0.62$) was observed. Time of sampling affected ($P < 0.01$) the net hepatic flux of acetone and 3-hydroxybutyrate; however, no effect ($P =$

Table 5. Blood plasma flow in hepatic artery, portal vein, and hepatic vein and net portal flux, net hepatic flux, and net splanchnic flux of oxygen, carbon dioxide, and metabolites

Item	Treatment ¹				SEM ²	P ³	
	Control	<i>Fusarium</i>	<i>Penicillium</i>	Propanol		Trt	Time
Blood plasma flows, L/h							
Hepatic artery	782	569	516	439	157	0.50	0.23
Portal vein	1,166	1,164	1,205	1,296	93	0.68	<0.01
Hepatic vein	1,944	1,733	1,720	1,755	167	0.68	0.01
Net portal whole blood flux, mmol/h							
Oxygen	-2,015	-2,100	-2,219	-2,320	164	0.62	0.57
Carbon dioxide	1,454	1,829	1,610	1,708	192	0.70	0.22
Net portal plasma flux, mmol/h							
Ethanol	113	145	118	41	19	0.08	<0.01
Propanol	28 ^a	18 ^b	17 ^b	42 ^c	4	<0.01	<0.01
2-Butanol	ND ⁴	ND	ND	ND			
Propanal	0.8	0.4	0.6	0.3	0.2	0.51	0.36
Acetone ⁵	80	108	87	89	14	0.51	<0.01
3-Hydroxybutyrate	176	182	200	199	17	0.65	<0.01
Glucose	-7	-26	-19	-10	8	0.43	<0.01
L-Lactate	158	153	169	187	12	0.09	0.50
Glutamate	0	3	7	5	2	0.30	0.06
Glutamine	-3	-4	-9	-5	5	0.84	0.40
Net hepatic whole blood flux, mmol/h							
Oxygen	-3,780	-3,448	-3,519	-3,551	218	0.86	<0.01
Carbon dioxide	1,911	2,010	1,609	1,487	467	0.66	0.67
Net hepatic plasma flux, mmol/h							
Ethanol	-131	-136	-124	-34	15	0.19	<0.01
Propanol	-29 ^a	-18 ^b	-17 ^b	-40 ^c	5	<0.01	<0.01
2-Butanol	ND	ND	ND	ND			
Propanal	-0.5	-0.2	-0.3	-0.2	0.3	0.70	0.62
Acetone	-82	-106	-81	-94	22	0.64	<0.01
3-Hydroxybutyrate	356	373	341	297	45	0.25	<0.01
Glucose	718	719	731	798	71	0.96	0.71
L-Lactate	-214	-196	-247	-178	38	0.39	<0.01
Glutamate	51	53	47	41	10	0.46	<0.01
Glutamine	2	9	17	17	7	0.53	0.06
Net splanchnic whole blood flux, mmol/h							
Oxygen	-5,803	-5,548	-5,739	-5,947	238	0.90	<0.01
Carbon dioxide	3,364	3,839	3,220	3,295	465	0.73	0.23
Net splanchnic plasma flux, mmol/h							
Ethanol	-18	10	-6	1	12	0.34	0.03
Propanol	-1	0	0	-2	1	0.26	0.77
2-Butanol	ND	ND	ND	ND			
Propanal	0.3	0.3	0.3	0.1	0.1	0.51	<0.01
Acetone	-2	3	6	0	12	0.87	0.39
3-Hydroxybutyrate	532	555	542	499	49	0.43	<0.01
Glucose	708	693	712	788	66	0.94	0.28
L-Lactate	-60	-43	-78	9	45	0.34	<0.01
Glutamate	52	56	54	45	11	0.67	<0.01
Glutamine	-1	6	9	6	7	0.75	0.33

^{a-c}Means within row without a common superscript differ ($P < 0.05$).

¹Diets contained corn silages selected to represent control and three qualities of problematic corn silage.

² $n = 4$ except for net hepatic flux and net splanchnic flux with the propanol treatment, where $n = 3$.

