THE EFFECTS OF DIFFERENT SOURCES OF LOW QUALITY ROUGHAGE ON SOYBEAN MEAL PROTEIN DEGRADATION IN THE RUMEN OF SHEEP

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SUMMARY

Three experimental rations containing concentrate feed mixture and soybean meal (SBM) along with one of three roughages, namely rice straw (RS), maize stalks (MS) or clover straw (CS) were formulated to be similar in CP% (13-14%). Three mature male ruminally canulated sheep were fed at 90% of their *ad libitum* intake from each experimental ration.

The DM and CP disappearance values of SBM were *in situ* determined at 3, 6, 9, 12, 18 h of incubation with each experimental ration and effective degradability was assessed. The *in situ* disappearance values showed that the DMD of SBM was significantly ($P<0.05$) increased when fed with CS from 3h up to 6hr than fed with RS or MS, while DMD continued to increase ($P<0.05$) from 6hr up to 18h when fed with MS. The DMD increased ($P<0.05$) when fed with CS than RS at 9h and 18h. The CP of SBM disappearance increased ($P<0.05$) at 3h up to 6h when fed with CS more than when fed with RS or MS. The CP disappearance increased ($P<0.05$) more when fed with MS or CS than with RS at 9h. It continued to increase ($P<0.05$) when fed with MS than with CS or RS from 12h up to 18h, while it increased ($P<0.05$) when fed with CS than with RS at the same period.

The effective degradability (ED) of DM was significantly increased ($P<0.05$) when fed with MS than when fed with RS or CS, while the ED of DM increased ($P<0.05$) when fed on CS than RS (57.57, 55.81 and 51.77% , respectively). The ED of the CP increased ($P<0.05$) when fed with MS than with CS and at the beast was with RS ration (45.65, 36.45 and 27.08% , respectively).

In conclusion, this study indicated that, although the crude protein (CP) is the most common method for expressing protein content, it fails to make important distinctions to the various contributors to poor-quality roughages. The simple measurements such as the degradable fractions (a, b and c) values are good indicators to distinguish between protein which degrades in the rumen and that which does not degrade. These different components of CP can be affected with the type of low quality roughage.

A clear understanding of these degradable fractions are needed to optimize the use of protein supplements with ruminants fed different low-quality forages.

Keywords : soybean meal, rice straw, maize stalks, clover straw, effective degradability.

INTRODUCTION

Protein is one of the important nutrients needed by animals for their growth and productivity.

Ruminants rations are supplied with oil seed cakes as cheap source of protein. Protein supplementation is needed when forage protein is too low to meet the ruminant requirements and / or

in instances when forage intake must be stimulated.

The requirements for protein are delineated into degradable crude protein; that is required by the rumen microbes, and metabolizable protein which is required by the host animal.

Degradable CP is a combination of protein that solubilizes in the rumen fluid and insoluble protein that is degraded by the microbes. Metabolizable protein is a combination of microbial protein synthesized in the rumen and the undegraded feed protein. Failure to meet the degradable protein requirement will result in a reduction of microbial protein synthesis, fermentation in the rumen and metabolizable protein supply. Protein source like soybean meal is excellent protein source because it contributes to degradable protein and can contribute to metabolizable protein when fed in excess of the degradable protein requirement.

Roughages play a major role as feed for ruminants. Seasonal patterns affect the availability and quality of these roughages, particularly during the dry season (Wanapat 1999). Not all indigenous fodder plants are readily palatable and acceptable by animals. Some have antinutritional factors and toxins. The differences between plant species are likely to be related to the differences in lignin structure, with gymnosperm lignin composed of coniferayl and sinapyl alcohols, and grass lignin of coniferyl, sinapyl, and P-coumaryl alcohols (Ladisch *et al*, 1983). The impact of lignin degradation on the biodegradability of the remaining carbon has not been extensively researched. The mathematical relationships developed by Van Soest (1994) are best used in a comparative sense, to help understand the differences in bioavailability of substrates of different composition.

The present study was conducted to predict the potential degradability of the dry matter and protein of soybean meal when supplemented to rice straw (RS), maize stalks (MS) and clover straw (CS) based diets using the *in situ* bag technique.

MATERIALS AND METHODS

The experimental work of the present study was conducted at the Agricultural Experimental Station and the Laboratories of Animal Production Department, Faculty of Agriculture, Mansoura University.

