Title: **Evaluation of rumen protected rapeseed expeller (NovaPro) as an alternative to soya bean meal in dairy cow diets**

Authors:

Philip C. Garnsworthy[a*], Neil Saunders[a], Jennifer R. Goodman[a] and Michael Marsden[b]

Affiliations:

[a]University of Nottingham School of Biosciences, Sutton Bonington Campus, Loughborough LE12 5RD, UK.

[b]AB Agri Limited, 64 Innovation Way, Peterborough Business Park, Lynch Wood, Peterborough, PE2 6FL, UK

[*]Corresponding Author: Phil.Garnsworthy@nottingham.ac.uk

ABSTRACT

There are environmental, social and economic pressures to reduce the use of soya bean meal in ruminant diets by using alternative protein sources, such as those derived from rapeseed. A new protected form of rapeseed (NovaPro) has been developed to provide similar quantities of digestible undegradable protein (DUP) compared to soya bean meal. NovaPro is hot pressed expelled rapeseed (no hexane solvent used), treated with a specific wood derived xylose-rich lignosulphonate in the presence of elevated moisture and heat to increase DUP. The objective of this study was to evaluate NovaPro as a protein supplement for high yielding dairy cows.

Four diets were formulated to supply similar quantities of metabolisable energy and protein but containing different dominant protein sources. The main protein sources were: Control – soya bean and rapeseed meals; NP1 – NovaPro and wheat distillers dried grains with solubles (DDGS); PR – protected solvent-extracted rapeseed meal and wheat-DDGS; NP2 - NovaPro and SoyPass. Diets were fed to 44 cows using a Latin square design with four feeding periods of 28 days each.

Milk yield was significantly higher when cows were fed on rapeseed treatment diets (mean 42.7 kg/d) than when fed on the control diet (mean 41.1 kg/d), as was energy-corrected milk (ECM) yield (mean 43.2 versus 41.7kg/d). Dry matter intake was higher when cows were fed on NP1 and NP2 (mean 25.0 kg/d) than when they were fed on the control diet (mean 23.9 kg/d); dry matter intake for PR was intermediate (mean 24.4 kg/d). Concentrations of milk fat and protein reflected differences in milk yield, and there was no difference between treatments in fat or protein yield, although fat plus protein yield was higher when cows were fed on rapeseed treatment diets (mean 2.84 kg/d) than when fed on the control diet (mean 2.72 kg/d).

Differences in rumen fluid and blood composition were commensurate with differences in diet composition, nutrient intake and milk yield. Retrospective calculation of metabolisable

energy and protein supplies showed that these were within 3% of requirements for observed responses. Calculation of amino acid profiles suggested that profiles, particularly methionine, were better for the rapeseed treatment diets.

Results of this study support the hypothesis that cows fed on NovaPro and other rumen protected rapeseed proteins will have similar or improved milk production compared to a control (soya-based) diet. Improved milk yield was accompanied by increased dry matter intake, but it is likely that intake was driven by milk yield rather than vice versa. The most likely explanation for improved milk yield when cows were fed on the rapeseed treatment diets is that amino acid balance was improved compared to control.

Keywords rapeseed meal, soybean meal, rumen protected protein, milk production

1. Introduction

Soya bean meal is widely used as a protein supplement in diets for dairy cows because of its high concentrations of crude protein (CP) and metabolisable energy (ME) compared to alternatives such as rapeseed (Canola) meal (Huhtanen et al., 2011). There are, however, environmental, social and economic pressures to reduce the use of imported soya bean meal in the European Union (EU), and to provide alternative forms of protein in ruminant diets.

The role of protein supplements in dairy diets is to ensure that metabolisable protein (MP) supply is adequate to meet requirements for maintenance and milk production. High-producing cows cannot meet MP requirements completely from microbial crude protein (MCP), and cows need additional MP in the form of digestible rumen undegraded protein (DUP). Soya bean meal has a higher DUP content than alternative oilseed meals, such as rapeseed meal, although heat and chemical treatment can enhance their DUP content.

Rapeseed is the largest EU-grown oilseed crop in terms of tonnage and hectares grown, and the co-product remaining after oil extraction (rapeseed meal) is widely used for animal feed.

Oil extraction method and subsequent processing affect the nutritive value of rapeseed meal. Oil extraction usually involves pre-heating seeds to around 35 °C, rupturing the seed coat by passing through rollers, conditioning the seeds by heating to 80-90 °C to rupture oil cells, crushing the seeds by passing through a series of screw presses, followed by solvent extraction with hexane, and then heat treatment to remove solvent and toast the meal and recover the hexane (Crawshaw, 2019). This heat treatment lowers rumen degradability of protein but can reduce protein digestibility further down the gastrointestinal tract (McKinnon et al., 1995). An alternative oil extraction method, which does not use solvents, involves heat treatment to condition seeds, followed by mechanical extraction in an expeller. Expeller meal has a higher ME concentration due to higher residual oil content (>80 g/kg compared with <40 g/kg for solvent extraction) and a higher digestibility of DUP due to lower temperatures especially during heat applied to recover hexane (Newkirk et al., 2003).

Soya bean and rapeseed meals can be rumen-protected by chemical treatment during manufacture to lower degradability of protein. For example, formaldehyde-treated soya bean meal has a lower protein degradability (0.21 versus 0.62 kg/kg) than untreated soya bean meal (O'Mara et al., 1997); xylose-treated soya bean meal (SoyPass®) had a lower protein degradability (0.27 versus 0.52 kg/kg) than untreated soya bean meal (Harstad and Prestløkken, 2000); lignosulfonate-treated rapeseed meal had a lower protein degradability than untreated rapeseed meal (0.29 versus 0.63 kg/kg, McAllister et al., 1993; 0.30 versus 0.71 kg/kg, Wright et al., 2005). Lower rumen degradability results in higher proportions of protein as DUP compared to untreated soya bean and rapeseed meals. Many studies have demonstrated benefits of replacing soya bean meal with rapeseed meal in protected and untreated forms, and a comprehensive summary of these benefits is provided in the Canola Meal Dairy Feed Guide (Canola Council of Canada, 2019).

A new rapeseed processing plant opened in 2019 near Stratford-upon-Avon, UK (www.yelo.com). The plant uses expeller technology without hexane extraction to produce high-quality rapeseed oil and rapeseed expeller. As well as an untreated rapeseed expeller,

the plant produces a rumen-protected rapeseed expeller branded as NovaPro. NovaPro is manufactured using a new process that combines hot pressing rapeseed followed by heat treatment with Xylig a specific xylose rich lignosulphonate. Xylig (Borregaard LignoTech, Sarpsborg, Norway) is a by-product of the wood pulping industry and delivers xylose, a reducing sugar which binds to amino acids in early Maillard reactions (Smith, 2016). Xylig is also used to protect soya bean meal in SoyPass®.

The objective of the current study was to evaluate NovaPro as a protein supplement for high yielding dairy cows. The specific aims were to: a) determine rumen degradation characteristics of NovaPro compared with conventional oil extracted rapeseed and soya bean meals; b) compare performance of dairy cows fed on balanced diets containing these protein sources. It was expected that the hot-pressed rumen protected rapeseed meal, NovaPro, would have improved digestibility compared to co-products produced from conventional hexane solvent extraction. Furthermore, it was expected that treatment with Xylig would protect rapeseed protein and supply a similar quantity of rumen by-pass protein as soya bean meal.

