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In vitro evaluation of agro-industrial by-products replacing barley in diets to dairy cows

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Introduction

A rapidly growing world population will demand a secure and increased global food supply in the future. Ruminant animals can utilize fibrous plant material not edible to humans efficiently and convert it into highly nutritious food for human consumption. A large number of by-products from the agricultural industry can thereby be suitable feed ingredients in diets to dairy cows. However, the use of agro-industrial by-products in feed rations to dairy cows have to be complementary to basal feed ingredients/efficient in terms of nutrient utilization and not lower production. Several *in vitro* techniques have been developed to enable rapid and cost-effective evaluation of feed resources as alternative to experiments with live animals. Recently, there has been great progress in the development of the automated gas *in vitro* technique, which enables comparison of treatment effects on diet digestibility, ruminal fermentation, digestion rate (k_d; Huhtanen et al., 2008) and CH_4 production (Ramin and Huhtanen, 2012). The aim of this study was to evaluate the effects of replacement of barley by some common agro-industrial by-products in diets based on grass silage on true organic matter digestibility (TOMD), volatile fatty acids (VFA) concentrations, diet k_d and CH_4 production *in vitro*.

Materials and Methods

Experimental diets for the gas *in vitro* incubation were composed from a basal diet of grass silage and rolled barley in the ratio 700:300 g/kg of diet dry matter (DM). Grass silage and barley were replaced by one of rolled barley, palm kernel cake (PKC), molasses, wheat bran or sugar beet pulp (SBP) in levels of 200 and 400 g/kg of diet DM. Replacements were such that the ratio of forage:concentrate was kept constant in all diets.

Two lactating Swedish Red cows fed a diet of 600 g/kg grass silage and 400 g/kg concentrate on DM basis *ad libitum* were used for the *in situ* incubation and for collection of rumen fluid for the *in vitro* incubations. Rumen fluid was collected from the same cows for all three *in vitro* incubations. The collected rumen fluid from each cow was strained separately through a double layer of cheesecloth into steel thermoses pre-heated to 39°C that had previously been flushed with CO_2. In the laboratory, rumen fluid was filtered through four layers of cheesecloth, mixed with a buffer-mineral solution (Menke and Steingass, 1988) and held in a water bath at 39°C under CO_2 saturation. The volume ratio of rumen fluid to buffer was 1:2. The experimental diets were subjected to *in vitro* incubations in which gas production was automatically recorded and corrected to normal atmospheric pressure (101.3 kPa; Cone et al., 1996). Dietary ingredients had previously been dried at 60ºC for 48 h and thereafter ground to pass a 1-mm screen using a Retsch SM 2000 cutting mill (Retsch GmbH, Haan, Germany). Diet samples of 1000 mg were weighed directly in 250 ml serum bottles (Schott, Mainz, Germany) and were incubated in 60 ml of buffered rumen fluid for 48 h. Incubations were conducted at 39°C and the bottles were continually agitated. All samples were incubated in

duplicate in three consecutive runs. All runs included triplicate bottles with blanks. Samples were randomly allocated to the different *in vitro* incubations flasks but never incubated in the same flasks in different runs. Mean blank gas production within run was subtracted from the sample gas production. Digestion rate was calculated from the cumulative gas production curve of each replicated experimental diet and predicted digestibility from a dynamic mechanistic rumen model as described by Huhtanen et al. (2008).

Gas samples were drawn from each bottle by a gas tight syringe (Hamilton, Bonaduz, Switzerland) at 2, 4, 8, 24, 32 and 48 h of incubation. Predicted *in vivo* CH_4 production was calculated as described by Ramin and Huhtanen (2012). Liquid samples of 0.6 ml for NH_3-N analysis were taken at 24 h of incubation and preserved with 0.024 ml of H_2SO_4. Another sample of 0.6 ml of buffered rumen fluid was collected at 48 h of incubation from the bottles and immediately stored at -20°C until processed for VFA determination. Samples for VFA analysis from the duplicate bottles in each run were pooled before analysis. The individual and total VFA productions were calculated by subtracting mean blank VFA concentration from the sample concentration. The TOMD was determined for all samples in all runs by analysing the neutral detergent fibre (NDF) concentrations in the residues after the 48 h incubations. Mean blank true *in vitro* digestibility within run was subtracted from the sample *in vitro* TOMD.