³Probability for the effect of treatment (Trt) and sampling time (Time).

⁴ND = not detected.

⁵Total concentration of acetone + acetoacetate in samples determined after decarboxylation of acetoacetate by incubation at 100°C for 60 min.

0.64 to $P = 0.25$, respectively) of treatment was observed. On average, the hepatic output of 3-hydroxybutyrate was 3.5 times higher than the hepatic uptake of acetone. The net hepatic flux of glucose was not affected ($P = 0.96$ to $P = 0.71$, respectively) by treatment or

sampling time. The hepatic uptake of L-lactate increased ($P < 0.01$) by approximately 50% after feeding, with the peak approximately 5 h after feeding (data not shown). There was no effect of treatment ($P = 0.39$) on the net hepatic flux of L-lactate. The net hepatic flux

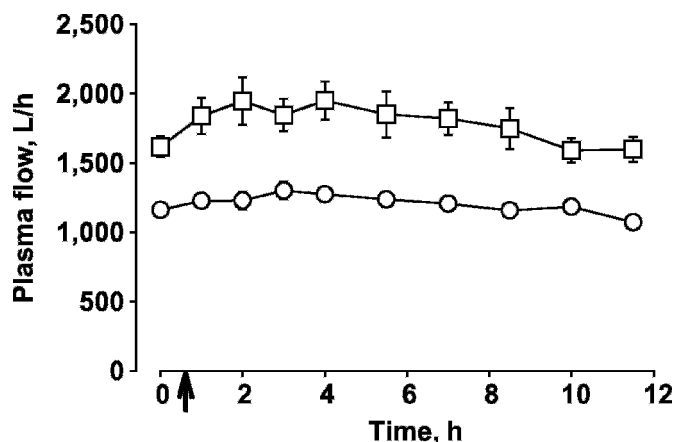


Figure 3. Blood plasma flows in the portal (○) and hepatic (□) vein in dairy cows sampled 0.5 h before as well as 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding. The arrow indicates the time of feeding. No effects ($P = 0.68$) of treatment were observed for portal and hepatic blood flows, and each data point is the mean of 16 observations \pm SE.

of glutamate increased ($P < 0.01$) after feeding and was approximately 60% higher 2.5 h after feeding compared with prefeeding. The net hepatic flux of glutamine tended ($P = 0.06$) to increase after feeding and was not affected ($P = 0.53$) by treatment.

Net Splanchnic Fluxes

The net splanchnic uptake of oxygen increased ($P < 0.01$), along with the increasing hepatic uptake of oxygen (see above). There was no effect of sampling time

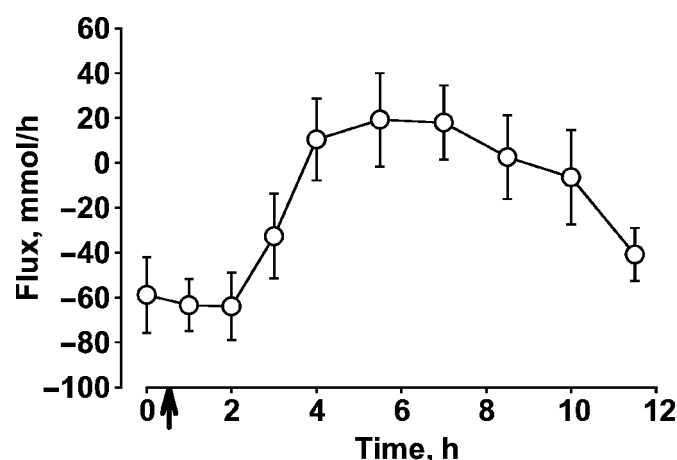


Figure 4. Net portal flux of glucose in dairy cows sampled 0.5 h before as well as 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding. The arrow indicates the time of feeding. No effect ($P = 0.43$) of treatment was observed for the net portal flux of glucose, and each data point is the mean of 16 observations \pm SE.

