Effect of Dry Heat Temperature and Moist Heat Pressure on Canola Meal for Ruminant Utilisation.
Part I: Nutritional, Protein Solubility and Degradability Characteristics

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Abstract

The effects of a range of barrel dry heat temperatures (20 to 180 $^{\circ}$C), and moist heat pressure (MHP) (120 $^{\circ}$C 15 min 192 kPa) on general nutritional, protein solubility, and *in vitro* protein degradability characteristics of canola meal were investigated. Increasing dry heat temperature was negatively correlated with meal crude protein (CP), soluble CP, neutral detergent fibre, acid detergent insoluble CP, and rapidly degradable (B1 Fraction) protein; and positively with NPN, intermediately degradable (B2 Fraction) protein, dry matter, lipid, carbohydrate, and *in vitro* rumen-undegradable protein. Relative to control meal, MHP increased *in vitro* rumen-undegradable protein, and *in vitro* CP digestibility; and decreased soluble protein, and 0.5% KOH solubility. Positive increases of Fraction A and B2, as B1 decreased, suggest barrel temperature induces protein hydrolysis and conversion of rapidly to intermediately degraded protein, respectively. The changes observed may have a great effect on ruminal degradation and supply of protein and AA for ruminant utilisation.

Keywords: *Brassica napus*, canola meal, dairy cow, expeller, rumen-undegradable protein

1. Introduction

Due to economic reasons, there is interest in increasing milk production per cow, through dietary modifications such as rumen-undegradable protein (RUP) supplementation (Paz et al., 2014; Thanh & Suksombat, 2015). Dietary RUP passes through the rumen to form a potential direct source of protein to satisfy post-ruminal AA requirements during lactation (NRC, 2001). Canola (rapeseed, *Brassica* spp. *napus*, *rapa*, and *juncea*) meal is a derivative of seed oil production utilised as a protein supplement in dairy cattle feed (Sánchez & Claypool, 1983), due to its desirable AA profile and digestibility (Santos, 2011). Current literature reports RUP in canola meal ranges from 10.1 to 75.0% CP (NRC, 2001; Purser & Woodroofe, 2004). Factors contributing to variation of RUP include species (Theodoridou & Yu, 2013), processing conditions (Newkirk et al., 2003), and physical and chemical treatments (McKinnon et al., 1991). To extract seed oil and generate meal, solvent-based and mechanical (e.g., cold-press, expeller, and extrusion) processing technologies exist. Expeller extraction utilises dry heat (95 to 135 $^{\circ}$C) (Newkirk, 2009), and, cold-press extraction mechanically presses seeds by frictional force ($\leq$ 65 $^{\circ}$C) (Leming & Lember, 2005). Deacon et al. (1988) proposed heat of expeller extraction establishes cross-linkages among and within peptides chains, and to carbohydrates to increase RUP. Toghyani et al. (2014) detailed the effects of expeller barrel dry heat temperature (90, 95, 100 $^{\circ}$C) on ileal AA digestibility of canola meal in broiler chickens.

To reduce ruminal degradation, and accordingly, increase the post-ruminal supply of canola protein, other heat treatments have been evaluated. Dry heating (125 $^{\circ}$C 10 min) of canola meal increased digestible RUP without compromising intestinal digestibility (McKinnon et al., 1995). Dry heating (125 $^{\circ}$C 20 min) of expeller canola meal reduced rumen degradability, and when fed to primiparous cows increased milk production (Jones et al.,

2001). Moist heat pressure (MHP, autoclaving, 117 kPa 127 ^{o}C 15 or 30 min) treatment of canola meal induced partial protein denaturation to decrease the ruminal protein degradability and increase ruminal bypass-AA for digestion and absorption in the small intestine (Moshtaghi Nia & Ingalls, 1992, 1995). Wright et al. (2005) reported dietary inclusion of MHP solvent-extraction canola meal (2% H_2O 100 ^{o}C 120 min) increased milk production in dairy cows by 0.5 kg per d. Although, Khalili et al. (1999) found the dietary inclusion of control and MHP rapeseed cold-press cake elicited similar milk responses in mid-lactation dairy cattle.

Limited knowledge exists of the effects of low, moderate and high expeller barrel dry heat, and the effects of MHP, on the general nutritional and protein degradability characteristics of canola meal for ruminant utilisation. The hypothesis tested in the current study was that the general nutritional, protein solubility and degradability of expeller-extracted canola meal would differ depending on the processing and treatment conditions. The objectives of this study were to investigate the effects of barrel dry heat temperature range and MHP on general nutritional, protein solubility and degradability characteristics of canola meal.

2. Method

2.1 Canola Meal and Suspension Preparation

2.1.1 Canola Seed

Commercial bulk-handling canola seed was provided by MSM Milling (Manildra, NSW, Australia). The heterogeneous seed lot was stored at room temperature (RT, ~21 ^{o}C), in an air-tight hessian polypropylene bag within a dark and dry cupboard.

2.1.2 Barrel Dry Heat and Moist Heat Pressure of Canola

To prepare canola meals, seed (~200 g) was passed separately through a primed bench-top screw-press expeller (Model.DSZYJ-200A/B, 220V, 50 Hz, 50 rpm) at a barrel dry heat temperature of either 20 ^{o}C (RT, cold-press) or, a pre-heated temperature of 60, 80, 100, 120, 140, 160 or 180 ^{o}C (expeller), then repeated two more times (n = 3 × 8, 24). The meals were individually ground in an electric mill (Breville Grinder, CG2B) and passed through a 1 mm sieve. The MHP treatment was completed by placing each meal (30 g) in a separate flat rectangle polypropylene container and autoclaving using a steriliser (Atherton Centenary Series, Melbourne, Australia) set on the Hard Goods Dry Cycle No. 1.1, for 15 min (192 kPa 120 ^{o}C). For each triplicate meal, an independent sterilising cycle was performed. The meals were stored in the dark at RT.

