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EFFECT OF FEEDING FREQUENCY ON DIGESTION, NITROGEN BALANCE AND RUMEN FERMENTATION IN OSSIMI SHEEP

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ABSTRACT: ***This study was designed to investigate the effect of increasing feeding frequency on nutrient digestibility, nitrogen balance and ruminal fermentation in sheep. Three fistulated Ossimi rams aged 3 years with an average body weight of 60 kg were used. The ration applied in the present study consists of Egyptian clover hay, 60% and concentrate feed mixture, 40%. The ration was offered either once, twice or three times per day. All animals had free access to fresh water. Rumen samples from each animal were collected before feeding and then at 1, 3 and 5 hrs after feeding via the ruminal fistulae. The results obtained revealed no differences were reported regarding DM intake. Digestion coefficients of CP, EE and CF were improved when animals were fed either twice or three-times a day over those fed once daily. Nutritive value (TDN) was not significantly different among the treatment groups. Increasing feeding frequency increased DCP%. Nitrogen retention was 5.16, 6.89 and 8.09 g/d, for 1x, 2x and 3x, respectively and Differences were significant. Biological value of the protein was improved as feeding frequency increased. Feeding frequency decreased the fluctuation in ruminal pH. Feeding the experimental ration more frequently lead to an increase in VFA production compared with feeding once. Molar proportion of acetic acid decreased when sheep was fed once a day compared with when sheep was fed twice or three times/d. Molar proportion of propionic acid took the opposite trend being low on 1x and higher on 2x and 3x feeding frequency.***

Key words: ***Feeding frequency, digestibility, N balance, rumen fermentation***

INTRODUCTION

Development in feeding frequency have made it possible to feed rations to farm animals in small portions at frequent intervals; promotional literature for these systems suggest that increased feeding frequency results in higher daily gain, digestibility and better reproductive performance (Taie, 1996; Mohy-El-Deen and Afify, 2003 and Afify *et al*., 2004). Increasing feeding frequency results not only in a more constant but also in a lower postprandial decrease of ruminal *p*H (Kaufmann, 1976 and Taie, 1996). Feeding ruminants more than once daily might decrease the risk of acidosis by minimizing

starch intake per meal and result in more stable ruminal conditions. Feeding twice daily decreased ruminal VFA concentration and increased the acetate: propionate ratio, which might affect the efficiency of energy utilization by limit-fed cattle, and thus improves performance (Soto-Navarro *et al*., 2000). Recently, Robles *et al*. (2007) suggested that it could be hypothesized that with more frequent feeding, intake would be more evenly spread throughout the day, moderating *p*H fluctuations and modifying feeding behavior. Therefore, the present study was designed to ascertain to what extent increasing feeding frequency could affect intake, nutrient digestibility and ruminal fermentation in sheep fed a high roughage ration.

MATERIALS AND METHODS

The present study was carried out at the Nutrition Laboratory and Animal Production Research Farm belonging to the Faculty of Agriculture, Menofiya University, Shebin El-Kom, Egypt during 2009. Three fistulated Ossimi rams aged 3 years with an average body weight of 60 kg were used to study the effect of feeding frequency (once, twice and three times) on water and feed intakes, nutrient digestibility, nitrogen balance and some rumen parameters. The experimental design was 3x3 Latin square. Rams were fed a high roughage ration. The experiment was designed in three periods; the first was considered as an adaptation period to each treatment, the second and third weeks were used for samples collection (repeated measurements). The experimental animals were kept individually in metabolic crates (1.60m x 0.53m) as described by Maynard *et al*. (1979) allowing separate collection of urine and feces.

The ration applied consisted of Egyptian clover (Berseem) hay, 60% and concentrate feed mixture (CFM), 40% and was offered either once (at 9.00h), twice (at 9.00h and 14.00h) or three times (at 9.00h, 14.00h and 18.00 h) per day. The DM allowance offered to the experimental animals was 3.5% of the body weight. Feed residuals were weighed daily every morning in the collection period and subtracted from the offered amount to obtain the actual feed consumed. All animals had free access to fresh water; water intake was measured daily.

The chemical composition of the ingredients and the experimental ration used in the formulation of the ration is presented in Table (1).

In order to verify the results, samples were collected during two successive weeks; during which, feces were quantitatively collected at 9:00 a.m. before feeding. A quantity of 10% from feces was withdrawn and dried to a constant weight in a forced air oven at 70ºC for 24 hrs. Dry fecal samples were ground to pass a 2mm screen and kept in plastic bags for later analysis. For the determination of nitrogen balance, every day during the collection periods, urine was quantitatively collected at 9:00 a.m. before feeding. Urine was collected in containers containing 50 ml of 0.1N HCl to maintain *p*H<2.00 in order to avoid N loss through ammonia volatilization and to avoid bacterial

growth in the urine. A quantity of 10% of the total urine from each ram was withdrawn and kept in glass bottles in the freezer for later determination of N content.