The hypothesis was that cows fed NovaPro and other protected rapeseed meals will have similar or improved milk production from a lower cost diet while excluding or reducing soya bean meal, compared to a typical (control) diet with soya bean meal and solvent-extracted rapeseed meal as protein supplements.

2. Materials and methods

All animal work was carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 under Project Licence number 30/3201. Procedures were approved by the University of Nottingham Animal Welfare and Ethical Review Body. Work was conducted at the University of Nottingham Centre for Dairy Science Innovation (Annual average milk yield 11,000 L per cow per calendar year).

2.1 Rumen degradation characteristics

Samples (2 kg) of seven commercially available protein supplements to be used in the animal performance study were supplied by KW Alternative Feeds, Peterborough, UK. These supplements were soya bean meal (HiPro), SoyPass, rapeseed meal (solvent extracted), rapeseed meal (expeller), protected rapeseed meal (solvent extracted and heat treated), NovaPro and wheat distillers dried grains with solubles (DDGS) from bioethanol production (Vivergo Fuels, Hull, UK). Protein supplements were stored on concrete floors within commercial feed stores in heaps varying in size from tens to hundreds of tonnes, so samples were collected from different parts of the heaps to ensure they were representative of the whole batch.

Rumen degradability of protein and dry matter was determined by a synthetic fibre bag technique based upon the method of Ørskov and McDonald (1979). Bags were incubated in the rumen of two non-lactating Holstein–Friesian dairy cows fed at maintenance level of feeding on grass hay (5 kg/d) and concentrates (2 kg/d).

For each protein source, the main sample was mixed thoroughly by hand and six sub-samples (approximately 20 g each) were crushed in a pestle and mortar to a size of <5 mm, sieved through a 55 µm screen to remove small particles, and weighed (four decimal places) into six pre-weighed 5 cm × 10 cm synthetic fibre bags with a 50 ± 10 micron porosity. (Ankom Technology, Macedon, USA). Bags were closed tightly with elastic bands, which were wound around the bag several times, and then several more times around the doubled-over end of the bag. Three bags per cow were incubated for each of six incubation periods (0, 4, 8, 12, 24 and 48 h). All bags, except time zero, were placed in the rumen at the same time, and removed after their respective time periods. For ease of placement and removal, batches of bags for each time point were contained in a plastic cage that was tied by string to an eyelet on the inside of the cannula stopper. After incubation, bags (and time-zero bags) were washed in a domestic washing machine (Super Spin, Indesit, Uxbridge, UK) at 30 °C for 20 minutes, and dried in an oven at 80 °C for 48 h. After drying, bags containing residues were weighed, and original weight of bags was subtracted to determine residue weight.

Residue weight was subtracted from original dry sample weight to give dry-matter disappearance. Nitrogen content of test samples and residues was determined using an elemental N analyser (NA 2000, Fisons Instruments, Crawley, Sussex). Nitrogen and dry matter degradability curves were fitted to disappearance data using the Nonlinear Models procedure of Genstat (18th Edition). The model fitted was:

$$\text{N or DM disappearance} = a + b(1 - e^{-ct})$$

where *a* is the washable fraction (time zero), *b* is the potentially degradable fraction, and *c* is the fractional rate of degradation of the *b* fraction with time *t* (Ørskov and McDonald, 1979). Solubility of nitrogen (sN) and dry matter (sDM) were determined using the method of Weisbjerg et al. (1990).

Effective degradability of nitrogen (edn) was calculated using the equation:

$$\text{edn} = (0.9\text{sN}/(0.9+\text{k})) + ((a-\text{sN})c/(c+\text{k}) + (bc/(c+\text{k}))$$

where k is the rumen outflow rate, which was assumed to be 0.08 for high-yielding dairy cows (Thomas, 2004).

Effective rumen degradable protein (ERDP; g/kg DM) was calculated as CP × edn.

DUP (g/kg DM) was calculated as (0.9(CP - ERDP) – 6.25ADIN), where ADIN is acid-detergent insoluble nitrogen (Thomas, 2004).

2.2 Animal performance study

2.2.1 Animals, housing and feeding system

Forty-four Holstein Friesian cows in early lactation (105 ±43 days in milk (DIM)), in parity 1 (n=16) or above (mean parity 3.6 ±1.07; n=28), were placed into one of 11 similar blocks of four cows according to parity, milk yield, DIM and live weight. Cows within blocks were then allocated randomly to one of four treatment groups, each containing 11 cows. Cows were housed in a freestall barn and milked individually at an automatic (robotic) milking station (AMS; Lely Astronaut A3; Lely UK Ltd., St Neots, UK). Feeding consisted of partial mixed rations (PMR), offered ad libitum, and a concentrate fed in the AMS during milking according to milk yield (0.45 kg/kg milk yield above 32 kg/d, up to maxima of 12 kg/d or 3 kg/AMS visit).

178 The AMS concentrate contained (kg DM/100 kg DM): sugar beet pulp, 19; wheat, 15;
179 rapeseed meal, 10.5; maize, 10; wheat feed meal, 10; wheat DDGS, 10; soya hulls, 7; cane
180 molasses, 6; barley, 5; SoyPass, 5; Megalac, 2.5. Cows had individual access (one cow at a
181 time) to electronic feed bins (Fullwood RIC feeders; Fullwood Ltd, Ellesmere, UK) containing
182 PMR. Each cow had free access to seven bins containing the PMR allocated to her
183 treatment group. The seven bins for each group were distributed randomly along a row of 28
184 bins to ensure no treatment bias due to bin position.

185 2.2.2 Experimental design and treatments

186 Cows in the four treatment groups were offered four PMR following a 4 x 4 Latin square
187 design, with four feeding periods each of 28 days. The four PMR were Control (C), NP1, PR,
188 and NP2 with each cow being offered each PMR over the course of the experiment. Diet
189 formulations are in Table 1 and laboratory analyses of diets and AMS concentrate are in
190 Table 2.

191 All PMR were formulated to provide metabolisable energy (ME) and metabolisable protein
192 (MP) requirements for Maintenance plus 32 L of milk per day with identical levels of forage
193 and mineral supplements, and all diets were formulated to the same ME and crude protein
194 supply.

195 PMR C was formulated as a balanced ration containing soya bean meal and rapeseed meal
196 as the main protein sources with no protected rapeseed. For PMR NP1, soya bean and
197 rapeseed meals were replaced by NovaPro; wheat DDGS and urea were included to
198 balance rumen degradable protein and keep the diet iso-nutrient. For PMR PR, soya bean
199 meal was replaced by a solvent-extracted, heat-treated rapeseed meal; wheat DDGS and
200 urea were included again to balance protein and keep the diet iso-nutrient. For PMR NP2,
201 soya bean meal was replaced by NovaPro and SoyPass, and no wheat DDGS was included.

