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In vitro evaluation of agro-industrial by-products in diets for cattle

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The objective of this *in vitro* batch culture study was to evaluate various agro-industrial by-products as feeds for cattle. In Experiment 1, a basal diet composed of grass silage and barley grain was replaced by additional barley grain, palm kernel cake (PKC), beet molasses (M), wheat bran (WB), or sugar beet pulp (SBP) at two levels of dietary supplementation. In Experiment 2, soybean meal (SBM) was compared with heat-treated rapeseed meal, dried distillers´ grains (DDG), rapeseed cake (RSC), and rapeseed meal (RSM) at two levels of dietary protein concentration in diets based on grass silage supplemented with barley grain or SBP. Propionate and branched chain volatile fatty acids decreased, and CH_4 production increased when energy by-products of higher fibre content replaced barley grain. Diets incorporating PKC and WB were less fermentable, while M and SBP did not alter digestibility when replacing barley. Generally, incremental levels of protein in Experiment 2 linearly increased digestibility, utilisable crude protein (uCP), isobutyrate and valerate *in vitro*. Utilisable crude protein increased for all by-products that replaced SBM, except for RSM in diets including SBP. There was a positive linear effect of by-product level on uCP and valerate, and a negative linear effect for acetate. Diet digestibility was equivalent for DDG and SBM in diets composed with barley as well as SBP. Overall, by-products provided more uCP than diets supplemented with SBM. However, the intestinal digestibility of uCP of the different feeds can vary. *In vivo* production experiments are needed to fully assess the potential of *in vitro* evaluated by-products as widely applicable alternative feeds in diets for cattle.

Key words: barley, by-products, methane, protein, soybean meal, utilisable crude protein

Introduction

The growing worldwide population, which is predicted to reach 11 billion people by the next century, will demand a secure and increased global food supply. Despite the increased food production in the past 50 years, not everyone has access to sufficient protein and energy within their diet (FAO et al. 2020). Additionally, food consumption and food trade patterns within developed countries have evolved, mainly towards an increased consumption of high-value foods with a larger environmental footprint, such as meat and refined dairy products. Currently, the environmental issues which are primarily raised within ruminant livestock production systems are related to greater use of agrochemicals and greenhouse gas emissions thereby contributing to global warming (Cederberg et al. 2019). This issue has launched an aim across several countries in Northern Europe to ensure, not only increased, but also sustainable production of food. It is likely that despite climate change, Northern Europe will also in the future have access to the most important natural resources such as agricultural arable land, pastures and water for food production (Olesen et al. 2011). Dairy foods have a beneficial nutrient composition and hold important characteristics such as hypotensive and muscle protein synthesis stimulation effects (Givens 2020). This suggests an important role from dairy farming systems in future food production.

Food production based on grass-fed ruminants can become more sustainable by developing grasslands through better management, feed evaluation, and improved varieties and through the implementation of efficient environmental goals (including CH_4 mitigation strategies), and targeted supplementation of forage-based diets with human-inedible resources (e.g. agro-industrial by-products) to improve human-edible output in the production (Ertl et al. 2015b, 2016, Whelan et al. 2017, Krizsan et al. 2021). To utilize the genetic potential for milk production by modern dairy cows, grass-based diets are supplemented with high energy and protein supplements to increase feed and nutrient intake. Many by-products from the agricultural and food industries can be used in dairy cow diets. However, few studies have been conducted where by-products relevant to Nordic conditions have been evaluated in wider comparisons simultaneously. *In vivo* studies are both expensive and laborious, but use of an automated *in vitro* gas production technique enables a large comparative screening of suitable by-products in cattle diets.

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Additionally, there has recently been significant progress in the development of the automated gas *in vitro* technique, which enables a complete evaluation of ruminant digestion (Huhtanen et al. 2008), CH_4 production (Ramin and Huhtanen 2012) and estimates of utilisable crude protein (uCP) (Edmunds et al. 2012).

The primary objective of this *in vitro* study was to compare a wide variety of agro-industrial by-products, that are readily available in Northern Europe for inclusion in ruminant diets. The use of agro-industrial by-products in diets to ruminants seems particularly suitable due to a generally high fibre content. There is a limited ability of monogastric livestock species to digest fibre supporting an improved feed utilisation when agro-industrial by-products are fed to ruminants. We hypothesized that currently and commonly used human-edible barley grain and soybean meal (SBM) can be replaced by agro-industrial by-products with no or limited food use in grass silage-based diets for dairy cows. The objective of this study was to evaluate the effect of several agro-industrial by-products as feed supplements in grass silage-based diets fed to dairy cows on digestibility and fermentation, feed value traits (e.g. uCP), and production of CH_4.

Materials and methods

The animals used for rumen fluid collection in this experiment were registered and cared for according to the guidelines approved by the Animal Care and Use Committee of Swedish University of Agricultural Sciences. The experiment was also carried out in accordance with the laws and regulations controlling experiments using live animals in Sweden.

Experimental samples and diets

To effectively evaluate energy and protein by-products, this study was conducted as two separate experiments. In the first experiment, energy by-products were evaluated (Experiment 1). In Experiment 1 diets were composed from grass silage and barley grain in a ratio of 700:300 g kg^{-1} of dietary dry matter (DM). The basal diet consisting of grass silage and barley grain was replaced with additional barley grain, palm kernel cake (PKC), beet molasses (M), wheat bran (WB), or sugar beet pulp (SBP) to inclusions of two levels of 200 and 400 g kg^{-1} of diet DM. Replacements were made to ensure that the grass silage to barley grain ratio remained constant across all diets (i.e. forage to barley grain ratio was 700:300, 560:240 and 420:180 of control diet and of the two inclusion levels of by-products) except when additional barley grain were used as replacement. Protein by-products were evaluated in the second experiment (Experiment 2). The diets used as controls were grass silage:barley grain and grass silage:SBP using a ratio 600:400 g kg^{-1} of dietary DM. Soybean meal (SBM) was used as the conventional crude protein (CP) source and was replaced with heat-treated rapeseed meal (Expro®; AAK Sweden AB, Karlshamn, Sweden), dried distillers´ grains with solubles (DDG) (Agrodrank™90; Lantmännen Agroetanol AB, Norrköping, Sweden), rapeseed cake (RSC) or rapeseed meal (RSM). Inclusions of protein by-products were made at two levels of CP in the diets to increase the CP concentration by 20 g kg^{-1} of diet DM per increment, aiming to give dietary CP concentration of 146, 166 and 186, and 126, 146 and 166 g kg^{-1} of diet DM for barley grain and SBP based diets, respectively. Replacements were made so that all treatments had the same grass silage:barley grain or grass silage:SBP ratio across all experimental diets in line with the composition of diets in Experiment 1.