2.1.3 Preparation of Canola Meal Suspensions

The meals were ground (< 5 μM) by placing 5 g of meal in a stainless-steel screw-top grinding jar (50 mL) with a ø 25 mm grinding ball. The jar was positioned in a mill (MM301, Retsch, GmbH, Hann, Germany) and shaken for 30 s (frequency 20 per s) followed by a 15 s rest, thrice. To generate suspensions, ground meal (200 mg) was added to deionised H_2O (10 mL) and shaken for 30 min using a Heidolph Multi Reax set at 10. The suspensions were stored in the dark at 4 ^{o}C.

2.2 General Nutritional Characteristics

The meals were analysed for dry matter (DM, AOAC 930.15), lipid (%DM, AOAC 992.06), CP (6.25 × N) by Leco Dumas N combustion (AOAC 992.23), and carbohydrate (%DM (Masuko et al., 2005)). To determine the quantity of carbohydrate (%DM), each meal suspension (40 μL) was added separately to deionised H_2O (10 μL), concentrated sulphuric acid (150 μL) and 5% phenol in deionised H_2O (30 μL) in a clear flat bottom non-absorbent 96 F Microwell microplate (Nunc, #269620). The plate was incubated for 5 min at 90 ^{o}C in a shallow water bath, rested for 5 min at RT, wiped dry, placed in a CLARIOstar 5.20 R5 microplate reader, shaken at 500 rpm for 10 s and measured for Abs_{490nm}. Values were corrected by deducting an average of blank measurements. A standard curve (0 to 10 nmol) was established with a 1 M stock solution of D-mannose (Sigma) prepared in deionised H_2O.

2.3 Protein Solubility and Fractionation

The meals were analysed for soluble protein (Licitra et al., 1996) and solubility in 0.5% KOH (Pastuszewska et al., 1998). The latter was performed by stirring samples of the meal (5 g) in 0.5% KOH (33.3 mL) for 20 min, centrifuging at 1250 ×g for 10 min, and quantifying the protein in the supernatant by Leco Dumas N combustion. The meals were analysed in duplicate (n = 2 × 8, 16) for NPN (tungstic acid (Licitra et al., 1996)), acid detergent fibre (ADF), neutral detergent fibre (NDF), ADIN and neutral detergent insoluble N (NDIN) by the Australian Oil Reference Laboratory (Department of Primary Industries, NSW, Australia). Results were utilised to calculate true protein: %, CP − NPN. The meals were partitioned into protein fractions based on characteristics of degradability according to the Cornell Net Carbohydrate and Protein System (CNCPS) as described (Sniffen et

al., 1992). Using CNCPS, Fraction A is NPN, Fraction B is degradable protein containing B1 (soluble protein, rapidly soluble in the rumen), B2 (intermediate degradation, Total CP – (A + B1 + B3 + C)), B3 (slowly degraded in the rumen, NDIN – ADIN), and Fraction C is undegradable protein (ADICP).

2.4 In Vitro Protein Degradability

2.4.1 Rumen Undegradable Protein

The meals were analysed for RUP utilising an *in vitro* simulated rumen proteolysis procedure by Krishnamoorthy et al. (1983) validated *in vivo* (R^2 = 0.61). The meal (0.5 g) was weighed into a 125 mL Erlenmeyer flask and incubated at 39 °C for 1 h in 40 mL borate-phosphate (BP) buffer (pH 8.0). *Streptomyces griseus protease* (Type XIV 5.4 U/mg protein, Sigma P-5147, St Louis, MO, USA) solution (0.33 U/mL, 10 mL BP-buffer) was added, and the meal was incubated at 39 °C for 18 h. All flasks were placed on ice to suspend proteolytic activity before filtering. The residue was collected on quantitative filter paper (22 µm pore, No. 541, Whatman), rinsed with distilled H_2O and air-dried overnight. Residual CP was determined by combusting the whole filter paper by Leco Dumas N combustion.

$$RUP(\% \ of \ CP) = (CP - Undegraded \ CP)/CP \times 100 \tag{1}$$

2.4.2 Intestinal Digestion of Protein in Ruminants

The meals were analysed in duplicate (n = 2 × 8, 16) for *in vitro* CP digestibility utilising the HCl-pepsin pre-digestion procedure of Calsamiglia & Stern, (1995) validated *in vivo* (r = 0.91). In a 50 mL Falcon tube sample (15 mg CP) was suspended in 10 mL pH 1.9, 0.1 N HCl solution of 1 g/L pepsin (Sigma P-7012), vortexed, and incubated at 38 °C for 1 h in a shaking H_2O bath. Pancreatin solution (13.5 mL: 0.5 M KH_2PO_4 pH 7.8 containing 3 g/L pancreatin, Sigma P-7545) and 1 N NaOH (0.5 mL) was added, and the tube was vortexed, incubated at 38 °C for 24 h in a shaking H_2O bath, vortexing every ~ 8 h. To cease the reaction TCA (3 mL) was added, the tube was vortexed, rested (15 min), and then centrifuged (10,000 ×g, 15 min). The supernatant was analysed for soluble N, as described. Results were utilised to calculate % pepsin-pancreatin digestion of protein:

