Effect of heat treatment on *in situ* rumen degradability and *in vitro* gas production of full-fat soyabeans and soyabean meal

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Abstract

The objective of this study was to determine the effect of the heat treatment of full-fat soyabean (FFSB) and solvent extracted soyabean meal (SBM) on the *in situ* dry matter (DM) and protein degradability, and *in vitro* gas production kinetics of the protein sources. Ruminal disappearance of DM and crude protein (CP), and *in vitro* gas production were determined after 0, 4, 8, 16, 24, 48 and 72 h incubation using the *in situ* ruminal degradation and *in vitro* gas production techniques, respectively. *In situ* DM and CP disappearances were fitted to the exponential equation $p = a + b\ (1\text{-}e^{-ct})$, where a is the rapid degradable fraction and b is the slow degradable fraction. *In vitro* gas production data were fitted to the equation, $y = A\ \{1 - exp\ [-\ b\ (t\text{-}T) - c\ (\sqrt{t} - \sqrt{T})]\}$. Where *b* and *c* are the initial gas production rate constant (h^{-1}) and later gas production rate constant ($h^{-1/2}$), respectively. The two protein sources were heat treated both with steam pressure in an autoclave at 120 °C and in an oven at 150 °C for 20 min. Heat treatment had a significant effect on effective DM degradability (EDMD), effective CP degradability (ECPD) and *in vitro* gas production. Although the heat treatments reduced the EDMD, ECPD and the amount of gas produced, the results were inconsistent between protein sources. The heat treatments applied in the autoclave and the oven reduced the $ECPD_{0.02}$ of FFSB by 12.5% and 10.9%, respectively. On the other hand, heat treatment applied through the autoclave decreased the $ECPD_{0.02}$ of SBM by 13.9%, but by 18.7% when heat was applied through the oven. Heat treatment of SBM using the oven seemed to be more effective than using autoclaving. Heat treatments in the autoclave and oven reduced the total gas production from FFSB by 7.25 and 7.32%, respectively, and from SBM by 12.69 and 7.91%, respectively. It was concluded that heat treatment is an effective method of altering the rumen degradation characteristics of DM and CP in SBM and FFSB. Both methods could be used to increase the proportion of the rumen non-degradable protein fraction in protein sources which would then reach the small intestines unaffected by ruminal fermentation.

Keywords: Full-fat soyabean, soyabean meal, heat treatment, *in situ* protein degradation, *in vitro* gas production
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Introduction

Recent European limitations on the use of animal protein sources in ruminant diets (94/7381/EC; 95/60/EC) have made it more difficult to meet the rumen non-degradable protein requirements in high producing ruminants. As an alternative, soyabean products with their high protein content and good profile of essential amino acids could be used to meet the protein requirements of these classes of ruminants (Gonzalez *et al*., 2002). However, the protein in soyabeans (SB) and soyabean meal (SBM) are utilised with relatively low efficiencies by ruminants because of extensive ruminal degradation. It is estimated that only 25 to 34% of the protein in SB and SBM escapes rumen fermentation (NRC, 1989). This limits the inclusion of these products in the diets of rapidly growing and high producing ruminants. Consequently, an improvement in the proportion of protein from these sources escaping ruminal fermentation would be of major importance to both beef and dairy producers.

Various methods have been used to alter the rate and extent of the ruminal degradation characteristics of proteins. These include extrusion, roasting, expeller, and lignosulfonate and formaldehyde treatments. Heat treatment is the most economical process with which the best results have been obtained in practice (Lin & Kung, 1999). The two most commonly used methods of heat treatment are roasting and extrusion.

Faldet *et al*. (1991) and Schroeder *et al*. (1995) reported a reduction in the ruminal degradation of the protein in heat treated oil-free soyabeans.