202

Table 1. Formulations of partial mixed rations containing different protein sources (kg/t DM basis)[1]

	Control	NP1	PR	NP2
Grass silage	256	256	256	256
Maize silage	232	232	232	232
Wholecrop wheat silage	139	139	139	139
Wheat straw	20	20	20	20
Wheat-rolled	143	88	87	135
Soya bean meal-HiPro	96			
Rapeseed meal-extracted	48			29
Protected Rape-expeller[2]		117		87
Protected Rape-extracted[3]			115	
SoyPass				19
Wheat DDGS[4]		78	77	
Sugar beet pulp	38	39	38	50
Butterfat extra (C16 rich > 85%)	13	16	19	16
Minerals & vitamins[5]	6	6	6	6
Limestone flour	5	5	5	5
Sodium bicarbonate	4	4	4	4
Urea[6]		2	2	3
	1000	1000	1000	1000

Control: soya bean meal and rapeseed meal as main protein sources; NP1: NovaPro and wheat DDGS as main protein sources; PR: protected rapeseed meal (solvent-extracted, heat-treated) and wheat DDGS as main protein sources; NP2: NovaPro and SoyPass as main protein sources.

[1] Formulations were calculated using lab-determined DM values (Table 2). A concentrate was fed in the AMS according to milk yield (0.45 kg/litre over 32 litres/day, up to maxima of 12 kg/d or 3 kg/AMS visit).

[2] NovaPro – hot pressed rapeseed expeller, rumen protected by heat treatment with Xylig.

[3] Solvent-extracted rapeseed meal, rumen protected by heat treatment.

[4] Dried distillers grains with solubles from bioethanol distillation using wheat.

[5] KW Complete Dairy 4; KW Alternative Feeds, Peterborough, UK

[6] KW Alternative Feeds, Peterborough, UK

2.2.3 Feeding and feed sampling

Feed bins were emptied and refilled with freshly mixed PMR between 07:00 and 08:00 daily. Treatment PMR were mixed using an automatic mixer system (MixFeeder; Skiold Mullerup, Ullerslev, Denmark), which mixed forages (grass silage, maize silage, wheat silage and straw) and then combined the forage mix with pre-mixed blends of the non-forage ingredients. Pre-mixed blends (one per treatment) were supplied by an accredited feed mill. Groups of cows were fed in a different order each day to avoid bias due to one group always being fed first.

Samples of each separate forage, concentrate blend and AMS concentrate were taken weekly, and samples were pooled at the end of each feeding period. Pooled samples were sent for analysis in commercial laboratories (Forages: Trouw Nutrition GB, Ashbourne, UK; blends: Sciantec Analytical, Cawood, UK). Forages were analysed using near-infrared (NIR) spectroscopy and Forage Analysis Assurance Group equations to predict nutrient contents (https://www.faagroup.co.uk/). Concentrate blends and AMS concentrates were analysed using wet chemistry. In addition, weekly samples of each forage were used for DM determination by oven drying at 80 °C for 48 h. Composition of the four PMR and the AMS concentrate are in Table 2.

Table 2. Laboratory analysis[1] of partial mixed rations containing different protein sources, and concentrate fed during milking

(g/kg DM, except where shown)	Control	NP1	PR	NP2	AMS concentrate
Dry matter (g/kg)	487	488	487	487	870
Crude protein	160	154	160	153	161
Metabolisable energy (MJ/kg DM)[2]	12.0	12.1	12.0	12.1	12.8
Starch	220	184	182	209	154
Sugars	25	28	28	27	64
Ash	65	63	66	63	56
Neutral-detergent fibre (aNDFom)	350	371	374	367	217
Oil-B (Acid hydrolysis)	52	65	61	60	41

Control: soya bean meal and rapeseed meal as main protein sources; NP1: NovaPro and wheat DDGS as main protein sources; PR: protected rapeseed meal (solvent-extracted, heat-treated) and wheat DDGS as main protein sources; NP2: NovaPro and SoyPass as main protein sources; AMS concentrate: concentrate fed during milking in an automatic milking station.

[1]Forages were analysed using NIR; non-forage components were analysed using wet chemistry.

[2]Calculated using NIR values for forages and the equation ME = [0.14NCGD + 0.25Oil] for non-forage components and robot concentrate, where NCGD is neutral cellulase gammanase digestibility.

2.2.4 Cow sampling and recording

Milk yield and live weight were recorded for each cow at each milking throughout the trial and converted to daily means. Milk samples were collected over three to five days in the last week of each feeding period, covering all milking times throughout the day and night (2 morning, 2

afternoon and 2 night samples per cow). Samples were collected automatically with a sampling shuttle attached to the AMS. The shuttle held up to 60 samples in 30 ml plastic pots and was programmed to sample nominated cows every time they visited the AMS. Pots were removed from the shuttle at 09:00 and 16:00 daily. If the shuttle was full of pots when a nominated cow visited the AMS, she would not be sampled. Therefore, the list of nominated cows was adjusted daily to ensure that all cows were sampled in the desired time windows. Individual milk samples were analysed for butterfat, protein and lactose using mid-infrared spectroscopy at the National Milk Laboratories, Wolverhampton, UK. Milk urea was determined by heating samples to 40 °C in a water bath before mixing and then deproteinising them with 10% w/v trichloroacetic acid (TCA) solution in the ratio of 2 parts milk to 1 part TCA solution. Samples were then centrifuged to provide a fat layer, an aqueous layer, and a proteinaceous precipitate. The aqueous layer was analysed for urea concentration using QuantiChrom^{TM} urea assay kits (DIUR-100; BioAssay Systems, Hayward, CA, USA). Milk urea concentration in milk was calculated by adjusting concentration in the aqueous layer for milk fat and protein concentrations. Daily mean concentrations of milk components were calculated as sum of milk component yield at sampled milkings divided by sum of milk yield at sampled milkings. Energy corrected milk yield (3.5% fat) was calculated using the equation described by Niu et al. (2018): ECM (kg/day) = 12.95 × fat yield (kg/day) + 7.65 × true protein yield (kg/day; i.e., crude protein-N minus urea-N × 6.38) + 0.327 × milk yield (kg/day).Rumination activity data were recorded throughout the trial by using sensor tags on neck collars (Lely Qwes system, Lely UK Ltd., St Neots, UK) and downloaded during each milking. Rumination was expressed as number of minutes per day spent ruminating, and data for the last seven days of each period were used in the analysis.

Body condition score was recorded for each cow weekly using a scale of 1 to 5 (Wildman et al., 1982). Blood samples (one per cow) were collected between 09:00 and 12:00 (one to five hours after fresh feed was given) on Day 23 or 24 of each feeding period via the jugular vein for determination of the following metabolites on a Bayer opera autoanalyzer (Bayer UK Ltd.,

279 Newbury, UK): non-esterified fatty acids (NEFA; Waiko kit NEFA-C), β-hydroxy butyrate
280 (BOHB; Randox kit Ranbut RB 1008), total protein (Bayer kit T01 130102), albumin (Bayer kit
281 T01 137702), globulin (total protein minus albumin), urea-N (Bayer kit T01 182356) and
282 glucose (Bayer kit T01 183356).

283 Rumen fluid samples were collected via stomach tube (Ruminator; www.profs-products.com)
284 at the same time as blood sampling on Day 23 or 24 of each feeding period for analysis of
285 volatile fatty acids (Playne, 1985) and ammonia (enzymatic UV method; Randox Laboratories
286 Ltd., Crumlin, UK).