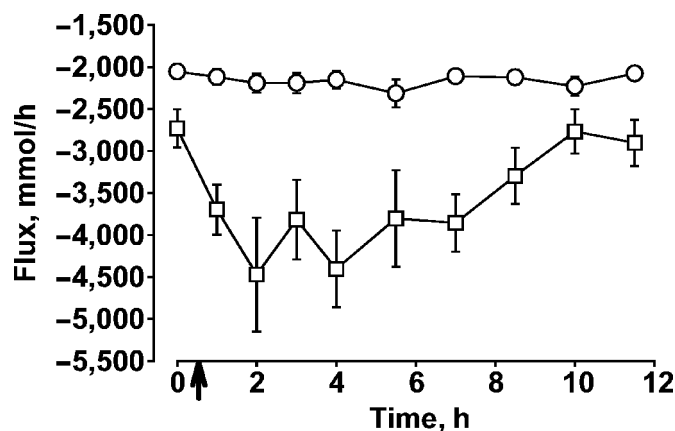


Figure 5. Net portal (○) and net hepatic (□) flux of oxygen in dairy cows sampled 0.5 h before as well as 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding. The arrow indicates the time of feeding. Each data point is the mean of 16 observations \pm SE.

on the net splanchnic flux of carbon dioxide ($P = 0.23$) and no effect ($P = 0.90$ and $P = 0.73$, respectively) of treatment on the net splanchnic flux of oxygen or carbon dioxide. The net splanchnic flux of ethanol increased ($P = 0.03$) after feeding; however, there was no effect ($P = 0.34$) of treatment, and the overall mean -4 ± 7 mmol/h was not different from 0 ($P = 0.60$). The net splanchnic flux of propanol was not affected ($P = 0.26$ and $P = 0.77$, respectively) by treatment or sampling time; however, the overall mean -0.7 ± 0.3 tended ($P = 0.07$) to be different from 0. The net splanchnic flux of propanol increased ($P < 0.01$) after feeding and was not affected ($P = 0.51$) by treatment, but overall, the net splanchnic flux was positive (0.2 ± 0.1 mmol/h; $P = 0.01$). The net splanchnic flux of acetone was not different ($P = 0.72$) from 0 (2 ± 5 mmol/h) and was not affected ($P = 0.87$ and $P = 0.39$, respectively) by treatment or sampling time (Table 5). The net splanchnic flux of 3-hydroxybutyrate increased ($P < 0.01$) from 291 ± 33 mmol/h before feeding to 702 ± 75 mmol/h 3.5 h after feeding. The net splanchnic flux of 3-hydroxybutyrate was not affected ($P = 0.43$) by treatment. The net splanchnic flux of glucose and glutamine was not affected ($P = 0.28$ to $P = 0.94$) by treatment or sampling time. The net splanchnic fluxes of L-lactate and glutamate were affected by sampling time ($P < 0.01$), with increased uptake of L-lactate and increased output of glutamate following feeding. The net splanchnic fluxes of L-lactate and glutamate were not affected ($P = 0.34$ and $P = 0.67$, respectively) by treatment.

Comparison Between Intake and Absorption of Alcohols

Overall, there was no difference between the intake and net portal flux of ethanol and propanol ($P = 0.91$

Table 6. Comparison of estimated 24-h intake and portal absorption of ethanol and propanol (mmol/d)

Item	Treatment ¹				SEM ²	P ³
	Control	<i>Fusarium</i>	<i>Penicillium</i>	Propanol		
Ethanol intake	5,004	3,490	2,498	1,531	594	0.91
Ethanol absorption ⁴	3,424	4,421	3,360	1,218	597	
Propanol intake	916	177	186	1,525	81	0.38
Propanol absorption	839	533	535	1,245	113	

¹Diets contained corn silages selected to represent the control and 3 qualities of problematic corn silage.

²n = 4.

³Test of the hypothesis that intake and absorption of the respective alcohol does not differ using a paired *t*-test (n = 16).

