Emirates Journal of Food and Agriculture. 2021. 33(10): 863-867
doi: 10.9755/ejfa.2021.v33.i10.2773
http://www.ejfa.me/

RESEARCH ARTICLE

Impacts of feeding urea on rumen fermentation, total number of bacteria and some blood parameters in shami goats

Salam Shaban Ibrahim[1], Jamal Abdul Rahman Tawfeeq Al-ani[2]*, Anmar Abdulghani Majeed Al-Wazeer[3], Rasha Mohamed Shaker[4]

[1]Office of Agricultural Research/Ministry of Agriculture, [2]Department of Animal Production, College of Agri. Engineering Sciences, Univ. of Baghdad, Baghdad, Iraq, [3]Department of Animal Production, College of Agri., Univ. of Kufa, Najaf, Iraq, [4]Biochemistry Section, College of Dentist, University of Baghdad, Baghdad, Iraq

ABSTRACT

To follow up the effects of feeding urea on rumen characteristics, rumen bacteria and some blood parameters in Shami goats, after 21 days of feeding 1.5% urea with concentrated feed, rumen fluid was collected at different times 0, 1, 2, 3 and 6 hours after morning feeding, pH were measured, some of rumen fluid was used for bacterial count, while 0.1N HCL was added to other part and kept in deep freeze for measuring rumen NH3-N and volatile fatty acids. Sixteen male Shami goats were distributed to T1 without urea and T2 1.5% urea. Rations fed at 3% of body weight as DM basis and alfalfa hay was fed ad-libitum. After 90 days, Jugular blood was sampled. Results showed no significant effects of feeding urea to Shami goats on blood parameters, while, rumen NH3-N decreased ($p<0.05$) for urea treatment after 3h of feeding with increasing volatile fatty acids (VFA) and pH values($p<0.05$) of rumen fluid at 3h and 6h after feeding. Bacteria counts decreased at 0, 3 and 6h after feeding at dilution 10^7 and 10^9 CFU/ml, due to ingestion large amounts of water with urea treatment and increase rate of passage of bacteria and rumen contents to other parts of alimentary canal.

Keywords: Urea; Soya protein; Goats; Blood traits; Rumen characteristics.

INTRODUCTION

Goat as ruminant is an important domesticated animal in agricultural sector due to its ability to withstand harsh conditions, especially Shami goats which highly production of meat and milk for human consumption, and to meet the highly needs of crude protein sources, urea was used as a source of non-protein nitrogen (NPN) that provides ammonia which required to produce microbial protein as true protein source for ruminants. The diamide carbonate, carbamide or urea, is the end product of protein metabolic catabolism for ureotelic animals like, terrestrial vertebrate and sharks. Commercially, it appears as solid odorless white crystals, contains 47% of nitrogen. In mammals bodies, it is produced from toxic compound "ammonia" in the liver by urea cycle to the blood and excreted as urine by kidney or with perspiration or milk or saliva and recycled in the rumen as source of non-protein nitrogen (NPN) by rumen microorganisms. Blood urea enhanced flow of water from all tissues into interstitial fluids and plasma leads to decreasing pressure in those tissues and increasing outflow of urine, that explain why ruminant drink much water and excreted much urine when feed urea as source of non-protein nitrogen. Rumen microorganisms break down urea to ammonia and using it with volatile fatty acids to form and hence microbial protein as true protein. In low quality forages, urea increased crude fiber digestibility, and crude protein content, that leads to increase intake and feed efficiency. Holder et al. (2015) found greater plasma ammonia concentration with increasing urea intake, and greater hepatic uptake of ammonia (NH_3) and increased urea synthesis, that leads to increase blood urea (Law et al., 2009). Puppel and Kuczynska (2016) refried to increase blood urea with increasing dietary nitrogen, and 40-80% of endogenously urea-N is returned to the gastrointestinal tract (Lapierre and Lobley, 2001), therefore, because of urea

***Corresponding author:**
Jamal Abdul Rahman Tawfeeq Al-ani, Department of Animal Production, College of Agri. Engineering Sciences, Univ. of Baghdad, Baghdad, Iraq.
E-mail: drjamalani@yahoo.com

