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The Effect of Heat or Heat-Xylose Processing on Nitrogen Fractions and *in Situ*/*In Vitro* Ruminal and Post-Ruminal Protein Disappearance of Guar Meal

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Abstract: Problem statement: Guar meal is a by-product consisting of hull and germ and is mostly used as a protein source in poultry and ruminant rations. The aim was to determine the effect of heat or heat-xylose processing on nitrogen fractions, *in situ* ruminal degradation parameters and *in situ*/*in vitro* ruminal and post-ruminal disappearance of guar meal. **Approach:** Samples were intact Guar Meal (GM), heat processed GM (GM_{hp}, GM was heated at 100°C for 45 minute using industrial heater) and heat-xylose processed GM (GM_{hx}, xylose was included in GM to give a final concentration of 10 g kg^{-1} DM, then was heat processed at 100°C for 45 minute using industrial heater). Ruminal degradation parameters of DM and Crude Protein (CP) were determined by *in situ* technique using four ruminally fistulated sheep. Post-ruminal disappearance of ruminal-undegradable CP was determined on residue from 16 h ruminal incubation of guar meal samples by three-step *in situ/in vitro* enzymatic procedure. **Results:** Non-protein nitrogen of the GM_{hx} and GM samples ranged from 218-319 (g kg^{-1} N). Acid detergents insoluble nitrogen concentration of both GM and GMhp was significantly lower than that of GM_{hx} (10.3, 11.29 and 18.53 g kg^{-1} nitrogen, respectively). *In situ* fractional degradation rate constant (c) of DM and CP was significantly decreased as a result of heat-xylose processing. Effective crude protein degradability of GM_{hp} and GM_{hx} was higher than that of GM. Post-ruminal disappearance of ruminal-undegradable CP of GM_{hx} (0.965) was significantly higher compared with GM and GM_{hp} (0.918 and 0.906, respectively). **Conclusion:** Results of the present study demonstrated that heat and heat-xylose processing might effectively protect the DM and CP of guar meal from ruminal degradation.

Key words: guar meal, degradability, nitrogen fractions, Trichloroacetic Acid (TCA), heat-xylose processed, NPN concentration, reducing sugars, pharmaceuticals manufacturing, germ plus, dry heat, monogastric animals, Crude Protein (CP), Dry Matter (DM), Ether Extract (EE)

INTRODUCTION

Guar (Cyamopsis Tetragonoloba) is a sub-tropic resistant legume that mostly is cultivated in India peninsula. The main purpose for growing this plant is production of guar bean's gum. Guar bean consist of hull (14-17% of bean), endosperm (35-42% of bean) and germ (43-47% of bean). This seed has a large endosperm which contains a significant amount of mannogalactan called gum. It has many industrial applications, such as paper making, cosmetics and pharmaceuticals manufacturing, explosives and producing textiles and carpets (Rahman and Leighton, 1968). Isolation of mannogalactan from guar bean yields a high protein by-product which consists of germ (high protein product) or germ plus hull and residual of gum (low protein product). This is mostly used as a source of protein in ruminant and monogastric animals (Conner, 2002). To remove some of the beans odour and gum residual from Guar Meal (GM), it is toasted being subjected to steam and dry heat (Rahman and Leighton, 1968). Feeding characteristics and other properties of GM have been well evaluated in poultry nutrition (Couch *et al*., 1967; Verma and McNab, 1982; Lee *et al*., 2003a; 2003b; 2005; Hassan *et al*., 2008). However, few experiments have been done to evaluate the chemical composition and nutritive value of different guar meals for ruminant nutrition. Different methods have been applied to decrease the rumen degradability of protein of various oilseed meals, including treatment with heat (Mir *et al*., 1984; Nakamura *et al*., 1994a, Vanhatalo *et al*., 1995),