Experimental design :

Three experimental rations were formulated to investigate the influence of the type of poor quality roughage (rice straw, maize stalks and clover straw) on soybean meal (SBM) protein degradation in the rumen. The experimental rations were formulated to be almost iso-nitrogenous and to contain slightly more than 12% crude protein (Table, 1) recommended by Ørskov *et al* (1972) to ensure maximal rate of fermentation in the rumen. The rice straw, maize stalks and clover straw were chopped to length of about 5 cm.

Experimental animals and their management.

Three healthy Rahmany rams were used. They were about 1.5-2 years old, with an average live body weight of 45 kg. They were fitted with wide permanent rumen cannula (4 cm diameter).

Each experimental ration was offered *ad lib.* at 8.00 am to the experimental animals. The animals were kept in individual pens for the first 21 days. Each animal was then kept in a metabolic cage for another 21 days as a preliminary period and the incubations were carried during the last two successive days. During the

fermentation studies, 90% of *ad lib.* intake was offered.

In situ disappearance :

The artificial fiber bag technique developed by Mehrez and Ørskov (1977) was applied for measuring rate of DM disappearance in the rumen. On each of the sampling days, 5 weighed dacron bags were incubated in the rumen of each sheep (3, 6, 9, 12 and 18 h incubation interval). Each bag contained about 5 grams DM of the tested soybean meal with each of the three experimental rations. The data of disappearance were fitted by the exponential equation derived by Ørskov and McDonald, (1979) to describe the relation between disappearance and elapse of time of incubation to predict the degradable potential of the tested material. In order to define and derive the portions of material which disappear from the bags during incubation in the rumen, they described the relationship between disappearance and elapse of time of incubation through an exponential equation :

$$P = a + b\,(1 - e^{-ct})$$

"P" Represents the percentage degradability at time T.

"a" Represents the readily soluble fraction which disappears irrespective to fermentation (the intercept with y axis).

"b" Represents the fermentable fraction which disappears with the elapse of incubation interval.

"c" Represents the undegradable fraction.

"t" Time (h).

Statistical analysis :

The data collected for dry matter and crude protein disappearance were analyzed as a factorial design, while the degradability constants for dry matter and crude protein of SBM were analyzed by one way ANOVA in order to ascertain whether the observed treatment effects were real and discernible from chance effects. The null hypothesis was tested by F. test of significance (Gomez and Gomez, 1984). The difference between treatment means were tested by Duncan's (1955) Multiple Range test.

RESULTS AND DISCUSSION

Effect of the tested roughage rations on dry matter disappearance (DMD) and crude protein disappearance (CPD) of soybean meal (SBM):

Table (2) shows the effect of tested roughages on DMD from 3h up to 18h of ruminal fermentation of SBM. The DMD of SBM was significantly ($P<0.05$) increased when fed with CS from 3h up to 6h than when fed with RS or MS, while DMD continued to increase ($P<0.05$) from 6h up to 18h when fed with MS. The DMD increased ($P<0.05$) when fed with CS than RS at 9h and 18h.

Table (3) shows the effect of tested roughages on CPD from 3h up to 18h of ruminal fermentation. The CPD of SBM was significantly ($P<0.05$) increased when fed with RS or CS from 3h up to 6h, while CPD continued to increase (P0.05) from 6h up to 9h and then from 12h up to 18h when fed with MS only. On the other hand, CPD increased ($P<0.05$) at 3h up to 6h when fed with CS than when fed with RS or MS, then CPD increased ($P<0.05$) when feeding with MS or CS than RS at 9h, but CPD increased ($P<0.05$) when fed with MS then CS or RS from 12h up to 18h, while CPD increased ($P<0.05$) when fed with CS than RS at the same period.

Dietary protein breakdown or protease activity is accomplished by a number of rumen microorganisms and in a series steps with each step involving different rumen microorganisms.

Table (1) : Formulation of the experimental rations.

Items	Formulation of rations (%)		
	RS ration (1)	MS ration (2)	CS ration (3)
RS	60	-	-
MS	-	60	-
CS	-	-	75
CFM	30	30	17
SBM	10	10	8

Table (2): Effect of the tested roughage rations at different incubation intervals on DM disappearance of SBM.

Items	Incubation interval				
	3h	6h	9h	12h	18h
RS rations	38.48^{l}	44.44^{i}	46.26^{h}	56.03^{e}	55.99^{e}
MS rations	38.34^{l}	43.24^{j}	57.34^{d}	63.70^{b}	65.27^{a}
CS rations	41.55^{k}	46.98^{g}	54.17^{f}	55.95^{e}	61.49^{c}

a,b,c,d, e, f, g, h, i,, j, k and l : Means within the same row and column with different superscripts are significantly different (P< 0.05)

Table (3): Effect of the tested roughage rations at different incubation intervals on CP disappearance of SBM.