⁴Absorption was calculated as the 24-h whole blood net portal flux of alcohols using the plasma recovery of alcohols added to whole blood (ethanol, 106 ± 3; propanol, 106 ± 1%) and the whole blood portal blood flow, calculated as plasma flow/[1 - (hematocrit/100)].

and $P = 0.38$, respectively), and there was no difference ($P = 0.32$ and $P = 0.20$, respectively) in the portal recovery of ethanol ($114 \pm 16\%$) or propanol ($255 \pm 68\%$) between treatments (Table 6).

DISCUSSION

A Danish survey of corn silage identified considerable variation in the alcohol content as well as the alcohol composition in corn silage. For the present experiment, we attempted to find silages that represented the highest levels of propanol; however, the feed obtained for the propanol treatment contained only about half of the propanol level that can be expected under the most extreme practical conditions, in which propanol contents as high as 2.5% of DM have been identified (N. B. Kristensen, unpublished data).

Ruminal Metabolism

Ethanol has been considered an insignificant intermediate metabolite of ruminal fermentation because of the low ruminal concentration and slow turnover rate in the rumen (Hungate, 1966). Previously reported ethanol concentrations in the rumen of dairy cows indicated that the levels were lower in cows not fed silage (0.26 to 0.43 mM; Pradhan and Hemken, 1970) compared with silage-fed cows (0.7 to 1 mM; Frederiksen and Ochia, 1970). Feeding or infusion of 500 to 875 g of ethanol/d to dairy cows was followed by only a small increase in ruminal ethanol (Orskov et al., 1967; Frederiksen and Ochia, 1970); however, infusion of 1 to 1.2 kg of ethanol/d to dairy cows increased the average ruminal ethanol concentration up to 87-fold (Pradhan and Hemken, 1970). Ruminal ethanol concentrations as high as 8 mM were observed in sheep after overfeeding with wheat (Allison et al., 1964). The lack of treatment effects on ruminal ethanol concentrations in the present study is in agreement with the previous studies

and indicates that ethanol integrates rapidly into rumen metabolism. Ethanol is an electron sink product that might be important for sequestration of redox electrons in situations in which the ruminal hydrogen pressure inhibits fermentation. In cocultures of ruminal fungi and methanogenic bacteria, the introduction of methanogens and the increased sequestration of redox electrons (hydrogen) in methane have been shown to reduce the proportions of other electron sink products (ethanol, lactate, and succinate; Teunissen et al., 1992). The high degree of thermodynamic control of ruminal fermentation (Ungerfeld and Kohn, 2006) and the presence of enzymatic pathways for both the production and degradation of alcohols in the rumen suggests that the concentration of ethanol in the rumen of animals consuming fermented feeds will depend on the hydrogen pressure.

Several studies using ruminal culture techniques have demonstrated that ruminal microbial metabolism of ethanol results primarily in acetate and methane (Chalupa et al., 1964; Czerkawski and Breckenbridge, 1972; Durix et al., 1991). In vivo, ethanol infusion has been followed by increasing ruminal acetate and caproate, by decreasing propionate and butyrate, and by variable responses in isovalerate and valerate among studies (Orskov et al., 1967; Frederiksen and Ochia, 1970; Pradhan and Hemken, 1970). To our knowledge, the present study is the first to report ruminal levels of ethyl acetate and propyl acetate. The decreased alcohol-ester proportions in the rumen, compared with feed, indicate that ruminal ester formation could be a biologically important pathway of alcohol metabolism. Esters of low molecular weight alcohols and VFA might have considerable biological activity and may modulate ruminal fermentation by selective inhibition of susceptible microorganisms. For example, ester-producing yeast inhibits molds in cocultures (Fredlund et al., 2004). The increase in ruminal propyl acetate following