The grass silage was harvested from a primary growth of a timothy (*Phelum pratense*) ley. The grass silage was harvested from a primary growth of a timothy (*Phleum pratense*) ley and ensiled using an acid-based additive (Promyr XR 630, Perstorp, Sweden), which was applied at a rate of 3.5 l $tonne^{-1}$. The grass was ensiled and stored in a bunker silo after wilting overnigh in the field. Samples of the agro-industrial by-products were provided by the feed manufacturer AB Västerbottens Fodercentral in Umeå, Sweden, if otherwise not stated.

In vitro and *in situ* incubations

Two rumen cannulated lactating Swedish Red cows fed *ad libitum*, a total mixed ration composed of 600 g kg^{-1} grass silage and 400 g kg^{-1} concentrate on DM basis, were used to collect rumen fluid for the *in vitro* incubations. Rumen fluid was collected from the same cows for all *in vitro* incubations at 2 h after morning feeding. Cows were separated individually by gates in alleys next to the collection area at the milking parlor. The rumen content was collected through the fistulas by grab samples from top to middle regions of the rumen (with vivid fermentation). The collected rumen content was squeezed and the fluid from each cow was strained separately through a double layer of cheesecloth into pre-heated (39 °C) insulated steel bottles that had been previously flushed with CO_2.

One thermos of almost 1500 ml of rumen fluid per cow was collected before each *in vitro* incubation and then immediately brought to the laboratory. In the laboratory, rumen fluid from the two cows was blended carefully in equal parts and filtered through four layers of cheesecloth into a bottle kept in a water bath at 39 °C and under constant CO_2 saturation.

Prior to the incubation of Experiment 2 the rumen fluid was pre-incubated for 3 h with a carbohydrate mixture according to the procedure described by Gidlund et al. (2018). In this procedure, a mixture of maltose (3.2 g), starch (1.6 g), xylose (1.6 g), pectin (1.6 g), and $NaHCO_3$ (2.8 g) dissolved in buffer as described by Menke and Steingaß (1988) was added to the rumen fluid, which was stirred for 10 min at 39 °C. After 30 min, the top layer of foam was removed with a vacuum pump and the stirrer was turned on again. The rumen fluid was then incubated at 39°C under a constant flush of CO_2 for an additional 2.5 h. Following pre-incubation, the rumen fluid was mixed with a low-N bicarbonate buffer (20:80 vol/vol), micro (0.24 ml in buffer solution) and macro minerals (482.35 ml in buffer solution) and resazurin (2.47 ml in buffer solution) according to the procedure described by Gidlund et al. (2018).

Diets of 1000 mg DM and 500 mg DM for Experiment 1 and 2, respectively, were previously weighed directly in 250-ml serum bottles (Schott, Mainz, Germany), which were flushed with CO_2 prior to incubation. All diets were incubated in 60 ml of the buffered rumen fluid for 48 h. Incubations were conducted at 39 °C in 36 bottles continually agitated in three separate water baths throughout the entire incubation. All diets were incubated in three consecutive runs, resulting in three replicates per diet, and treatments were randomly allocated between different in vitro flasks. All runs included triplicate bottles with blanks divided equally in the three separate water baths. The fully automated gas *in vitro* system used in both experiments was a custom made device obtained from Wagening University in the Netherlands and have been described earlier by Ramin (2013) and by Gidlund (2017).

The same two cows were used for a 288 h in situ incubation according to Huhtanen et al. (1994) to determine the concentration of indigestible neutral detergent fibre (iNDF) in all feed samples. Samples of 2 (±0.1) g were weighed in polyester bags of 11 µm pore size (07-11/5 Sefar Petex; Sefar AG, Heiden, Switzerland) as described by Krizsan et al. (2015). All samples were incubated in duplicates.

Sample collection and calculations

Gas production was automatically recorded and corrected to normal atmospheric pressure (101.3 kPa; Cone et al. 1996). 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 based on digestibility using a dynamic mechanistic rumen model, as described by Huhtanen et al. (2008). A 2-pool Gompertz model was fitted to the cumulative gas production profile and parameter estimates were used in a dynamic mechanistic rumen model (Huhtanen et al. 2008) to determine digestibility of potentially digestible DM. The digestibility equation by Allen and Mertens (1988) was solved for digestion rate using a rumen residence time of 50 h distributed between the two compartments in a ratio of 40:60.

In Experiment 1, gas samples were drawn from each bottle using a gas tight syringe (Hamilton, Bonaduz, Switzerland) at 2, 4, 8, 24, 32 and 48 h of incubation, while only at 24 and 48 h of incubation in Experiment 2. Methane production was calculated as described by Ramin and Huhtanen (2012). In brief, concentration of CH_4 gas was determined by injecting 0.2 ml of collected gas into a star 3400 (CX series) gas chromatograph (Varian Chromatography, Palo Alto, CA, USA) equipped with a thermal conductivity detector. Calibration gas of a mixture of CO_2 and CH_4 (100 mmol mol^{-1}) prepared by AGA Gas AB (Sundbyberg, Sweden) was used. Mean blank gas production within run was subtracted from sample gas production. In vivo predicted CH_4 production was calculated as:

CH_4 (ml) = 265 (ml) x CH_4 concentration (ml l^{-1}) + total gas production (ml) x CH_4 concentration (ml l^{-1}) x 0.55

where CH_4 represents cumulative CH_4 produced at a given time point, 265 is the total headspace volume, and 0.55 is the ratio of CH_4 emissions in the outflow gas from the *in vitro* system. A mean retention time of 50 h (20 h in the first compartment and 30 h in the second compartment), corresponding to the maintenance level of feed intake, was used in model simulations of *in vivo* predicted CH_4 in Experiment 1.