Items	Incubation interval				
	3h	6h	9h	12h	18h
RS rations	11.52^{i}	21.20^{gh}	22.88^{gh}	27.24^{fg}	33.14^{def}
MS rations	16.84^{hi}	24.59^{g}	39.33^{bcd}	43.93^{b}	55.38^{a}
CS rations	23.94^{g}	31.04^{ef}	34.82^{de}	36.47^{cde}	42.09^{bc}

a,b,c,d, e, f, g, h and 1 : Means within the same row and column with different superscripts are significantly different (P< 0.05)

Table (4): Effect of the tested roughage rations at different incubation intervals on DM degradability constants (%) of SBM.

Items	a	b	c	a+b	ED
RS rations	29.78^{l}	33.54^{m}	0.096	63.32^{m}	51.77^{m}
MS rations	20.91^{m}	52.06^{k}	0.122	72.97^{k}	57.57^{k}
CS rations	32.39^{k}	36.41^{l}	0.096	68.80^{l}	55.81^{l}

k, l and m: Means within the same column with different superscripts are significantly different (p< 0.05)

Table (5): Effect of the tested roughages rations at different incubation intervals on CP degradability constants (%) of SBM.

Items	a	b	c	a+b	ED
RS rations	3.10^{l}	37.87^{l}	0.099	40.97^{l}	27.08^{m}
MS rations	1.76^{l}	75.73^{k}	0.069	77.49^{k}	45.65^{k}
CS rations	16.07^{k}	29.36^{l}	0.108	46.03^{l}	36.45^{l}

k, l and m: Means within the same column with different superscripts are significantly different (p< 0.05)

Hobson and Stewart (1997) reported that 30 to 50% of the bacteria isolated from rumen fluid possess extracellular proteolytic activity . *Ruminobacter amylophillis* is one of the most active proteolytic species. Other predominant proteolytic bacteria include: *Butyrivibrio fibisolens, Prevotella mminicola, Clostridium* and *Eubacterium.*

Maklad and Mohamed (2000) made a comparison among the effects of clover hay (CH), corn silage (CS) and silage without ears (S) as feed ingredients on bacteria strains in the rumen of sheep. The mean values of *Bacteroids amylophilus* were 17, 75 and 42% for CH, CS and S rations, respectively. The *B. Succinogenes* values were 75, 58 and 50% when fed with CH, CS and S, respectively The total viable bacteria count (CFU/ml) × 10 values were 18.85, 29.30 and 17.09 with feeding on CH, CS and S rations, respectively.

The quality and quantity of carbohydrates fermented in the rumen influences the growth of rumen microorganisms (Nocek and Russell, 1988). The energy availability from forage is limited by fiber concentration because fiber is slowly and incompletely digested, whereas cell solubles are almost completely digested (Buxton and Redfearn, 1997). Thus the proportion of fiber to cell solubles are major determinant of energy availability in forages. Grasses normally have more fiber than legumes, especially in leaves. Grasses fiber is more digestible than that of legume but legume's fiber is digested at a faster rate. Ruminants digest 40-50% of legume fiber and 60-70% of grass fiber.

Blade *et al* (1993) reported that leguminous hays had a fast degradation rate and that their asymptote reach a plateau after 24 to 48 hr incubation, but for graminaceous hays the higher DM degradation was the result of a slower, but continuous rate of fermentation where the plateau was not reached until about 72 h incubation. Under *in vitro* conditions, such a low rate of degradation time led to a higher apparent DMD.

Effect of the tested roughages rations on the degradability constant (a, b, c) and effective degradability of DM and CP for SBM:

The effect of the tested roughages rations on the degradability constants a, b, c and effective degradability (ED) of DM of SBM is presented in Table (4). The soluble fraction (a) of DM was significantly higher ($P<0.05$) when fed with CS than when fed with MS or RS, but the higher ($P<0.05$) when fed with on MS than RS. The degradable fraction (b) was significantly increased ($P<0.05$) when fed with MS than when fed with RS or CS, while the DM degradable fraction (b) was higher ($P<0.05$) when fed with CS than with RS, and the same trends were observed with the DM degradability (a+b). There was no significant effect on the rate constant (c) when fed on RS, MS or CS.

Table (5) shows the effect of the tested roughages rations on the degradability constants a, b, c and effective degradability of CP of SBM. The soluble fraction (a) of CP was significantly higher ($P<0.05$) when fed with CS than when fed with MS or RS, while the CP degradable fraction (b) was significantly increased ($P<0.05$) when fed with MS than when fed with RS or CS. There was no significant treatment effect on the rate constant (c). The CP degradability (a+b) and effective degradability (ED) were higher ($P<0.05$) when feeding on MS than RS or CS.

