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EFFECT OF OSCILLATING CRUDE PROTEIN CONTENT ON NITROGEN UTILIZATION, MILK PRODUCTION AND PERFORMANCE OF SHEEP

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SUMMARY

The objective of this study was to determine the effects of oscillating dietary crude protein on milk milk yield, composition, and N metabolism in ewes, and lambs performance. Twenty Barki ewes ewes rearing single lamb (36.7 ± 1.78 kg and 6.38 ± 0.12 kg initial body weights of the ewes and and their lambs, respectively) were used in a completely randomized design during a 10-week period of lactation. Ewes were fed a diet containing different levels of crude protein, 11.2% (low), 14.1% (Medium), 17.3% (High), or oscillating (Low and High crude protein diets oscillated for 3 days and 4 days, respectively). Dry matter intake did not differ among diets, but CP intake (g/d) differed (P<0.05) from 179 (low) to 230 (medium), 272 (high), and 229 (oscillating) g/ d. However, dry matter digestibility was not varied among diets, while that of crude protein was significantly differed (P<0.05) in which the highest value was for the high diet (69.6%) and the lowest one for the low crude protein diet (56.5%). An intermediate value was recorded for ewes fed the medium and oscillating crude protein diets (64.7%). Nitrogen retention was higher (P<0.05) in ewes fed high (14.6 g/d) and oscillating (12.8 g/d) diets than that in the ewes fed medium (10.0 g/ d) and low (8.0 g/d). Urinary urea N not differed (P<0.05) between ewes fed medium (12.1 g/d) and oscillating (11.6 g/d) but was lowest for those fed low (8.2 g/d) and greatest for those fed high (16.1 g/d). The pH and total VFA values did not differ among diets, but ammonia-N differed (P < 0.05) from 5.11 (low) to 10.63 (medium), 14.95 (high), and 12.37 mg/dl (oscillating). Microbial protein synthesis was higher (P < 0.05) in ewes fed oscillating (1.69) and high (1.64) than that reported in the ewes fed medium (1.49) or low (1.27 g/ 100 ml rumen liquid). Milk yield and composition did not differ among diets. Initial and final body weights of lambs are similar, but average daily gain (g/ d) significantly ($P<0.05$) differed (186, 194, 203 and 208 g/d for low, medium, high and oscillating, respectively). In conclusion, these data indicated that ewes fed oscillating diet varying in crude protein concentrations had an improvement in the crude protein digestibility, microbial protein synthesis and N retention compared with those fed low and medium dietary protein diets.

Keywords*: Sheep, nitrogen utilization, milk yield, milk composition and urea.*

INTRODUCTION

Crude protein is the most expensive component and considered the key element in formulating rations for ruminants. However, more attention has been paid to control the N excretion by ruminants to reduce the environmental pollution (Islam *et al*., 2002). Excess dietary protein is usually broken down into ammonia and then lost by ruminants.

It is well-known that ruminants have low efficiency nitrogen utilization compared to the non-ruminants. This low efficiency has many implications, not only for its negative effects on production performance and economic efficiency but also for the emission of contaminants to the environment. Several studies have demonstrated that feeding diets with oscillating CP concentrations on a 2-d basis can enhance nitrogen retention in growing sheep (Kiran and Mutsvangwa, 2009) and finishing cattle (Cole *et al*., 2003; Ludden *et al*., 2003). Overall, oscillating the CP concentration in growing ruminant diets can improve N retention and reduce N excretion, potentially decreasing the effects of N emission to the environment and increasing nitrogen utilization efficiency (NUE).

Recycling of N back to the rumen can increase the proportion of dietary N secreted in milk (Marini and Van Amburgh, 2003). Urea can be recycled back to the rumen either directly through transporters or through the saliva (Stewart *et al*., 2005). The recycled urea can be degraded and used to make microbial protein may be critical for the maintenance of acceptable levels of milk and meat production by rumen bacteria and increase the NUE (Kennedy and Milligan, 1980; Reynolds and Kristensen, 2008). Ruminant microbes become more efficient in capturing recycled urea when dietary concentrations of N are low compared to when dietary N concentrations are high because of the lower amount of N fed and physiological changes accompanying low dietary N concentrations (Leng *et al*., 1984; Isozaki *et al*., 1994; Ford and Milligan, 1970).