$$\%IVCPD = TCA - Soluble \ N/initial \ N \times 100 \tag{2}$$

2.5 Statistical Analysis

Statistical analyses of data were performed using the statistical software OriginLab v 95E (Origin, Northampton, MA, USA). To establish differences, the one-way ANOVA mathematical model used for analysis was:

$$Y_{ij} = \mu + T_j + e_{ij}j \tag{3}$$

where, Y_{ij} is an observation on the dependent variable ij; μ is the population mean for the variable, T_j is the effect of treatment (i = MHP and/or barrel dry heat temperature), as a fixed effect. The independent barrel runs at each temperature were experimental replications and e_{ij} value is the random error associated with the observation ij. A post-hoc Fisher Least Significant Difference test was performed to determine the statistical significance of differences between individual means, declared at P < 0.05. Normal distribution was established by performing an Anderson-Darling test, $P > 0.05$. The Spearman correlation coefficient (r_s) with a two-tailed test of significance ($P < 0.05$) was used to define strength and association of relationships between dry heat temperature and dependent variables. Polynomial regression was performed to determine the Coefficient of Determination (R^2), using the equation:

$$Y_i = \beta_0 + \beta_1 x_i + \beta_2 x^2_i + \varepsilon_i \tag{4}$$

3. Results

The effects of dry heat (20 to 180 °C) with MHP on general nutritional characteristics of canola meal are presented in Table 1 (Table A1 and Figure A1). The DM content of MHP meals (r_s = 0.78) and control meals (R^2 = 0.95) increased with temperature and the average DM content was higher ($P < 0.05$) in MHP meals (94.1%) than in the control (93.3%) meals (without MHP). The CP content of the all meals decreased with temperature (R^2 = 0.90, 0.89); however, the average CP content did not differ ($P > 0.05$) between the control (33.2%) and MHP meals (34.1%). Greatest CP for control meal was at 60 and 80 °C, and from 60 to 100 °C for MHP meals. Lipid content also increased with dry heat temperature (R^2 = 0.98, 0.95). The average lipid content was similar ($P > 0.05$) in MHP (19.7%) and control meals (17.3%). Lipid extraction was most efficient at 80 and 100 °C for control, and from 60 to 100 °C for MHP meals. Carbohydrate content increased with temperature (r_s = 0.95, 0.50, $P < 0.05$), with the average carbohydrate content similar in the control (11.5%) and MHP meals (11.5%).

BP-buffer pH 6.7 soluble protein content was least at 160 and 180 °C in control meals, and similar in all MHP treatment meals (Table 1). The buffer soluble protein content was lower ($P < 0.01$) in the MHP meals (14.2%)

compared to the control (75.1%) and decreased with temperature in control meals ($R^2 = 0.83$). The effect of increasing dry heat temperature (20 to 180 ^{o}C) on 0.5% KOH soluble protein was similar in the control and MHP canola meals ($r_s = -0.08$ and -0.16, respectively), and average 0.5% KOH soluble protein was higher ($P < 0.01$) in the control (55.3%) than MHP meals (33.2%). For all meals RUP content increased with dry heat temperature ($R^2 = 0.87$ and $r_s = 0.68$, respectively), and the average RUP was lower ($P < 0.01$) in the control (32.3%) than MHP meals (64.4%). For all meals IVCPD did not correlate ($P > 0.05$) with dry heat temperature ($r_s = 0.34$ and 0.08, respectively); and, IVCPD was lower ($P < 0.01$) in the control (10.1%) than MHP meals (12.0%).

Table 1. General nutritional composition, soluble protein, and *in vitro* protein degradability of canola meals produced at increasing barrel dry heat (20 to 180 ^{o}C) with moist heat pressure (MHP)

| | MHP | Barrel Dry Heat (°C) | | | | | | | | SEM | P_{BT} | P_{MHP} | r_s | R^2 |
		20	60	80	100	120	140	160	180					
DM (% AsIs)	−	92.2^e	92.3^e	92.6^e	92.8^{de}	93.3^{cd}	93.8^{bc}	94.4^b	95.1^a	0.212	**	NS	0.77*	0.95
	+	92.2^{cd}	92.3^{cd}	92.6^{abcd}	92.8^{bcd}	93.1^{abc}	93.2^{bc}	94.4^b	95.2^a	0.193	*		0.78*	0.37
CP (% DM)	−	33.1^{bcde}	34.7^{acd}	35.1^{ac}	34.4^{cd}	33.8^{de}	32.8^e	31.2^{fg}	30.4^g	0.473	**	NS	−0.62*	0.90
	+	33.2^{bc}	34.7^{ab}	35.4^a	34.6^{ab}	33.9^b	32.6^c	30.9^d	30.5^d	0.076	**		−0.61*	0.89
Lipid (% DM)	−	15.6^d	13.8^e	12.6^f	13.1^{ef}	15.1^d	18.4^c	21.6^b	27.9^a	1.022	**	NS	0.70*	0.98
	+	19.2^c	16.1^d	16.2^d	16.7^d	19.3^c	20.7^c	23.5^b	26.1^a	0.711	**		0.75*	0.95
Carbohydrate (% DM)	−	11.3^{de}	11.4^{de}	11.4^e	11.4^e	11.5^{cd}	11.5^{cb}	11.6^{ab}	11.7^a	0.025	**	NS	0.95*	0.93
	+	11.5^{bc}	11.4^c	11.5^{abc}	11.4^{bc}	11.6^{ab}	11.5^{abc}	11.5^{ab}	11.6^a	0.022	*		0.50*	0.32
Soluble protein (% CP)	−	77.1^a	78.4^{ab}	75.3^{ab}	76.2^{ab}	77.2^{ab}	74.4^b	71.7^c	67.9^c	4.222	**	**	−0.75*	0.83
	+	15.8	16.3	13.1	14.6	13.0	14.7	11.6	14.8	4.129	NS		−0.24*	0.09
Solubility 0.5% KOH (% CP)	−	57.2	54.9	56.2	53.6	55.1	52.8	54.2	58.3	2.874	NS	**	−0.08*	0.24
	+	36.0	34.0	32.8	29.4	33.4	35.2	33.2	31.6	2.945	NS		−0.16*	0.11
RUP (% CP)	−	26.4^f	25.8^f	27.4^{ef}	32.2^c	29.1^{cd}	30.0^d	39.0^b	46.9^a	2.423	**	**	0.79*	0.87
	+	66.6^a	65.4^{ab}	65.3^{ab}	65.9^a	64.4^{ab}	64.1^{ab}	63.5^{ab}	60.1^a	2.448	**		0.68*	0.00
IVCPD (%)	−	9.78	10.1	10.7	9.82	10.8	9.80	10.4	10.5	0.180	NS	**	0.08	0.05
	+	11.8	11.9	11.9	11.6	12.1	11.6	10.9	11.6	0.007	NS		0.34	0.11