Effective degradability (ED), is the degradability to be anticipated *in vivo* as

c. fractional outflow rate). The actual retention time in the rumen and hence the outflow rate depends on the type of the basal ration and level of feeding (Ørskov & McDonald, 1979). In addition, Mehrez (1981) demonstrated clearly that the effective degradation of several protein supplements varied when fed either with concentrate or roughage basal diets specialy of plant origin. To demonstrate the effect of the tested roughages on the ED, the equation developed by McDonald, (1981) was applied, being :

$$ED = a + (bc/c+k)$$

The fractional outflow rate from the rumen (k) was considered as 0.05, as described by Michalet-Doreau *et al* (1987) as shown in Tables (4 and 5).

Barry (1995) used exponential and logistic equations for respective processes. This integration allows estimation of extent of ruminal digestion of substrate for various feedstuffs. He reported that the sensitivity to variations in passage rate in these exponential rates was found to be relatively low (+/- 0.01/h). This method enables to combine the effects of multiple physical processes with differing kinetic behavior. Khazaal *et al.* (1995) showed that intake from a food is mostly explained by the rate at which the food is degraded (c) and the effect of this on the rate of passage of food through the rumen, whereas apparent digestibility is strongly associated with potential (a+b) degradability of the food.

The factional outflow rate, from the rumen was assumed at wide range from 0.01 to 0.12. The obtained possible ED values are presented in Table (6). It was clear that ED of DM of SBM was not greatly affected by type of roughage at any given factional outflow rate. On the other hand, ED of CP of SBM was markedly higher when fed with MS than the other tow roughages with the tendency for higher values when fed with CS than RS. This would indicate quite clearly that SBM protein is more success stable for degradation when fed with MS compared with the other tow roughages.

Mehrez *et al* (2003) showed that the CS was the lowest in NDF than MS and RS, while MS was the lowest in ADF and lignin than RS and CS. Beauchemin and Roode (2001), showed that the microbial activity is directly related to the NDF content of a feed. As plants mature, the proportion of lignin relative to the total fiber content increases. This means that the rumen bacteria are able to digest less fiber and the host animal will have to spend more time ruminating feed to allow it to pass out of the rumen.

Nonstractural carbohydrates are almost completely digested in the rumen within 24 h post feeding (Nocek and Russell, 1988). Stokes *et al* (1991) reported increased microbial protein yield *in vitro* when the proportion of NSC was increased.

Implications :

Protein supplements fed to ruminants consuming low-quality forage can simultaneously maximize the proportion of nutrients obtained from the forage. However, it has also been observed that the response to protein supplementation can be quite variable.

The crude protein (CP) is the most common method for expressing protein content. However, because of the method by which CP is determined, it fails to make important distinctions of the various contributors to poor-quality roughages. The simple measurements such as the degradable fraction (a, b and c) values are good indicators to distinguish between protein which degrades in the rumen (degradable intake protein, DIP) and that which does not degrade in the rumen (undegradable

Table (6) : The effect of low quality roughages rations on effective degradability of dry matter and crude protein of soybean meal at different out flow rates.

K values	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10	0.11	0.12
ED of dry matter												
RS rations	60.83	58.20	55.98	54.10	52.47	51.06	49.81	48.71	47.73	46.84	46.05	45.32
MS rations	68.91	65.45	62.47	59.86	57.57	55.54	53.72	52.09	50.61	49.27	48.05	46.93
CS rations	65.10	62.12	59.66	57.58	55.81	54.28	52.95	51.77	50.72	49.78	48.93	48.16
ED of crude protein												
RS rations	36.89	33.69	31.09	28.92	27.09	25.51	24.14	22.93	21.86	20.90	20.03	19.25
MS rations	67.89	60.46	54.53	49.68	45.66	42.25	39.34	36.82	34.61	32.67	30.94	29.40
CS rations	43.45	41.28	39.44	37.84	36.45	35.23	34.15	33.18	32.31	31.53	30.82	30.17

intake protein, UIP). These different components of CP can be affected by the type of low-quality roughage depending mainly on its cell wall components.

A clear understanding of these degradable fraction, are needed to optimize the use of protein supplements with ruminants fed low-quality forages. Further studies are needed to determine the outflow rate of protein supplements when fed with different roughages to accurately assess and recommend optimal level of other supplement.