Studies evaluating the effects of oscillating dietary CP have not been conducted with ewes. We hypothesized that oscillating dietary CP concentrations to equal the average concentration of a diet limited or higher in CP for ewes will improve milk yield and nitrogen utilization because oscillating the CP concentration could increase recycling of N back to the rumen. The objective of the experiment was to determine the effect of oscillating dietary CP on digestibility, performance, milk yield, and milk protein as well as N excretion into milk and urine.

MATERIALS AND METHODS

This study was carried out at the Maryout Research Station (located 35 km South West of Alexandria, Latitude 31.02 ºN, Longitude 29.80 ºC), Desert Research Center, Egypt.

Animals, feeding and experimental design

Twenty nursing Barki ewes, each with a single lamb (36.7 ± 1.78 kg and 6.4 ± 0.12 kg initial body weights for the ewes and their lambs, respectively) were selected for the experiment based on lambing date. Ewes with their lambs were housed individually in shaded pens (1.5m × 1m) and fed their diets, daily at 9:00 h. Four dietary treatments consisted of 11.2% CP (Low), 14.1% CP (Medium), 17.3% CP (High), or oscillating dietary CP diets. The oscillating dietary treatment fed in two different sequences, for example 3 days of low CP followed by 4 days of high CP in a week cycle and for 10 weeks. Based on NRC (2007), the medium crude protein diet was calculated to met the crude protein requirement of lactating ewes, whereas the low and high crude protein diets were below and above crude protein requirements, respectively. however, the total crude protein intake in ewes fed the oscillating crude protein diet over a 7-d oscillating cycle as similar to that of ewes fed the medium diet. The ingredient and chemical compositions of the Low, Medium, High and Oscillating diets are presented in Table 1. Ewes were given 2 weeks for adaptation period, followed by 8 weeks for measurement period. Ewes and lambs were weighed separately biweekly before feeding throughout the study and had free access to water.

Nutrient digestibility and chemical analyses

Feed intake was recorded daily by weighting the feed leftover from the previous day. The daily samples were mixed thoroughly, composited, and then subsample for each ewe was taken. Ewes were placed on the metabolic cages at the last 10 days of the experiment: 3 days for adaptation to the metabolic cages and 7 days for collecting total faces and urine. Total daily fecal output for each ewe was collected, mixed and weighed. A daily sub-sample (10%) was taken daily and dried at 60°C in a forced-air oven for 48 h and composited by each ewe. Feed and fecal samples were ground through a 1-mm screen and analyzed for DM, ash, and nitrogen according to AOAC (1997). Neutral detergent fiber (NDF) was determined by the procedure of Van Soest *et al*. (1991) without the use of an alpha-amylase but with sodium sulfite. Acid detergent fiber (ADF) was analyzed according to AOAC (1997).

For urine, a plastic bag collector was glued around the vulva of each ewe and connected to tubing that directed each day’s urine to plastic container containing 5 ml of 50% sulphuric acid. Total urine output was recorded daily. A 10% sub-sample of urine was composited by each ewe and stored at −20°C until later analysis of N (AOAC, 1997) and urea-N (Paton and Crouch, 1977).

Sampling and analysis of rumen fluid

On the last day of the collection period, rumen content was sampled at 3 h after the morning feeding. Approximately 100 ml of rumen fluid were collected manually from the ventral sac by using a stomach tube, strained through 4 layers of cheesecloth. The pH of rumen fluid was measured immediately with a pH meter (Accumet Model 15, Fisher Scientific, USA). A 50 ml was immediately acidified with 2.5 ml of 6 N HCl and frozen (–20°C) for subsequent determination of ammonia-N concentration (AOAC, 1997), and total volatile fatty acid analysis by titration, after steam distillation of a 5-mL sample (Annison, 1954).

The method of tungstic acid (TA) - sulphuric acid precipitation was used to determine microbial protein synthesis in rumen liquid according to winter et. al. (1964) as follow: a 16 ml of filtrated fluid was added to 4 ml of 10% (wt/vol) sodium tungstate and 4 ml of 1.07 N sulfuric acid. After allowing the tubes to stand at 5°C for 4 h, the samples were centrifuged at 9,000×g for 15 min., and the supernatant was analyzed for TA N.