Liquid samples of 0.6 ml for NH_3-N analysis were taken from each bottle using a plastic syringe at 24 and 48 h of incubation in Experiment 1, and at 8, 16, 24, and 30 h after incubation in Experiment 2. The liquid samples were preserved with 0.024 ml of 18 M H_2SO_4. Utilisable CP at 16 h was calculated as described by Edmunds et al. (2012):

uCP (g kg^{-1}) = (NH_3-N_{blank} + N_{sample} – NH_3-N_{sample}) / (sample weight [mg DM]) × 6.25 × 1000

In both experiments, samples of 0.6 ml of rumen fluid were collected at 48 h of incubation from the bottles and immediately stored at –20 °C until being processed for volatile fatty acid (VFA) determination. Total VFA production was calculated as a sum of individual VFA by subtracting the mean value of blank within run and multiplying it with 60 ml (the volume of buffered rumen fluid).

After 48 h incubation, all flasks were removed from the baths and placed into crushed ice to prevent fermentation. In vitro incubation residues were quantitatively transferred to the same 11-µm polyester bags that were used for the *in situ* incubation. *In vitro* true organic matter digestibility (TOMD) was determined for all diets in all runs by analyzing the ash-free NDF concentrations in the residues after the 48 h incubations and subtracting that from the amount of OM at the start of incubation according to Van Soest (1992). Mean blank true *in vitro* digestibility within run was subtracted from the sample *in vitro* TOMD.

Chemical analyses

All feed and by-product samples were dried at 60 °C for 48 h and were ground through a 1.0-mm screen using a cutting mill Retsch SM 2000 (Retsch GmbH, Haan, Germany) prior to chemical analysis and *in vitro* incubations. Samples for *in situ* incubations were ground with mortar and pestle, and manually sieved through a 2.5-mm sieve.

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 samples at 500 °C for 4 h. The samples, and *in vitro* and *in situ* residues were analyzed for NDF including sodium sulphite and 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. Concentration of N was determined by Kjeldahl digestion of 1.0 g sample in 12 M H_2SO_4 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). Crude protein (CP) was determined from N concentrations multiplied by 6.25. 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 nr G-102-93 multitest MT7) using an Auto Analyzer 3 (SEAL Analytical Ltd., Mequon, WI, USA). The protein by-products were analyzed in a commercial laboratory (Dairy One, Ithaca, NY, USA) for Cornell protein fractions according to Higgs et al. (2015), and for neutral and acid detergent insoluble CP.

Statistical analysis

Data were analyzed using the GLM procedure (SAS Inc. 2002–2003, Release 9.2; SAS Inst. Inc., Cary, NC, USA) of SAS with a model correcting for the fixed effects of run and experimental diet. Orthogonal polynomial contrasts were used to evaluate linear and quadratic responses to level of by-products, and to compare by-product diets with the control diet in both experiments (barley vs. by-products or SBM vs. by-products). *p*-values less than 0.05 were considered statistically significant and less than 0.10 indicated a tendency for significance.

Results

Experiment 1

Chemical composition of experimental feed ingredients for Experiment 1 is presented in Table 1. *In situ* iNDF values indicated a potential digestibility of the NDF fraction ranging between 705 and 920 g kg^{-1} for energy by-products (PKC and SBP, respectively) compared with 824 g kg^{-1} for barley. For protein by-products this ranged between 563 and 781 g kg^{-1} (RSM and DDG, respectively) compared with 975 g kg^{-1} for SBM. Crude protein concentrations were higher in PKC and WB compared with barley, while both M and SBP displayed lower concentrations. Further, non-fibre carbohydrate concentrations were much higher for M and SBP compared to PKC and WB. Dietary feed composition of incubated experimental diets in Experiment 1 is given in Appendix 1.

Table 1. Chemical composition of experimental dietary ingredients (g kg^{-1} DM unless otherwise stated)

						By-products							
						Energy (Experiment 1)				Protein (Experiment 2)			
Item	Silage[1]	Silage[2]	Barley	SBP[3]	SBM	PKC	M	WB	SBP[4]	Expro®	DDG	RSC	RSM
DM, g kg^{-1}	259	255	779	917	854	922	718	896	917	906	877	921	911
OM	919	842	972	848	925	948	877	936	924	837	827	859	840
CP	143	157	129	78	496	179	101	139	79	387	315	378	392
NDF	552	611	239	339	237	606	NA	487	339	322	288	251	270
NFC[5]	200	286	582	578	688	88	773	267	495	584	589	670	641
iNDF	85	102	42	30	6	179	NA	106	27	129	63	109	118

[1]Grass silage used in Experiment 1; [2]Grass silage used in Experiment 2; [3]Sugar beet pulp used in basal diet in Experiment 2; [4]Sugar beet pulp used as by-product in Experiment 1; [5]Calculated using tabulated values of ether extracts for all feeds and for NDF for molasses from NRC (2001) and Alimon (2004). SBM: soybean meal; PKC: palm kernel cake; M: beet molasses; WB: wheat bran; SBP: sugar beet pulp; Expro®: heat-treated RSM; DDG: dried distillers' grains; RSC: rapeseed cake; RSM: rapeseed meal; NA: not analysed; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; NFC: non-fibre carbohydrate; iNDF: indigestible neutral detergent fibre

Measurements derived from the gas in vitro incubation of the basal diet (grass silage and barley), and with the replacements of barley and by-product feed ingredients at two levels of inclusion are presented in Table 2. *In vitro* TOMD was lower ($p \leq 0.02$) in diets including PKC and WB compared with barley. Ammonia-N measured in buffered rumen fluid at 8 h after start of the incubation was lower ($p \leq 0.01$) for M and SBP compared with barley, and lower at 24 h for M compared to barley. Furthermore, NH_3-N at 24 h decreased ($p \leq 0.02$) with increased barley and by-product inclusion.