Table (1): Chemical composition of the ingredients used in the formulation of the experimental ration

Item	CFM*	Clover hay	The experimental ration
	----------% ----------		
Dry matter, DM%	90.79	87.76	88.97
	---------- on DM basis ----------		
Organic matter, OM	86.77	89.22	88.24
Crude protein, CP	11.65	13.41	12.71
Ether extract, EE	1.84	2.75	2.39
Nitrogen-free extract, NFE	61.89	42.86	50.47
Crude fiber, CF	11.39	30.2	22.67
Ash	13.23	10.78	11.76

*** CFM, concentrate feed mixture consisted of wheat bran; yellow corn; wheat straw; soybean cake; extracted bran; molasses; limestone and common salt (production of EL-Gaafrawy feeds).**

Following each collection period, rumen samples from each animal were collected before feeding and at 1, 3 and 5 hrs after feeding via the ruminal fistulae. Ruminal *p*H was measured immediately after collection using a digital *p*H meter (Sophisticated microprocessor *p*H meter). Rumen contents were strained through four layer of cheesecloth, and the fluid portion was acidified with 7.2 N H_2SO_4 at the rate of 1 ml of acid/100 ml of strained ruminal fluid. Strained samples were stored frozen (-10ºC) until analyzed for ammonia-N and total volatile fatty acid.

The determination of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen-free extract (NFE by difference) and ash in the feed and feces were carried out according to the official methods of AOAC (1990). Urinary N was also determined by the AOAC (1990) method. Ruminal ammonia-nitrogen determination was carried out as soon as possible using the steam distillation method described by Ahmed (1976). Total volatile fatty acids (VFA) were measured according to AOAC (1990). Data were analyzed using the general linear model of SPSS v13 (2003).

RESULTS AND DISCUSSION

Data of dry matter and water intake of sheep fed the tested ration as affected by feeding frequency are presented in Table (2). No differences were reported regarding DM intake either from roughage or concentrate portion. Intake of roughage was 1250, 1232 and 1248g/d for sheep fed once, twice and three times/d.

Table (2): Intake of DM and water of sheep fed the tested ration as affected by feeding frequency

Items	Treatments (feeding frequency)			SEM	Sig
	Once	Twice	Thrice		
Roughage, g/d	1250	1232	1248	8.32	NS
Concentrates, g/d	830	821	828	5.22	NS
Total DMI*, g/d	2080	2053	2076	9.31	NS
Water intake, L/d	6.5[a]	7.3[b]	7.7[b]	0.04	0.05

* DMI, total dry matter intake; SEM, standard error of means
[a,b] means within each row with different superscript differ significantly

It is worthy to note that the amount of feed offered to the experimental sheep was equal and restricted at 3.5% of the body weight; therefore, it was not expected for DMI to change. Abdel-Rahman and Suleiman (1994) reported that intake of DM, starch equivalent and DCP did not differ with Najdi male sheep fed 1x, 4x and 8x per day. Taie (1996) reported similar results when sheep was fed at the same total feed intake.

Earlier studies (Prior, 1976 and Coleman *et al*., 1984) did not observe any differences in DM intake due to frequency of feeding. Recently, Elseed (2005) also reported that intake of DM was not influenced by feeding frequency of protein supplement.

Water intake increased linearly as the frequency of feeding increased; water intake was 6.5, 7.3 and 7.7 L/d for sheep fed 1x, 2x and 3x, respectively. The increase in water intake with the increase in frequency of feeding was also reported by Taie (1996).

Nutrients digestion coefficients of the experimental ration are shown in Table (3). Data indicated that the digestion coefficients of CP, EE and CF were improved ($P<0.05$) when animals were fed either twice or three-times a day over those fed once daily. Differences regarding digestibility of DM, OM and NFE were not significant among the treatment groups. Abdel-Rahman and Suleiman (1994) revealed that digestibility of all nutrients increased ($P<0.05$) with animals fed 4x and 8x daily. Results of Taie (1996) indicated that apparent digestibility of DM, CP and CF was highest for 2x followed by 3x and least for 1x.