The gas production technique has proven to be a potentially useful technique to evaluate the nutritive value of feedstuffs (Beuvink *et al*., 1992; Blümmel & Ørskov, 1993) since it gives an estimate of the potential rate and extent of nutrient fermentation in the rumen (Groot *et al*., 1996; Cone *et al*., 1997). However, this technique is measuring gas produced by the fermentation of energy containing components in feeds, and not only that of protein. There is, in fact, a lack of information regarding the effect of heat treatment on effective dry matter degradability (EDMD), effective crude protein degradability (ECPD) and *in vitro* gas production kinetics of protein sources. The objective of this study was to determine the effect of heat treatment of SBM and full-fat soyabeans (FFSB) on their EDMD, ECPD and *in vitro* gas production kinetics.

Materials and Methods

The two protein sources used in this experiment, raw FFSB and solvent extracted SBM, were subjected to two heat processing treatments, heat treated in an autoclave at 120 °C with steam pressure and heated in an oven at 150 °C for 20 min. The *in situ* ruminal degradation properties and gas production kinetics of these treated samples were compared with those of the untreated products.

The chemical composition of the products was determined. Ash content was determined by igniting the dried samples in a muffle furnace at 525 °C for 8 h (AOAC, 1990), nitrogen (N) levels were measured using the Kjeldahl method (AOAC, 1990) to calculate crude protein (CP) (N x 6.25), and crude fibre (CF) and ether extract (EE) levels were determined by the AOAC (1990) methods. All chemical analyses were carried out in triplicate.

The *in situ* DM degradation analysis was carried out according to the procedure described by Mehrez & Ørskov (1977). Five gram samples, milled through a 3 mm sieve, were placed in nylon bags with a pore diameter of 35-40 µ, and incubated for 4, 8, 16, 24, 48 and 72 h in three rumen fistulated sheep. The 0 h samples were washed in cold water to determine water solubility. A complete randomized block design was used. The sheep were fed twice a day on a 60% lucerne hay and 40% concentrate diet. After removal from the rumen, the nylon bags were washed thoroughly in running cold water until no further coloured liquid could be extruded, and dried at 60 °C for 48 h. Dry matter and CP losses for each incubation period were determined. The DM and CP degradation data were fitted to the exponential equation:

$p = a + b (1-e^{-ct})$ (Ørskov & McDonald, 1979),

where p is DM or CP disappearance in rumen at time t, a is the rapid degradable fraction, b is the insoluble but fermentable fraction and c = the constant rate of degradation of b (% per h).

Effective DM degradability (EDMD) was calculated applying the equation of Ørskov & McDonald (1979): EDMD = a + (bc / (c+k)), where k is the rumen outflow rates of 2, 5 and 8% per h.

To measure *in vitro* gas production, samples milled through a 1 mm sieve were incubated with rumen fluid in calibrated glass syringes, according to the procedures of Menke & Steingass (1988). Rumen fluid was obtained from three fistulated sheep fed twice daily on a diet containing lucerne hay (60%) and concentrates (40%). A dry sample (0.2 g) was weighed in triplicate into a calibrated glass syringe of 100 mL. The syringes were prewarmed at 39 °C before 30 mL of a rumen fluid-buffer mixture (in a 1 : 2 ratio) were injected into each syringe, followed by incubation at 39 °C in a water bath. Readings of the volume of gas produced were recorded before incubation (0) and at 4, 8, 16, 24, 48 and 72 h of incubation. Total gas values were corrected for the blank incubation. Cumulative gas production data were fitted to the model of France *et al*. (1993) using the MLP (Most Likelihood Program), (Ross, 1987):

$$y = A \{1 - exp [- b (t-T) - c (\sqrt{t} - \sqrt{T})]\}$$

where *y* represents the cumulative gas production (mL), *t* the incubation time (h), *A* the asymptote (total gas production, mL), *T* the lag time (h), *b* and *c* are the initial and later gas production rate constants (h^{-1}) and ($h^{-1/2}$), respectively. Estimated values of four parameters, *A, T, b* and *c* were determined from a time course experiment of 96 h incubation. The model postulates that the fractional degradation rate (µ, h^{-1}) is not constant, but varies with time along the fermentation period: $\mu = b + c / (2\sqrt{t});\ t \geq T$. Therefore, the gas production rates (µ, h^{-1}) were calculated after 4, 8 and 16 h of incubation.