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 PKC, WB and SBP diets were most fermentable at the 200 g kg^{-1} inclusion level. All by-product ingredients resulted in a fermentation with higher ($p < 0.01$) molar proportion of acetate than diets with barley, which was also reflected in less ($p \leq 0.05$) propionate except for diets with WB inclusion. There was a linear decrease ($p < 0.01$) in propionate and increase ($p < 0.01$) in butyrate with increased dietary inclusion levels of PKC. All by-product ingredients induced a fermentation that was lower ($p < 0.01$) in molar proportion of butyrate when compared with diets including barley, except with PKC that had a higher ($p = 0.03$) molar proportion. Further, molar proportions of branched-chain VFA increased ($p \leq 0.03$) quadratically giving the highest molar proportion at 200 g kg^{-1} diet DM inclusion. The by-products PKC, M and SBP were lower ($p \leq 0.03$) in molar proportion of isobutyric acid than barley. Additionally, M and SBP were also lower ($p < 0.01$) in molar proportion of isovaleric acid compared with barley. Molar proportions of valeric acid was lower ($p < 0.01$) in M and SBP than in diets including barley. Molar proportions of caproic acid increased ($p < 0.01$) linearly with increased barley and PKC inclusion level. All by-product ingredients were lower ($p \leq 0.04$) in molar proportion of caproic acid, except PKC which was higher ($p < 0.01$), than diets with barley.

Predicted CH_4 production increased ($p < 0.01$) quadratically with higher dietary supplementation of barley and by-product ingredients. The by-products PKC and WB gave less ($p < 0.01$) *in vivo* predicted CH_4 than diets including barley, while M inclusion increased ($p < 0.01$) *in vivo* predicted CH_4 compared with barley.

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), beet molasses (M), wheat bran (WB) and sugar beet pulp (SBP) (Experiment 1)

Item	Basal diet	200 g kg^{-1} diet DM					400 g kg^{-1} diet DM					SEM	*p*-value[1]					
		B	PKC	M	WB	SBP	B	PKC	M	WB	SBP		C1	C2	C3	C4	Lin	Quad
TOMD, g kg^{-1}	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^{-1}	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^{-1}	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, mmoles mole^{-1}																		
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	0.06	<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, h^{-1}	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[2] ml g^{-1} DM	35.3	39.0	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. [1]C1 = B vs. PKC; C2 = B vs. M; C3 = B vs. WB; C4 = B vs. SBP; Lin = linear effect of supplementary inclusion level (significant if at least one treatment display a linear effect); Quad = quadratic effect of supplementary inclusion level (significant if at least one treatment display a quadratic effect); [2]Predicted CH_4 *in vivo*

Experiment 2

In Experiment 2, levels of CP in the by-products ranged between 315 and 392 g kg^{-1} DM (DDG and RSM) compared to 496 g CP kg^{-1} DM in SBM (Table 3). Inclusions of protein by-products were made at two levels of CP in the diets, which resulted in dietary CP of 146, 166, and 186 g kg^{-1} DM, respectively, for diets based on silage and barley, and dietary CP of 126, 146 and 166 g kg^{-1} DM, respectively, in diets based on silage and beet fibre. Dietary feed composition of incubated experimental diets in Experiment 2 is given in Appendix 2.

Table 3. Protein fractions (g kg^{-1} CP) according to Higgs et al. (2015), and neutral and acid detergent insoluble crude protein of soybean meal and protein by-products (g kg^{-1} DM)

Item	SBM[1]	Expro	DDG	RSC	RSM
Cornell protein fractions[2]					
A1	55	100	307	145	131
A2	2	27	35	152	212
B1	673	600	243	431	344
B2	128	164	123	180	213
C	142	109	291	92	100
Fibre-bound protein fractions[3]					
NDICP	127	104	127	102	122
ADICP	67	42	89	35	39
NDICP – ADICP	60	62	38	67	83

[1]SBM= soybean meal; Expro®= heat-treated RSM; DDG = dried distillers' grains; RSC = rapeseed cake; RSM = rapeseed meal; [2]A1 = ammonia; A2 = soluble true protein; B1 = insoluble true protein; B2 = fibre-bound protein; C = indigestible protein; [3]NDICP = neutral detergent insoluble crude protein; ADICP = acid detergent insoluble crude protein

Table 3 reports the protein fractions according to Higgs et al. (2015), and neutral detergent in soluble CP (NDICP) and acid detergent insoluble CP (ADICP) of SBM and protein by-products. There were some differences in protein fractions among the different supplements. Dried distillers´ grains were the highest in ammonia (A1) and indigestible protein (C), while SBM and Expro were clearly higher in insoluble true protein (B1). Moreover, RSC and RSM were the supplements highest in soluble true protein (A2). The differences in fibre bound protein fractions were relatively small between the protein supplements, but SBM and DDG were highest in ADICP among the feeds. This rendered the smallest value for DDG of the difference between NDICP and ADICP.

The effect of two inclusion levels of protein by-products replacing SBM on digestibility, uCP, fermentation parameters, and CH_4 production in diets based on grass silage supplemented with barley grain or SBP are provided in Tables 4 and 5, respectively. The results indicated that higher dietary CP concentration linearly increased (p= 0.03) TOMD in diets composed of barley and SBM. All by-product inclusions except DDG decreased ($p\leq$ 0.05) TOMD compared to SBM, except for RSC in the diet based on SBP. Generally, uCP increased linearly ($p<$ 0.01) with increased CP concentration of the diets. All by-product inclusions gave higher ($p<$ 0.01) estimates of uCP than SBM, except for RSM in the diet based on SBP.