Received: 27 August 2021; **Accepted:** 11 Ocboter 2021

can across cell membranes down a concentration gradient, feeding low protein hay leads to entering blood urea pool into the rumen and little excreted in urine (Abdoun et al., 2010). In ruminants, urea breakdown to ammonia via a wide range of ureolytic bacteria strains, which can produce urea amidohydrolases, EC 3.5.1.5 (urease), Urease induced by the presence of urea (Armbruster et al., 2014), while in non-ruminants, releasing ammonia in later parts of small and large intestine, leads to damage of mucosa, decrease absorption, feed efficiency and growth of animal. Endogenous urea (EU) form 75% of crude protein intake and 71% of EU recycled internally (Batista et al., 2017). When feed large amounts of urea, rumen ammonia increases and absorbed into the blood as NH3 or NH4+, because the disability of microflora to capture all ammonia to growth and produce microbial protein, that's may lead to ammonia toxicity when liver can't convert excessive ammonia to urea (non-toxic), because of ammonia, rumen pH increase and increasing more than 7.5 leads to promote urea poisoning, so, giving acetic acid slow down the production of ammonia. Zhang et al. (2016) showed that supplementation of urea increased both pH and protozoa population after twenty-four hours of feeding. Therefore, excess urea and ammonia circulated in the blood, causing poisoning, for these reasons, the objective of this study was to investigate the effects of moderate level of urea (1.5%) on rumen fermentations, total number of bacteria and some blood characteristic in Shami goats.

MATERIALS AND METHODS

Experimental animal and management

Sixteen Shami male goats aged 9-10 months penned in individual cages 1.5×2m., animals were randomized distributed to two levels of urea 0% (T1) and 1.5% (T2) replaced with soya bean meal protein. All animals were provided concentrate, alfalfa hay, clean water, vaccines and kept continuous veterinary supervision all experimental period. Concentrate feed offered at 7.00 AM at 3% of live body weight as DM basis, while alfalfa hay offered *ad-libitum* with remaining. After 21 days, rumen fluid was collected and after 90 days of the experiment, blood samples were collected.

Blood sampling

Blood samples were collected from the jugular vein after three months of experimental feeding by using a 10ml syringe needle before morning feeding. All samples analyzed in the laboratory by using Biosystem, design BTS-300 Germany origin.

Estimation of blood parameters

liver function enzymes

A blood test was carried out for the experimental parameters after blood was drawn from animals in a volume of 10ml from the jugular vein, and the examination was done with Bio systems - BTS - 300, to monitor liver disorders, the functions of the liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were examined.

Blood glucose

The level of blood glucose was measured using a ready-made kit produced by the French company Spin react, which is an enzymatic method in which glucose was analyzed after it is oxidized enzymatically in the presence of the enzyme glucose oxidase.

Blood urea

Estimation was carried out using ready kit from Randox Company, using the enzymatic - colorimetric method.

In-vitro dry matter digestibility (IDMD)

In-vitro dry matter digestibility (IDMD) was implemented by active sample of rumen fluid collected from slaughtered Shami goat and incubated in water bath at 38°C following Tilley and Terry (1963) procedure.

Rumen fluid

Rumen fluid samples were collected using oral stomach tube, at different times after feeding, 0, 1, 2, 3, 6 h. The pH values were directly measured with portable pH meter (HANNA Instrument), then, some of rumen fluid used to determine total bacterial count were kept at 4ºC and 0.1N HCl was added to another part of rumen fluid and kept in deep freeze to determine the total volatile fatty acids (TVFA) and ammonia nitrogen cementations (NH_3-N).

pH values

The pH value of rumen fluid was measured immediately after being withdrawn, using a portable pH meter from HANNA Instrument, while pH value of feeds was measured by weight 1g of feed then adding 10ml of distilled water. After 10 minutes, filtering the sample with a cheese cloth, and measure it with a portable pH meter from HANNA Instrument (Tawfeeq and Hassan, 2014)

Rumen ammonia nitrogen

After thawing the frozen rumen fluid samples, withdrawing 5 ml of it into a Kjeldahl digestion tube, then adding 0.5g of magnesium oxide MgO and 0.5 ml of calcium chloride at a concentration of 25% and 10 ml of distilled water, then placed in the Kjeldahl apparatus. The resulting ammonia is received with 5 ml of receiving solution consisting of 2% boric acid and drops of the mixture (methyl red 0.099 g and bromocresol green 0.066 g dissolved in 100 ml ethyl alcohol), then titrated with 0.05 HCL to calculate ammonia concentration (mg/100ml rumen fluid):

Nitrogen Conc. (mg/100ml) = 14.008 × 0.05 × (titration volume of HCL for sample – titration volume for blank) × 100/5ml.

Rumen volatile fatty acids

Volatile fatty acids were measured after thawing frozen rumen fluid: Take 5ml rumen fluid into Kjeldahl digestion tube, then adding 1ml orthophosphoric acid, washing the tube with a little distilled water, receiving flask containing drops of phenol dye (50ml of absolute ethanol + 1g of phenolphthalein + 50ml of distilled water + drops of NaOH 0.05M), collect 50ml, then titrate with sodium hydroxide 0.1M (Warner, 1964). Concentrations of total volatile fatty acids (mmol/100ml):

Total volatile fatty acids (mmol/100ml) = 0.1M × (titration volume of NaOH sample – titration volume for blank) × 100/5ml.