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formaldehyde (Mir *et al*., 1984), lignosulfonate (McAllister *et al*., 1993) and reducing sugars such as xylose (Wallace and Falconer, 1992; McAllister *et al*., 1993; Harstad and Prestlokken, 2000; Can and Yilmaz, 2002; Tunce and Sacaki, 2003). Heat processing has the advantage of increasing protein resistant to rumen degradability and enhance the amount of protein entering small intestinal. However, heating above the optimal temperature might result in the over-protection of protein to ruminal degradation and intestinal digestion (Dakowski *et al*., 1996). Heat-xylose treatment might decrease ruminal protein degradation that occurs via Maillard reaction between aldehydes group of xylose and amino acid (Van Soest, 1989). The aim of the present study was to evaluate the effect of heat or heat-xylose processing on chemical composition, nitrogen fractionations, *in situ* ruminal Dry Matter (DM) and Crude Protein (CP) degradation and ruminal and post-ruminal CP disappearance using three-step *in situ*/*in vitro* enzymatic procedure (3-step) of guar meal.

MATERIALS AND METHODS

Commercial production of guar gum is normally undertaken by using the process of roasting, differential attrition, sieving and polishing. Selected guar split is screened to clean and then soaked to pre-hydrate in a double cone mixer. Soaked splits, which have reasonably high moisture content, are passed through a flaker. The flaked guar split is ground to the desired particle size, followed by drying of the material. The powder is then screened through rotary screens to deliver required particle size. Using heating, grinding and polishing process, the husk is separated from the endosperm halves and the refined guar gum split is obtained. This isolation from the beans yields a by-product named intact Guar Meal (GM). In many industrial, it has been exposed to the further heat processing to extract the guar residual.

Experimental samples were intact GM, heat processed GM (GMhp, GM was processed at 100°C for 45 min using industrial heater) and heat-xylose processed GM (GMhx, xylose was included in GM to give a final concentration of 10 g kg^{-1} DM and heat processed as 100°C for 45 minute using industrial heater).

Samples were ground to pass through a 2 mm screen and then analyzed for Organic Matter (OM), Nitrogen (N), Ether Extract (EE) and ash (AOAC, 1990). Neutral Detergent Fibre (NDF) not assayed with a heat stable amylase and expressed exclusive of residual ash and acid detergent fibre exclusive of residual ash (ADF) were determined using the method of Van Soest *et al*. (1991). Nitrogen fractions [Non-Protein Nitrogen (NPN), buffer insoluble nitrogen, Neutral Detergent Insoluble Nitrogen (NDIN) and Acid Detergent Insoluble Nitrogen (ADIN) were determined as proposed by Licitra *et al*. (1996).

In situ rumen degradability of DM and CP of the samples were determined using polyester bag procedure. Approximately, 5 g DM of each sample was placed in a bag (9×17 cm; pore size of 52 μm, 10 bags per each sample) and incubated in the rumen for 2, 4, 8, 12, 16, 24 and 48 h. For 0.0 h, bags were washed with cold tap water (5 minute with gently hand pressure) until water was clear. Four sheep (49.5±2.5 kg) fitted by rumen fistulae were used. The animals were fed with 1.5 kg DM of Lucerne hay and 0.4 kg DM concentrates (165 g CP kg^{-1} of DM) per head per day. After removal the bags from the rumen, were washed under tap water and subsequently dried using an oven dryer (60°C, 48 h), then weighed to determine DM disappearance. Then, N concentration of rumen un-incubated and incubated samples was determined using Kjeldahl method (Kjeltec 2300 Auto analyzer, Foss Tecator AB, Hoganas, Sweden.