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تأثير نوع مادة العلف الخشنة الفقيرة على درجة تكسير بروتين كسب فول الصويا فى كرش الأغنام

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أجرى هذا البحث بهدف دراسة تأثير أنواع مختلفة من مواد العلف الخشنة الفقيرة على خصائص تكسير المادة الجافة وبروتين كسب فول الصويا فى الكرش. حيث تم إختيار ثلاثة مواد علف خشنة فقيرة شائعة الاستعمال وهى قش الأرز وحطب الأذرة وتبن البرسيم حيث كونت علائق بإستخدام بعض الاضافات وهى علف مصنع وكسب فول صويا وهى على النحو التالى:

٦٠% قش أرز + ٣٠% علف مصنع + ١٠% كسب فول صويا
٦٠% حطب أذرة + ٣٠% علف مصنع + ١٠% كسب فول صويا
٧٥% تبن برسيم + ١٧% علف مصنع + ٨% كسب فول صويا

وكانت الخلطات متساوية تقريبا فى محتواها من البروتين. وتم قياس معدلات إختفاء المادة الجافة وبروتين كسب فول الصويا بطريقة تحضين أكياس فى الكرش على فترات ٣، ٦، ٩، ١٢، ١٨ ساعة بعد التغذية. ومن أهم النتائج المتحصل عليها:

زادت درجة إختفاء المادة الجافة لكسب فول الصويا معنويا (على مستوى ٥%) عند التغذية على علائق بها تبن برسيم وذلك خلال الفترة من ٣ – ٦ ساعات مقارنة بالتغذية على قش الأرز أو حطب الذرة بينما استمر زيادة إختفاء المادة الجافة معنويا (على مستوى ٥%) خلال الفترة من ٦ – ١٨ ساعة عند التغذية على حطب الذرة مقارنة بالتغذية على قش الأرز وتبن البرسيم مع ملاحظة أن المادة الجافة زاد درجة إختفائها معنويا (على مستوى ٥%) عند الفتر ٩ ساعات عند التغذية على تبن البسيم مقارنة بقش الأرز.

زاد إختفاء بروتين كسب فول الصويا معنويا (على مستوى ٥%) عند ٣ساعات ، ٦ ساعات عند التغذية على علائق تبن برسيم مقارنة مع علائق قش الأرز أو حطب الذرة بينما زاد إختفاء بروتين كسب فول الصويا معنويا (على مستوى ٥%) عند الفترة ٩ ساعات عند التغذية على علائق تبن البرسيم أو حطب الذرة مقارنة بعليقة قش الأرز ثم زاد معنويا (على مستوى ٥%) خلال الفترة ١٢ – ١٨ ساعة . التغذية على عليقة حطب الذرة مقارنة بالتغذية على بالتغذية على عليقة قش الأرز أو تبن البرسيم ولكن كان درجة إختفاء بروتين كسب فول الصويا أعلى معنويا (على مستوى ٥%) عند التغذية على تبن البرسيم مقارنة بعليقة قش الأرز خلال تلك الفترة.

زاد المهضوم من المادة الجافة معنويا (على مستوى ٥%) عند التغذية على حطب الذرة مقارنة بالتغذية على قش الأرز أو تبن البرسيم كما زاد المهضوم معنويا (على مستوى ٥%) من المادة الجافة عند التغذية على تبن البرسيم مقارنة بالتغذية على قش الأرز. كما زاد المهضوم معنويا (على مستوى ٥%) لبروتين كسب فول الصويا عند التغذية على عليقة حطب الذرة ثم علقة تبن البرسيم مقارنة بعليقة قش الأرز حيث كانت القيم هى ٤٥,٦٥، ٣٦,٤٥، ٢٧,٨% على التوالى.

تشير الدراسة الى أن تقدير خصائص تكسر البروتين بالكرش مثل c, b , a يمكن أن تعطى دلالة جيدة للقيمة الغذائية للبروتين المضاف للخلطات وخاصة عند التغذية على مواد علف خشنة فقيرة كعليقة اساسية لما لذلك من تأثير على درجة تكسير البروتين بالكرش بالدرجة التى تؤثر على نمو ونشاط الكائنات الحية الدقيقة سلبا .

من هذا فانه يمكن إستخدام مثل هذه الطرق لتوفير الاحتياجات الغذائية للكائنات الحية الدقيقة (البروتين المهضوم بالكرش) وذلك عند تركيب الخلطات العلفية لمعظمة تخمرات الكرش وخاصة عند تغذية حيوانات اللبن على مواد علف خشنة فقيرة .