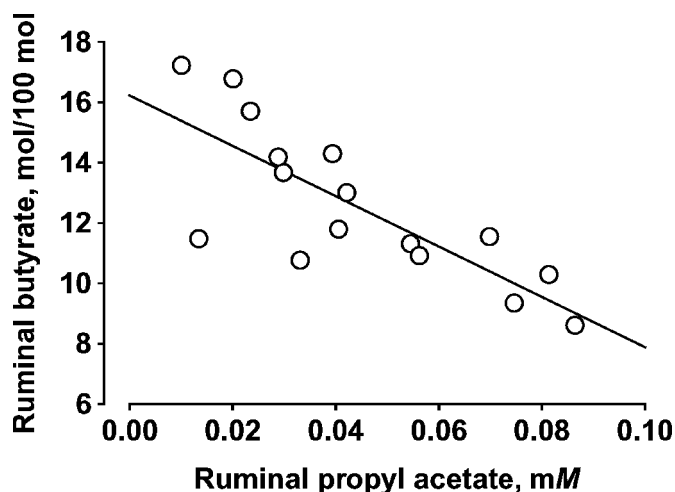


Figure 6. Relationship between the mean concentration of propyl acetate and the mean molar proportion of butyrate in the rumen of dairy cows. Each data point (16) is the mean of 10 sampling times ($r = -0.79$, $P < 0.01$).

increased propanol intake was negatively correlated with the molar proportion of butyrate in the rumen ($r = -0.79$, $P < 0.01$, $n = 16$; Figure 6). Which microorganisms might be more susceptible to inhibition by propyl acetate are not known. However, *Butyrivibrio fibrisolvens* is a major producer of butyrate and vaccenic acid in the rumen (Stewart et al., 1997), and if inhibition of these bacteria is responsible for the decrease in butyrate by being susceptible to propyl acetate, one could hypothesize that intake of propanol or intake of other substrates metabolized into propanol in the rumen (e.g., propylene glycol; Raun et al., 2005) would reduce both butyrate and vaccenic acid production in the rumen. Data from the present study point to the conclusion that even relatively low alcohol intakes could modulate ruminal fermentation through selective inhibition or stimulation of microorganisms in the rumen, or both.

Arterial Variables

No treatment effects were observed for any of the arterial variables. The peak concentration of arterial ethanol varied considerably between cows; however, even the highest concentrations in the present study, approximately 1.5 mM, were considerably lower than the level previously observed in ethanol-intoxicated calves (60 to 70 mM; Wijayasinghe et al., 1984). The large variation among cows might be related to differences in the eating rate, and whether similar variation can be expected between cows under normal production conditions is not known. Orskov et al. (1967) observed blood ethanol concentrations of 3.2 to 5.7 mM with an

intraruminal infusion of 875 g of ethanol/d. In the present study, arterial ethanol and propanol peaked at approximately 2.5 h after feeding and reached background concentrations 7 h after feeding, indicating that despite the high tolerance and metabolic capacity of the cow, ethanol intake will affect the systemic concentration for several hours after feeding, with a risk of transfer to milk and a negative impact on milk quality (Randby et al., 1999). Strong effects of sampling time were observed for all arterial nutrient variables as well as for blood pH and blood carbon dioxide. The arterial concentration of all metabolites except glucose increased after feeding; however, blood pH, blood carbon dioxide, and plasma glucose decreased. It is noteworthy, however, that plasma concentrations of nonalcohol metabolites peaked 1 to 2 h after the peak in alcohols, and the relative changes were smaller. Plasma glucose decreased from 4.00 ± 0.05 mM before feeding to 3.85 ± 0.06 mM 5 h after feeding and was not related to the net portal flux of glucose, which was highest (positive) 5 h after feeding. Plasma concentrations of glucose have previously been shown to decrease after feeding (Sutton et al., 1988; Plaizier et al., 2005), which could be caused by increased insulin levels after feeding induced by feed intake and by the stimulatory effects of other nutrients on the pancreas (Brockman, 1986). However, neither net hepatic flux nor net splanchnic output of glucose were affected by sampling time, so the decreased arterial concentration of glucose does not reflect quantitatively major changes in glucose metabolism by the splanchnic tissues.

