



Proceedings of the 8th Nordic Feed Science Conference, Uppsala, Sweden

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences Department of Animal Nutrition and Management Rapport 296 Report

Uppsala 2017 ISSN 0347-9838 ISRN SLU-HUV-R-296-SE Published by: Organising committee of the 8th Nordic Feed Science Conference Department of Animal Nutrition and Management Swedish University of Agricultural Sciences (SLU) SE- 753 23 Uppsala, Sweden

Copyright © 2017 SLU

All rights reserved. Nothing from this publication may be reproduced, stored in computerised systems or published in any form or any manner, including electronic, mechanical, reprographic or photographic, without prior written permission from the publisher (SLU).

The individual contributions in this publication and any liabilities arising from them remain the responsibility of the authors.

Organising Committee: Peter Udén Rolf Spörndly Bengt-Ove Rustas Torsten Eriksson Johanna Karlsson Kamyar Mogodiniyai Kasmaei

Edited by: P. Udén T. Eriksson R. Spörndly B-O. Rustas K. Mogodiniyai Kasmaei M. Liljeholm

Printed by: SLU Repro SE-75007 Uppsala, Sweden

Distributed by: Department of Animal Nutrition and Management, Box 7024, SE-75323 Uppsala, Sweden www.slu.se/animal-nutrition-management

This conference proceedings is available as PDF file updated with corrections (where applicable) at <u>http://www.slu.se/nordicfeedscienceconference</u> Menu item: Contributions

In vitro evaluation of agro-industrial by-products replacing barley in diets to dairy cows S.J. Krizsan¹, M. Ramin¹, F. Fatehi², R. Spörndly³ & P. Huhtanen¹

¹Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, S-901 83 Umeå, Sweden; ²University of Tehran, Campus of Agricultural and Natural Resources, Department of Animal Science, Karaj, Tehran, Iran 31587-77871. ³Swedish University of Agricultural Sciences, Department of Animal Nutrition & Management, Box 7024, SE-750 07 Uppsala, Sweden. Correspondence: Sophie.Krizsan@slu.se

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 CH₄ 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 CH₄ 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 CO₂. 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 CO₂ 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 60°C 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* CH₄ production was calculated as described by Ramin and Huhtanen (2012). Liquid samples of 0.6 ml for NH₃-N analysis were taken at 24 h of incubation and preserved with 0.024 ml of H₂SO₄. 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 ANKOM²⁰⁰ 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 NH₃ 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.

			By-product feeds							
	Silage	Barley	РКС	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				

Table 1 Chemical	composition of	f avnarimantal	diatary ina	radiants (a/ka DN	M)
Table I Chemical	composition of	i experimentar	uletary mg	redients (g/kg Dr	VI)

PKC = palm kernel cake; SBP = sugar beet pulp; NA = not analysed; ^aCalculated 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 NH₃-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 ($P \le 0.04$). Predicted CH₄ 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 ($P \le 0.02$). 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 CH₄ in vivo. The higher concentration of acetate (P<0.01) and generally lower concentration of butyrate (except PKC; P <0.01) for the byproduct 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.

References

Alimon, A.R., 2004. The nutritive value of palm kernel cake for animal feed. Palm oil Dev. 40, 12-14.

Cone, J.W., Van Gelder, A.H., Visscher, G.J.W, & Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Tech. 61, 113-128.

Ertl, P., Knaus, W., Metzler-Zebeli, B.U., Klevenhusen, F., Khiaosa-Ard, R. & Zebeli, Q., 2015. Substitution of common concentrates with by-products modulated ruminal

fermentation, nutrient degradation, and microbial community composition in vitro. J. Dairy Sci. 98, 4762-4771.

Huhtanen, P., Seppälä, A., Ots, M., Ahvenjärvi, S. & Rinne, M., 2008. In vitro gas production profiles to estimate extent and effective first-order rate of neutral detergent fiber digestion in the rumen. J. Anim. Sci., 86, 651-659.

Krizsan, S.J., Rinne, M., Nyholm, L. & Huhtanen, P., 2015. New recommendations for the ruminal *in situ* determination of indigestible neutral detergent fibre. Anim. Feed Sci. 205, 31-41.

Menke, K.H. & Steingass. H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28, 7-25.

Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J. & Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with Refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85, 1217-1240.

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy of Sciences, Washington, DC.

Puhakka, L., Jaakkola, S., Simpura, I., Kokkonen, T., & Vanhatalo, A., 2016. Effects of replacing rapeseed meal with fava bean at 2 concentrate crude protein levels on feed intake, nutrient digestion, and milk production in cows fed grass silage-based diets. J. Dairy Sci. 99, 7993-8006.

Ramin, M., & Huhtanen, P., 2012. Development of an in vitro method for determination of methane production kinetics using a fully automated in vitro gas system-A modelling approach. Anim. Feed. Sci. Technol., 174, 190-200.

Tedeschi L.O., Fox D.G., & Russell, J.B., 2000. Accounting for ruminal deficiencies of nitrogen and branched-chain amino acids in the structure of the Cornell net carbohydrate and protein system. Proceedings of Cornell Nutrition Conference for Feed Manufacturers; New York: Cornell University; 2000.

Posters

	Basal	200 g/kg diet DM					400 g/kg diet DM						P-value ^a					
Item		В	РКС	М	WB	SBP	В	РКС	М	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	e/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
CH4 ^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

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)

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 CH₄ *in vivo*.

DISTRIBUTION: Sveriges Lantbruksuniversitet Institutionen för husdjurens utfodring och vård Box 7024 750 07 UPPSALA Tel.018/672817

Margareta.Norinder@slu.se