Note. MHP = moist heat pressure (120 ^{o}C 15 min 192 kPa), DM = dry matter, CP = crude protein, RUP = *in vitro* rumen-undegradable protein, IVCPD = *in vitro* crude protein digestibility. Means in rows with unlike superscripts differ ($P < 0.05$). SEM = standard error of mean; r_s = pair-wise Spearman correlation coefficient; R^2 = coefficient of determination; P_{BT} = difference between barrel temperatures; P_{MHP} = difference between non- and MHP treatment samples; **$P < 0.01$; *$P < 0.05$; NS = not significant.

Table 2. Cornell Net Carbohydrate and Protein System protein fractions of canola meals produced at increasing barrel dry heat (20 to 180 ^{o}C)

| | Barrel Dry Heat (°C) | | | | | | | | SEM | P_{BT} | r_s | R^2 | Population Mean |
	20	60	80	100	120	140	160	180					
NPN (% DM)	0.095^c	0.087^{cd}	0.082^d	0.083^d	0.095^c	0.107^b	0.114^b	0.119^a	0.004	**	0.73*	0.87	0.10
ADF (% DM)	17.5	18.7	16.6	17.1	18.0	17.6	16.5	16.8	0.254	NS	−0.41	0.13	17.3
NDF (% DM)	22.5^{ac}	23.4^{ab}	21.2^d	19.6^e	23.0^{ab}	20.7^d	20.8^d	19.7^e	0.369	**	−0.61*	0.37	21.4
ADICP (% DM)	1.73	1.88	1.57	1.62	1.80	1.70	1.51	1.61	0.037	NS	−0.53*	0.17	1.68
NDICP (% DM)	2.03^{abcd}	2.16^{ab}	1.86^{bcd}	1.81^{cd}	2.07^{bc}	1.80^{cd}	1.89^c	1.93^{abcd}	0.034	**	−0.33	0.19	1.94
A (% CP)	0.042^f	0.039^{ef}	0.038^e	0.037^c	0.042^d	0.046^c	0.049^b	0.053^a	0.001	**	0.74*	0.92	0.04
B1 (% CP)	77.1^d	78.3^a	75.3^{bc}	77.5^b	75.9^c	75.5^c	71.6^e	69.0^f	0.768	**	−0.76*	0.86	75.1
B2 (% CP)	16.8^e	15.5^f	19.5^c	17.2^{de}	17.8^{cd}	18.6^c	22.2^b	24.7^a	0.738	**	0.81*	0.82	19.0
B3 (% CP)	0.894	0.720	0.849	0.437	0.893	0.308	1.30	0.982	0.103	NS	0.23	0.14	0.80
C (% CP)	5.21	5.43	4.39	4.81	5.36	5.24	4.86	5.24	0.106	NS	0.05	0.06	5.07

Note. NPN = Non-protein nitrogen, ADF = acid-detergent fibre, ADICP = acid-detergent insoluble crude protein, NDF = neutral-detergent fibre, NDICP = neutral-detergent insoluble crude protein. The protein fractions were calculated as previously described (Sniffen et al., 1992), whereby B3 (NDIN − ADIN), and B2 (Total CP − (A + B1 + B3 + C)). A = non-protein N, B1 = rapidly degraded true protein, B2 = intermediately degraded true protein, B3 = slowly degraded true protein, C = undegradable true protein. Means in rows with unlike superscripts differ ($P < 0.05$). SEM = standard error of mean; r_s = pair-wise Spearman correlation coefficient; R^2 = coefficient of determination; P_{BT} = difference between barrel temperatures; **$P < 0.01$; *$P < 0.05$; NS = not significant.

For all meals ADF and ADICP contents were similar between dry heat temperatures, whereas NPN, NDF and NDICP contents varied ($P < 0.01$, Table 2). Overall, NPN ($R^2 = 0.87$) was positively and NDF ($r_s = -0.61$) and ADICP ($r_s = -0.53$) were negatively associated with dry heat temperature, but ADF ($r_s = -0.41$) and NDICP ($r_s = -0.33$) were not ($P > 0.05$). Dry heat did not alter ($P > 0.05$) protein Fraction B3 (0.80%) and C (5.07%) but did alter ($P < 0.01$) A (0.04%), B1 (75.1%) and B2 (19.0) ($P < 0.01$, Table 2). Fraction A ($R^2 = 0.92$) and B2 ($R^2 = 0.82$) were positively and B1 ($R^2 = 0.86$) was negatively associated with dry heat temperature.