Table (3): Digestion coefficients and nutritive values of the experimental ration as affected by feeding frequency

Nutrients	Treatments (feeding frequency)			SEM	Sig
	Once	Twice	Thrice		
Digestibility, %:					
DM	63.59	64.94	65.14	0.57	NS
OM	65.13	66.50	66.82	0.58	NS
CP	58.04[b]	62.82[a]	63.88[a]	1.27	0.05
EE	77.07[b]	81.06[a]	80.79[a]	0.77	0.05
NFE	71.11	68.18	68.28	1.31	NS
CF	54.52[b]	63.29[a]	63.77[a]	1.98	0.05
Nutritive value, %:					
TDN	59.78	61.11	61.39	0.51	NS
Improvement	-	2.22	2.69		
DCP	7.37[b]	7.98[ab]	8.11[a]	0.16	0.05
Improvement	-	8.28	10.04		
Nutritive ratio 1:	7.11	6.67	6.57	0.15	NS

SEM, standard error of means
[a,b] means within each row with different superscript differ significantly

Differences regarding the nutritive value (calculated as TDN) were not significant among the treatment groups (Table 3). Values of TDN were 59.78, 61.11 and 61.39% for 1x, 2x and 3x, respectively. Feeding frequency significantly (P<0.05) increased DCP from 7.37% when fed once to 7.98% when fed twice and 8,11% when fed three-times daily, respectively compared feeding once. Nutritive value as DCP improved by 8.28 and 10.04 percentage units for 2 and 3 meals/d, respectively. Taie (1996) reported better nutritive value (TDN and DCP) for the experimental diet when fed more frequently.

Data of nitrogen balance as affected by feeding frequency for sheep fed on the tested ration are shown in Table (4). Data indicated that the nitrogen intake (NI) was almost equal being 42.15, 41.08 and 42.15 g/d for animals fed once, twice or three-times a day. Nitrogen excreted in feces (FN) was lower (P<0.05) with sheep fed twice and three-times than those fed once a day. Values were 17.68, 15.23 and 15.23g/d for 1x, 2x and 3x, respectively. These

data are in complete agreement with the digestibility of CP (Table 3). The respective values for nitrogen excreted in urine (UN) were 19.30, 18.96 and 18.87 g/d for the same respective groups. Differences were not significant. Nitrogen retention (NB) was 5.16, 6.89 and 8.09 g/d, for 1x, 2x and 3x, respectively. Differences were significant (P<0.02). Feeding sheep twice and thrice improved NB by 33.52 and 55.81% over those fed once/d. Biological value of the protein was 21.12, 26.49 and 29.88% for the same respective groups; differences were significant (P<0.01). Improvement in BV for sheep fed twice and three-times/d was 25.42 and 41.47%, respectively compared with feeding once. Values of NB and BV increased in a linear manner as the feeding frequency increased.

Table (4): Nitrogen balance (g/d) of sheep fed the experimental ration as affected by feeding frequency

Items*	Treatments (feeding frequency)			SEM	Sig
	Once	Twice	Thrice		
Nitrogen intake, NI	42.15	41.08	42.15	1.07	NS
Fecal nitrogen, FN	17.68[a]	15.23[b]	15.23[b]	1.55	0.05
Urinary nitrogen, UN	19.30	18.96	18.87	.98	NS
Nitrogen balance, NB	5.16[b]	6.89[ab]	8.04[a]	1.48	0.02
Improvement, %	-	33.52	55.81		
Biological value, BV	21.12[b]	26.49[a]	29.88[a]	4.25	0.01
Improvement, %	-	25.42	41.47		

* BV = 100*NB/(NI-FN)
[a,b] means within each row with different superscript differ significantly
SEM, standard error of means

Prior (1976) reported that N retention was significantly improved by feeding the sheep on a natural diet 12 times/day compared with twice daily. Abdel-Rahman and Suleiman (1994) found that growing male Najdi lambs on 4x and 8x feeding frequency retained significantly (P<0.05) more nitrogen than those fed once daily. Taie (1996) reported that nitrogen retention, daily gain and feed efficiency were improved with increasing feeding frequency. Elseed (2005) found that N retention and microbial N yield was higher (P<0.01) in sheep offered supplemental protein twice a day.

Ruminal *p*H values of sheep as affected by the feeding frequency are presented in Table (5). Before feeding, *p*H values were 7.06, 6.92 and 6.93 for 1x, 2x and 3x, respectively. Values of *p*H decreased after feeding to reach the

lowest values at 3h after feeding with 2x being 6.37 and continued to decrease up to 5h for 1x being 5.91 and 3x being 6.23. The recorded *p*H values are within the reported ranges for normally functioning rumen (El-Sheikh, 2007) being from 6 to7. Roth and Kirchgessner (1976) reported that with higher frequency of feeding, variations of *p*H were less. Taie (1996) indicated that diurnal variation in ruminal *p*H was less dramatic with sheep fed 2x and 3x daily. Castro *et al*. (2002) measured rumen fermentation using three rumen-cannulated Merino sheep in a 3x3 Latin square design to study the effect of either once or twice daily concentrate supplementation and found that concentrate supplementation decreased (P<0.01) ruminal *p*H.