287 Methane emissions were recorded automatically during each milking using the online
288 monitoring system developed at the University of Nottingham (Garnsworthy et al., 2012). This
289 system monitors methane concentration in the AMS feed bin at one-second intervals using a
290 non-dispersive infrared gas analyser (Guardian, Edinburgh Instruments, Edinburgh). Peaks in
291 methane concentration due to eructations by cows are used to estimate daily methane
292 emissions with an established calibration against respiration chambers (Garnsworthy et al.,
293 2012).

294 Faecal samples were collected from each cow on Monday and Thursday of each recording
295 week for digestibility determination. Samples were collected by grab sampling between 09:00
296 and 12:00 and oven dried at 80 °C until constant weight, which was reached after 3 to 5 days.
297 Acid insoluble ash (AIA) concentration in feed and faeces was determined by the method of
298 Van Keulen and Young (1977), and dry matter digestibility was determined from the ratio of
299 AIA in feed and faeces. Nitrogen concentration in feed and faeces was determined using a
300 Thermo Scientific Flash 2000 elemental analyser, and nitrogen digestibility was determined
301 from ratios of AIA and N in feed and faeces.

302 *2.3 Statistical analysis*

303 One cow was removed from the trial during the first feeding period due to problems accessing
304 the feed bins, so all her data were removed from the analysis. One cow was removed in the
305 second period due to chronic mastitis. Two cows were removed in the third period; one due to

mastitis and one due to lameness. All data for these cows were declared missing for the relevant periods in the statistical analysis.

For all cows, recordings made during the fourth week of each feeding period were checked for outliers in the daily values and then averaged before statistical analysis. Six cow-days were found to be statistical outliers with explainable causes. One cow developed mastitis on day 5 of the recording week, so data from days 6 and 7 were discarded; one cow was in oestrus during the recording week and had low intake and milk yield for one day, so data for that day were discarded. Three cow-days had an abnormal daily milk yield due to timing of milking around midnight (instead of 3 milkings per day, there were 2 one day and 4 the next day; mostly this did not affect the daily mean for the week, but it did on these 3 occasions).

Data were analysed using the Latin Square design of the ANOVA procedure in Genstat (18th Edition). The fixed effect was treatment diet, and the random effects were feeding period and individual cow.

A retrospective calculation of ME and MP supplies versus requirements was performed in Ultramix Professional (AGM Systems, Romsey, UK) using Feed into Milk (Thomas, 2004) equations, applied to mean observed performance for each treatment and laboratory analysis of feed ingredients.

3. Results

3.1 Rumen degradation characteristics

Soya products had higher concentrations of crude protein than rapeseed products and wheat-DDGS (Table 3). The three protected products had crude protein concentrations similar to their untreated equivalents. Solvent-extracted rapeseed products had crude protein concentrations slightly higher than expeller rapeseed products.

Degradability of nitrogen was similar for soya bean meal and solvent-extracted rapeseed meal, but slightly lower for expeller rapeseed meal. The three protected products had numerically lower nitrogen degradability than their untreated equivalents, although Xylig treatment

appeared to lower degradability more than heat treatment. Nitrogen degradability of NovaPro was similar to that of SoyPass.

Rumen degradable and undegradable protein concentrations varied according to crude protein concentration and nitrogen degradability. Digestible undegraded protein as a proportion of crude protein was lowest for solvent-extracted rapeseed and DDGS, higher for soya bean meal, heat-treated rapeseed meal and expeller rapeseed meal, and highest for SoyPass and NovaPro.

Table 3. Rumen degradation characteristics and nutrient composition of supplementary protein sources manufactured from soya bean and rapeseed, and wheat-based dried distillers grains with solubles

	Soya bean meal (solvent)	SoyPass (solvent + Xylig)	Rape meal (solvent)	Rape meal (expeller)	Protected rape (solvent + heat)	NovaPro rape (expeller + Xylig)	Wheat DDGS
DM, g/kg	889	866	890	913	880	922	891
ME[1], MJ/kg DM	14.0	13.5	11.8	13.2	12.2	12.9	13.4
NDF[1], g/kg DM	80	299	305	351	303	351	322
Starch[1], g/kg DM	70	55	85	67	68	56	22
Sugar[1], g/kg DM	119	103	16	79	99	79	11
Oil[1], g/kg DM	21	16	34	96	36	93	56
Ash[1], g/kg DM	72	57	79	73	80	73	56
CP, g/kg DM	520	528	378	343	381	323	352
ADIN[2], g/kg DM	2.20	2.20	3.66	3.66	3.66	3.66	7.00
sDM	0.331	0.215	0.234	0.198	0.198	0.201	0.323
aDM	0.416	0.393	0.245	0.527	0.426	0.444	0.659
bDM	0.574	0.499	0.602	0.356	0.498	0.454	0.240

cDM	0.050	0.095	0.105	0.058	0.093	0.026	0.067
sN	0.214	0.042	0.156	0.195	0.143	0.116	0.242
aN	0.309	0.048	0.273	0.446	0.145	0.297	0.710
bN	0.682	0.860	0.674	0.412	0.755	0.557	0.224
cN	0.053	0.028	0.074	0.060	0.060	0.030	0.066
edn	0.51	0.26	0.52	0.43	0.46	0.30	0.49
ERDP, g/kg DM	266	139	196	149	174	95	171
DUP, g/kg DM	217	338	143	154	166	184	121
DUP/CP	0.42	0.64	0.38	0.45	0.44	0.57	0.34

DDGS, dried distillers grains with solubles from bioethanol distillation; DM, dry matter; ME, metabolisable energy; NDF, neutral detergent fibre; CP, crude protein; sDM, soluble DM proportion; aDM, bDM, cDM, constants of the degradability curve equation for DM; sN, soluble nitrogen (N) proportion; aN, bN, cN, constants of the degradability curve equation for N; edn, effective N degradability; ERDP, effective rumen degradable protein at rumen outflow rate 0.08; DUP digestible undegraded protein at rumen outflow rate 0.08.

[1] Typical analytical value from KW Data Sheet (www.kwalternativefeeds.co.uk).

[2] Values for ADIN (g/kg DM) were from the Feed into Milk (FiM) feed database (Thomas, 2004).

3.2 Feed Intake

Intakes of total dry matter and PMR dry matter were higher when cows were fed on treatment diets NP1 and NP2 than when they were fed on the control diet, but intake of AMS concentrate was not affected by diet (Table 4). Intakes of ME and nutrients reflected differences in dry matter intake and also differences in diet composition. Intake of ME was higher when cows were fed on treatment diets NP1 and NP2 than when they were fed on the control diet. Intake of starch was higher when cows were fed on treatment diets NP1 and PR than when they were fed on the control or NP2 diets. Intakes of sugars, oil and NDF were higher when cows were fed on rapeseed treatment diets than when they were fed on the control diet. Intake of

crude protein was not affected by diet, but intake of effective rumen degradable protein (ERDP) was higher, and intakes of DUP and MP were lower, when cows were fed on the control and PR diets than when they were fed on diets NP1 and NP2. Metabolisable energy and protein intakes were at or above requirements for observed performance (range 1.00 to 1.03 of requirements) for all diets.

Digestibility of dry matter was not affected by treatment (Table 4). Nitrogen digestibility was higher when cows were fed on control or treatment diet PR than when they were fed on treatment diet NP1 (Table 4).

