# STUDIES ON THE DEGRADABILITY OF SOME PROTEIN SOURCES IN MIXED DIETS

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# ABSTRACT

Three females Barki sheep with an average body of weight 45 kg were used in this study in order to evaluate the degradability of dry matter (DM), organic matter (OM) and crude protein (CP) of four protein sources. Four mixed diets supplemented with one of four protein sources; soybean meal (SBM), linseed meal (LSM) undecorticated cotton seed cake(UDCSC) or urea as protein sources., Meat and bone meal (MBM) was added to all diet The degradability of whole diet was also studied. Roughage: concentrate ratio was 1:1 for all diets which were almost iso-caloric and iso nitrogenous. Data could be summarized as follow

1-The dry matter degradability of sources ranged between  $41.47\pm0.28$  for MBM and  $60.39\pm0.47$  for SBM.Significant differences were found (p<0.05).

2- The organic matter degradability of protein sources ranged between  $33.47\pm0.46$  for UDCSC and  $58.72\pm0.34$  for SBM. Significant differences were found. (p<0.05).

3- The crude protein degradability of protein sources ranged between46.45 $\pm$ 0.38 for MBM and 59.37 $\pm$ 0.41 for SBM. Significant differences were found (p< 0.05).

4- The dry matter degradability of the whole diet ranged between  $41.09\pm0.23$  for UDCSC and  $49.17\pm0.39$  for SBM. Significant differences were found (p< 0.05).

5- The organic matter degradability of the whole diet ranged between  $39.13\pm0.44$  for UDCSC and  $46.40\pm0.67$  for SBM with significant differences (p< 0.05).

6-- The crude protein degradability of the whole diet ranged between  $40.66 \pm 0.25$  for UDCSC and  $60.43 \pm 0.16$  for MBM with significant differences (p< 0.05).

Key words: Protein Degradability, SBM, LSM, UDCSC, MBM, Urea and WHFR

# INTRODUCTION

The recent protein evaluation systems are based on the amount of rumen degradable (RDP) and rumen undegrable protein (RUDP), in relation to the amount of microbial protein synthesis in the rumen (Lindberg 1985). The amount of the (RUDP) is the most important source of amino acid supply to the intestine in addition to the microbial protein (Messman *et al.*, 1996).

Protein composition and levels may affect the amount of degradable and undegradable protein in the rumen (Fahmy *et al* 1991), where the presence of cross-link bond may lead to increase the amount of undegradable protein (by pass protein) .These factors may lead to a change in the value of the calculated effective degradability (ED). Many other factors such as carbohydrates content, rate of outflow through the rumen (Michalet –Doreau and Ould –Bah 1992), may affect the (ED) value of different feeds, in addition the particle size can greatly affect the dry matter disappearance from nylon bags in the rumen (Mehrez and <sup>L</sup>rskov, 1978). Cell wall composition and concentration may also affect degradability. The objective of the present investigation is to study the effect of some protein sources (plant and animal proteins) on the dry matter, organic matter and crude protein degradability of whole mixed diets. Also, the total nitrogen reaching the post ruminal tract was calculated.

## MATERIALS AND METHODS

The present work was carried out at the Noubaria Experimental Station, Animal Production Research Institute, Ministry of Agriculture .The proximate analysis were carried out at the Department of Animal Production ,Faculty of Agriculture, Alexandria University (El Shatby).

Three female Barki sheep with an average body weight of 45 kg were used in the present work. They were, each fitted with a permanent rumen fistulae were used for the *in situ* study. Animals were offered four mixed diets which basically consisted of 35% water hyacinth fibrous residues (WHFR) as the most efficient percentages (Borhami and EL-Shazly,1984), and corn stalks (15%)were used as roughages . The concentrate : roughage ratio was 1:1. (Table 1) .Meat and bone meal (MBM 5%) was added as an animal protein source, in order to study the effect of mixed diets on animal protein degradability , also to supplement diets with some amino acids, in addition to one of four plant protein sources (soybean meal, linseed meal undecorticated cottonseed cake or urea), as protein supplement. Diets were formulated to be fairly isonitrogenous and isocaloric. Animals were fed the tested diets *ad.lib*. Each animal was fed the four diets.

Ingredients and chemical composition of experimental diets are presented in Table (1).

The *in situ* study was carried out by the incubation of nylon bags containing samples of the four basal diets and the four tested protein sources in sheep rumen for consuming the corresponding diet 2, 4, 8, 12, 24 and 48 hours in order to calculate the effective degradability (ED) of dry matter, organic matter and crude protein. Obtained data were fitted to a non linear regression model (exponential model) as developed by Ørskov and McDonald (1979) and modified by Dhanoa (1988).