4. Discussion

In this study, the effects of expeller barrel dry heat and MHP on general, protein solubility, and *in vitro* protein degradability characteristics of canola meals were investigated. Initial analysis of the general nutritional characteristics of cold-press (20 °C) and expeller (100 °C) meals, respectively, were similar values to those published for CP (33.1 *vs.* 36.4 to 45.0% (Leming & Lember, 2005; Seneviratne et al., 2011), 33.2 *vs.* 31.6 to 38.4% (AOF, 2007; Seneviratne et al., 2011)), DM (92.2 *vs.* 90.4 to 93.7% (Seneviratne et al., 2011), 93.3 *vs.* 88.3 to 98.2% (AOF, 2007; Leming & Lember, 2005)), and lipid levels (16.9 *vs.* 9.60 to 24.4% (AOF, 2007; Leming & Lember, 2005; Seneviratne et al., 2011), 18.4 *vs.* 8.5 to 17.0% (AOF, 2007)). Leming and Lember (2005) reported cold-press canola meal (expelled at 60 °C) contained less DM (91.7 *vs.* 95.3%), CP (30.6 *vs.* 36.1%), and more lipid (17.8 *vs.* 11.6%) compared to meal expelled at barrel temperatures of 98 to 112 °C. Here, DM, lipid and CP were similar between meals produced at 60 °C compared to 100 °C. Higher barrel temperatures would remove a higher amount of moisture and contribute to increases in DM. A reduction in moisture by heat evaporation may further catalyse the Maillard browning reaction (S. S. Bharate & S. B. Bharate, 2014). A decline in CP at higher temperatures ($\geq$ 160 °C, $P < 0.01$) is indicative of increased retention of CP in extracted oil (thereby decreasing the CP content of the meal), or the degradation of thermolabile proteins at higher temperatures (Jung et al., 2012). A reduction of carbohydrate (11.5%) levels compared to a prior report (15% (Newkirk, 2009)), may be explained by a variance of carbohydrate levels among canola and rapeseed types (Naczk & Shahidi, 1990).

These results differed from other studies reporting little association between barrel dry heat and general nutritional characteristics (Toghyani et al., 2014) and decreases in meal lipid at higher processing temperatures (Clandinin et al., 1956). While MHP had no effect on CP, lipid and carbohydrate contents, DM increased (control 93.3% *vs.* MHP 94.1%, $P < 0.05$). In comparison, Samadi et al. (2013) reported MHP of canola seed had no effect on DM and CP, but reduced carbohydrate and increased lipid (control 94.9%, 25.2%, 29.0%, 41.8% *vs.* autoclave (120 °C 1 h) treated 94.9%, 25.0%, 26.4%, 44.6%, respectively). Increased lipid in meal processed at higher barrel temperatures may have further catalysed Maillard reactions since lipid is a known proactive contributor to these reactions in other systems (Farmer, 1996).

Although protein solubility of expeller and cold-press meals in 0.5% KOH or borate buffer were similar, Smulikowska et al. (2006) reported a reduction of protein solubility of expeller meal compared to cold-press rapeseed meal. A negative association of BP-buffer protein solubility with dry heat temperature suggests the formation of insoluble complexes at higher temperatures. Based on the soluble protein classifications outlined by Pastuszewska et al. (2003), the expelled meals (55.3%) were very well processed and of high nutritional value (55 to 60% solubility); however, the MHP meals (33.2%) were over processed and declined in nutritional value (< 45% solubility). The *in vitro* RUP values of the cold-press (26.4%) and expeller (average 32.2%) meals were higher than previous reports by Kaldmäe et al. (2010) (10.8%) and Shannak et al. (2000) (17.8%), respectively, particularly at higher dry heat temperatures. Heat during the expelling process can induce the formation of insoluble peptide chain and carbohydrate complexes, which contribute to greater RUP in these meals (Deacon et al., 1988). Consequently, the positive association of RUP with dry heat temperature ($P < 0.01$) suggests the formation of insoluble complexes under higher temperature conditions. The application of MHP (15 min 192 kPa 120 °C) considerably increased the formation of RUP (32.3 *vs.* 64.4%, $P < 0.01$). Moshtaghi Nia and Ingalls (1992) similarly reported the application of MHP (15 min 117 kPa 127 °C) increased RUP (69.9 *vs.* 25.6%). Heating of meal was theorised to promote protein denaturation and reduce solubility to favour more rumen escape of un-denatured protein to the lower gastrointestinal tract (Van Soest, 1994). Levels of IVCPD were similar to those reported by (Mustafa et al., 2000) (9.78% *vs.* 16.4% CP). Notwithstanding dry heat temperature associated variances in ruminal protein availability, changes were not reflected in IVCPD values, which remained similar among dry heat temperatures. Increases in IVCPD after MHP were less than those reported for toasting (11.7 *vs.* 49.1%), but still suggest protein denaturation and formation of insoluble protein complexes with irreversible bonds by MHP, in turn, reducing available protein (Toghyani et al., 2014). Levels of ADF and NDF were similar to previous reports (CCC, 2015; Newkirk, 2009), while ADICP content was similar, and NDICP was reduced relative to feed library values (DairyOne, 2016; NRC, 2001). These results imply lower

levels of digestible fibre associated protein, known to contribute to RUP (Chrenkova et al., 2014). Compared to a previous report of Shannak et al. (2000) for expeller rapeseed meal, protein fraction levels of Fraction A (NPN) and B2 were lower, B1 was higher, and B3 and C were similar. These results imply lower levels of digestible fibre associated protein. Positive increases of Fraction A and B2 as B1 decreased imply increased dry heat temperature induces protein hydrolysis and conversion of rapidly to intermediately degraded protein, respectively. Future studies may aim to test different expeller barrel types and monitor the barrel retention time and exit temperature of meal.