Molar proportions of acetate decreased ($p<$ 0.01) linearly with increased dietary CP concentration in diets composed of barley and SBP. Notably, DDG generated less ($p<$ 0.01) acetate and butyrate, and more ($p<$ 0.01) propionate than SBM in diets with both barley and beet fibre. Expro and RSM also resulted in higher ($p\leq$ 0.03) propionate than SBM in diets with beet fibre. Molar proportions of butyrate and valerate increased linearly ($p\leq$ 0.05) with higher dietary CP concentration in diets with barley and SBP. In diets with barley, isobutyrate also increased ($p<$ 0.01) linearly with increased dietary CP concentration. All by-products gave more ($p\leq$ 0.03) valerate than SBM in diets with barley, but only with Expro and DDG when diets were composed with SBP. In diets with barley, DDG and RSC gave less ($p\leq$ 0.01) CH_4. In diets with SBP, Expro, DDG and RSM produced less ($p\leq$ 0.04) CH_4 compared with diets with SBM.

Table 4. Effect of increasing level of agro-industrial by-products replacing soybean meal (SBM) on digestibility, estimated utilisable crude protein, fermentation parameters and CH_4 production in diets based on silage and barley (Experiment 2)

Item	Basal diet 146 g CP kg^{-1} DM	Diets 166 g CP kg^{-1} DM					Diets 186 g CP kg^{-1} DM					SEM	*p*-value[1,2]				
		SBM	Expro	DDG	RSC	RSM	SBM	Expro	DDG	RSC	RSM		C1	C2	C3	C4	Lin
TOMD, g kg^{-1}	849	856	849	864	852	857	869	849	859	855	853	3.3	<0.01	0.60	0.01	0.02	0.03
uCP, g kg^{-1} DM	150	158	164	165	161	163	167	179	182	172	171	0.8	<0.01	<0.01	<0.01	<0.01	<0.01
Total VFA, mmole	3.54	3.54	3.28	3.48	3.58	3.62	3.58	3.57	3.43	3.27	3.34	0.118	0.28	0.37	0.27	0.52	0.37
Molar proportions, mmoles mole^{-1}																	
Acetate	591	590	586	585	594	591	589	587	579	584	590	1.9	0.15	<0.01	0.89	0.66	0.01
Propionate	228	226	229	233	221	228	227	227	241	227	224	1.9	0.47	<0.01	0.18	0.71	0.41
Butyrate	111	111	107	105	110	109	108	109	102	107	108	1.1	0.20	<0.01	0.42	0.30	<0.01
Isobutyrate	19	20	20	19	20	19	20	20	20	21	20	0.4	0.94	0.12	0.33	0.91	<0.01
Valerate	27	27	29	31	29	28	28	31	31	32	30	0.5	<0.01	<0.01	<0.01	0.03	<0.01
Isovalerate	25	26	28	27	26	25	27	26	27	29	28	1.1	0.60	0.90	0.50	0.91	0.10
k_d, h^{-1}	0.073	0.073	0.072	0.074	0.077	0.077	0.077	0.068	0.077	0.077	0.082	0.0028	0.09	0.80	0.42	0.10	0.26
CH_4, ml g^{-1} DM	53.5	53.3	52.1	50.9	49.6	51.8	53.9	51.4	49.8	51.9	52.9	1.06	0.09	<0.01	0.01	0.24	0.33

CP = crude protein; Expro® = heat-treated rapeseed meal; DDG = dried distillers' grains; RSC = rapeseed cake; RSM = rapeseed meal; SEM = standard error of mean; TOMD = true organic matter digestibility; uCP = utilisable crude protein; Total VFA = volatile fatty acids (sum of all individual acids); k_d = diet digestion rate. [1]C1 = SBM vs. Expro; C2 = SBM vs. DG; C3 = SBM vs. RSC; C4 = SBM vs. RSM; Lin = linear effect of supplementary inclusion level (significant if at least one treatment display a linear effect). [2]Quadratic term was not significant.

Table 5. Effect of increasing level of agro-industrial by-products replacing soybean meal (SBM) on digestibility, estimated utilisable crude protein, fermentation parameters and CH_4 production in diets based on silage and beet fibre (Experiment 2)

Item	Basal diet 126 g CP kg^{-1} DM	Diets 146 g CP kg^{-1} DM					Diets 166 g CP kg^{-1} DM					SEM	*p*-value[1,2]				
		SBM	Expro	DDG	RSC	RSM	SBM	Expro	DDG	RSC	RSM		C1	C2	C3	C4	Lin
TOMD, g kg^{-1}	841	850	837	856	838	839	855	845	846	846	842	6.0	0.05	0.85	0.09	0.05	0.33
uCP, g kg^{-1} DM	140	151	152	155	152	151	158	167	171	162	162	1.0	<0.01	<0.01	<0.01	0.08	<0.01
Total VFA, mmole	3.56	3.73	3.41	3.50	3.73	3.45	3.89	3.68	3.58	3.60	3.74	0.158	0.11	0.09	0.37	0.18	0.08
Molar proportions, mmoles mole^{-1}																	
Acetate	633	628	622	623	627	623	623	619	613	620	622	2.6	0.07	<0.01	0.45	0.28	<0.01
Propionate	230	222	228	231	222	228	224	225	238	228	226	1.6	0.03	<0.01	0.17	0.02	0.70
Butyrate	81	85	84	80	85	83	86	86	81	83	86	1.3	0.50	<0.01	0.20	0.34	0.05
Isobutyrate	17	18	19	18	18	18	18	19	17	18	18	0.7	0.24	0.28	0.86	0.70	0.32
Valerate	22	24	26	26	25	24	25	28	27	26	26	0.6	<0.01	0.01	0.30	0.54	<0.01
Isovalerate	24	23	26	23	23	25	24	23	24	25	22	1.1	0.30	0.94	0.58	0.95	0.73
k_d, h^{-1}	0.076	0.077	0.074	0.081	0.079	0.077	0.078	0.077	0.082	0.088	0.083	0.0030	0.54	0.24	0.06	0.43	0.05
CH_4, ml g^{-1} DM	52.8	52.1	52.0	50.9	49.8	51.9	55.6	51.6	51.8	54.9	51.1	1.10	0.04	0.01	0.14	0.02	0.36