Analysis of variance (ANOVA) was carried out to determine the effect of heat treatment on the *in situ* DM, CP degradation and *in vitro* gas production, using the General Linear Model (GLM) of Statistica for

windows (1993). Significant differences between individual means were identified using the Tukey's multiple range test (Pearse & Hartley, 1966). Mean differences were considered significant at $P < 0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

Results

The proximate chemical composition of the protein sources is presented in Table 1. In general, the heat treatments had little effect on the chemical composition of the products, though the two protein sources differed substantially in chemical compositions with the FFSB samples containing an average of 316 g CP and 194 g fat/kg DM and the SBM samples 479 g CP and 25 g fat/kg DM.

Table 1 The mean organic matter (OM), crude protein (CP), ether extract (EE), ash and crude fibre (CF) composition (g/kg DM) of the protein sources used in the study

Protein source	OM	CP	EE	Ash	CF
FFSB	931.7	317.9	192.6	68.3	44.0
$FFSB_A$	931.0	311.3	194.6	69.0	45.9
$FFSB_O$	931.5	316.4	193.8	68.5	42.6
SBM	923.2	486.9	26.6	76.9	95.0
SBM_A	927.9	476.2	25.4	72.1	94.7
SBM_O	920.7	474.6	24.1	79.3	94.5

FFSB - Raw full-fat soyabeans; $FFSB_A$ - Full-fat soyabeans heated in autoclave; $FFSB_O$ - Full-fat soyabeans heated in oven; SBM - Solvent extracted soyabean meal; SBM_A - Solvent extracted soyabean meal heated in autoclave; SBM_O - Solvent extracted soyabean meal heated in oven

The effects of heat treatment on percentage *in situ* DM disappearance are presented in Table 2. Heat treatment had a significant effect on the DM disappearances of both FFSB and SBM. Although heat treatments did not affect DM disappearances of FFSB and SBM at the early stages of incubation (4, 8 and 16 h), both the oven and autoclave treatments decreased ($P < 0.05$) DM disappearances at 24, 48 and 72 h incubation.

Table 2 The effect of heat treatment of raw full-fat soyabeans (FFSB) and solvent extracted soyabean meal (SBM) on the *in situ* dry matter disappearance (%) in the rumen

	Incubation times (h)						
Treatment	0	4	8	16	24	48	72
FFSB	30.3	39.6	46.8	60.7	72.8^b	81.5^b	87.6^b
$FFSB_A$	31.4	38.0	45.5	57.9	66.2^a	72.4^a	74.0^a
$FFSB_O$	30.7	34.4	45.7	57.2	64.6^a	72.0^a	76.7^a
s.e.m.	0.629	0.924	1.055	1.205	1.200	1.346	0.579
P	NS	NS	NS	NS	***	***	***
SBM	29.6	35.6	50.3	62.5	74.5^c	81.9^c	86.7^c
SBM_A	30.6	33.6	45.2	54.0	60.6^a	66.6^a	72.1^a
SBM_O	29.7	35.2	48.4	57.2	65.5^b	70.8^b	75.5^b
s.e.m.	0.823	1.350	1.252	2.179	0.843	0.960	0.505
P	NS	NS	NS	NS	***	***	***

$^{a\,b\,c}$ Column means with common superscripts did not differ ($P > 0.05$); s.e.m. - Standard error of mean
P - Within protein source, within column: NS - Non significant; *** $P < 0.001$
$FFSB_A$ - Full-fat soyabeans heated in autoclave; $FFSB_O$ - Full-fat soyabeans heated in oven
SBM_A - Solvent extracted soyabean meal heated in autoclave; SBM_O - Solvent extracted soyabean meal heated in oven