Table (1): Ingredients and chemical composition of experimental diets (% DM basis).

Item	Low	Medium	High
Ingredients			
Berseem hay	50	50	50
Corn grain	33.7	26.7	19.7
Soybean meal	0	8	17
Wheat bran	14.6	13.6	11.6
Limestone	0.9	0.9	0.9
Salt	0.5	0.5	0.5
Vitamin mineral premix[1]	0.3	0.3	0.3
Chemical composition			
DM	92.8	92.7	92.2
OM	89.6	90	90.7
CP	11.2	14.1	17.3
NDF	37	38.6	38
ADF	27.5	26.7	27.6
NE[2] (Mcal/ kg)	1.49	1.50	1.51

[1]*composition/ kg vitamin mineral premix feed: 8.0 g of Ca; 6.0 g of P; 33.3 g of Mg; 0.2 g of Co; 0.1 g of Se; 1.7 g of I; 6.7 g of Cu; 18.7 g of Fe; 20 g of Zn; 23.3 g of Mn; 3,000,000 IU of vitamin A; 1,000,000 IU of vitamin D3, 3,000 IU of vitamin E.*

[2] *NE: Net energy was calculated according to NRC (2007).*

Sampling and analysis of blood plasma

A blood sample (about 10 mL) was collected from a jugular vein (at the same time as rumen fluid sampling) into tubes containing EDTA, and plasma was separated by centrifugation at 3000×*g* for 10 min. and stored at −20°C until analysis of plasma urea N according to the method of Crocker (1967).

Milk sampling and milk composition

After the adaptation period (i.e., two weeks), milk measurements were taken weekly for the following six weeks. Prior to milking, lambs were separated from their dams for 12 hours overnight (8 pm – 8 am) and were only reintroduced to their dams after milking. Milk yield was recorded. Milk yield was then multiplied by 2 to calculate daily milk yield and, consequently, multiplied by 60 days to calculate milk intake kg/ lamb. Milk samples were analyzed for fat, protein, and lactose using infrared spectrophotometry (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark).

Statistical analyses

Statistical analysis for the obtained data was carried out using the general linear model producers of SAS (SAS Inst. Inc. Cary, NC, 2008) in a complete randomized design. Comparison among means was carried out using Fisher's least significant difference test. Treatment effects were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

As mentioned in the materials and methods section, the experimental diets had a similar composition of nutrients but with different CP concentrations (Tables 1).

Intakes and digestibility of DM, OM, CP, NDF and ADF are presented in Table (2). Ewes fed the oscillating diet had similar DM and OM intake when compared to those fed the low, medium or high CP diets. It is likely that DM intake was not differed among diets because of the restricted feeding. Also, no significant differences existed among diets in digestibility of DM and OM. Our results agree with Cole (1999) who reported that DM digestibility was not affected by oscillating dietary protein concentrations in lambs between 10 and 15% at 24-h or 48-h intervals, and with Ludden *et al*. (2002) in lambs fed a diet, containing a different CP concentration between 13 and 17% at 48-h intervals. Moreover, kiran and Mutsvangwa (2009) demonstrated that oscillating dietary protein concentrations between 7.5 and 13.5% on 48 h basis in lambs did not affect DM digestibility.

Intakes of NDF and ADF did not differ (P<0.05) between diets (Table 2). Digestibility of NDF and ADF were different (P<0.05) in ewes fed oscillating and high CP diets compared to those fed low and medium diets. In our experiment, fiber digestibility was increased with increasing dietary crude protein and with oscillating treatment. This increase may be related with greater nitrogen availability for fiber fermentation. Similarly, Ludden *et al*. (2002) and kiran and Mutsvangwa (2009) reported that NDF and ADF digestibilities were affected by increasing dietary protein concentrations in lambs.

Table (2): Nutrient intake and digestibility for ewes fed low, medium, high and oscillating dietary crude protein content.