These findings will likely benefit producers of canola meal by further detailing the effects of barrel dry heat temperature and MHP on canola meal structural and ruminal digestibility characteristics. Further knowledge of the nutritional characteristics of canola meal will enhance ration formulations and the predictions of animal performance.

Acknowledgements

References

AOF. (2007). *Final Report: Canola Meal Value Chain Quality Improvement Project* (Project Code: 1B-103-0506). Retrieved from http://www.porkcrc.com.au/Final_Report_1B-103.pdf

Bharate, S. S., & Bharate, S. B. (2014). Non-enzymatic browning in citrus juice: chemical markers, their detection and ways to improve product quality. *Journal of Food Science And Techology, 51*(10), 2271-2288. https://doi.org/10.1007/s13197-012-0718-8

Calsamiglia, S., & Stern, M. D. (1995). A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. *Journal of Animal Science, 73*(5), 1459-1465. https://doi.org/10.2527/1995.7351459x

CCC. (2015). *Canola Meal Feed Industry Guide*. Winnipeg, Manitoba, Canada. Retrieved from http://www.canolacouncil.org/media/516716/2015_canola_meal_feed_industry_guide.pdf

Chrenkova, M., Ceresnakova, Z., Weisbjerg, M., Formelova, Z., Polacikova, M., & Vondrakova, M. (2014). Characterization of proteins in feeds according to the CNCPS and comparison to *in situ* parameters. *Czech Journal of Animal Science, 59*(6), 288-295. Retrieved from http://www.agriculturejournals.cz/publicFiles/125751.pdf

Clandinin, D. R., Renner, R., & Robblee, A. R. (1956). Rapeseed oil meal studies. 1. Effects of variety of rapeseed, growing environment and processing temperatures on the nutritive value and chemical composition of rapeseed oil meal. *Poultry Sci., 38*, 1367-1372. https://doi.org/10.3382/ps.0381367

DairyOne. (2016). *Interactive Feed Composition Library Accumulated Crop Years 5/1/2000-4/30/2016*. Retrieved from http://www.dairyone.com/analytical-services/feed-and-forage/feed-composition-library/

Deacon, M., De Boer, G., & Kennelly, J. (1988). Influence of Jet-Sploding and extrusion on ruminal and intestinal disappearance of canola and soybeans. *Journal of Dairy Science, 71*(3), 745-753. http://doi.org/10.3168/jds.S0022-0302(88)79614-9

Farmer, L. J. (1996). Chapter 5: Interactions Between Lipids And The Maillard Reaction, *Flavor-Food Interactions, ACS Symposium Series* (1st ed., pp. 48-58). American Chemical Society, https://doi.org/10.1021/bk-1996-0633.ch005

Jones, R. A., Mustafa, A. F., Christensen, D. A., & McKinnon, J. J. (2001). Effects of untreated and heat-treated canola presscake on milk yield and composition of dairy cows. *Animal Feed Science and Technology, 89*(1-2), 97-111. https://doi.org/10.1016/S0377-8401(00)00219-4

Jung, S., Maria, J., Nobrega de Moura, L., Campbell, A. K., & Johnson, L. A. (2012). Enzyme-assisted aqueous extraction of oilseeds. *Enhancing Extraction Processes In The Food Industry* (1st ed., pp. 570). CRC Press, Boca Raton. Retrieved from https://www.taylorfrancis.com/books/9781439845950

Kaldmäe, H., Leming, R., Kass, M., Lember, A., Tölp, S., & Kärt, O. (2010). Chemical composition and nutritional value of heat-treated and cold-pressed rapeseed cake. *Veterinarija Ir Zootechnika, 49*(71), 55-60. Retrieved from http://vetzoo.lsmuni.lt/data/vols/2010/49/pdf/kaldmae.pdf

Khalili, H., Kuusela, E., Saarisalo, E., & Suvitie, M. (1999). Use of rapeseed and pea grain protein supplements for organic milk production. *Agricultural and Food Science in Finland, 8*(3), 239-252. Retrieved from https://journal.fi/afs/article/view/5626

Krishnamoorthy, U., Sniffen, C., Stern, M., & Van Soest, P. (1983). Evaluation of a mathematical model of rumen digestion and an *in vitro* simulation of rumen proteolysis to estimate rumen-undegraded nitrogen content of feedstuffs. *British Journal of Nutrition, 50*, 555-568. https://doi.org/10.1079/BJN19830127

Leming, R., & Lember, A. (2005). Chemical composition of expeller extracted and cold-pressed canola meal. *Journal Of Agricultural Sciences, 16*, 103-109. https://doi.org/10.17605/OSF.IO/BR749

Licitra, G., Herdandez, T., & Van Soest, P. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology, 57*, 347-358. https://doi.org/10.1016/0377-8401(95)00837-3