CP = crude protein; Expro® = heat-treated rapeseed meal; DDG = dried distillers' grains; RSC = rapeseed cake; RSM = rapeseed meal; SEM = standard error of mean; TOMD = true organic matter digestibility; uCP = utilisable crude protein; Total VFA = volatile fatty acids (sum of all individual acids); k_d = diet digestion rate. [1]C1 = SBM vs. Expro; C2 = SBM vs. DDG; C3 = SBM vs. RSC; C4 = SBM vs. RSM; Lin = linear effect of supplementary inclusion level (significant if at least one treatment displays a linear effect). [2]Quadratic term was not significant, except for propionate ($p < 0.01$) and CH_4 production ($p = 0.02$).

Discussion

The aim of the current *in vitro* study was to explore the various effects of ingredients included in whole diets, because ruminants are fed multiple ingredients that interact and complement each other. Therefore, single feeds were not incubated *in vitro*, but dietary composition was restricted to include a single by-product to elucidate the most suitable dietary by-product inclusions. Since by-products originate from agricultural crops and human food processing industries, their chemical composition varies markedly between, as well as potentially within by-products (Rinne et al. 2014, García-Rodríguez et al. 2019). Feeds used in this study originated only from one batch of that particular by-product, and to obtain a reliable feed value, a manufacturer should consistently repeat analyses to monitor batch-to-batch uniformity of the product. However, all current feeds were within the typical range of that particular feed material when compared with published values in feed tables, e.g. Feedipedia (2025), Luke (2025) and NorFor (2025), and the products were obtained from commercial sources so that they represent the options available for practical farms.

Experiment 1

The energy by-products used in this study originated from food milling or extraction processes that generated fibrous residues except for molasses. Wheat bran and SBP are the most widely used nonforage fibre sources (NFFS) derived from agro-industries that are used in Swedish ruminant production systems (Swedish Board of Agriculture 2022) while PKC is imported by feed industry to be included in commercial concentrate mixtures. The feeds chosen for the experiment showed a wide range in their fibre content and quality most clearly described by their iNDF content, which was highest in PKC, intemediate in WB and lowest in SBP, while M did not contain any fibre. Starch content of the samples was not analysed as the study focused on by-products low in starch concentration, but in general, starch is a key dietary component in commercial concentrate feeds to ruminants.

Increasing diet fibre content via by-product addition increased acetate production in the incubation medium, and CH_4 production (per g DM) decreased in line with the lower TOMD. All energy by-products in this study exhibited a higher proportion of acetate when replacing barley, but PKC decreased molar proportion of propionate and increased that of butyrate. Only M increased molar proportion of propionate, while WB and SBP induced no change. Ertl et al. (2015a) observed a lower acetate to propionate ratio *in vitro* for a diet supplemented with by-products compared with a control concentrate mixture and attributed this to more easily fermentable fibre in the by-products. This was assumed to stimulate propionate formation and may be beneficial, particularly during early lactation, through improved energy efficiency. Molasses was clearly highest in NFC of all energy by-products included in this study. The higher NFC in M and SBP can also explain the lower NH_3 in Experiment 1, which is also in line with the lower CP concentration in these energy by-products.

Ertl et al. (2015b) completely replaced cereals and pulses with agro-industrial by-products in diets to organic cows, which generated a slightly lower energy and CP concentration in the diet, but did not affect any production traits except milk urea concentration that was decreased when cows were fed by-products. Based on the results obtained by Ertl et al. (2015b), Ertl et al. (2016) conducted an experiment where cereals and field beans were replaced with wheat bran and SBP without any additional protein supplementation. They concluded that no production traits were affected, but that the results were too limited to be considered valid for post-peak lactating cows and that there were trade-offs in the use of nonforage fibre sources (NFFS) and current efficiency criteria of dairy production. Moreover, Guinguina et al. (2021) reduced starch content in the diets of early lactating cows from 170 to 53 g kg^{-1} DM by replacing cereals with fibrous by-products. In accordance with Dann et al. (2014) and the *in vitro* results of PKC and WB in this study, TOMD decreased, but also digestibility of NDF, neutral detergent solubles, and CP when fed with by-products. There were no effects on DM intake or any production traits, but daily enteric CH_4 production decreased by 10% (Guinguina et al. 2021).

Replacement of barley with NFFS has been reported to result in greater milk yield, an effect explained by an improved silage DM intake (e.g. Huhtanen 1993) or with no change in intake (Huhtanen 1987). It has been speculated that greater milk yield with fibrous supplements, despite a lower intake of energy, may be related to positive associative effects from a combination of different carbohydrate sources compared with barley (Huhtanen 1991). Carbohydrates fermented at different rates compared with barley can improve microbial protein synthesis in grass silage-based diets (Huhtanen 1987). Additionally, the CP content of NFFS is sometimes higher than that of barley, which could explain a general increase in milk yield (Huhtanen 1993). Dann et al. (2014) partly replaced ground corn with wheat middlings and beet pulp to evaluate the effect of increasing fibre content of the diets from 342 to 380 g kg^{-1} DM for dairy cows without any effects on production traits except a slight decrease in milk urea.

The high fibre diet also decreased TOMD and increased the molar proportion of butyrate in rumen fluid. In accordance with Dann et al. (2014), the more fibrous by-products, i.e., PKC and WB in this study decreased *in vitro* TOMD. The lower digestibilities of PKC and WB compared with barley are in line with their high NDF and iNDF concentrations.