Portal and Hepatic Blood Flows

The estimated ME intake in the present study was 180 MJ/d and the hepatic whole blood flow ($2,403 \pm 128$ L/h) was higher than predicted ($1,909$ L/h) from the linear equation presented by Reynolds (1995). However, data from lactating cows were much more variable than data from other cattle, and our data are within the presented range from dairy cattle (Reynolds, 1995). Contrary to most studies on splanchnic metabolism, we observed a postprandial increase in portal and hepatic blood flows. This was probably caused by the restricted feeding and intake of a large proportion of the feed allowance during a short time span after feeding, which gives larger diurnal variation compared with more meals and smaller meal sizes (Sutton et al., 1988). In meal-fed sheep, portal blood flow responds to feeding, in agreement with the present study (Kristensen et al., 1996a).

Splanchnic Oxygen Metabolism

The postprandial increase in hepatic oxygen consumption was striking when compared with the rela-

tively constant oxygen uptake by the PDV. This difference in the pattern of oxygen consumption is in line with the liver being the primary organ responding to a changing nutrient availability and with a relatively small proportion of absorbed nutrients being metabolized by the PDV in the first pass (Kristensen and Harmon, 2006; Reynolds, 2006). If the PDV had metabolized a large fraction of absorbed nutrients during absorption, as previously suggested (Bergman and Wolff, 1971; Tagari and Bergman, 1978), we would have expected increased oxygen consumption after feeding. The PDV and liver used 40 and $60 \pm 2\%$ of the net splanchnic oxygen uptake, respectively, which is in agreement with observations by Reynolds et al. (1988).

Net Fluxes of Glucose and L-Lactate

Despite an overall net portal flux of glucose close to zero (-16 ± 8 mmol/h), there was a marked diurnal variation with increasing (i.e., less negative) fluxes from 2.5 h after feeding, with the highest net portal fluxes observed 5 h after feeding (Figure 4). Similar diurnal variation was observed by Benson et al. (2002). Integrating the postprandial increase in net portal glucose flux (less negative flux) as the area over the background, as defined by the mean of the 3 first samples and the 24-h values as 2 times the sampled 12-h window, the total glucose absorption to portal blood was 201 g/d. Assuming a 50% recovery of glucose available in the small intestine (Kreikemeier and Harmon, 1995), this would be equivalent to 8% of the starch intake with the TMR, which is in good agreement with estimates of the ruminal bypass of 7 to 10% of starch in corn silage (Jensen et al., 2005).

Net Fluxes of 3-Hydroxybutyrate and Acetone

Rapid decarboxylation of acetoacetate into acetone and CO_2 (recoveries of 50 and 67% after 4 d at $+4$ or -20°C , respectively; Työppönen and Kauppinen, 1980) implies that immediate analysis of acetoacetate in blood plasma has to be performed to obtain the correct value. A lack of sufficient personnel to simultaneously oversee both samplings and multiple chemical analyses on experimental days often precludes the inclusion of acetoacetate. The method applied in the present study allows for storage of the sample because the remaining acetoacetate in the sample is decarboxylated to acetone upon analysis (Felby and Nielsen, 1994). The strong correlation between acetone and acetoacetate in blood and milk (Enjalbert et al., 2001) ensures that very little information is lost by pooling acetone and acetoacetate. Ruminal microbes reduce acetone transferred from the blood to the rumen into isopropanol (Bruss and Lopez,

2000); therefore, the net portal flux of total acetone measured in the present experiment had a small bias because it was a combination of a numerically small negative net portal flux of acetone and a positive net portal flux of acetoacetate. However, the acetone concentration in the blood of nonketotic cows is very low, and the error introduced was considered insignificant under the conditions of the present study. The relative net portal flux of acetone-acetoacetate and 3-hydroxybutyrate in the present study (0.44 ± 0.09) was comparable to the previously reported values of 0.28 to 0.35 (Lomax and Baird, 1983) and 0.30 to 0.55 (Krehbiel et al., 1992).