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.-I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Analytical Biochemistry, 339*(1), 69-72. https://doi.org/10.1016/j.ab.2004.12.001

McKinnon, J. J., Olubobokun, J. A., Christensen, D. A., & Cohen, R. D. H. (1991). The influence of heat and chemical treatment on ruminal disappearance of canola meal. *Canadian Journal of Animal Science, 71*, 773-780. https://doi.org/10.4141/cjas91-092

McKinnon, J. J., Olubobokun, J. A., Mustafa, A. F., 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. *Animal Feed Science and Technology, 56*(3-4), 243-252. https://doi.org/10.1016/0377-8401(95)00828-4

Moshtaghi Nia, S. A., & Ingalls, J. R. (1992). Effect of heating on canola meal protein degradation in the rumen and digestion in the lower gastrointestinal tract of steers. *Canadian Journal of Animal Science, 72*, 83-88. https://doi.org/10.4141/cjas92-009

Moshtaghi Nia, S. A., & Ingalls, J. R. (1995). Influence of moist heat treatment on ruminal and intestinal disappearance of amino acids from canola meal. *Journal of Dairy Science, 78*, 1552-1560. https://doi.org/10.3168/jds.S0022-0302(95)76777-7

Mustafa, A. F., Christensen, D. A., McKinnon, J. J., & Newkirk, R. (2000). Effects of stage of processing of canola seed on chemical composition and *in vitro* protein degradability of canola meal and intermediate products. *Canadian Journal of Animal Science, 80*, 211-214. http://doi.org/10.4141/A99-079

Naczk, M., & Shahidi, F. (1990). Chapter 12: Carbohydrates of Canola and Rapeseed. *Canola And Rapeseed: Production, Chemistry, Nutrition And Processing Technology* (1st ed., pp. 211-220). Springer, Boston, MA, USA. https://doi.org/10.1007/978-1-4615-3912-4_12

Newkirk, R. W. (2009). *Canola Meal: Feed Industry Guide* (4th ed. pp. 1-47). Canadian International Grains Institute, Winnipeg, MB, Canada. Retrieved from https://cigi.ca/wp-content/uploads/2011/12/2009-Canola_Guide.pdf

Newkirk, R. W., Classen, H. L., & Edney, M. J. (2003). Effects of prepress-solvent extraction on the nutritional value of canola meal for broiler chickens. *Animal Feed Science and Technology, 104*, 111-119. https://doi.org/10.1016/S0377-8401(02)00331-0

NRC. (2001). *Nutrient Requirements of Dairy Cattle* (7th ed., pp. 1-381). National Academy Press, Washington, DC, USA. http://doi.org/10.17226/9825

Pastuszewska, B., Buraczewska, L., Ochtabinska, A., & Buraczewski, S. (1998). Protein solubility as an indicator of overheating rapeseed oilmeal and cake. *Journal of Animal and Feed Science, 7*, 73-82. https://doi.org/10.22358/jafs/69199/1998

Pastuszewska, B., Jabłecki, G., Buraczewska, L., Dakowski, P., Taciak, M., Matyjek, R., & Ochtabinska, A. (2003). The protein value of differently processed rapeseed solvent meal and cake assessed by *in vitro* methods and in tests with rats. *Animal Feed Science and Technology, 106*, 175-188. http://doi.org/10.1016/S0377-8401(03)00005-1

Paz, H. A., Klopfenstein, T. J., Hostetler, D., Fernando, S. C., Castillo-Lopez, E., & Kononoff, P. J. (2014). Ruminal degradation and intestinal digestibility of protein and amino acids in high-protein feedstuffs commonly used in dairy diets. *Journal of Dairy Science, 97*(10), 6485-6498. https://doi.org/10.3168/jds.2014-8108

Purser, D., & Woodroofe, J. (2004). Milk production and protein concentration are enhanced by replacing mechanically extracted canola meal with commercially treated canola meal in dairy diets *Animal Production in Australia, 25*, 136-139. https://doi.org/10.1071/SA0401035

Samadi, S. A., Theodoridou, K., & Yu, P. (2013). Detect the sensitivity and response of protein molecular structure of whole canola seed (yellow and brown) to different heat processing methods and relation to protein utilization and availability using ATR-FT/IR molecular spectroscopy with chemometrics. *Spectrochimica Acta Part A: Molecular And Biomolecular Spectroscopy, 105,* 304-313. https://doi.org/10.1016/j.saa.2012.11.096

Sánchez, J. M., & Claypool, D. W. (1983). Canola meal as a protein supplement in dairy rations. *Journal of Dairy Science, 66*(1), 80-85. https://doi.org/10.3168/jds.S0022-0302(83)81756-1

Santos, J. E. P. (2011). Chapter 5: Nutritional Management Of Lactating Dairy Cows*. Dairy Production Medicine* (1st ed., pp. 33-72). John Wiley & Sons, Inc. http://doi.org/10.1002/9780470960554.ch5

Seneviratne, R. W., Beltranena, E., Newkirk, R. W., Goonewardene, L. A., & Zijlstra, R. T. (2011). Processing conditions affect nutrient digestibility of cold-pressed canola cake for grower pigs. *Journal of Animal Science, 89*(8), 2452-2461. https://doi.org/10.2527/jas.2010-3569

Shannak, S., Südekum, K., & Susenbeth, A. (2000). Estimating ruminal crude protein degradation with *in situ* and chemical fractionation procedures. *Animal Feed Science and Technology, 85*(3-4), 195-214. http://doi.org/10.1016/S0377-8401(00)00146-2