The different estimates of (ED) were calculated from (*a*) the proportion of water soluble dry matter, organic matter and N of the tested feeds, (*b*) fraction, the proportion of potentially degradable dry matter, organic matter and N and *c* the fractional rumen degradation rate per hour of feed dry matter organic matter, and nitrogen and at rate of outflow k value 0.05 through the rumen, as described by Michalet-Doreau *et al* (1987).

Nylon bags (6x10cm) with 52ö m average poor size were prepared. Bags were dried at 80 C<sup>0</sup> over night in a forced air oven before being weighed. Samples of feeds were ground through 20 mm screen and 5.0 g from each tested feed was weighed in two bags for each incubation time and for each animal.

Bags were withdrawn, dried for 24 hours at 80 C<sup>0</sup> in a forced air oven then placed in a dessicator to cool and weighed. Analysis of feed and residual material, dry matter, organic matter and nitrogen disappearance (Mehrez and

 $\varnothing$  rskov, 1977).Feed and residual material nitrogen were determined according to the A.O.A.C (1970) methods.

Results of nitrogen balance, feed evaluation and microbial nitrogen synthesis were obtained from a previous study (Borhami etal, 2002), microbial nitrogen synthesis was calaculated as described by Borhami *et al.* 1992.

Data were statistically analyzed using the general model procedure (SAS 1990). Significance of results was tested by the least square means F score (p < 0.05).

### RESULTS

#### Degradation kinetics of protein sources:

Results of dry matter degradability of protein sources are shown in Table (2). Rapidly soluble fraction (*a*) of DM was higher (p < 0.05) for SBM and LSM than MBM and UDCSC. The UDCSC had the lowest value (p < 0.05). The (*b*) fraction was higher for LSM and SBM compared with MBM and UDCSC. No significant difference either between LSM and SBM or MBM and UDCSC were detected. The rate of degradation per hour(*c*) of fraction (*b*) was significantly higher (P<0.05) for UDCSC than the other three protein sources.

Effective degradability (ED %) of DM significantly varied

( P< 0.05) among the four protein sources tested. It was the highest for SBM(  $60.39\% \pm 0.27$ ), intermediate for LSM ( $55.22\% \pm 1.01$ ), MBM(  $41.47\% \pm 0.28$ ), and the lowest for UDCSC ( $35.30\% \pm 0.19$ ).

The degradation pattern of organic matter (OM) of the protein sources are presented in Table (2), the same trend for (*a*) fraction observed with DM was recorded for OM. Values of (*b*) fraction were higher with SBM, intermediate with LSM and the lowest (p< 0.05) was observed with UDCSC and MBM. The rate of degradation (*c*) recorded higher value with UDCSC followed by MBM in comparison to SBM and LSM.(P<0.05).

Regarding the ED% of OM there were significant differences among sources. The lowest figure was recorded with UDCSC ( $33.47\% \pm 0.46$ ) and the highest value was for SBM ( $58.72\% \pm 0.34$ ).

The degradation characteristics of crude protein (CP) for the different sources are presented in Table (2). The MBM had the highest (*a*) fraction than other protein sources, though it had the lowest value of (*b*) fraction, while the highest value was observed for SBM, followed by UDCSC (P < 0.05).

Concerning CP degradation MBM had the higher rate of degradation than other sources (P < 0.05).

The highest value for effective degradability (ED%) was observed for SBM (59.37 $\pm$ 0.41) and the lowest value was recorded for UDCSC , MBM ( 48.20 $\pm$  2.29 and 46.45 $\pm$  0.38) respectively .

The intermediate value was obtained with LSM ( $53.43 \pm 1.03$ ). The variances were significant (p<0.05).

#### Degradation kinetics of whole tested diets:

In addition to the degradability of protein sources, each mixed(basal diet+ protein source) diet was incubated in the rumen of sheep to measure DM, OM and CP degradability of the whole diet. Table (3) illustrates the ED (%) of mixed diet. The SBM supplemented diet showed the highest DM soluble fraction (P< 0.05), while the lowest value was obtained with UDCSC. The difference was significant.

Ruminal ED values of mixed diets were,49.17±0.39, 46.66±0.67,43,75±0.66 and 41.09±0.23 for SBM ,urea, LSM and UDCSC containing diets respectively with significant difference.