Table (5): Rumen fermentation in sheep as affected by feeding frequency

Feed frequency	Sampling time, h			
	0	1	3	5
pH				
1x	7.06	6.31	6.21	5.91[b]
2x	6.92	6.4	6.37	6.46[a]
3x	6.93	6.55	6.44	6.23[a]
Sig	NS	NS	NS	0.05
VFA, meq/dl				
1x	9.34	10.32[c]	10.99[c]	10.73[c]
2x	9.47	11.68[b]	13.17[b]	12.93[b]
3x	10.0	14.16[a]	16.60[a]	15.87[a]
Sig	NS	0.05	0.01	0.05
NH3-N, mg/dl				
1x	28.77	30.50	25.20	25.10
2x	26.37	30.06	26.78	25.27
3x	26.78	30.90	27.37	25.50
Sig	NS	NS	NS	NS
VFA molar proportion				
Feeding frequency	Acids, %			
	Acetic	Propionic	A:P ratio	Others
1x	52.53[a]	30.09[b]	1.75:1[a]	17.38[b]
2x	47.39[b]	35.57[a]	1.33:1[b]	17.04[b]
3x	44.19[c]	35.18[a]	1.26:1[b]	20.63[a]
Sig	0.05	0.05	0.05	0.05

[a ,b ,c] **means within each column with different superscript differ significantly**

The results in Table (5) showed that concentration of total VFA was low before feeding and then increased in animals under all the experimental categories post-feeding. Before feeding, VFA was 9.34, 9.47 and 10.0 meq/dl in rumen of sheep fed 1x, 2x and 3x, respectively; differences were non-significant. Feeding the experimental ration more frequently lead to an increase in VFA production than feeding once; at 1h post-feeding VFA was 10.32, 11.68 and 14.16 meq/dl for 1x, 2x and 3x, respectively. Differences were significant (P<0.05). The respective values at 3h were 10.99, 13.17, 16.60 meq/dl. Differences were highly significant (P<0.01). At 5h VFA was 10.73, 12.93 and 15.87 for the same respective order. Differences were significant (P<0.05).

Giving the diets to farm animals more frequently leads to a more stabled ruminal environment; which in turn lead to a better fermentation and higher VFA production. Michalowski (1979) reported that total VFA were 74 to 131 mmol/liter rumen fluid in 2 wethers fed once or twice daily. Taie (1996) studied the effect of feeding frequency on ruminal VFA in sheep. Results indicated a sharp increase in production of VFA was detected up to 3h post-feeding then steadily increased at 6-7h post-feeding for 2x and 3x daily.

Data in Table (5) presented the molar proportion of acetate, propionate and acetate: propionate (A:P) ratio in the rumen of sheep fed the experimental diet at 3hr post-feeding. The molar proportion of acetic acid decreased from 52.53% when sheep was fed once a day to 47.39 and 44.19% when sheep was fed twice or three times/d; differences were significant (P<0.05). The molar proportion of propionic acid took the opposite trend being low on 1x (30.09) and higher on 2x (35.57) and 3x (35.18) feeding frequency. The A:P ratio was 1.75:1 for 1x, 1.33:1 for 2x and 1.26:1 for 3x; differences were significant (P<0.05). El-Sheikh (2007) reported that roughages increased molar proportion of acetic and decreased that of propionic acid leading to a higher A:P ratio. Sutton *et al*. (1986) gave concentrates in 2 and 5 or 6 meals and the hay in 2 meals daily. Increasing the proportion of concentrates in the diet reduced the proportion of acetic acid and increased the proportions of propionic and n-valeric acid. Sato *et al*. (1987) found that acetic to propionic acid ratio in rumen fluid decreased frequently with feeding twice daily. Soto-Navarro *et al*. (2000) evaluated the effects of feeding frequency and reported that total VFA concentration was greater (P<0.01) at 9 h after the 0800 feeding when feed was offered once vs. twice daily. Feeding twice daily increased (P<0.05) the molar proportion of acetate and decreased (P<0.05) the molar proportion of propionate.