Generally, the variability in feed value can be assumed to be greater if individual by-products are used rather than mixtures consisting of several by-products. Huhtanen (1993) and Huhtanen et al. (1995) suggested that variable production responses in dairy cows fed NFFS supplements can be explained by the rumen fermentation profile. This is in line with the differences observed in *in vitro* fermentation when barley was replaced by different agro-industrial by-products in this study. Ruminal branched-chain VFA (i.e. isobutyric and isovaleric acid) and valeric and caproic acid primarily originate from degradation of dietary protein (Tedeschi et al. 2000), and in the current study they generally decreased in diets containing by-products compared with diets supplemented only with barley, especially for molasses and SBP. Changes in NH_3-N in buffered rumen fluid, particularly for M, can be difficult to explain and can, in addition to diet degradation, be a result of degradation of feed particles from the rumen fluid medium, or at later time points, be due to microbial lysis and degradation.

Experiment 2

Several *in vivo* studies have demonstrated that SBM can be successfully replaced with RSM in grass silage-based diets for dairy cows without compromising production (e.g. Shingfield et al. 2003, Huhtanen et al. 2011, Martineau et al. 2013, Gidlund et al. 2015). Although the Finnish ruminant livestock sector has not used soya bean-based feeds since 2018 (Rinne et al. 2023), there is still a substantial import of soybean meals to Northern Europe. Farmers are also exposed to fluctuations in feed prices on the global market, and there is a low marginal efficiency of SBM of just 10% in milk production (Huhtanen et al. 2011). However, the national supply of RSM may be insufficient under Nordic conditions. For example, in Sweden, RSM comprises 74% of the agro-industrial by-products fed to ruminants and imported RSM comprises 17% of the total amount of agro-industrial by-products used in ruminant production systems, while there is a surplus of DDG being exported. Further, according to feed companies, imported SBM comprises 88% of the total SBM used for cattle in Sweden (Swedish Board of Agriculture 2022). Increasing cultivation of rapeseed will likely not meet the demand of protein feed to livestock in Northern Europe, due to the necessity for other crops before rapeseed can return to the crop rotation. Oilseeds (such as rapeseed or turnip rape) are valuable crops in grain-dominated crop rotations but should not occur more than every five to six years, as they carry risks especially for fungal diseases (Bernes and Gustavsson 2016).

All protein supplements evaluated in this study were within a CP concentration ranging from 315 g kg^{-1} DM in DDG to 392 g kg^{-1} DM in RSM, and DDG differed from the other protein by-products because it did not decrease TOMD, which can be explained by its relatively low proportion of iNDF compared with the other by-products. DDG also showed the highest uCP concentration of the protein feeds evaluated. Dairy cow studies have however shown reduced concentration of milk protein and total tract CP digestibility of DDG compared to PKC and RSM (Karlsson et al. 2018, Pang et al. 2018). Pang et al. (2018) explained the reduction in CP digestibility by a potential heat damage of the protein in DDG during the drying process. Gaillard et al. (2017) gradually replaced SBM and canola cake with DDG to dairy cows, and observed a negative effect on both milk yield and protein with increased inclusion of DDG.

Supplementation with DDG affected rumen fermentation differently than other protein feeds by increasing the proportion of propionate and decreasing that of butyrate with a subsequent decrease in CH_4 production. A reason for this could be that propionate and CH_4 production requires H_2, and since propionate production increased, less hydrogen was available for CH_4 production. There is a high correlation between CH_4 production and digestibility (Ramin and Huhtanen 2013) and this was evident in the RSM diet, where NDF digestibility decreased, TOMD tended to decrease, and CH_4 production also decreased. The same pattern was also observed when SBM was replaced by RSC in diets based on silage and barley, in accordance with the results by Jentsch et al. (2007).

By-products provided more uCP in comparison to SBM, which could be explained by the higher proportions of ammonia and soluble true protein (fractions A1 and A2; Table 2), which may supply a greater amount of substrate to produce microbial protein flowing to the duodenum. The A1 fraction was particularly high in DDG potentially explaining the good evaluation as protein supplement in vitro. Further, DDG having a greater proportion of indigestible protein (both C fraction and ADICP) than all other protein supplements is in line with the reported decrease in CP digestibility by Karlsson et al. (2018) and Pang et al. (2018). Finally, the subtraction of the ADICP from NDICP was shown to be greater in RSC and RSM than in SBM, and it may contribute to more potentially available fermentable substrate for ruminal bacteria to produce microbial protein. Generally, reduced digestibility

was more prominent in the diets based on silage and barley than the diets based on silage and SBP. This could be explained by a better rumen environment when digestible fibre from the beet-based feed replaced the starch in barley (Huhtanen 1993).

The increase in uCP in response to incremental levels of dietary CP concentration suggested that all by-products qualified as potential protein feed sources to ruminants. A high uCP level, defined as the sum of microbial protein (MP) and rumen undegraded protein (RUP) (Edmunds et al. 2012), indicates that there is a higher proportion of utilizable protein substrate available in the duodenum. In the *in vitro* uCP estimation, RUP and MP are simultaneously estimated and cannot be differentiated. According to Edmunds et al. (2012), validation using in vivo data is recommended, and based on the evaluation of Gidlund et al. (2018), the uCP method ranked the feeds similarly as in vivo data measuring the flow of protein into duodenum. Although uCP was used, it is not possible to differentiate between RUP and MP, and it is likely that Expro, RSC, and RSM have a relatively high proportion of RUP due to decreased TOMD when those by-products replaced SBM in this in vitro evaluation. On top of total amount of amino acids entering the small intestine, the profile also plays a role and there are e.g., indications that rapeseed based feeds may provide a more balanced amino acid profile to dairy cows than those based on soya bean protein (Rinne et al. 2015).