The effects of heat treatment on estimated parameters of DM degradation are presented in Table 3. Heat treatment had a significant effect on the estimated parameters of FFSB and SBM. Only autoclaving had a clear effect on the rate of DM disappearance of FFSB whereas heat treatment had no effect on the rate of DM disappearance of SBM. Heat treatment decreased the rapid degradable fraction (a), slow degradable fraction (b) and EDMD of FFSB and SBM at outflow rates of 0.02, 0.05 and 0.08/h. However, heat treatment due to autoclaving had no effect on the rapid degradable fraction (a) of SBM. Heat treatment of FFSB in the autoclave and oven reduced the EDMD by 10.84 and 11.12, respectively the outflow rate of 0.02/h. Heat treatment of SBM in autoclave reduced the EDMD by 17.78% whereas heat treatment of SBM using an oven reduced the EDMD by 10.78%.

Table 3 The effect of heat treatment of raw full-fat soyabeans (FFSB) and solvent extracted soyabean meal (SBM) on the *in situ* dry matter (DM) degradation kinetics (units in footnote)

	Estimated parameters					
Treatment	c	a	b	$EDMD_{0.02}$	$EDMD_{0.05}$	$EDMD_{0.08}$
FFSB	3.9^{a}	33.9^{b}	57.4^{c}	71.9^{a}	59.3^{b}	52.9^{b}
$FFSB_{A}$	5.5^{b}	31.9^{a}	43.9^{a}	64.1^{b}	54.9^{a}	49.8^{a}
$FFSB_{O}$	4.8^{ab}	30.5^{a}	47.4^{b}	63.9^{b}	53.6^{b}	48.2^{a}
s.e.m.	0.289	0.378	0.746	0.545	0.561	0.500
P	*	***	***	***	***	***
SBM	6.4	23.3^{a}	63.2^{b}	71.4^{c}	58.7^{c}	51.3^{c}
SBM_{A}	5.5	24.7^{a}	46.4^{a}	58.7^{a}	49.0^{a}	43.6^{a}
SBM_{O}	5.8	29.2^{b}	46.3^{a}	63.7^{b}	54.2^{b}	48.8^{b}
s.e.m.	0.351	0.532	0.831	0.601	0.599	0.557
P	NS	***	***	***	***	***

$^{a\,b\,c}$ Column means with common superscripts did not differ (P > 0.05); s.e.m. - Standard error of mean
P - Within protein source, within column: NS – non significant; * P < 0.05; *** P < 0.001
$FFSB_{A}$ - Full-fat soyabeans heated in autoclave; $FFSB_{O}$ - Full-fat soyabeans heated in oven
SBM_{A} - Solvent extracted soyabean meal heated in autoclave; SBM_{O} - Solvent extracted soyabean meal heated in oven
c - rate of DM disappearance (%); a – rapid degradable fraction of DM (%); b - slow degradable fraction of DM (%)
$EDMD_{0.02}$, $EDMD_{0.05}$, $EDMD_{0.08}$ – Effective DM degradability at outflow rates of 0.02, 0.05 and 0.08/h, respectively

Table 4 The effect of heat treatment of raw full-fat soyabeans (FFSB) and solvent extracted soyabean meal (SBM) on the *in situ* crude protein (%) disappearance in the rumen