The ED values of mixed diets OM degradability and different parameters *a*, *b* and *c* are shown in Table (3). Fraction (*a*) showed the highest value with the urea diet , while the lowest value was obtained with the UDCSC and LSM diets, respectively with a significant difference (P<0.05). Fraction (*b*) was higher with SBM and urea diets followed by LSM diet, while UDCSC recorded lower value with a significant difference (P<0.05). Even though, LSM and UDCSC diets expressed the higher (P<0.05) value of fraction (*c*) followed by SBM containing diet, than did the urea diet where it was the lowest value (P<0.05).

The ED % of OM degradability showed the highest value for SBM containing diet  $46.40\pm0.67$  followed by urea diet  $46.04\pm0.73$  then LSM diet  $41.77\pm0.63$ , while the lowest value was found with the UDCSC diet  $39.13\pm0.44$ . With a significant differences (P < 0.05).

Degradation parameters of CP of the experimental diets are given in Table (3). The urea containing diet exhibited the highest saline solution solubility of CP. The lowest value was recorded with the UDCSC diet, while SBM and LSM diets showed intermediate values.

Slowly degradable of CP was the lowest in UDCSC diet, while urea diet showed the highest. The lowest value of *c* was associated with the SBM diet, while the highest figure was observed with LSM diet followed by UDCSC diet. Urea diet had a comparable rate of degradation. The ED values ranged between  $60.43\pm0.16$  for urea diet and  $40.66\pm0.25$  for UDCSC diet with a significant difference (P< 0.05).

Table 4 illustrates the effect of experimental diets supplemented with different protein sources on the utilization of nitrogen in sheep, where the highest value of microbial nitrogen synthesis (g/day) was obtained with the diet containing LSM followed by SBM, UDCSC and urea diets .different was significant (P<0.05) Data was obtained from a previous study (Borhami etal , 2002). Total nitrogen reaching the hind gut consisted from undegradable nitrogen and the microbial nitrogen synthesis, was highest with the diet containing UDCSC followed by LSM, SBM and urea diets.

# DISCUSION

It is well known that the dietary protein reaching the rumen are either degraded by rumen micro-organisms or escapes ruminal degradation and enter to the abomasums Effective degradability is a function of different parameters such as a, b and c with a rate of outflow k (0.05). Type of protein source, protein nature and amino acids composition which extremely affect degradability. The potential degradable fraction of plant protein usually is higher than that of animal origin. The latter has high content of collagen that makes it more resistant to degradation, while plant protein has more N in the form of potential degradable nitrogen fraction (b).(Bach *et al.,* 1998).

In the present study, the degradation fractions and effective degradability (ED) for different protein sources tested and their respective mixed diets were measured. Lower CP solubility (*a*) fraction was recorded for UDCSC, this may be attributed to its high content of nitrogen associated with cell wall, the highest *a* fraction for MBM may be related to the heat treatment during the manufacturing process that induce gelatinization of collagen and increase its solubility (Bach *et al.*, 1998). The slightly low value of fraction *a* for SBM and LSM proteins and giving rise to other fraction.

The effective degradability of dietary CP of mixed diet was affected by protein source. They had the same trend of their supplemented protein sources.

The comparison between the values of ED of protein in relation with *u* fraction calculated as  $\{u=100 - (a+b)\}$  seems to be in a good agreement with the values published in INRA tables of feed degradability (1988) and with those published by the AFRC (1993).

Otherwise, when the value obtained of u fraction increase, the value of NB as % of NA increase which seems to be logic. A better utilization of protein in the hindgut depends on the supply of amino acids to the duodenum (Merchen and Titgemeyer ,1992).

Results obtained in this study illustrated that when the microbial nitrogen synthesis in the rumen was less and the undegradable nitrogen expressed as a percentage of nitrogen balance (NB) from nitrogen intake (NI) was high Table (4), the nitrogen utilization was better which is the case of UDCSC which could be due to the high soluble fraction (*a*) in addition to the lack of some amino acids in comparison with other diets, however diet containing urea did not confirm this view.

This study revealed that there was a positive effect due to the interaction between animal and plant protein sources, this effect was more clear with UDCSC and SBM supplemented diets. These diets exhibited better nitrogen utilization. Cecava *et al.*,(1991), declared that the greater utilization for dietary nitrogen was associated with the combination of animal and plant protein sources. This result could be related to the complementary effect of amino acids profile. Oil seed cake and meals have good quality protein with badly balanced amino acid.