Item	Diet				SEM	*P*-value
	Low	Medium	High	Oscillating		
Nutrient intake, g/d						
DM	1600	1649	1577	1600	29.04	0.8923
OM	1434	1473	1431	1450	26.29	0.9627
CP	179^{c}	230^{b}	272^{a}	229^{b}	9.86	0.0181
NDF	593	631	599	603	11.31	0.7305
ADF	440	436	443	442	7.92	0.9832
Digestibility, %						
DM	65.70	66.32	68.24	66.71	0.45	0.3397
OM	68.05	68.18	70.81	69.30	0.42	0.1293
CP	56.45^{c}	63.32^{b}	69.56^{a}	64.67^{b}	1.53	0.0140
NDF	43.35^{b}	43.40^{b}	46.66^{a}	47.80^{a}	0.68	0.0472
ADF	34.76^{b}	36.05^{ab}	39.87^{a}	39.53^{a}	0.67	0.0455

$^{a, b, c}$ means within a row with different superscripts differ (P<0.05).
SEM: Standard error of means.

Ewes fed high CP diet had a higher (P<0.05) in CP intake and digestibility compared to those fed medium and oscillating CP diets, then low diet recorded the lowest value (Table 2).

Nitrogen utilization values are presented in Table (3). Nitrogen intake and urinary N execration (g/d) were greater (P<0.05) for the ewes fed high compared to those fed oscillating, medium, and low CP diets.

Table (3): Nitrogen utilization in ewes fed low, medium, high and oscillating dietary crude protein content.

Item	Diet				SEM	*P*-value
	Low	Medium	High	Oscillating		
N utilization, g/d						
N intake	28.66^{c}	36.83^{b}	43.75^{a}	36.65^{b}	0.79	0.0083
Fecal N	12.45	13.82	12.91	12.25	0.27	0.3930
Urine N	8.24^{c}	13.22^{ab}	16.09^{a}	11.56^{b}	0.83	0.0018
N retention	7.98^{b}	9.95^{b}	14.57^{a}	12.84^{a}	0.63	0.0246
N retention % N intake	16.61^{c}	23.32^{b}	30.66^{a}	24.40^{b}	0.83	0.0169

$^{a, b, c}$ means within a row with different superscripts differ (P<0.05).
SEM: Standard error of mean.

Ewes fed high and oscillating CP diets had a greater (P<0.05) nitrogen retention (g/d) compared with those fed the medium and low CP diets. Results are going with those reported in Table (2) for the digestibility trail. In agreement, previous studies showed that oscillation of the dietary CP improved N retention in ruminants (Collins and Pritchard, 1992; Cole, 1999; Cole *et al*., 2003). This improvement in the N retention may provide a viable means to reduce the release of N, especially ammonia, into the environment. Cole (1999) hypothesized that the improvement in N retention by oscillating the dietary CP is due to improved recycling of N to the rumen in the form of urea, stimulating the increase in total entry of urea N back to the gastrointestinal tract.

Values of pH, ammonia-N, total VFA, microbial protein, urinary urea and plasma urea are presented in Table (4). The pH and total VFA values were not varied among treatments. Ammonia-N and microbial protein synthesis were higher (P<0.05) in ewes fed the high and oscillating diets compared to the low CP diet, while an intermediate values were recorded for those fed the medium diet. The effects of feeding oscillating dietary protein levels compared to feeding static dietary CP on microbial protein production in ewes is the primary objective of the current study. Microbial protein flow to the small intestine was higher in ewes fed the oscillating diet compared to those fed the medium diet, although a similar CP intake was recorded for ewes fed both diets (Table 2). Cole (1999) reported that the improvement in N retention observed in ruminants when dietary CP was oscillated could be due to the stimulating for the increased urea-N recycling, where it (urea-N) can potentially increase intestinal microbial protein supply. Although we did not measure the incorporation of recycled urea into microbial protein, microbial protein was greater in ewes fed the oscillating compared with those fed the medium diet.

Table (4): Rumen metabolites, microbial protein, urinary urea and plasma urea in ewes fed low, medium, high and oscillating dietary crude protein content.