	Incubation times (h)						
Treatment	0	4	8	16	24	48	72
FFSB	27.3^{b}	35.7^{b}	48.2^{b}	58.8	71.2^{b}	79.9^{b}	84.7^{b}
$FFSB_{A}$	22.8^{a}	31.6^{ab}	41.7^{a}	55.9	63.8^{a}	68.9^{a}	71.3^{a}
$FFSB_{O}$	21.0^{a}	30.7^{a}	46.1^{b}	57.5	63.3^{a}	72.3^{a}	74.6^{a}
s.e.m.	0.647	1.055	0.613	1.927	0.974	1.235	1.455
P	***	*	***	NS	***	***	***
SBM	29.8^{b}	42.7^{b}	57.7^{b}	67.6^{b}	76.4^{b}	83.7^{c}	88.0^{c}
SBM_{A}	22.4^{a}	33.2^{a}	46.3^{a}	55.9^{a}	61.7^{a}	67.8^{a}	72.7^{a}
SBM_{O}	23.0^{a}	34.3^{a}	43.9^{a}	56.1^{a}	64.7^{a}	73.7^{b}	76.5^{b}
s.e.m.	0.557	1.329	1.128	1.587	1.102	0.682	0.578
P	***	***	***	***	***	***	***

$^{a\,b\,c}$ Column means with common superscripts did not differ (P > 0.05); s.e.m.- Standard error of mean
P - Within protein source, within column: NS - Non significant; * P < 0.05; *** P < 0.001
$FFSB_{A}$ - Full-fat soyabeans heated in autoclave; $FFSB_{O}$ - Full-fat soyabeans heated in oven
SBM_{A} - Solvent extracted soyabean meal heated in autoclave; SBM_{O} - Solvent extracted soyabean meal heated in oven

Heat treatment had a significant effect on the CP disappearance of FFSB and SBM by reducing the disappearance at almost all incubation times except for the 16 h incubation time (Table 4). However, heat treatment in the oven had no effect on the CP disappearance of FFSB after 8 h incubation.

The effect of heat treatment on estimated parameters of CP degradation is presented in Table 5. Heat treatment had a significant effect on the estimated parameters of CP degradation of FFSB and SBM. Heat treatment increased the rate (c) of CP disappearance of FFSB whereas heat treatment in the oven decreased the rate of CP disappearance of SBM. Heat treatment decreased the rapid degradable fraction (a), the slow degradable fraction (b) and ECPD of FFSB and SBM at outflow rates of 0.02, 0.05 and 0.08/h.

The heat treatment in the autoclave reduced the $ECPD_{0.02}$ of FFSB by 12.5%, whereas heat treatment in the oven reduced the $ECPD_{0.02}$ of FFSB by 10.9%. On the other hand, the autoclave treatment decreased the $ECPD_{0.02}$ of SBM by 13.9%, whereas heat treatment in the oven decreased the $ECPD_{0.02}$ of SBM by 18.7%.

Table 5 The effect of heat treatment of raw full-fat soyabeans (FFSB) and solvent extracted soyabean meal (SBM) on the *in situ* crude protein (CP) degradation kinetics (units in footnote)

	Estimated parameters					
Treatment	c	a	b	$ECPD_{0.02}$	$ECPD_{0.05}$	$ECPD_{0.08}$
FFSB	4.2^{a}	30.8^{b}	58.5^{b}	70.4^{b}	57.5^{b}	50.9^{b}
$FFSB_{A}$	6.8^{b}	24.2^{a}	48.4^{a}	61.6^{a}	52.2^{a}	46.5^{a}
$FFSB_{O}$	6.1^{b}	24.3^{a}	50.9^{a}	62.7^{a}	52.3^{a}	46.4^{a}
s.e.m.	0.294	0.291	1.173	0.867	0.784	0.693
P	***	***	***	***	***	***
SBM	6.5^{ab}	33.1^{c}	54.4^{b}	74.7^{c}	63.8^{b}	57.4^{b}
SBM_{A}	6.9^{b}	23.9^{a}	47.6^{a}	60.7^{a}	51.4^{a}	45.8^{a}
SBM_{O}	4.4^{a}	31.1^{b}	48.1^{a}	64.3^{b}	53.7^{a}	48.2^{a}
s.e.m.	0.544	0.434	0.736	0.546	0.798	0.851
P	*	***	***	***	***	***