Smulikowska, S., Mieczkowska, A., Czerwiński, J., Weremko, D., & Nguyen, C. V. (2006). Effects of exogenous phytase in chickens fed diets with differently processed rapeseed expeller cakes. *Journal of Animal and Feed Science, 15*, 237-252. https://doi.org/10.22358/jafs/66896/2006

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science, 70*, 3562-3577. http://doi.org/10.2527/1992.70113562x

Thanh, L. P., & Suksombat, W. (2015). Milk production and income over feed costs in dairy cows fed medium-roasted soybean meal and corn dried distiller's grains with solubles. *Asian-Australasian Journal of Animal Sciences, 28*, 4. https://doi.org/10.5713/ajas.14.0685

Theodoridou, K., & Yu, P. (2013). Effect of processing conditions on the nutritive value of canola meal and presscake. Comparison of the yellow and brown-seeded canola meal with the brown-seeded canola presscake. *Journal of the Science of Food and Agriculture, 93*(8), 1986-1995. https://doi.org/10.1002/jsfa.6004

Toghyani, M., Rodgers, N., Barekatain, M. R., Iji, P. A., & Swick, R. A. (2014). Apparent metabolizable energy value of expeller-extracted canola meal subjected to different processing conditions for growing broiler chickens. *Poultry Science, 93*(9), 2227-2236. https://doi.org/10.3382/ps.2013-03790

Van Soest, P. J. (1994). *Nutritional Ecology of the Ruminant* (2nd ed., pp. 1-488). Cornell University Press, Ithaca, NY, USA. Retrieved from http://www.cornellpress.cornell.edu/book/?GCOI=80140100037050

Wright, C. F., von Keyserlingk, M. A., Swift, M. L., Fisher, L. J., Shelford, J. A., & Dinn, N. E. (2005). Heat- and lignosulfonate-treated canola meal as a source of ruminal undegradable protein for lactating dairy cows. *Journal of Dairy Science, 88*(1), 238-243. https://doi.org/10.3168/jds.S0022-0302(05)72681-3
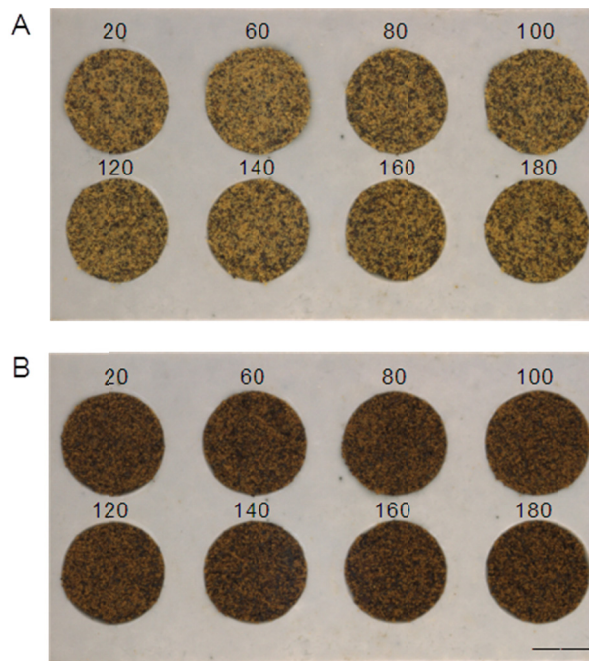
Appendix A

Figure A1. Representative photographs of expeller canola meal processed at increasing barrel dry heat (20 to 180 $^{\circ}$C) before (A) and after (B) moist heat pressure (MHP) treatment (120 $^{\circ}$C 15 min 192 kPa). A black scale bar represents 10 mm

Table A1. Linear and polynomial equations to describe the effect of increasing barrel dry heat (20 to 180 $^{\circ}$C) and moist heat pressure on general nutritional, protein solubility and degradability characteristics of canola meals

	MHP	Equation
DM (% AsIs)	−	$Y = 92.29149 - 0.00723x + 1.28488 \times 10^{-4}x^2$
	+	$Y = 92.89642 + 0.01082x$
CP (% DM)	−	$Y = 32.10474 + 0.06784x - 4.42899 \times 10^{-4}x^2$
	+	$Y = 32.23222 + 0.0687x - 4.54799 \times 10^{-4}x^2$
Lipid (% DM)	−	$Y = 19.27546 - 0.18641x + 0.00128x^2$
	+	$Y = 20.96246 - 12475x + 8.65724 \times 10^{-4}x^2$
Carbohydrate (% DM)	−	$Y = 11.23977 + 0.00219x$
Soluble protein (% CP)	−	$Y = 76.99929 + 0.05349x - 5.45297 \times 10^{-4}x^2$
RUP (% CP)	−	$Y = 29.51216 - 0.1371x + 0.00123x^2$
	+	$Y = 62.9791 + 4.44766 \times 10^{-4}x$
NPN (% DM)		$Y = 0.10202 - 4.62846 \times 10^{-4}x + 3.24676 \times 10^{-6}x^2$
NDF (% DM)		$Y = 23.21729 - 0.01731x$
ADICP (% DM)		$Y = 1.79581 - 0.00111x$
A (% CP)		$Y = 0.04458 - 1.80992 \times 10^{-4}x + 1.28359 \times 10^{-6}x^2$
B1 (% CP)		$Y = 75.51017 + 0.07307x - 5.90513 \times 10^{-4}x^2$
B2 (% CP)		$Y = 17.95254 - 0.05438x + 4.92388 \times 10^{-4}x^2$

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