This stage of the experiment followed the 3-step procedure proposed by Danesh Mesgaran and Stern (2005). Ruminal disappearance of CP from the samples was determined using *in situ* bag procedure. Approximately, 5 g DM of each sample (15 bags per each feed) was placed in polyester bag (9×17 cm, with pore size of 52 μm) and incubated in the rumen of the sheep for 16 h. Bags were placed simultaneously in the rumen before the morning feeding and after removal from the rumen, they were washed under tap water until water was clear and subsequently dried using an oven dryer (60°C, 48 h). Samples from the incubated ruminal residues were taken for N analysis using Kjeldahl method (Kjeltec 2300 Auto analyzer, Foss Tecator AB, Hoganas, Sweden). Then, a part of ruminal un-degradable CP from the *in situ* bags was weighed into a 50 mL polypropylene centrifuge tube (each sample contained 15 mg of N, 4 tubes per each sample). Two blank tubes were also prepared to correct the N contribution of the enzymes. Ten ml of pre-warmed (37°C) HCl-pepsin solution [2 g of pepsin (Merck M-785) dissolved into 1 L of 0.1 N HCl] was placed in each tube. Tubes were vortexes and incubated for 1 h in a shaking incubator at 38.6°C (Parsazma, Iran). After 1 h of incubation, 0.5 mL of 1 N NaOH solution was added to each tube and then vortexes. The procedure continued by adding 13.5 mL of phosphate-pancreatin buffer (68 g of KH_2PO_4 per 1 L of distilled water, 37°C). The pH was adjusted to 7.8 with strong NaOH, followed by the addition of 6 g of pancreatin (Merck, M-7130). Tubes were vortexes and incubated for 24 h in a shaking incubator at 38.6°C. After incubation, 3 mL of Trichloroacetic Acid (TCA) solution (100 g of TCA 100 mL^{-1} of distilled water) was added to each

tube, then vortexes. The tubes were left for 15 min and then centrifuged at 10,000 g for 15 min. A part of the supernatant (5 mL) was pipetted from each tube to determine the N concentration using the Kjeldahl method (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden).

Calculation and statistical analysis: *In situ* disappearance of the DM and CP was calculated as the difference between the un-incubated and those remained after the incubation.

In situ disappearance data were fitted using an exponential equation (Fathi Nasri *et al*., 2006) of P= a+ b (1-e^{-ct}); where P is potential of degradability, a is quickly degradable fraction, b is slowly degradable fraction; c is constant rate of degradation and t is incubation time (h). Effective Degradability (ED) of DM and CP was calculated using the parameters of a, b and c at ruminal outflow rate (kp) of 0.05 and 0.08 h^{-1}, according to the equation described by Orskov and McDonald (1979); ED= a+ b.kp/(c+ kp). Data of *in situ*/ *in vitro* enzymatic procedure were used to calculate the ruminal and post-ruminal CP disappearance as described by Calsamiglia and Stern (1995). Data were subjected to statistical analysis using the GLM procedure of SAS with the flowing statistical model (SAS Institute, 1999); Yij= µ+ Ti+ Eij; where Yij = depended variable, µ= overall mean, Ti = effect of processing and Eij = residual error. Tukey test was used for the comparing of the means at $p<0.05$.

RESULTS

The chemical composition and nitrogen fractions of the guar meal samples used in the present experiment are shown in Table 1 and 2, respectively. Heat processed GM (GM_{hp}) and GM_{hx} had higher ($p<0.05$) EE (75.2 and 71.9 g/kg DM, respectively) and CP (580 and 594 g/kg DM, respectively) than those of GM (36.1 and 556 g/kg DM, respectively). Heat and heat-xylose processing caused an increase ($p<0.01$) in the NDF-NDIP and ADF concentration of GM_{hp} and GM_{hx} compared with GM (Table 1).

Non-protein nitrogen value of GM_{hx} was lower ($p<0.01$)) than those of GM and GM_{hp} (218, 319 and 297 g kg^{-1} N, respectively). The GM_{hx} and GM_{hp} samples had greater proportion ($p<0.01$) of N in the buffer insoluble N fraction compared with GM (16.2 and 9.76 %, respectively). Neutral detergent insoluble nitrogen content of GM_{hp} and GM_{hx} was higher ($p<0.01$) than that of GM (Table 2). Heat-xylose processing caused an increase ($p<0.01$) of the ADIN concentration of GM_{hx} compared whit those of GM and GM_{hp} (18.5, 10.3 and 11.3 g kg^{-1} N, respectively).