$^{a\,b\,c}$ Column means with common superscripts did not differ (P > 0.05); s.e.m. - Standard error of mean
P - Within protein source, within column: NS - Non significant; * P < 0.05; *** P < 0.001
$FFSB_{A}$ - Full-fat soyabeans heated in autoclave; $FFSB_{O}$ - Full-fat soyabeans heated in oven
SBM_{A} - Solvent extracted soyabean meal heated in autoclave; SBM_{O} - Solvent extracted soyabean meal heated in oven
c - rate of CP disappearance (%); a – rapid degradable fraction of CP (%); b- slow degradable fraction of CP (%)
$ECPD_{0.02}$, $ECPD_{0.05}$, $ECPD_{0.08}$ – Effective CP degradability at outflow rates of 0.02, 0.05 and 0.08/h, respectively

Heat treatment had a significant effect (P < 0.05) on the gas production of FFSB and SBM (Table 6). The lag time for samples was very low and close to zero. Therefore, lag time was ignored and not included in Table 6. Heat treatment reduced the gas production of FFSB and SBM at almost all incubation times. However, the reduction in gas production was considerably higher (P < 0.05) when the heat treatment was applied through the autoclave than in the oven.

Heat treatment had a significant effect on the estimated parameters of FFSB (Table 7). Heat treatment applied through both the oven and the autoclave reduced (P < 0.05) total gas production of FFSB without changing the rate of gas production. Heat treatment had no effect on the time to produce 50% of the total gas produced from the incubation of FFSB, though heat treatment increased (P < 0.05) the time to produce 95% of total gas produced from FFSB. Total gas production was decreased (P < 0.05) by 7.25 and 7.32% in the autoclave and oven treated FFSB, respectively, compared to the untreated sample, while the reduction in total gas production from SBM due to heat treatment in the autoclave and oven was 12.59 and 7.91%, respectively.

Table 6 The effect of heat treatment of raw full-fat soyabeans (FFSB) and solvent extracted soyabean meal (SBM) on the *in vitro* gas production (mL/g OM)

	Incubation times (h)					
Treatment	4	8	16	24	48	72
FFSB	135.2^{b}	161.0^{b}	239.2^{b}	289.5^{b}	367.7^{b}	389.8^{b}
$FFSB_A$	112.4^{a}	129.0^{a}	203.9^{a}	250.0^{a}	308.3^{a}	352.6^{a}
$FFSB_O$	130.2^{ab}	149.6^{ab}	235.6^{b}	265.7^{ab}	329.4^{ab}	370.0^{ab}
s.e.m.	4.791	6.110	5.880	7.95	10.06	7.41
P	*	*	**	*	*	*
SBM	185.4^{b}	265.8^{b}	320.1^{c}	393.2^{c}	434.6^{c}	451.2^{b}
SBM_A	140.3^{a}	216.1^{a}	250.2^{a}	305.2^{a}	344.4^{a}	381.2^{a}
SBM_O	152.7^{a}	229.4^{a}	292.6^{b}	344.3^{b}	386.2^{b}	405.0^{a}
s.e.m.	6.484	9.082	4.608	7.753	6.609	9.28
P	***	**	***	***	***	*

$^{a\,b\,c}$ Column means with common superscripts did not differ (P > 0.05); s.e.m. - Standard error of mean
P - Within protein source, within column: NS - Non significant; * P < 0.05; ** P < 0.01; *** P < 0.001
$FFSB_A$ - Full-fat soyabean heated in autoclave; $FFSB_O$ - Full-fat soyabean heated in oven
SBM_A - Solvent extracted soyabean meal heated in autoclave; SBM_O - Solvent extracted soyabean meal heated in oven

Table 7 The effect of heat treatment of raw full-fat soyabeans (FFSB) and solvent extracted soyabean meal (SBM) on the *in vitro* gas production kinetics