Table 4. Intake of dry matter, metabolisable energy and nutrients, and digestibility of dry matter and nitrogen, in cows fed on diets containing different protein sources

	Treatment					
Intake	Control	NP1	PR	NP2	sed	P
Dry matter (DM; kg/d)	23.9^{a}	25.1^{b}	24.4^{ab}	24.9^{b}	0.39	0.012
PMR (kg DM/d)	17.5^{a}	18.5^{b}	17.8^{ab}	18.4^{b}	0.35	0.013
AMS Concentrate (kg DM /d)	6.42	6.62	6.63	6.48	0.152	0.434
Metabolisable energy (MJ/d)	293^{a}	309^{c}	299^{ab}	304^{bc}	4.6	0.004
Crude protein (kg/d)	4.16	4.25	4.25	4.19	0.065	0.387
ERDP (kg/d)	2.68^{a}	2.58^{b}	2.66^{a}	2.54^{b}	0.040	<0.001
DUP (kg/d)	1.21^{a}	1.35^{b}	1.25^{a}	1.34^{b}	0.020	<0.001
Metabolisable protein (kg/d)	2.71^{a}	2.83^{b}	2.75^{a}	2.86^{b}	0.043	0.006
Starch (kg/d)	5.16^{a}	4.75^{b}	4.59^{b}	5.16^{a}	0.079	<0.001

Sugars (kg/d)	0.98[a]	1.07[b]	1.05[b]	1.04[b]	0.016	<0.001
Oil (kg/d)	1.25[a]	1.56[c]	1.45[b]	1.45[b]	0.023	<0.001
NDF (kg/d)	7.95[a]	8.77[b]	8.56[b]	8.61[b]	0.138	<0.001
DM digestibility (kg/kg)	0.708	0.698	0.701	0.698	0.0054	0.217
Nitrogen digestibility (kg/kg)	0.684[a]	0.658[b]	0.674[a]	0.669[ab]	0.0076	0.010

Control: soya bean meal and rapeseed meal as main protein sources; NP1: NovaPro and wheat DDGS as main protein sources; PR: protected rapeseed meal (solvent-extracted, heat-treated) and wheat DDGS as main protein sources; NP2: NovaPro and SoyPass as main protein sources.

SED, standard error the difference between treatment means; P, F-ratio probability; PMR, partial mixed ration; AMS concentrate, concentrate fed during milking in an automatic milking station; ERDP, effective rumen degradable protein; DUP digestible undegraded protein; NDF, neutral detergent fibre.

3.3 Milk production, live weight, and body condition score

Milk yield, ECM yield and lactose yield were higher when cows were fed on all rapeseed treatment diets than when they were fed on the control diet (Table 5). Feed conversion efficiency (1.73 ±0.038 kg ECM/kg DMI) was not affected by treatment. Milk protein and lactose concentrations were lower when cows were fed on treatment diets NP1 and NP2 than when they were fed on the control diet. Butterfat and urea concentrations, and yields of fat and protein, were not affected by treatment. Number of milkings per day (3.25 ±0.06) was not affected by treatment. There was no effect of treatment on live weight (691 ±6.62 kg), or body condition score (2.62 ±0.021).

393

394 Table 5. Milk yield, milk composition, and component yields in cows fed on diets containing
395 different protein sources

	Treatment					
	Control	NP1	PR	NP2	sed	P
Milk yield (kg/d)	41.1[a]	42.8[b]	42.5[b]	42.7[b]	0.57	0.009
ECM yield (kg/d)	41.7[a]	43.2[b]	43.3[b]	43.2[b]	0.63	0.033
Butterfat (g/kg)	35.1	34.5	35.1	34.5	0.60	0.448
Protein (g/kg)	32.9[a]	32.5[b]	32.7[ab]	32.5[b]	0.16	0.004
Lactose (g/kg)	47.5[a]	47.2[b]	47.3[ab]	47.1[b]	0.10	0.004
Urea (mg/dl)	33.6 [a]	29.7 [b]	31.9 [ab]	31.8 [ab]	1.34	0.040
Fat yield (kg/d)	1.43	1.46	1.48	1.45	0.026	0.327
Protein yield (kg/d)	1.35	1.39	1.39	1.38	0.020	0.141
Fat + Protein yield (kg/d)	2.72 [a]	2.84 [b]	2.85 [b]	2.82 [b]	0.045	0.035
Lactose yield (kg/d)	1.95[a]	2.02[b]	2.01[b]	2.01[b]	0.028	0.039

396 Control: soya bean meal and rapeseed meal as main protein sources; NP1: NovaPro and
397 wheat DDGS as main protein sources; PR: protected rapeseed meal (solvent-extracted,
398 heat-treated) and wheat DDGS as main protein sources; NP2: NovaPro and SoyPass as
399 main protein sources.
400 SED, standard error the difference between treatment means; P, F-ratio probability; ECM,
401 energy-corrected milk.
402
403
404
405
406 *3.4 Rumen and blood Parameters*

Methane output (g/d) and methane intensity (g/kg ECM) were not affected by treatment, but methane yield (g/kg DMI) was higher when cows were fed on control than when they were fed on all rapeseed treatment diets and was lower when cows were fed on NP1 and NP2 than when fed on PR (Table 6). Rumination time and total volatile fatty acid concentration were not affected by treatment (Table 6). Rumen pH was higher when cows were fed on treatment diet NP1 than when they were fed on treatment diet PR. Rumen ammonia concentration and molar proportion of acetate were higher when cows were fed on treatment diet PR than when they were fed on treatment diet NP2. Molar proportion of butyrate was lower when cows were fed on control or treatment diet NP2 than when they were fed on treatment diet NP1. Molar proportion of iso-butyrate was higher when cows were fed on control than when they were fed on treatment diets NP1 and PR. Molar proportion of iso-valerate was higher when cows were fed on control than when they were fed on all rapeseed treatment diets. Ratio of acetate plus butyrate to propionate was higher when cows were fed on control or treatment diet NP2 than when they were fed on treatment diets NP1 and PR. Molar proportions of propionate, valerate and caproate were not affected by treatment diet.

Table 6. Rumination time, methane emissions, rumen pH, total rumen volatile fatty acid and ammonia concentrations, and molar proportions of volatile fatty acids, in cows fed on diets containing different protein sources

	Treatment					
	Control	NP1	PR	NP2	sed	P
Methane (g/d)	346	346	346	338	3.6	0.072
Methane (g/kg DMI)	14.8^{a}	14.0^{c}	14.5^{b}	14.0^{c}	0.28	0.009
Methane (g/kg ECM)	8.6	8.2	8.6	8.1	0.30	0.178
Rumination (min/d)	489	504	499	491	7.5	0.168
Rumen pH	6.63^{ab}	6.69^{a}	6.56^{b}	6.67^{ab}	0.048	0.049

Ammonia (μmol/l)	4609^{ab}	4653^{ab}	5321^{a}	4130^{b}	404.2	0.036
Total VFA (mmol/l)	111	104	112	103	5.8	0.269
Acetic (mol%)	57.6^{ab}	57.6^{ab}	56.8^{a}	58.2^{b}	0.440	0.018
Propionic (mol%)	23.9	23.3	24.1	23.5	0.381	0.127
Butyric (mol%)	14.2^{a}	15.0^{b}	14.9^{ab}	14.3^{a}	0.328	0.020
Iso-butyric (mol%)	0.81^{a}	0.76^{b}	0.73^{b}	0.77^{ab}	0.023	0.010
Valeric (mol%)	1.76	1.71	1.78	1.67	0.054	0.171
Iso-valeric (mol%)	1.28^{a}	1.13^{b}	1.13^{b}	1.13^{b}	0.047	0.004
Caproic (mol%)	0.51	0.47	0.51	0.46	0.029	0.242
Acetate+Butyrate / Propionate	5.93^{a}	5.50^{b}	5.54^{b}	5.88^{a}	0.161	0.012

Control: soya bean meal and rapeseed meal as main protein sources; NP1: NovaPro and wheat DDGS as main protein sources; PR: protected rapeseed meal (solvent-extracted, heat-treated) and wheat DDGS as main protein sources; NP2: NovaPro and SoyPass as main protein sources. DMI: dry matter intake; ECM: energy-corrected milk yield.