Rumen total bacteria count

One ml of each sample was taken and 9 ml of physiological salt solution was added and the dilutions were completed in test tubes containing 9 ml of dilution solution to reach the dilution that gives the appropriate numbers between 25-300 colonies of microorganisms. Then 1 ml of the appropriate dilution for each of the samples was transferred to empty Petri dishes using a sterile pipette, then the culture media was poured out Chocolate Agar or Nutrient agar, and after mixing the medium well into the dishes, it was placed inside the anaerobic containers in an inverted form with several anaerobic conditions (Gas pak) to make the conditions anaerobic and then the dishes were incubated at 37 °C for 48 hours. After development on each of the media, the number of colonies was calculated for each of them, as in (Roberts and Greenwood, 2003).

Chemical analyses

Ingredients and chemical composition of concentrated feeds and alfalfa (Table 1.) as AOAC (2005).

Statistical analysis

All data were statistically analyzed using completely randomized design, One-way ANOVA analysis was performed using statistical program (SAS, 2012) and Duncan's multiple range test was used to determine the significant differences ($p<0.05$) among treatments (Duncan, 1955) using following formula $Yij = \mu + ti + \delta ej$.

RESULTS AND DISCUSSION

The effects of 1.5% urea on blood glucose, liver functions enzymes and blood urea were examined in Shami goats (Table 2.), blood samples were collected from the jugular vein before morning feeding which represented 24 hours after morning feeding. Results showed no effects of feeding urea on blood parameters under studied, there were insignificantly decreases for AST and ALT with urea treatment, that's mean no a destruction of hepatocytes and better conditions for liver, skeletal muscle, kidney and heart with urea intake, the same results for blood glucose and blood urea, there were insignificant increases, +6.62 and +23.61 for glucose and urea, respectively.

Table 1: Ingredients and chemical composition of concentrate feeds and alfalfa (% as DM basis)

Ingredients	T1	T2	alfalfa
Soya bean meal	9.0	0.0	
Urea	0.00	1.5	
Barley	49.0	49.0	
Wheat bran	30.00	37.50	
Corn	10.0	10.0	
Min. & Vit.	2.0	2.0	
Total	100	100	
Approximate analysis			
Dry matter (DM) %	93.80	94.74	29.37
Organic matter (OM) %	93.26	94.21	93.71
Crude protein (CP) %	14.76	14.87	17.89
Ether extract (EE) %	6.14	5.62	2.33
Crude fiber (CF) %	8.32	7.68	23.11
Inorganic matter (ash) %	6.74	5.79	6.29
Nitrogen free extract (NFE)	64.04	66.04	50.38
*Metabolic energy (MJ/kg DM)	12.90	12.90	10.90
pH value	7.44	7.78	6.69
IDMD (%)**	78.58	83.51	

*Metabolic energy (MJ/kg DM) = 0.012 × crude protein + 0.031 × ether extract + 0.005×crude fiber + 0.014×nitrogen free extract (MAFF, 1975).
First experiment: T1 = 0% urea; T2 = 1.5 urea
** IDMD = In-vitro dry matter digestibility (Tilley and Terry, 1963).

Table 2: Effects of urea on some blood parameters in Shami goats (Mean ± standard error)

Treatments	blood glucose (mg/dL)	blood AST (mg/dL)	blood ALT (mg/dL)	blood urea (mg/dL)
T1	37.75±6.03	109.5±16.39	34.0±4.63	36.0±3.91
T2	40.25±2.32	74.5±8.96	25.5±0.95	44.5±2.90
Significance	NS	NS	NS	NS

T1 = without urea, T2 = with 1.5%urea. NS = non-significant differences

Successful feeding of urea needs more attention for few days adaptation period, good mixing of ingredients, frequency of feeding, temperate climate, don't exceed 2% in the component of daily feed intake, efficient management and continuous monitoring, in generally, make attention to all previous steps leads to successful utilization of NPN compounds. Our results agreed with Adiwinarti et al. (2018) who referred to insignificant increase of goat's blood urea with increasing rumen degradability of protein, it was 48.1mg/dL with soya bean meal at zero time in contrast with 40.4mg/dL with fish meal and 34.4 mg/dl with roughage or natural grass, Barbosa et al. (2012) referred to same results and insignificant increase of blood urea when fed urea to goats.