Item	Diets				SEM	*P*-value
	Low	Medium	High	Oscillating		
pH	6.32	6.28	6.49	6.32	0.04	0.2131
NH_3-N, mg/ dl	5.11[b]	10.63[ab]	14.95[a]	12.37[a]	1.19	0.0425
Total VFA, mg/ dl	3.87	3.99	4.58	4.40	0.13	0.2411
Microbial protein g/ 100 ml rumen liquid	1.27[c]	1.49[b]	1.64[a]	1.69[a]	0.05	0.0371
Urinary urea-N, g/d	7.87[c]	12.06[b]	17.10[a]	11.24[b]	0.83	0.0010
Plasma urea-N, mg/dl	12.45[c]	30.81[b]	46.23[a]	29.04[b]	0.88	0.0029

[a, b, c] means within a row with different superscripts differ (P<0.05). SEM: Standard error of means.

On the other hand, ewes fed the high dietary protein diet had a higher (P<0.05) urinary urea-N and plasma urea-N (Table 4), when compared to those fed the oscillating and medium CP diets. Our results are in agreement with the findings of Cole (1999) and kiran and Mutsvangwa (2009). These observations are likely attributable to the higher N intakes that were observed in ewes fed the high diet compared to those fed the oscillating and medium CP diet. A similar CP intake (Table 2) and similar values for urea N in urine or plasma were observed for ewes fed medium and oscillating CP diets that are in agreement with Cole (1999) and Archibeque *et al*. (2007).

Milk yield and milk composition are presented in Table (5). Milk yield (g/ 12h) and its composition, fat, protein, and lactose, were similar among dietary treatments. Also, there were no differences among diets regarding to milk composition (%).

Table (5): Milk yield and milk composition in ewes fed low, medium, high and oscillating dietary crude protein content.

Item	Diets				SEM	*P*-value
	Low	Medium	High	Oscillating		
Milk, g/ 12 h						
Yield	234	257	297	285	10.31	0.1705
Fat	7.44	9.43	10.85	9.67	0.45	0.1073
Protein	9.84	11.21	13.64	13.74	0.67	0.1149
Lactose	13.75	12.95	16.07	16.30	0.66	0.1946
Milk composition, %						
Fat	3.19	3.73	3.69	3.36	0.10	0.2342
Protein	4.22	4.37	4.61	4.72	0.10	0.2896
Lactose	5.93	5.06	5.38	5.77	0.18	0.3970

SEM: Standard error of means.

Performance of ewes and lambs are presented in Table (6). Similar initial or final body weight for ewes and lambs was reported. Lambs fed the high and oscillating diets had higher (P<0.05) milk intake (kg/ lamb) when compared to those fed the medium and low diets. Average daily gain of the lambs in oscillating diet had higher (P<0.05), when compared to those in the low diet. Lambs in the medium and high diets had intermediate. The effects of oscillating dietary crude protein on performance in the present study tend to agree with results of Kiran and Mutsvangwa (2009), who also noted numerically greater average daily gain in lambs fed oscillating crude protein concentrations than in lambs fed the same quantity of crude protein on a continuous basis.

Table (6): Performance of lambs and ewes fed low, medium, high and oscillating dietary crude protein content.

Item	Diet				SEM	*P*-value
	Low	Medium	High	Oscillating		
No. of ewes	5	5	5	5		
Initial weight, kg	38.76	38.36	39.16	38.06	1.78	0.4545
Final weight, kg	36.98	37.78	37.92	38.76	0.49	0.6467
No. of lambs	5	5	5	5		
Milk intake, kg/ lamb	28.03^{b}	30.82^{ab}	35.60^{a}	34.22^{a}	1.24	0.0494
Initial weight, kg	6.36	6.46	6.08	6.62	0.12	0.8557
Final weight, kg	17.54	18.08	18.24	19.08	0.27	0.2419
Average daily gain, g/ d	186^{b}	194^{ab}	203^{ab}	208^{a}	2.72	0.0130

$^{a, b}$ means within a row with different superscripts differ (P<0.05). SEM: Standard error of means.

CONCLUSIONS

In conclusion, compared to feeding the same CP diet on a daily basis, feeding oscillating dietary CP concentrations (low and high) in a week cycle improves the CP utilization by nursing ewes in which the digestibility of CP, microbial protein synthesis, and N retention were significantly greater in comparison with those reported by ewes fed a similar CP intake (medium diet).