Plasma concentrations of BOHB, NEFA, globulin and total protein were not affected by treatment (Table 7). Plasma glucose concentration was highest when cows were fed on control, intermediate when cows were fed on treatment diet NP2, and lowest when cows were fed on treatment diets NP1 and PR. Plasma albumin and urea concentrations were lower when cows were fed on all rapeseed treatment diets than when they were fed on the control diet

Table 7. Plasma concentrations of metabolites and nutrients in cows fed on diets containing different protein sources

	Treatment					
	Control	NP1	PR	NP2	sed	P
BOHB (mmol/l)	0.586	0.614	0.608	0.571	0.025	0.284
NEFA (mmol/l)	0.15	0.16	0.14	0.15	0.012	0.320
Glucose (mmol/l)	3.7[a]	3.5[c]	3.5[c]	3.6[b]	0.05	<0.001
Albumin (g/l)	36.2[a]	35.4[b]	35.3[b]	35.6[b]	0.28	0.008
Globulin (g/l)	42.3	42.5	42.1	42.4	0.79	0.956
Total protein (g/l)	78.6	77.9	77.4	78	0.81	0.547
Urea (mmol/l)	5.3[a]	4.7[b]	4.7[b]	4.5[b]	0.11	<0.001

Control: soya bean meal and rapeseed meal as main protein sources; NP1: NovaPro and wheat DDGS as main protein sources; PR: protected rapeseed meal (solvent-extracted, heat-treated) and wheat DDGS as main protein sources; NP2: NovaPro and SoyPass as main protein sources.

4. Discussion

The main finding of this study was that cows consumed more dry matter (mean +0.9 kg/d) and produced greater volumes of milk (mean +1.6 kg/d) and ECM (mean +1.0 kg/d) when fed on the rapeseed treatment diets than when fed on the control diet. This is in agreement with the review and meta-analysis of 43 published studies performed by Huhtanen et al. (2011) who found that forage intake responses to dietary protein concentration (kg DMI/g CP) were significantly greater when protein concentration was altered by using canola meal (0.023) and heat-treated canola meal (0.032) than when using soya bean meal (0.011); similarly, they found that milk yield responses to protein intake (kg milk/kg CP intake) were significantly greater for diets containing canola meal (3.41) and heat-treated canola meal (3.73) than for diets containing soya bean meal (2.09). Broderick et al. (2015) also found that cows had higher dry matter intake (25.2 v 24.8 kg/d) and milk yield (40.3 v 39.3 kg/d) when fed on diets containing canola meal than when fed on diets containing soya bean meal. Brito and Broderick

(2007), however, found an increase in dry matter intake (24.9 v 24.2 kg/d) when cows were fed on canola meal compared with soya bean meal, but the difference in milk yield (41.1 v 40.0) was not significant. In the current study, protected rapeseed meals were blended with either wheat DDGS or SoyPass. Martineau et al. (2019) performed a metanalysis on 22 studies where responses to canola meal were compared with responses to canola meal blended with other protein sources, and concluded that blending canola meal with other protein sources did not increase the positive production responses to canola meal alone. This suggests that responses in the current study can be attributed mainly to protected rapeseed meal, although the other protein sources were necessary to produce balanced diets.

In their review, Huhtanen et al. (2011) found no difference in milk composition between protein sources. In the current study, however, milk protein and lactose concentrations were higher when cows were fed on the control diet than when fed on treatment diets NP1 and NP2; milk protein and lactose concentrations were intermediate for treatment diet PR, so were not significantly different from any other diet. The higher milk protein concentration counteracted the lower milk yield, so there was no effect of treatment on milk protein yield. This is in contrast to Huhtanen et al. (2011), where there was no effect on milk protein concentration, so higher milk yield with canola compared with soya bean meal translated into increased milk protein yield. In the current study, differences in milk protein concentration were small, so probably result from dilution effects because means for milk protein concentration mirror differences in mean milk yield.

In the current study, mean ME and MP intakes closely matched requirements for observed performance according to FiM equations. Metabolisable energy and protein intakes were between 100 and 103% of requirements for all treatments. These values were calculated retrospectively from observed performance and laboratory analyses, and give confidence that observed responses were in agreement with FiM (Thomas, 2004) predictions. This suggests that differences between treatments were due to responses in either feed intake or milk production. It is not possible to say whether cows produced more milk on the rapeseed

487 treatment diets than on the control diet because they ate more feed, or they consumed more
488 of the rapeseed treatment diets than the control diet because they produced more milk.

489 Increased feed intake and milk yield when cows were fed rapeseed treatment diets cannot be
490 explained by digestibility of dry matter or energy because these coefficients did not differ
491 between diets. This concurs with the review of Huhtanen et al. (2011) who found no difference
492 in dry matter digestibility between protein sources. Other possible explanations discussed by
493 Huhtanen et al. (2011) were effects of dietary protein concentration and protein digestibility.
494 Although dietary protein concentration varied slightly between diets in the current study, the
495 two diets with the higher protein concentration (Control and PR) had lower mean intakes of
496 dry matter than the other two diets (NP1 and NP2), whereas the relationship between protein
497 concentration and dry matter intake is usually positive (Sinclair et al., 2014). Similarly, nitrogen
498 digestibility was higher for Control and PR than for NP1 and NP2, whereas Brito and Broderick
499 (2007) found that canola meal had a higher nitrogen digestibility than soya bean meal. Ratios
500 of ERDP to MCP were above 1.0 in all diets, which indicates that rumen degradable nitrogen
501 was not limiting microbial protein synthesis in the rumen for any diet in the current study.
502 Diets were formulated to provide similar levels of ME and MP, but some compositional
503 differences were necessary in order to achieve this objective. Based on laboratory analysis,
504 the most noticeable differences between control and rapeseed treatment diets were that
505 control had lower neutral-detergent fibre concentration but higher oil and starch
506 concentrations. Collectively and individually, these differences are unlikely to explain the
507 higher intakes for the rapeseed treatment diets compared with control. Neutral-detergent fibre
508 concentration is usually negatively related to feed intake in diets with NDF concentration
509 greater than 250 g/kg DM (Allen, 2000), so higher NDF concentrations of rapeseed treatment
510 diets (349 v 333 g/kg DM) would be expected to reduce intake and cannot explain responses
511 observed. Increasing dietary oil concentration (52 v 60 g/kg DM) with fatty acids from oilseeds
512 or hydrogenated fat, as in the current study, might either have no effect on feed intake or might
513 depress feed intake (Allen, 2000), which would be the opposite of observations in the current