Hume et al. (1970) referred the best rumen ammonia concentration about 8.8 mg/dL, while Satter and Slyter

Table 3: Effects of urea on rumen characteristics in Shami goats (Mean±standard error)

Treatments	0 h	1h	2h	3h	6h
Ammonia nitrogen (NH_3-N) concentration (mg/dL)					
T1	10.068±1.31	10.769±1.22	12.432±1.92	21.888a±3.07	14.008±2.60
T2	13.57±0.83	14.884±1.51	15.847±1.86	11.819b±1.10	13.921±2.15
Significance	NS	NS	NS	*	NS
Total volatile fatty acids (VFA) concentration (mg/dL)					
T1	0.6525±0.01	0.6575±0.01	1.025±0.01	0.7175b±0.01	0.5750b±0.01
T2	0.5800±0.01	0.9025±0.01	0.905±0.01	1.2700a±0.01	1.3225a±0.01
Significance	NS	NS	NS	*	*
Ruminal pH					
T1	6.92±0.06	5.97a±0.08	5.15±0.09	5.17b±0.07	5.20b±0.05
T2	6.85±0.12	5.05b±0.06	5.02±0.06	5.75a±0.05	5.73a±0.06
Significance	NS	*	NS	*	*

Different litters in same column means significant differences; * Significant differences at level 0.05 T1 = without urea, T2 = with 1.5%urea.

Table 4: Effects of urea on total count of rumen bacteria in Shami goats at 10^7 and 10^9 CFU/ml (Mean±standard error)

Treatments	0 h	1h	2h	3h	6h
10^7 CFU/ml					
T1	199.50a±6.84	236.50±14.72	256.50b±10.04	268.00a±6.32	272.00a±8.64
T2	176.75b±4.51	258.00±11.19	269.50a±10.27	209.25b11.34	215.75b±5.37
Significance	*	NS	NS	*	*
10^9 CFU/ml					
T1	188.50a±7.32	227.50±13.27	239.00±6.85	245.50a±4.99	263.50a±4.57
T2	88.00b 19.93	239.00±11.09	232.00±7.34	123.00b21.00	137.50b±20.88
Significance	*	NS	NS	*	*

Different litters in same column means significant differences; * Significant differences at level 0.05
T1 = without urea, T2 = with 1.5%urea.

(1974) found maximum microbial protein production at a rumen ammonia level of 5mg/dL. and more than 140 mg/dL indicated ammonia toxicity (Lewis, 1960). The effects of feeding 1.5% urea to Shami goats on rumen characteristics showed in Table 3., ureolytic bacteria rapidly hydrolyzed urea to ammonia, so, we found increased ammonia nitrogen for urea treatment at 0 time, 1h. and 2h., which meaning increased the activity of rumen microflora even with alfalfa hay, before morning concentrated feeding, and after 1h. and 2h., despite non-significant increasing, that is meaning hydrolysis urea 100% within about two hours after feeding, so, we found decreased rumen ammonia after 3h ($p<0.05$) and 6h. for urea treatment in contrast with soya bean meal treatment (T1) which degraded slowly in contrast with urea, and we found that rumen ammonia concentrations more than 8.8mg/dl as referred by Hume et al. (1970) and below the toxic level (140mg/dL) as reported by Lewis (1960). Rumen VFA increased ($p<0.05$) after 3h. and 6h., which mean increase microflora activity for degraded carbohydrate, without any significant for 0, 1h and 2h. after feeding. However, total VFAs in goat fed urea synchronized with ammonia, pH and total bacteria count after 1h, and 2h., we found increasing total bacteria count and low pH values which means increasing VFAs production and increasing ammonia, all these conditions referred to synchronization between source of protein and energy to enhance the production of rumen microbial protein. Additionally, Xu et al. (2019) and Li et al. (2020) agreed with our results and referred to high ammonia concentration with increasing level of urea. Wang et al. (2018) showed that urea-N could be detected only by 1h. post incubation, after that, urea treatment leads to decrease total count of bacteria, it's may the deficiency of urea nitrogen after first two hours.

The total number of rumen bacteria also decreased at 0, 3 and 6 h after feeding at two levels of dilution 10^7 and 10^9 CFU/ml, which may be due to ingestion of large amounts of water with urea treatment and then an increase in the rate of passage of microbial protein and rumen contents to the other parts of alimentary canal.