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تأثير تذبذب محتوي البروتين الخام على الاستفادة من النيتروجين، انتاج اللبن والآداء في الأغنام

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اجريت هذه التجربة لدراسة تأثير تذبذب البروتين الخام في علائق الاغنام على إنتاج والتركيب الكيماوي للبن والإستفادة من النيتروجين وأداء النعاج والحملان. استخدم 20 نعجة برقي مع مواليدها (بمتوسط وزن الجسم 36,7 ± 1,78 كجم و6,38 ± 0,12 كجم للنعاج والحملان على التوالي) قسمت فى الدراسة الى أربع مجموعات وغذيت تغذية فردية علي إحدى العلائق التجريبية الأربعة الأتية:

1- عليقة منخفضة في محتواها من البروتين الخام (11,2 % بروتين خام)
2- عليقة متوسطة في محتواها من البروتين الخام (14,1 % بروتين خام)
3- عليقة مرتفعة في محتواها من البروتين الخام (17,3 % بروتين خام)
4- عليقة متذبذبة في محتواها من البروتين الخام وفيها يتم التغذية 3 أيام على العليقة المنخفضة في محتواها من البروتين الخام يليها 4 أيام يتم فيها التغذية على العليقة المرتفعة في محتواها من البروتين الخام يتكرر ذلك أسبوعيا ولمدة 10 أسابيع.

وكانت النتائج كالتالي:

1- لم تكن هناك فروق معنوية بين العلائق على كمية المادة الجافة المأكولة، في حين اختلفت كمية البروتين الخام المأكولة وتراوحت القيم بين 179، 230، 272 و229 جم/ يوم للعلائق المنخفضة، المتوسطة، العالية والمتذبذبة علي التوالي.
2- لم تكن هناك فروق معنوية بين العلائق على معامل هضم المادة الجافة، في حين اختلف معامل هضم البروتين الخام معنويا وسجلت العليقة المرتفعة أعلي القيم 69.6%، وأقلها للمنخفضة 56.5%، في حين كانت قيم العلائق المتوسطة والمتذبذبة متشابهة ومتوسطة 63,3 و64,7% على التوالي.
3- كمية النيتروجين المحتجز جم/يوم كان أعلي في النعاج المغذاة على العليقة المرتفعة والمتذبذبة بالمقارنة بالنعاج المغذاة علي العليقة المتوسطة ثم العليقة المنخفضة في محتواها من البروتين الخام.
4- لم يكن هناك فروق معنوية بين العلائق في pH والأحماض الدهنية الطيارة الكلية بالكرش، في حين اختلفت كمية الأمونيا بالكرش معنويا بين العلائق وكانت اعلي القيم في العلائق المرتفعة والمتذبذبة 14,9 و12,4 على التوالي ثم العليقة المتوسطة 10,6 وأقلهم العليقة المنخفضة 5,1 مجم/ 100 مل سائل الكرش.
5- ارتفعت معنويا كمية البروتين الميكروبي المنتج في الكرش في العلائق المتذبذبة والمرتفعة 1,7 و1,6علي التوالي ثم العليقة المتوسطة 1,4 وأقلهم العليقة المنخفضة 1,3 جم/ 100 مل سائل الكرش.
6- لم يكن هناك تأثير معنوي على كمية اللبن المنتجة والتركيب الكيماوي للبن.
7- سجلت معدل الزيادة اليومية للحملان زيادة معنوية للعليقة المتذبذبة عن العليقة المنخفضة وكانت القيم 208، 186 جم/ يوم علي التوالي، في حين كانت للعلائق العالية والمتوسطة قيم متوسطة 203، 194 جم/ يوم علي التوالي.

وعلى ذلك تشير النتائج الي أن العليقة المتذبذبة في مستويات البروتين الخام أدت الي تحسن في معامل هضم البروتين، معدل تخليق البروتين الميكروبي، الاستفادة من النيتروجين بالمقارنة بالعلائق المنخفضة والمتوسطة والعالية في مستوي البروتين الخام وتساوت تقريبا مع العليقة المرتفعة فى مستوى البروتين.