514 study. High starch concentration might depress feed intake if it induced SARA, but rumen pH
515 was not lower for control than for any treatment, so this possible explanation can also be
516 discounted.
517 Because it is difficult to explain the results in terms of known dietary effects on feed intake,
518 the more likely explanation is that diet composition induced a response in milk yield, which
519 then drove feed intake. Milk composition and blood results suggest that glucose supply to the
520 mammary gland was more than adequate for the control treatment; milk lactose, milk protein
521 and blood glucose concentrations were all higher for control than for rapeseed treatment diets.
522 This is most likely due to the higher starch and lower oil concentrations of the control diet
523 compared with rapeseed treatment diets, but could simply reflect the greater drain on blood
524 glucose for lactose synthesis when rapeseed treatment diets were fed. In a study of dietary
525 energy sources and fertility in dairy cows (Garnsworthy et al., 2008a), cows fed on the highest
526 starch diet (starch 231 g/kg DM) produced numerically highest milk lactose concentration,
527 although there was no treatment effect on milk protein or blood glucose concentrations in that
528 study. In concordance with the current study, however, blood urea concentration was
529 significantly higher for the highest starch diet than for all other diets. At the time, it was noted
530 that the positive relationship between urea-N and dietary starch concentration is unusual;
531 normally high-starch diets improve rumen ammonia capture and decrease plasma urea-N
532 concentrations (Reynolds, 2006). In the current study, rumen ammonia concentration was not
533 related consistently to dietary concentration of starch or any other nutrient. Therefore, the
534 current study provides another observation of a positive association between dietary starch
535 and blood urea but does not provide an explanation. It is possible that a high starch diet
536 induces a degree of insulin resistance, thereby increasing catabolism of protein for glucose
537 synthesis and raising blood urea concentration, although differences in dietary starch
538 concentration were small in the current study.
539 In the current study blood albumin concentration was higher for the control than for any of the
540 rapeseed treatment diets. This concurs with the results of a study involving two levels of MP
541 and two levels of leucine (Garnsworthy et al., 2008b). In that study, blood albumin

concentration was higher for cows on low MP diets, and tended to be higher for cows on imbalanced (low leucine) diets. Although all diets in the current study were formulated to supply adequate MP, perhaps the amino acid balance was better for the rapeseed treatment diets. In their review, Huhtanen et al. (2011) hypothesised that intake and milk yield responses could be related to a more balanced supply of amino acids for canola diets compared with soya diets. To examine the relative balance of amino acids, data from the Evonik Aminodat 4.0 database were used to calculate concentrations of individual amino acids in the control and rapeseed treatment blends. Values were adjusted to measured crude protein concentration from true protein sources (i.e. excluding urea) (Table 8). This analysis suggests that blends NP1 and PR might have supplied more methionine and cystine than other diets. All other amino acids had lower concentrations in rapeseed treatment blends than in the control blend.

Table 8. Concentration of amino acids in protein of control (C) and treatment blends (NP1, PR, NP2) relative to crude protein (CP) and lysine

	Treatment			
	Control	NP1	PR	NP2
g/kg CP				
Lysine	542	403	377	431
Methionine	156	171	160	153
Cysteine	184	210	197	186
Threonine	392	372	348	346
Histidine	269	244	228	227
Leucine	745	676	633	599
g/100 g lysine				
Methionine	29	43	42	36
Cysteine	34	53	52	44
Threonine	72	97	92	81
Histidine	50	60	60	54
Leucine	137	170	168	139

Control: soya bean meal and rapeseed meal as main protein sources; NP1: NovaPro and wheat DDGS as main protein sources; PR: protected rapeseed meal (solvent-extracted, heat-treated) and wheat DDGS as main protein sources; NP2: NovaPro and SoyPass as main protein sources.

Higher concentrations of methionine in rapeseed treatment diets might explain the milk yield response because all diets were formulated to be marginal for methionine supply. The threshold value for metabolisable methionine in FiM (Thomas, 2004) is 2.1 g/100g MP. Metabolisable methionine concentrations (g/100g MP) calculated from actual protein intakes, were 2.13 for C, 2.26 for NP1, 2.26 for PR and 2.22 for NP2. This supports the hypothesis of a milk yield response to methionine as diet C was marginal and all other diets were above the threshold.

Following the ideal protein concept used in non-ruminants, amino acids can be expressed relative to lysine (Table 8). When expressed relative to lysine, most amino acids were present at greater concentrations in rapeseed treatment blends than in the control blend.

Differences in amino acid supply and balance might explain the milk yield response when rapeseed treatments are compared to control; not only methionine, as discussed above, but also leucine was greater for rapeseed treatment diets than for control. Previous studies have reported responses to leucine (Allison and Garnsworthy, 2002; Garnsworthy et al., 2008b). Comparison of amino acid balance between treatments, however, does not provide such strong support for this hypothesis because the profile of NP2 matched that of the control more closely than that of NP1 and PR (Table 8). Leucine as a percentage of MP was 7.45% for control and averaged 7.36% for rapeseed treatment diets. Furthermore, differences in concentration of branched-chain volatile fatty acids in the rumen do not match differences in relative proportions of branched-chain amino acids in diets. It is unlikely that inclusion of urea in the rapeseed treatment diets might explain the milk yield response through an increase in RDP for microbial protein synthesis, because ERDP did not limit microbial protein synthesis for any diet.

Lower methane yields when cows were fed on rapeseed treatment diets, particularly NovaPro diets, compared with control agrees with Brask et al. (2013) who found that rapeseed expeller cake (oil content 173 g/kg DM) reduced methane yield by dairy cows compared with conventional rapeseed meal (oil content 55 g/kg DM), but methane output and methane

intensity were not different. In contrast, Gidlund et al. (2015) found that methane intensity decreased more when dietary protein concentration increased in heat-treated rapeseed diets compared with soya bean meal diets, but responses in methane output and methane yield were not affected by protein source. Furthermore, Beauchemin et al. (2009) found that crushed canola seeds reduced methane output, methane yield and methane intensity when canola replaced calcium salts of long-chain fatty acids, which they speculated was due to reduced protozoal numbers in the rumen when cows were fed canola seeds. Clearly effects of rapeseed meals on methane emissions are variable, depending on experimental conditions, but seem to be either neutral or beneficial in terms of reducing emissions.

5. Conclusions

- Results of this study support the hypothesis that cows fed NovaPro and other rumen protected rapeseed products will have similar or improved milk production compared to a control (soya-based) diet.
- Improved milk production was accompanied by increased dry matter intake, but it is likely that intake was driven by milk yield rather than vice versa.
- The most likely explanation for improved milk yield when cows were fed on the rapeseed treatment diets is that amino acid balance was improved compared to control.

Acknowledgements

We thank Bethany May, Laura Tennant, Emma Gregson, Sarah Potterton and Lindsay Billsborrow for technical assistance, the staff of the University of Nottingham Centre for Dairy Science Innovation for care of the animals, and Jim Craigon for statistical advice. This study was funded by AB Agri, the Agriculture Group of Associated British Foods plc.