The values obtained of the amount of nitrogen leaving the rumen showed the highest value with the diet containing UDCSC followed by LSM containing diet. On the other hand the highest value of microbial nitrogen synthesis was obtained with LSM containing diet. The difference between microbial nitrogen synthesis and the total amount of nitrogen leaving the rumen between the two diets could be due to the high amount of nitrogen escaping degradability in the diet containing UDCSC. This may be due to the treatment of the UDCSC during processing or to the availability of different amino acids in this material which lead to low effective degradability of UDCSC compared with LSM.

In conclusion the present results cleared that the better utilization of basal diet containing water hyacinth fibrous residues could be achieved when an intermediate degradable plant protein source (UDCSC) was supplemented with an animal protein source (MBM).

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دراسات على تحلل بعض مواد العلف فى العلائق المخلوطة برهامى عز العرب, جواد عماد على, وائل جلال فهمى, منير العدوى, حسام الدين كامل قسم الانتاج الحيوانى كلية الزراعة جامعة الاسكندرية (الشاطبى)

اجريت هذه الدراسة بأستخدام عدد ثلاث من اناث الاغنام البرقى المزودة بفتحات مستديمة فى الكرش لدراسة تحلل بعض مواد العلف النباتية مثل كسب فول الصويا و كسب الكتان و كسب القطن الغير مقشور اضافة الى مسحوق اللحم و العظم كمصدر حيوانى و قد تم تغذية الحيوانات على اربع علائق احتوت بشكل ساسى على تفل ورد النيل بنسبة ٣٥% و حطب الذرة بنسبة ٢٥% كمواد مائلة و ٥% مسحوق لحم و عظم فى كل العلائق و دعمت كل عليقة بأحد مصادر البروتين و هى علىالتوالى كسب فول الصويا , كسب الكتان , كسب القطن الغير مقشور و اليوريا وذلك لاستقصاء الثاثيرات المتزامنة لمثل هذةالمصادر مع المصدر الحيوانى. و قد تم اجراء تجارب التقييم الغذائى لهذةالعلائق فى تجربة سابقة.و قد غذيت الحيوانات الى حد الإشباع لمدة ثلاثة اسابيع فترة لجمع العينات للتقيم اضافة الى ١٠ ايام فترة تمهيدية.

و قُدُّتم تحضين كل مصدر من مصادر البروتين مع االاربع علائق لدراسة تحلل كل مصدر على حدة اضافة الى تحضين الاربع علائق المخلوطة لدراسة تحلل كل عليقة عندما غذيت الحيوانات على نفس العليقة و قد اظهرت النتائج ما يلى:

١-اعلى معدل تحلل للمادة الجافة و المادة العضوية (٦٠,٣٩,٥٨,٧٢) كان لكسب فول الصويا و
 كان اقلها (٣٥,٣٥,٣٣,٤٧) لكسب القطن الغير مشور مع وجود فروق معنوية بين جميع العلائق.

٢- سجلت اعلى قيمة لمعدل تحلل لبروتين الخام كان اعلى قيمة مع كسب افول الصويا ٥٩,٣٧) و اقل قيمة سجلت مع مسحوق اللحم و العظم على التوالى (٤٦,٤٥,٤٨,٢٠) و كانت الفروق معنوية.

٣- معدل تحلل المادة الجافة فى العلائق المخلوطة سجل نفس الاتجاة حيث كانت اعلى قيمة فىعليقة كسب فول الصويا (٤٩,١٧) متبوعة بعليقة البوريا (٤٦,٦٦%) اما اقل عليقة فكانت عليقة كسب القطن الغير مقشور (٤٩,١٧) مع وجود فروق معنوية.

٤ --سلكت العلائق نفس المسلك في تحلل المادة العضوية حيث سجلت عليقة كسب فول الصويا اعلىمعدل تحلل للمادة العضوية (٤٦,٤٠ %) متبوعة بعليقة البوريا (٤٦,٠٤ %) في حين كانت عليقة كسب القطن الغير مقشور هي اقل العلائق (٣٩,١٣ %) مع وجود فروق معنوية.

 - تحلل البروتين الخم لعلائق المخلوطة اظهر اعى قَيمة لعليقة اليوريا (٦٠,٤٣) متبوعة بعليقة كسب فول الصويا (٩٣,٠٥%) و كانت اقل العلائق (٤٣,٦٦) مع و جود فروق معنوية.