Absorption and Metabolism of Alcohols

To our knowledge, this is the first paper to present the quantitative portal absorption and hepatic metabolism of alcohols in dairy cattle. Across treatments, the intake of alcohol could be accounted for by absorption into the portal blood. However, although the portal recovery did not differ among treatments, the net portal flux, especially of propanol, appeared more similar among treatments than expected from the content in the TMR. That is, the TMR with the highest propanol contained >9 times more propanol than the lowest; however, the net portal flux of propanol with the highest propanol intake was only approximately 2.5 times the net portal flux with the lowest propanol intake. In the rat and human, a first-pass mucosal metabolism of ethanol is assumed to occur in the gastrointestinal tract (Seitz and Pöschl, 1997). This might also be the case in ruminants and, to the extent that a first-pass metabolism of alcohols occurs, the net portal flux will underestimate the net ruminal output of alcohols. The hepatic extractions of ethanol and propanol were comparable to the extraction ratio typically observed for butyrate (Kristensen and Harmon, 2005). There were no indications of saturation of the hepatic alcohol uptake with the present levels of alcohol absorption. We assume that the liver will primarily metabolize alcohols via alcohol and aldehyde dehydrogenases and that the hepatic extraction ratios observed indicate that the liver has a high affinity toward ethanol and propanol compared with, for example, propylene glycol, with a hepatic extraction ratio of approximately 8% (Raun et al., 2004). If the hepatic oxidation of ethanol and propanol (to acetate and propionate, respectively) is coupled with the reduction of NAD^+ to reduced NAD (NADH), there might be a risk of a reduced hepatic extraction of alcohols in ketotic animals because of the higher hepatic NADH: NAD^+ ratio (Zammit, 1990). The possible interaction between the hepatic redox state and alcohol metabolism could be involved in the greater sensitivity of

fresh cows consuming silage with a high alcohol content. We would hypothesize that if less alcohol could be oxidized by the liver, increased peripheral blood alcohol concentrations would result and increased concentrations of aldehydes would also be possible.

Aldehydes, which are derived from the oxidation of alcohols, are more reactive than alcohols and are regarded as key metabolites in mediating the toxic properties of alcohol consumption in monogastrics (Lieber, 1997; Holownia et al., 1999). Data from the present study indicated a numerically small net propanal output from splanchnic tissues and a numerically small splanchnic uptake of propanol, indicating that extra-splanchnic tissues were exposed to propanal and that this propanal was partly reduced to propanol. Overall, the metabolic capacity of splanchnic tissues in dairy cows is able to handle the silage propanol; however, alcohols and aldehydes are not completely cleared by the liver. A certain ratio between alcohols and aldehydes is expected in the liver and hepatic vein blood because of a close equilibrium with the NADH:NAD⁺ ratio in the liver (Krebs, 1969). In situations in which propanol intakes were higher, and if the hepatic redox status were to lead to a higher splanchnic release of propanal and acetaldehyde, these compounds would likely be toxic to dairy cows. Preliminary data from a study with intraruminal dosing of propylene glycol showed an increased arterial concentration of propanal following dosing of cows with 650 g of propylene glycol (Raun et al., 2005); however, this was not followed by any signs of acute intoxication. The ME intake with alcohols was relatively low (approximately 2 to 5% of ME), and alcohols will therefore be expected to supply less than 10% of the energy absorbed as VFA in cattle. Volatile fatty acid absorption accounts for approximately 55% of ME (Kristensen et al. 2005).

CONCLUSIONS

Silage alcohols are partly metabolized into low molecular weight esters in the rumen, and even natural levels of alcohols induced changes in the ruminal concentrations of esters that were correlated with changes in VFA fermentation in the rumen. Dairy cows under conditions of low metabolic stress are capable of metabolizing alcohols present in silage without apparent side effects on splanchnic metabolism; however, alcohol intake affects systemic blood concentrations of alcohols for at least 5 h after feeding.

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