Results concerning NH_3-N concentration are presented in Table (5). Concentration of NH_3-N increased to reach the highest level at 1h post feeding being on the average 30.5 mg/dl for all groups; NH_3-N decreased thereafter to reach the lowest levels at 5h post feeding being on the average 25.3 mg/dl. Differences were non-significant regarding all treatments at all sampling times. Ruminal ammonia concentrations were in the range of those

reported to be required for maximum ruminal microbial activity. Sutoh *et al*. (1991) revealed that ammonia nitrogen was greatly reduced with 12 feeds daily. Afify *et al*. (2004) using buffalo heifers fed once, twice or three times daily. They reported non-significant differences in ammonia-N concentration among groups. However, feeding frequently reduced the ruminal fluctuation in rumen parameters.

It could be concluded that increasing feeding frequency from once up to 3 times lead to a better microbial activity (more VFA production) with constant ruminal *p*H. Digestibility of protein, CF and ether extract (energy source) was also better with increased frequency of feeding. More NH_3-N was available in the rumen for better microbial activity. It is well known that microbial protein synthesis within the rumen is correlated positively with the VFA production. This leads to more N retained within the animal body leading to better growth performance. Frequency of feeding leads to a more stable ecosystem in the rumen and less diurnal fluctuation in *p*H, ammonia concentrations and volatile fatty acid production. Molar proportion was in favor of propionate leading to decreased in energy loss and increase the efficiency of energy utilization.

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تأثير عدد مرات التغذية على الهضم وتخمرات الكرش في الأغنام

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الملخص العربي

صُمّمتْ هذه الدراسةِ بهدف التعرف على تأثيرِ عدد مرات التغذية على كميةِ الغذاء والماء المستهلك، ومعاملات الهضم ، ميزان النتروجينِ وتخمرات الكرشِ في الأغنام الأوسيمي. دامتْ التجربة لمدة ١٨ إسبوعِ. استخدم فيها ثلاثة أغنام عند عمرِ ٣ سَنَواتِ بمتوسط وزنِ جسمِ ٦٠ كيلوجرامِ مزودة بفتحة مستديمة بالكرش. إستخدم عليقة مكونة من البرسيمِ المصريِ (٦٠%) ومخلوط العلف المركزَ (٤٠%). قدمت الوجبة الغذائية أمّا مرّة، مرّتين أَو ثلاث مراتِ بِاليوم. وقد كان الماء متاحا لكُلّ الحيوانات؛ وقدرت كمية الماءِ المستهلكة يومياَ. بعد كُلّ فترة تجريبية تم جمع عينات من سائل كرش الحيوانات قبل التغذية ثم بعد ذلك عند ١، ٣ و٥ ساعاتِ بعد التغذية عن طريق فتحاتا النواسيرِ. أوضحت النَتائِجُ عدم وجود أية إختلافاتَ معنوية بخصوص كمية الغذاء المستهلك من المادة الجافة. زادتْ كمية الماءِ المستهلكة بشكل خطيّ مع زيادة عدد مرات التغذية اليومية. تحسنت معاملات الهضمِ للبروتين والدهن والألياف الخام معنوياً مع الحيواناتَ التي غُذّيتْ أمّا مرّتين أَو ثلاثة مرات مقارنة بالتغذية مرّة واحدة يومياً. المواد المهضومة الكلية لم تختلف باختلاف عدد مرات التغذية بينما تحسن البروتين الخام المهضوم.

كان النتروجينِ المحتجز ٥,١٦، ٦,٨٩ و٨.٠٩ جم/اليوم للحيوانات المغذاة مرة أو مرتين أو ثلاثة مرات يوميا على التوالي. أدت تغذية الأغنام مرّتين أوثلاث مرات إلى تحسن معنوي في كمية الاتزان النيتروجيني بنسبة ٣٢,٥٢ و٥٥,٨١ % على الأغنام المغَذاة مرّة واحدة يوميا كما تحسنت القيمة الحيوية للبروتينِ مع زيادة عدد مرات التغذية . أدت زيادة عدد مرات الإطعام إلى نَقصَ التقلّبَ في حموضة الكرش. كما أدت إلى زيادةِ في إنتاجِ الأحماض الدهنية الطيارة. نَقصتْ نسبة حامضِ الخليك في الأغنام التي غُذّيتْ مرة كل يوم عِنْ تلك التي غُذّيتْ مرّتين أَو ثلاث مراتِ يوميا، واتخذت نسبة حامضِ البروبيونيك الإتجاهَ المعاكسَ حيث كانت أقل تركيز عند التغذية مرة واحدة وأعلى عند التغذية مرتين أو أكثر يومياِ.