Table 1: Chemical composition of the guar meal (g kg^{-1} of DM)

	Feed samples*				
Nutrients	GM	GM_{hp}	GM_{hx}	SEM	P-value
Organic matter	948.0	948.5	948.5	0.27	0.04
Crude protein	566.0[a]	580.0[b]	594.0[c]	4.10	0.04
Ether extract	36.1[a]	75.2[b]	71.9[b]	1.30	<0.01
NDFom- NDIP [t]	218.0[a]	238.0[b]	238.0[b]	6.07	<0.01
ADFom [t t]	131.0[a]	140.0[b]	140.0[b]	2.00	<0.01
Ash	52.0[a]	50.9[b]	51.7[ab]	0.27	0.04

GM = intact guar meal; GM_{hp} = overheat processed GM; GM_{hx}= overheat-xylose processed GM' NDFom- NDIP = neutral detergent fiber not assayed with a heat stable amylase and expressed exclusive of residual ash which corrected for neutral detergent insoluble crude protein.[t]ADFom = acid detergent fibre expressed exclusive of residual ash. [a, b, c] Means with difference letter in each row were significant

Table 2 Nitrogen fractionations of the guar meal samples

	Feed sample *				
Nitrogen fractions	GM	GM_{hp}	GM_{hx}	SEM [t]	P-value
Non-protein nitrogen (g kg^{-1} N)	319.0[a]	297.0[a]	218.0[b]	8.76	<0.01
Buffer insoluble nitrogen (g kg^{-1} N)	666.0[a]	731.0[b]	774.0[c]	9.10	<0.01
Neutral detergent insoluble nitrogen (g kg^{-1} N)	55.0[a]	104.0[b]	118.0[c]	3.20	<0.01
Acid detergent insoluble nitrogen (g kg^{-1} N)	10.3[a]	11.3[a]	18.5[b]	1.20	<0.01

*GM= intact guar meal; GM_{hp}= overheat processed GM; GM_{hx}= overheat-xylose processed GM [t] SEM = standard error of means [a, b, c] Means with difference letter in each row were significant

Table 3: *In situ* ruminal Dry Matter (DM) and Crude Protein (CP) degradation parameters of the guar meal samples (mean±SEM)

	Feed sample*		
Parameters	GM	GM_{hp}	GM_{hx}
DM degradability parameters			
a	0.20±0.03	0.23±0.03	0.21±0.02
b	0.80± 0.03	0.77± 0.04	0.79±0.03
c (h^{-1})	0.12±0.01[a]	0.09±0.01[b]	0.07±0.01[c]
R^2	0.89	0.94	0.95
Effective degradability (kp= 0.05 h^{-1})	0.73	0.69	0.64
Effective degradability (kp = 0.08 h^{-1})	0.64	0.60	0.54
CP degradability parameters			
a	0.10±0.01	0.09±0.04	0.07±0.04
b	0.90±0.05	0.91±0.06	0.93±0.06
c (/h)	0.11±0.01[a]	0.08±0.01[b]	0.06±0.01[c]
R^2	0.87	0.93	0.92
Effective degradability (kp = 0.05 h^{-1})	0.71	0.65	0.57
Effective degradability (kp = 0.08 h^{-1})	0.60	0.54	0.46

GM = intact guar meal; GM_{hp}= overheat processed GM; GM_{hx}= overheat-xylose processed GM [**]: a= quickly degradable fraction; b= non-soluble degradable fraction; c= constant rat of degradation (h^{-1}). [a, b, c]: Means with difference letter in each row were significant ($p<0.05$). [Ŧ] kp= ruminal out flow rat. SEM = Standard Error of Means

In situ DM and CP degradation parameters of the samples are presented in Table 3. The fractional degradation rate (c) of DM was decreased ($p<0.05$) as a result of both heat and heat-xylose processing (GM= 0.12, GM_{hp}= 0.09 and GM_{hx}=0.07). In addition, applying of heat and heat-xylose processing on the samples decreased the effective degradability of DM.

Table 4: Ruminal (after 16 h incubation) and post-ruminal disappearance of ruminal un-degraded crude protein of the guar meal samples

	Feed sample				
Items	GM*	GM_{hp}	GM_{hx}	SEM †	P-value
Ruminal disappearance	0.960[a]	0.840[b]	0.700[c]	0.03	<0.01
Post-ruminal disappearance of ruminal-undegraded	0.918[a]	0.906[a]	0.965[b]	0.008	<0.01
Total tract disappearance	0.997[a]	0.985[b]	0.990[b]	0.001	<0.05

*: GM= intact guar meal; GM_{hp}= overheat processed GM; GM_{hx}= overheat-xylose processed GM † SEM = Standard Error of Means; [a, b]: Means with difference letter in each row were significant

Heat and heat-xylose processing did not have any effects ($p>0.05$) on both a and b fractions of CP (Table 3), while the rate of CP degradation of GM_{hx} was lower ($p<0.05$) than those of the other samples (Table 3). Effective CP degradability (calculated for kp = 0.05 h^{-1}) of GM_{hp} and GM_{hx} were 8.5 and 20% less than of GM. This effect was more obvious when kp was assumed at 0.08 h^{-1} (90 and 76% of GM, for GM_{hp} and GM_{hx}, respectively).