	Estimated parameters					
Treatment	*A*	T_{50}	T_{95}	μ_4	μ_8	μ_{16}
FFSB	406.8^{b}	10.2	67.8^{a}	3.8	2.7	1.9
$FFSB_A$	377.3^{a}	11.4	74.7^{b}	3.5	2.5	1.7
$FFSB_O$	377.0^{a}	10.5	80.1^{b}	4.0	2.9	2.0
s.e.m.	4.74	0.572	1.060	0.213	0.153	0.106
P	*	NS	**	NS	NS	NS
SBM	442.3^{b}	6.23	42.51^{a}	5.2	3.7	2.6
SBM_A	386.6^{a}	7.58	63.32^{b}	5.3	3.7	2.6
SBM_O	407.3^{a}	6.66	44.43^{a}	4.9	3.5	2.5
s.e.m.	6.038	0.293	2.312	0.384	0.270	0.191
P	*	NS	*	NS	NS	NS

$^{a\,b\,c}$ Column means with common superscripts did not differ (P > 0.05); s.e.m. - Standard error of mean
P - Within protein source, within column: NS - Non significant; * P < 0.05; ** P < 0.01;
$FFSB_A$ - Full-fat soyabeans heated in autoclave; $FFSB_O$ - Full-fat soyabeans heated in oven;
SBM_A - Solvent extracted soyabean meal heated in autoclave; SBM_O - Solvent extracted soyabean meal heated in oven;
A - total gas production (mL/g OM); T_{50} - time (hour) to produce 50% of the gas; T_{95} - time (hour) to produce 95% of the gas; μ_4, μ_8 and μ_{16} - the rate (%) of gas production at 4, 8 and 16 h incubation, respectively

Discussion

The chemical compositions of SBM and FFSB were consistent with those reported by Prestlokken *et al*. (1999), Gonzalez *et al*. (2002) and Deniz *et al*. (2004), though the CP level of FFSB was lower than that reported by Gonzalez *et al*. (2002). The differences in the chemical composition could possibly be associated with differences in the different industrial processing methods employed in this experiment. These results were consistent with findings of Gonzalez *et al*. (2002) who found that the CP levels of SBM and FFSB varied with processing methods.

The estimated parameters (c, a and b) of CP for SBM were consistent with finding of Deniz *et al.* (2004), but the estimated parameters (b and c) were lower than those obtained by Gorgulu *et al.* (1999) and Woods *et al*. (2003). On the other hand, the rapid degradable fraction (a) of SBM obtained in this experiment was higher (P < 0.05) than that reported by Gorgulu *et al*. (1999) and Woods *et al*. (2003). The estimated

parameters a and c of SBM and FFSB obtained in this experiment were consistent with the findings of Gonzalez *et al*. (2002) but the estimated parameter, b, of SBM and FFSB was lower than that reported by Gonzalez *et al.* (2002). Therefore, the estimated EDMD and ECPD were lower than those reported by Gonzalez *et al*. (2002).

The differences between the present experiment and published results might be due to differences in protein sources, pore size of the nylon bags, milling screen sizes, fistulated animals used and manufacturing processes employed when protein sources were obtained. In this experiment, pore size of the nylon bags and sample size were 35-40 µ and 3 mm, respectively, whereas in the experiment carried out by Woods *et al.* (2003) the same parameters were 50 µ and 2 mm, respectively. In the current experiment fistulated sheep were used, while cows were used in the experiment conducted by Woods *et al.* (2003). The variation between laboratories in the determination of protein degradability could therefore be associated with differences between laboratories in the methods used in sample preparation and processing, and in the characteristics of the bags used for incubation (Madsen & Hvelpund, 1994).