٦- -اما بالنسبة لتحلل ابروتين الخام مع الاتزان الازوتى كنسبة مئوية من الازوت الممتص فقد اوضحت النتائج انة كلما زاد تحلل البروتين كلما انخفضت قيمة الاتزان الازوت كنسبة مئوية من الازوت الممتص فقد بلغت فى عليفة البوريا ١٢,١٥% فى كانت ٢٢,٩٠% فى عليقة كسب القطن الغير مقشور .

كنتيجة لهذة الدراسة يمكن توضيح ان استخدام تفل نبات ورد النيل يمكن ان يكون مجدى في علائق الحيوانات المجترة خاصة اذا تم استبدال جزء من البروتين النباتي في العليقة بجزء من البروتين الحيواني و ان كانت هذة النقطة تحتاج الى مزيد من الدراسة.

 Table (1): Composition of mixture diets fed to sheep (%, on dry matter basis).

				Diet
Ingredients	SBM	LSM	UDCSC	UREA
WHFR*	35	35	35	35
ChopedCorn Stalks	15	15	15	15
Yellow corn	29	29	20	37.5
MBM**	5	5	5	5
SBM***	10			0
LSM****		10		0
UDCSC*****			19	0
Urea				1.5
Molasses	3	3	3	3
Limestone	2	2	2	2
Common salt	1	1	1	1
Chemical composition, % DM				
OM	87.06	87.64	88.28	87.55
CP	15.65	14.60	15.12	14.81
CF	18.79	18.96	19.89	19.59
EE	3.49	3.49	3.85	3.23
NFE	49.13	50.59	49.42	49.92
Ash	12.94	12.36	11.72	12.45

\*Water hyacinth fibrous residues\*\*Meat and bone meal \*\*\*Soybean meal \*\*\*\* Linseed mea \*\*\*\*\* Undecortictaed cottonseed cake

 Table (2): Degradation kinetics of dry matter (DM), organic matter (OM) and crude protein of protein sources used

		in the experimental diets.					
	a.	b.	С	$U^1$	ED%		
Protein source							
DM							
SBM	34.19± 1.05 <sup>a</sup>	42.27± 1.65 <sup>a</sup>	0.101± 0.014	23.54±1.35°	60.39± 0.47		
LSM	32.63± 0.34 ª	48.25± 4.78ª	0.051± 0.007	19.12±2.56 <sup>d</sup>	55.22± 1.01		
UDCSC	15.45± 0.31 °	26.28± 1.41 <sup>b</sup>	0.017± 0.027 a	58.20±0.88ª	35.30± 0.1 9		
MBM OM	21.64±0.74 <sup>b</sup>	30.04±0.91 <sup>b</sup>	$0.098 \pm 0.005^{b}$	48.32±0.92 <sup>b</sup>	41.47±0.28°		
SBM	31.15± 0.46ª	43.96± 1.90ª	0.099± 0.014	24.89±1.18 <sup>d</sup>	58.72± 0.34		
LSM	32.18± 0.41 ª	36.11±2.97 <sup>b</sup>	0.102± 0.015	31.17±1.68°	54.33± 0.77		
UDCSC	13.80± 0.27 °	25.74± 1.04 °	0.019± 0.030	60.46±0.79 <sup>a</sup>	33.47± 0.46		
MBM	25.76±0.72 <sup>b</sup>	25.53± 1.11 °	0.158± 0.008	48.71±0.91 <sup>b</sup>	44.38±0.32°		
СР							
SBM	22.51±0.74°	55.59± 2.01ª	0.099± 0.057	21.90±1.46°	59.37± 0.41		
LSM	24.61± 0.59 <sup>b</sup>	47.57± 3.49 <sup>b</sup>	0.085± 0.0096 <sup>b</sup>	27.82± 3.53	53.43± 1.03		
UDCSC	15.10± 0.17 <sup>d</sup>	51.37± 1.41 <sup>ab</sup>	0.080± 0.0048 <sup>b</sup>	33.53± 1.35	48.20±2.29℃		
MBM	26.84± 0.20 <sup>a</sup>	26.79± 0.38°	0.155± 0.0022ª	46.37± 0.37ª	46.45±0.38°		

a, b, c and d Means in the same column for each consitituent with different superscripts are differ significantly (p

1 *u* : Undegradable fraction calculated from (100- (a+b))

# Table (3): Degradation kinetics of dry matter (DM), organic matter (OM) and crude protein (CP) of mixed diets supplemented with different protein sources.