Residual moisture of all feed samples was determined by oven drying for 16 h at 105°C. Ash concentration was determined by ignition of the dried sample at 500°C for 4 h. Indigestible NDF (iNDF) concentration was determined by a 12-d *in situ* ruminal incubation according to the procedure of Krizsan et al. (2015). Samples were analyzed for NDF using a heat stable α-amylase (Mertens et al., 2002) in an ANKOM^{200} Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA). Values of NDF and iNDF were expressed on an ash-free basis. Concentrations of N were determined by Kjeldahl digestion of 1.0 g sample in 12 *M* sulfuric acid using Foss Tecator Kjeltabs Cu (Höganäs, Sweden) in a Block Digestion 28 system (SEAL Analytical Ltd., Mequon, WI, USA) with determination of total N by continuous flow analysis using an Auto Analyzer 3 (SEAL Analytical Ltd., Mequon, WI, USA). Individual VFA concentrations in rumen fluid samples were determined using a Waters Alliance 2795 UPLC system as described by Puhakka et al. (2016), and NH_3 according to the method provided by the SEAL Analytical (Method no. G-102-93 multitest MT7) using the AutoAnalyzer 3.

Data was analysed using the GLM procedure (SAS Inc. 2002-2003, Release 9.2; SAS Inst., Inc., Cary, NC, USA) by a model correcting for effect of run and experimental diet. Polynomial contrasts were included for evaluation of linear and quadratic responses to level of barley and by-product ingredient in the experimental diet, and diets with barley vs. by-products.

Results and Discussion

Chemical composition of experimental feed ingredients is presented in Table 1. Silage, barley and by-product ingredients displayed chemical composition within expected ranges (NRC, 2001; Alimon, 2004). *In situ* iNDF values indicated a potential digestibility of the NDF fraction of 705, 782 and 920 g/kg for PCK, wheat bran and SBP compared with 824 g/kg for barley. Crude protein concentrations were higher in PCK and wheat bran compared to barley, while both molasses and SBP displayed lower concentrations. Further, non-fibre

carbohydrates concentrations were much higher for molasses and SBP compared to PKC and wheat bran.

Table 1 Chemical composition of experimental dietary ingredients (g/kg DM)

			By-product feeds			
	Silage	Barley	PKC	Molasses	Wheat bran	SBP
Dry matter	259	779	922	718	896	917
Organic matter	919	972	948	877	936	924
Crude protein	143	129	179	101	139	79
Neutral detergent fibre (NDF)	552	239	606	NA	487	339
Non-fibre carbohydrates[a]	200	582	88	773	267	495
Indigestible NDF	NA	42	179	NA	106	27

PKC = palm kernel cake; SBP = sugar beet pulp; NA = not analysed; [a]Calculated using tabulated values of ether extracts for all feeds and for NDF for molasses from NRC (2001) and Alimon (2004).

Measurements derived from the gas *in vitro* incubation of the basal diet (grass silage and barley) and with replacement of grass silage and barley by barley and by-product feed ingredients at two levels of inclusion are in Table 2. Ammonia-N measured in buffered rumen fluid at 24 h after start of the incubation decreased (P=0.02) with increased barley and by-product inclusion. Changes in NH_3-N in buffered rumen fluid can be difficult to explain and can, in addition to diet degradation, be a result of degradation of feed particle from the rumen fluid medium, or at later time points, be due to microbial lysis and degradation. There was a quadratic increase (P=0.04) in total VFA production with the replacement of basal diet with barley and by-product feed ingredients indicating that diets were more fermentable at the 200 g/kg inclusion level. There was a linear decrease (P<0.01) in propionate with increased dietary inclusion level. As a result of the lower propionate, there was an increase in molar proportion of butyrate (P<0.01) with increased dietary inclusion level. Further, molar proportions of branched-chain VFAs increased quadratically and caproic acid increased linearly (P<0.01) with increased barley and by-product dietary inclusion level (P≤0.04). Predicted CH_4 production increased quadratically (P<0.01) with greater dietary supplementation. The PKC and wheat bran diets were lower in TOMD when compared to barley diets (P≤0.02). Otherwise, the lower potential digestibility of NDF in PKC and wheat bran, and the higher NFC in molasses and SBP than when compared to barley, were reflected in the fermentation profile and predicted CH_4 *in vivo*. The higher concentration of acetate (P<0.01) and generally lower concentration of butyrate (except PKC; P <0.01) for the by-product supplemented diets reflect a shift in fermentation when exchanging starch in barley to either more fibre or sugar containing dietary ingredients. Ertl et al. (2015) reported lower butyrate production from feeds containing hemicelluloses and pectins compared to those containing starch. Ruminal branched-chain VFA (i.e. isobutyric, isovaleric) and valeric and caproic acid primarily originate from dietary protein according to Tedeschi et al., (2000), and was generally decreased in by-product containing diets compared to diets only supplemented with barley, especially for molasses and SBP (P<0.01).