Data of ruminal (16 h incubation), post-ruminal and total tract CP disappearances are presented in Table 4. Results of the present study indicate that heat and heat-xylose procedures cause a decrease ($p<0.01$) in ruminal CP disappearance of GM (GM= 0.96, GM_{hp}= 0.84, GM_{hx}= 0.70). Post-ruminal disappearance of ruminal undegradable CP of GM_{hx} was higher ($p<0.01$) than those of the other samples (Table 4). It was also indicated that heat and heat-xylose processing decreases ($p<0.01$) in total tract CP disappearance of GM_{hp} and GM_{hx} (GM = 0.997, GM_{hp}= 0.985, GM_{hx}= 0.990).

DISCUSSION

Heat and heat-xylose processing applied in the present study caused a significant increase in CP, EE and NDF content of final products (Table 1). Approximately, 35-42% of guar bean is mannogalactan gum while the germ concentration is 43-45% (Conner, 2002). In contrast to the other compartments of guar bean, germ has a high CP and EE concentration (0.55 and 0.052 per kg DM, respectively). In gum production industry, mannogalactan residual is extracted from guar meal by subjecting it to steam and dry heating over than initial heat process which applied to extract gum (Rahman and Leighton, 1968). Therefore, steam and dry heat processing of guar meal tended to increase the proportion of germ and hull in the residual which caused to increase proportion of CP, EE and NDF in the guar meal.

The solubility of any feed protein source in acidic or basic solution is a practical procedure which is proposed by Cornell Net Carbohydrate and Protein system (Sniffen *et al*., 1992; Licitra *et al*., 1996) to evaluate the value of ruminant feedstuffs. Results of the present study indicate that heat-xylose processing is an effective procedure to decrease NPN concentration of GM as previously observed by Fathi Nasri *et al*. (2008) used whole soybeans. The reduction in NPN concentration due to heat and heat-xylose processing was likely due to the loss of amino acid. Ljokjel *et al*. (2000) and Dakowski *et al*. (1996) reported that heat processing caused to loss of labile essential and non-essential amino acids. The NPN concentration in GM_{hx} was almost 32.5 and 26.5% lower ($p<0.01$) than those of GM and GM_{hp}, respectively. Results of the present experiment indicate that NPN concentration of the guar meals ranged from 21-32%. These values are higher than the values reported for soybean meal (2.4-22.5, percent of total N) as a reference protein source (NRC, 2001).

Less in the buffer soluble N fraction of GM_{hx} and GM_{hp} in contrast with GM (-47.8 and -24.2%, respectively) suggested that buffer soluble N fraction was probably more responsive to heating and heat-xylose processing. An increase in the neutral detergent insoluble nitrogen fraction as a result of heat and heat-xylose processing was probably due to the denaturation and Maillard reaction of the proteins as this has been shown to reduce their solubility (Van Soest, 1994).

Heat processing applied in the present study caused to 1.8 times increase in ADIN concentration of guar meal, but. It has been proposed that xylose is a reducing sugar which causes to occur Maillard reaction and increase in ADIN concentration when combined with a feed protein source (Nakamura *et al*., 1994b). It has been assumed that ADIN might use as an indicator to estimate the ruminal and post-ruminal unavailable nitrogen (Goering *et al*., 1972; Yu and Thomas, 1976; Pichard and Van Soest, 1977; Sniffen *et al*., 1992). However, various workers indicated that ADIN was a poor indicator of nitrogen digestibility in forage and concentrate feedstuffs (Nakamura and Britton, 1994b; Jahani-Azizabadi *et al*., 2007).