Declaration of interests

MM was an employee of the funder, whose divisions Trident and KW Alternative Feeds market all of the protein supplements used in this study. The other authors declare that they have no conflict of interests.

References

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83, 1598-1624.

Allison, R.D., Garnsworthy, P.C., 2002. Increasing the digestible undegraded protein intake of lactating dairy cows by feeding fishmeal or a rumen protected vegetable protein blend. Anim. Feed Sci. Technol. 96, 69–81.

Beauchemin, K.A., McGinn, S.M., Benchaar, C., Holtshausen, L., 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. J. Dairy Sci. 92, 2118-2127.

Brask, M., Lund, P., Weisbjerg M.R., Hellwing, A.L.F., Poulsen, M., Larsen, M.K., Hvelplund, T., 2013. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. J. Dairy Sci. 96, 2356–2365.

Brito, A.F., Broderick, G.A., 2007. Effects of feeding different protein supplements on milk production and nutrient utilization in dairy cows. J. Dairy Sci. 90, 1816–1827.

Broderick, G.A., Faciola, A.P., Armentano, L.E., 2015. Replacing dietary soybean meal with canola meal improves production and efficiency of lactating dairy cows. J. Dairy Sci. 98, 5672–5687.

Canola Council of Canada, 2019. Canola Meal Dairy Feed Guide, 6th Edition. Canola Council of Canada, Winnipeg, Canada. www.canolacouncil.org

Crawshaw, R. 2019. Co-Product Feeds in Europe: Animal feeds derived from industrial processing. Lulu.com. ISBN 978-0-244-20922-3.

638 Garnsworthy, P.C., Craigon, J., Hernandez-Medrano, J.H., Saunders, N., 2012. On-farm
639 methane measurements during milking correlate with total methane production by
640 individual dairy cows J. Dairy Sci. 95, 3166-3180.

641 Garnsworthy, P.C., Gong, J.G., Armstrong, D.G., Newbold, J.R., Marsden, M., Richards, S.E.,
642 Mann, G.E., Sinclair, K.D., Webb, R., 2008b. Nutrition, Metabolism, and Fertility in
643 Dairy Cows: 3. Amino Acids and Ovarian Function. J. Dairy Sci. 91, 4190–4197.

644 Garnsworthy, P.C., Lock, A., Mann, G.E., Sinclair, K.D., Webb, R., 2008a. Nutrition,
645 Metabolism, and Fertility in Dairy Cows: 1. Dietary Energy Source and Ovarian
646 Function. J. Dairy Sci. 91, 3814–3823.

647 Gidlund, H., Hetta, M., Krizsan, S.J., Lemosquet, S., Huhtanen, P., 2015. Effects of soybean
648 meal or canola meal on milk production and methane emissions in lactating dairy cows
649 fed grass silage-based diets. J. Dairy Sci. 98, 8093-8106.

650 Harstad, O.M., Prestløkken, E., 2000. Effective rumen degradability and intestinal
651 indigestibility of individual amino acids in solvent-extracted soybean meal (SBM) and
652 xylose-treated SBM (SoyPass®) determined in situ. Technol.Anim. Feed Sci. Technol.
653 83, 31-47.

654 Huhtanen, P., Hetta, M., Swensson, C., 2011. Evaluation of canola meal as a protein
655 supplement for dairy cows: A review and a meta-analysis. Canadian J. Anim. Sci. 91,
656 529-543.

657 Martineau R., Ouellet D.R., Lapierre, H., 2019. Does blending canola meal with other protein
658 sources improve production responses in lactating dairy cows? A multilevel mixed-
659 effects meta-analysis. J. Dairy Sci. 102, 5066–5078.

660 McAllister, T.A., Cheng, K.-J., Beauchemin, K.A., Bailey, D.R.C., Pickard, M.D., Gilbert. R.P.,
661 1993. Use of lignosulfonate to decrease the rumen degradability of canola meal
662 protein. Canadian J. Anim. Sci. 73, 211–215.

McKinnon, J.J., Olubobokun, J.A., Mustafa, A., Cohen, R.D.H., Christensen, D.A., 1995. Influence of dry heat treatment of canola meal on site and extent of nutrient disappearance in ruminants. Anim. Feed Sci. Technol. 56, 243-252.

Newkirk, R.W., Classen, H.L., Scott, T.A., Edney, M.J., 2003. The availability and content of amino acids in toasted and non-toasted canola meals. Canadian J. Anim. Sci. 83, 131–139.

Niu, M., Kebreab, E., Hristov, A., Oh, J., Arndt, C., Bannink, A., Bayat, A.R., Brito, A.F., Boland, T., Casper, D., Crompton, L.A., Dijkstra, J., Eugène, M.A., Garnsworthy, P.C., Haque, M.N., Hellwing, A.L.F., Huhtanen, P., Kreuzer, M., Kuhla, B., Lund, P., Madsen, J., Martin, C., McClelland, S.C., McGee, M., Moate, P.J., Muetzel, S., Muñoz, C., O'Kiely, P., Peiren, N., Reynolds, C.K., Schwarm, A., Shingfield, K.J., Storlien, T.M., Weisbjerg, M.R., Yáñez-Ruiz, D.R., Yu, Z, 2018. Prediction of enteric methane production, yield and intensity in dairy cattle using an intercontinental database. Glob. Chang. Biol., 24, 3368–3389.

O'Mara, F.P., Murphy, J.J., Rath, M., 1997. The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. J. Anim. Sci. 75, 1941–1949.

Ørskov,E.R., McDonald, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., Camb. 92,499-503.

Playne, M.J., 1985. Determination of Ethanol, Volatile Fatty Acids, Lactic and Succinic Acids in Fermentation Liquids by Gas Chromatography. J. Sci. Food Agric. 36, 638-644.

Reynolds, C.K., 2006. Production and metabolic effects of site of starch digestion in dairy cattle. Anim. Feed Sci. Technol. 130, 78–94.

687 Sinclair K.D., Garnsworthy, P.C., Mann, G.E., Sinclair, L.A., 2014. Reducing dietary protein in
688 dairy cow diets: implications for nitrogen utilization, milk production, welfare and
689 fertility. Animal 8, 262-274.

690 Smith, Ted, 2016. Bypass protein for high producing dairy cows. Available at
691 https://www.lignotechfeed.com/Bypass-Protein

692 Thomas, C., 2004. Feed into Milk: A New Applied Feeding System for Dairy Cows. Nottingham
693 University Press, Nottingham.

694 Van Keulen, J, Young, B.A., 1977. Evaluation of acid-insoluble ash as a natural marker in
695 ruminant digestibility studies. J. Anim. Sci. 44, 282–287.

696 Wildman, E.E., Jones, G.M., Wagner, P.E., Bowman, R.L., 1982. A dairy cow body condition
697 scoring system and its relationship to selected production characteristics. J. Dairy Sci.
698 65, 495-501.

699 Wright, C.F., von Keyserlingk, M.A.G., Swift, M.L., Fisher, L.J., Shelford, J.A., Dinn, N.E.,
700 2005. Heat- and lignosulfonate-treated canola meal as a source of ruminal
701 undegradable protein for lactating dairy cows. J. Dairy Sci. 88, 238–243.