Conclusions

When comparing the energy by-products, replacing barley with M and SBP in grass silage-based diets did not decrease diet TOMD in buffered rumen fluid in vitro. However, both M and SBP inclusion changed rumen fermentation profile towards more acetate and less butyrate. Inclusion of a mixture of NFFS and M in a concentrate is more likely to not induce changes in the rumen fermentation profile.

In the protein by-product experiment, DDG had the same digestibility performance as SBM in both diets. Diets with DDG decreased acetate and butyrate, while propionate proportion increased compared with diets with SBM. Utilisable CP was, on average, higher in diets based on silage and barley than silage and SBP. Overall, by-products provided more uCP than diets supplemented with SBM. However, the amino acid profile and intestinal digestibility of uCP of the different feeds can vary and is necessary to fully evaluate the true protein value of the diet. Further, *in vivo* production experiments are needed to fully assess the potential of inclusions of in vitro evaluated by-products as widely applicable alternative feeds in beef cattle and dairy cow diets.

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Appendix 1. Dietary feed composition in Experiment 1

		Diets[1]									
		First supplementation level					Second supplementation level				
Dietary composition	Basal diet	B	PKC	M	WB	SBP	B	PKC	M	WB	SBP
Grass silage	695	554	554	560	561	559	420	420	420	425	421
Barley (B)	305	446	243	246	246	245	580	184	184	186	185
Palm kernel cake (PKC)	-		203	-	-	-		396	-	-	-
Molasses (M)	-		-	194	-	-		-	395	-	-
Wheat bran (WB)	-		-	-	193	-		-	-	389	-
Sugar beet pulp (SBP)	-		-	-	-	196		-	-	-	395
Chemical composition											
Dry matter, g kg^{-1}	418	491	520	476	510	516	561	617	536	604	536
Organic matter	935	943	938	924	935	933	950	940	912	935	916
Crude protein	139	137	147	131	139	127	135	155	124	139	130
Neutral detergent fibre	457	412	487	368	462	433	370	516	276	468	182

[1]The basal diet consisting of grass silage and barley grain was replaced with additional barley grain, palm kernel cake (PKC), beet molasses (M), wheat bran (WB), or sugar beet pulp (SBP) to inclusions of two levels of 200 and 400 g kg^{-1} of diet DM.

Appendix 2. Dietary feed composition in Experiment 2 (g kg^{-1} DM if not otherwise stated)

		Diets[1]									
		First supplementation level					Second supplementation level				
Dietary composition	Basal diet	SBM	Expro	DDG	RSC	RSM	SBM	Expro	DDG	RSC	RSM
Grass silage	614	577	563	538	561	564	540	511	462	508	513
Barley	386	362	353	337	352	354	339	321	290	319	322
Soybean meal (SBM)	-	61	-	-	-	-	121	-	-	-	-
Expro	-	-	84	-	-	-	-	169	-	-	-
Dried distillers´grains (DDG)	-	-	-	125	-	-	-	-	248	-	-
Rapeseed cake (RSC)	-	-	-	-	86	-	-	-	-	173	-
Rapeseed meal (RSM)	-	-	-	-	-	82	-	-	-	-	165
Chemical composition											
Dry matter, g kg^{-1}	457	481	495	509	497	494	505	533	561	537	532
Organic matter	874	877	871	868	873	871	880	868	862	871	868
Crude protein	146	167	166	167	166	166	188	187	188	186	187
Neutral detergent fibre	467	454	455	445	449	451	440	443	423	430	435

[1]The diets used as controls were grass silage:barley grain. Soybean meal (SBM) was used as the conventional crude protein (CP) source and was replaced with heat-treated rapeseed meal (Expro®; AAK Sweden AB, Karlshamn, Sweden), dried distillers´ grains with solubles (DDG) (Agrodrank™90; Lantmännen Agroetanol AB, Norrköping, Sweden), rapeseed cake (RSC) or rapeseed meal (RSM). Inclusions of protein by-products were made at two levels of CP in the diets to increase the CP concentration by 20 g kg^{-1} of diet dry matter per increment, aiming to give dietary CP concentration of 146, 166 and 186 g kg^{-1} of diet dry matter.

Appendix 3.

Dietary composition	Basal diet	Diets[1]									
		First supplementation level					Second supplementation level				
		SBM	Expro	DDG	RSC	RSM	SBM	Expro	DDG	RSC	RSM
Grass silage	605	571	559	539	557	560	537	512	473	510	515
Beet fibre	395	373	365	352	364	365	351	334	308	333	336
Soybean meal (SBM)	-	56	-	-	-	-	113	-	-	-	-
Expro	-	-	76	-	-	-	-	153	-	-	-
Dried distillers´grains (DDG)	-	-	-	109	-	-	-	-	219	-	-
Rapeseed cake (RSC)	-	-	-	-	79	-	-	-	-	158	-
Rapeseed meal (RSM)	-	-	-	-	-	75	-	-	-	-	149
Chemical composition											
Dry matter, g kg^{-1}	516	536	546	556	548	546	555	576	595	580	575
Organic matter	844	849	843	842	845	844	853	843	840	846	843
Crude protein	126	147	146	147	146	146	168	166	167	166	166
Neutral detergent fibre	504	489	490	480	484	486	474	476	457	464	469

[1]The diets used as controls were grass silage:sugar beet pulp. Soybean meal (SBM) was used as the conventional crude protein (CP) source and was replaced with heat-treated rapeseed meal (Expro®; AAK Sweden AB, Karlshamn, Sweden), dried distillers´ grains with solubles (DDG) (Agrodrank™90; Lantmännen Agroetanol AB, Norrköping, Sweden), rapeseed cake (RSC) or rapeseed meal (RSM). Inclusions of protein by-products were made at two levels of CP in the diets to increase the CP concentration by 20 g kg^{-1} of diet DM per increment, aiming to give dietary CP concentration of 126, 146 and 166 g kg^{-1} of diet dry matter.