Conclusions

Replacing barley with molasses and SBP in grass silage-based diets did not decrease diet TOMD in buffered rumen fluid *in vitro*. However, both molasses and SBP inclusion changed

rumen fermentation profile towards more acetate and less butyrate, which might affect the production by dairy cows and beef cattle.

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Table 2 Measurements derived from the automated gas *in vitro* system of basal diet (grass silage and barley) replaced in two levels of diet dry matter (DM) with barley (B), palm kernel cake (PKC), molasses (M), wheat bran (WB) and sugar beet pulp (SBP)

	Basal	200 g/kg diet DM					400 g/kg diet DM						P-value[a]					
Item		B	PKC	M	WB	SBP	B	PKC	M	WB	SBP	SEM	C1	C2	C3	C4	Lin.	Quadr.
TOMD, g/kg	862	867	829	869	829	888	878	823	911	837	867	15.0	<0.01	0.25	0.02	0.73	0.83	0.57
NH_3-N_8, mg/l	247	407	329	210	377	219	313	385	153	426	215	53.0	0.96	<0.01	0.44	0.01	0.49	0.35
NH_3-N_{24}, mg/l	555	455	542	657	583	433	364	274	527	303	525	70.0	0.98	0.02	0.64	0.33	0.02	0.26
Total VFA, mmoles	1.95	2.24	2.12	2.20	2.12	2.58	2.22	1.82	2.25	1.90	2.24	0.150	0.10	0.99	0.16	0.24	0.78	0.04
Molar proportions, mmole/mole																		
Acetate	601	587	598	595	595	615	581	598	600	601	623	4.5	<0.01	<0.01	<0.01	<0.01	0.91	0.38
Propionate	242	236	220	238	232	228	230	201	244	227	224	3.4	<0.01	0.03	0.38	0.05	<0.01	0.24
Butyrate	117	132	136	128	127	116	144	151	122	128	116	2.4	0.03	<0.01	<0.01	<0.01	<0.01	0.06
Isobutyric acid	9.2	10.8	9.9	8.6	11.0	9.9	10.7	9.5	7.3	10.7	8.6	0.45	0.03	<0.01	0.81	<0.01	0.69	0.03
Isovaleric acid	7.4	9.0	8.1	6.7	9.2	7.7	8.5	7.6	5.1	8.7	6.6	0.50	0.10	<0.01	0.66	<0.01	0.44	0.04
Valeric acid	19.3	21.0	20.4	19.2	20.8	19.0	20.9	20.5	17.6	20.3	18.0	0.49	0.31	<0.01	0.41	<001	0.83	0.07
Caproic acid	4.0	5.1	7.5	4.6	4.6	4.6	5.6	12.0	4.4	4.6	4.7	0.32	<0.01	0.02	0.03	0.04	<0.01	0.60
k_d, 1/h	0.117	0.104	0.101	0.117	0.097	0.118	0.119	0.091	0.139	0.102	0.145	0.0100	0.12	0.12	0.24	0.06	0.54	0.14
CH_4[b], ml/g DM	35.3	39	36.1	40.5	38.1	40.0	40.9	36.7	43.5	36.3	41.8	0.53	<0.01	<0.01	<0.01	0.09	<0.01	<0.01

SEM = standard error of mean; TOMD = true organic matter digestibility, NH_3-N_8 = ammonia N in sampled rumen fluid 8 h after start of incubation; NH_3-N_{24} = ammonia N in sampled rumen fluid 24 h after start of incubation; Total VFA = volatile fatty acids (sum of all individual acids); k_d = diet digestion rate. [a]C1 = B vs. PKC; C2 = B vs. M; C3 = B vs. WB; C4 = B vs. SBP; Lin.= linear effect of supplementary inclusion level; Quadr. = quadratic effect of supplementary inclusion level; [b]Predicted CH_4 *in vivo*.

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