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Diet					
	а	b.	С	$U^{1}$	ED%
DM					
SBM	19.18± 0.45 <sup>a</sup>	44.31±1.65	0.107± 0.009 <sup>ab</sup>	36.519±1.05	49.17± 0.39ª
LSM	15.12± 0.44 °	41.33±1.18	0.123± 0.005 <sup>a</sup>	43.55±0.81°	43.75± 0.66 °
UDCSC	13.11±0.24 <sup>d</sup>	41.90±1.21	0.106± 0.007 <sup>ab</sup>	44.99±0.72 <sup>a</sup>	41.09± 0.23°
Urea <b>OM</b>	18.02±0.31 <sup>b</sup>	46.6±2.34	0.087±0.009 <sup>b</sup>	35.38±1.32 <sup>b</sup>	46.66±0.67 <sup>b</sup>

SBM	13.16± 1.02 <sup>b</sup>	48.57± 1.04ª	0.109± 0.007 <sup>ab</sup>	38.27±1.03°	46.40± 0.67 <sup>a</sup>
LSM	9.42± 0.27 °	44.81± 1.69 <sup>ab</sup>	0.142± 0.0019ª	45.77±0.98 <sup>b</sup>	41.77±0.63 <sup>b</sup>
UDCSC	9.07±0.28°	41.38±0.75	0.134±0.008ª	49.55±0.51ª	39.13± 0.44 °
Urea	15.85±0.20 <sup>a</sup>	46.26±1.30 a	$0.094 \pm 0.003^{b}$	37.89±0.75℃	46.04±0.73 <sup>a</sup>
СР					
SBM	14.27± 0.24 <sup>b</sup>	58.84± 1.81ª	0.085±0.006 °	26.89± 1.70 °	50.97±0.22 <sup>b</sup>
LSM	9.73±0.28℃	55.92±1.13 a	0.129± 0.007 ª	34.35± 1.29 <sup>b</sup>	48.95± 1.03 °
UDCSC	7.79± 0.05 <sup>d</sup>	47.27±0.82	$0.114 \pm 0.003$	44.94± 0.80 ª	40.66± 0.25 <sup>d</sup>
Urea	19.38±0.23 <sup>a</sup>	59.53±0.58 a	0.111±0.002 <sup>b</sup>	21.09±0.50 <sup>d</sup>	60.43±0.10 <sup>a</sup>

a, b ,c and d Means in the same column for each constituent with different superscripts are differ significantly (p <0.05).

1 - u: Undegradable fraction = (100 - (a+b))

# Table (4): Effect of diets supplemented with different protein sources on nitrogen utilization.

				Diets
Item	SBM	LSM	UDCSC	Urea
Dry matter intake (g/day)	1392.03±10.98	1231.10±14.6	1261.73±15.12	1005.9±14.81°
	а	0 <sup>b</sup>	b	
Crude protein %	15.65	14.60	15.12	14.81
Nitrogen intake (g)	34.63±0.27 <sup>a</sup>	28.76±0.99 <sup>b</sup>	30.53±0.78 <sup>b</sup>	23.84±1.81°
Ruminal undegradable	26.8±1.70℃	34.51±0.29 <sup>b</sup>	44.93±0.80 <sup>a</sup>	21.09±0.50°
nitrogen %				
Ruminal undegradable	9.37±0.59 <sup>b</sup>	9.87±0.37 <sup>b</sup>	13.71±0.24 <sup>a</sup>	5.02±0.12°
nitrogen (g)				
Microbial N synthesis (g)	9.27±0.06 <sup>a</sup>	9.99±0.02ª	7.61±0.13℃	5.29±0.09 <sup>d</sup>
Total N leaving the rumen	18.64±0.33 <sup>b</sup>	19.79±0.20 <sup>a</sup>	21.32±0.19 <sup>a</sup>	10.31±0.11°
(g/day)				
Total ruminal undegradable	53.79±1.22 <sup>b</sup>	68.88±0.20 <sup>a</sup>	69.83±0.24 <sup>a</sup>	43.28±0.06 <sup>c</sup>
nitrogen and microbial N				
synthesis as % nitrogen				
intake as%				
Nitrogen balance from	13.4±0.67ª	12.46±0.14ª	12.50±0.06 <sup>a</sup>	7.88±0.66 <sup>b</sup>
Nitrogen intake %				
Nitrogen balance (NB) as %	19.29±0.41 <sup>b</sup>	17.36±0.20 <sup>c</sup>	22.90±0.09 <sup>a</sup>	12.15±0.85 <sup>d</sup>
from nitrogen absorbed (NA)				

a, b,c and d means in the same column for each constituent with different superscripts are differ significantly (p<0.05)