The lower *in situ* DM and CP degradation in the heat treated samples *vs*. the untreated samples suggested effective protection against ruminal degradation induced by heat. This result was in agreement with findings of Mustafa *et al*. (2003) who found that a moist heat treatment decreased ruminal DM and CP degradability of sunflower seed by 17% and 19%, respectively. These reductions in DM and CP degradation were higher than those recorded in the present experiment. Faldet *et al*. (1991) and Schroeder *et al*. (1995) also reported reductions in ruminal degradations of DM and CP when protein sources were heat treated. Heat facilitates the Maillard or nonenzymatic browning reaction between sugar aldehyde groups and free amino acid groups of protein to yield an amino-sugar complex (Lin & Kung, 1999). This complex is more resistant than normal peptides to enzymatic hydrolysis and reversibility of this reaction is dependent on temperature and duration of heat exposure (Lin & Kung, 1999). However, some precautions must be taken when heat treatments are employed because the Maillard reactions might render the protein and the carbohydrate unavailable in the small intestine if excess heat was employed (Lin & Kung, 1999). In the Maillard reaction excess heat causes losses of sugar and amino acids, especially lysine, which can be 5 to 15 times greater than for the other amino acids (Andrian, 1974). Therefore, heat treatment should be kept to a minimum due to its cost and the possibility of destroying essential amino acids such as lysine and methionine and reducing the availability of other nutrients (Kratzer *et al*., 1990; Van der Poel *et al*., 1995; Qin *et al*., 1998). The effect of heat treatment on the rumen degradability of protein sources was not consistent. As can be seen from Table 5 the heat treatment of SBM in an oven seems to be more effective than that in the autoclave. On the other hand, heat treatment of FFSB using an oven or autoclave gave similar responses.

The nutrients in protein supplements which are resistant to microbial degradation in the rumen, yet available for absorption in the small intestine, may help to supply the extra energy and amino acids needed by high producing dairy cows (Faldet & Satter, 1991). Feed efficiency of producing milk was improved when dairy cattle were fed diets containing heat treated SB and SBM (Schingoethe *et al*., 1988; Faldet *et al*., 1991; Nakamura *et al*., 1992).

The gas production method accentuated the differences between SBM and FFSB at all incubation times whereas the *in situ* nylon bag method was not sensitive enough to record differences between these protein sources in terms of DM disappearance. This could be due to the loss of DM and CP from the nylon bags which were not available to the rumen microorganisms. In addition the high fat content would inhibit fermentation of some of the fibre (Zinn, 1989; Doreau & Chilliard, 1997), and thus the amount of gas produced. This negative effect of fat on fibre fermentation would not be noticeable in the open *in situ* method. As can be seen from Table 7 the total gas production of SBM was considerably higher than that of FFSB. The *in vitro* gas production method would be more reliable in detecting inhibitory compounds in feeds because it is a closed system with a limited supply of rumen liquor. Any inhibitory compounds in feeds are likely to affect the activity of the rumen microbes. On the other hand, the *in situ* method is associated with a dilution effect which results from an open system within a large rumen environment and the copious supply of rumen fluid to nylon bag content (Apori *et al*., 1998). This study revealed a general problem of overestimation of degradability by the *in situ* nylon bag technique. As can be seen from Table 2 overestimation was especially noticeable after the short incubation. Dewhurst *et al*. (1995) found that the *in situ* method overestimated fermentation, and was strongly correlated with the carbohydrate composition of feeds, particularly during the shorter incubations. This suggested that it was caused mainly by a rapid

fermentable fraction which was lost from bags before it was fermented. However, heat treatment developed a similar pattern of reduction in EDMD and total gas production. Although the heat treatment of SBM in the autoclave seemed to be more effective in reducing EDMD and total gas production, heat treatment of SBM in the oven seemed to be more effective in reducing ECPD.

Conclusion

Heat treatment of the protein sources, SBM and FFSB, was an effective method of altering the extent of ruminal degradation of their DM and CP by increasing the rumen non-degradable protein fractions in the products. Heat treatment had a significant effect but showed inconsistent results between protein sources. Heat treatment of SBM using an oven was more effective than when using an autoclave. On the other hand, heat treatments of FFSB using either an oven or an autoclave gave similar results. Therefore, both methods of applying the heat treatment could be used to increase the proportion of rumen non-degradable protein to the small intestine, provided that caution is taken that the availability of amino acids in the small intestine is not affected.

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