As it was expected, the changes produced by heat and heat-xylose processing in the chemical composition of the samples evaluated caused an alter in the ruminal degradation parameters of the samples. Quickly degradable fraction (a) of the guar meal samples was slightly affected ($p>0.05$) with heat and heat-xylose processing.

Decrease in the fractional degradation rate (c) and effective DM and CP degradability of heat and heat-xylose processed guar meal samples might be a response of formation of low-digestible lignin-like polymers (Van Soest and Mason, 1991). Furthermore,

the formation of soluble phenolic compounds which have a depressing effect on ruminal DM degradation might be the other alternative (Lopez *et al*., 1995). Present results indicate that heat and heat-xylose processing caused to decrease the amount of material available to rumen microbes and this confirmed the findings of Pereira *et al*. (1998). In addition, results obtained by the present experiment confirmed the previous results suggesting that reducing sugars had a potential to alter ruminal degradability of protein sources such as soybean and canola meals (McAlister *et al*., 1993; Stanford *et al*., 1995; Tuncer and Sacakli, 2003; Rooke, 1985).

The lower effective CP degradability of both GM_{hp} and GM_{hx} demonstrated that the chemical and physical changes produced by heat and heat-xylose processing increased the proportion of intermediate and slowly degradable fractions of nitrogen (Table 2) which confirmed the results of previous workers (Broderick and Craig, 1980; Pereira *et al*., 1998; Tunce and Sacakli, 2003). Heat and heat-xylose processing, which stimulate Maillard reaction between aldehydes group of reducing sugars and free amino group (Martins *et al*., 2001), might increase resistance of the GM_{hx} protein to ruminal degradation. In the study of Tunce and Sacakli (2003), in which the effect of different levels of xylose (0.5, 1 and 2% of DM) on degradation of soybean and canola meals was studied, ruminal CP degradability (24 h incubation) was lower than the untreated sample (at the rate of 37-67% for soybean meal and 1-21% for canola meal). However, the effect of heat-reducing sugar such as xylose on the extent of ruminal protein degradation depends on feed type and the concentration of reducing sugar (Hashiba, 1982), moisture content, both treatment temperature and time applied (Cleale *et al*., 1987; Broderick *et al*., 1993), amount of lysine content and epsilon amino group of lysine (Windschitl and Stern, 1988).

Results of the present experiment demonstrated that heat-xylose processing might decrease ruminal disappearance of CP with a positive effect on post-ruminal disappearance of ruminal undegradable protein. Chalupa (1974) suggested that Maillard reaction between sugar aldehydes group and free amino group might decrease ruminal degradation of protein without adversely affecting post-ruminal protein disappearance. Van Straalen *et al*. (1993) evaluated ruminal and post-ruminal CP disappearance of the grass and clover and reported that within forages lower ruminal degradability was recompensed by higher post-ruminal digestion (Jahani-Azizabadi *et al.*, 2009). Ruminal, post ruminal and total tract CP disappearance of guar meal samples evaluated by the 3-step *in situ*/*in vitro* enzymatic procedure showed that the value reported were higher than those of the other plant meals (Danesh Mesgaran and Stern, 2005; Danesh Mesgaran *et al*., 2008). Despite the significant difference in post-ruminal CP disappearance of GM_{hx} with GM, the amount of total tract indigestible CP of GM_{hx} was very low (10 g kg^{-1} CP). Therefore, the results of the present study indicated that CP of various guar meals evaluated was almost completely digestible in gastrointestinal tract of ruminants.

CONCLUSION

In conclusions, it has been indicated that the protein content in guar meal is well digestible in ruminant gastrointestinal tract. However, GM has higher ruminal degradability and NPN content compared with the heat and heat-xylose processed samples. Therefore, present results indicate that both heating and heating-xylose treatments are effective methods to alter the rumen degradation characteristics of DM and CP of GM. Results obtained by the present study indicate that the ruminal and post-ruminal disappearance of GM protein are influenced by the physical and physico-chemical methods applied. However, there is a need to evaluate more physical and chemical methods to alter the ruminal degradation of GM without any negative effect on the post-ruminal disappearance of ruminal un-degradable protein.

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