Total bacterial count was examined in Shami goats with two levels of dilution 10^7 and 10^9 CFU/ml at different times after morning feeding (Table 4.), at zero time (before morning feeding), soya bean meal treatment had superiority increasing for total bacteria count ($P<0.05$) at all dilutions, while, after 1h. and 2h., there were unsignificant increasing for urea treatments in contrast without urea T1, then significantly decreased ($P<0.05$) at 3h. and 6h. after morning feeding. It's well known that the feeding urea leads to increasing water intake, that may lead to dilution rumen fluid and increasing rate of passage of feeds and microbial protein, so, decreasing total count of bacteria by increasing

the time after feeding, Wanapat et al. (2016) referred to increase total count of bacteria and cellulolytic bacteria when fed rice straw supplemented with urea in contrast with control, Zhang et al. (2016) showed that microbial protein production was increased with increasing urea from 0% to 2% and no difference between 2% and 3%.

CONCLUSIONS

In the rumen, urea is hydrolysed quickly within two hours by ureolytic bacteria to ammonia, which enhance microbial protein, feed digestibility, crude protein contents. The 1.5% urea had no effects on blood urea and live functions enzymes, there were big chances to be synchronize between ammonia nitrogen and volatile fatty acids within first two hours after feeding. Urea in the diet has no adverse effect on animal health under experimental condition. However, more trials involving diagnosing types of ureolytic bacteria strains and combination between soyabean meal and urea are suggested prior to have final recommendations.

ACKNOWLEDGEMENTS

The authors are gratefully to the Agricultural Research Service, Ministry of Agriculture and College of Agricultural Engineering Sciences, University of Baghdad, and higher studies laboratories for their valuable material support and assistance during the experimental period.

CONTRIBUTIONS OF AUTHORS

All authors read and commented on draft versions, and there are no conflicts of interest.

REFERENCES

Abdoun, K., F. Stumpff, I. Rabbani and H. Martens. 2010. Modulation of urea transport across sheep rumen epithelium *in vitro* by SCFA and Co_2. Am. J. Physiol. Gastrointest. Liver Physiol. 298: G190-G202.

Adiwinarti, R., I. G. S. Kustantinah, B. Rusman and E. Indarto. 2018. Profile of rumen fermentation and blood urea nitrogen concentration of Kacang goat fed total mixed ration vs. roughage. Earth Environ. IOP Conf. Ser. 119: 012049.

AOAC. 2005. Official Methods of Analysis. 18th ed. Washington, DC, USA, Association of Official Analytical Chemists.

Armbruster, C. E., S. N. Smith, A. Yep and H. L. Mobley. 2014. Increased incidence of urolithiasis and bacteremia during *Proteus mirabilis* and *Providencia stuartii* coinfection due to synergistic induction of urease activity. J. Infect Dis. 209: 1524-1532.

Barbosa, J. G., R. G. Cosat, A. N. de Medeiroes, R. C. R. Queiroga, A. M. V. Batista, G. R. de Medeiros and E. M. B. Filho. 2012. Use of different urea levels in the feeding of Alpine goats. Rev. Bras. Zootec. 41: 1713-1719.

Batista, E. D., E. Detmann, S. C. Valadares Filho, E. C. Titgemeyer and R. F. D. Valadares. 2017. The effect of crude protein concentration in the diet on urea kinetics and microbial usage of recycled urea in cattle: A meta-analysis. Animal. 11: 1303-1311.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1-42.

Holder, V. B., J. M. Tricarico, D. H. Kim, N. B. Kristensen and D. L. Harmon. 2015. The effects of degradable nitrogen level and slow-release urea on nitrogen balance and urea kinetics in Holstein steers. Anim. Feed Sci. Technol. 200: 57-65.

Hume, I. D., R. J. Moir and M. Somers. 1970. Synthesis of microbial protein in the rumen. I. Influence of the level of nitrogen intake. Aust. J. Agric. Res. 21: 283-296.

Lapierre, H. and G. Lobley. 2001. Nitrogen recycling in the ruminant: A review. J. Dairy Sci. 84: E223-E236.

Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. Wylie and C. S. Mayne. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. J. Dairy Sci. 92: 1001-1012.

Lewis, D. 1960. Ammonia toxicity in the ruminant. J. Agric. Sci. 55: 111-117.

Li, Z, C. Mu, Y. Xu, J. Shen and W. Zhu. 2020. Changes in the solid-, liquid-, and epithelium-associated bacterial communities in the rumen of Hu lambs in response to dietary urea supplementation. Front. Microbiol. 11: 244.

MAFF. 1975. Ministry of Agriculture Fisheries and Food Department of Agriculture and Fisheries for Scotland Energy Allowances and Feed Systems for Ruminants, Technical Bulletin, pp. 33.

Puppel, K. and B. Kuczynska. 2016. Metabolic profiles of cow's blood. A review. J. Sci. Food Agric. 96: 4321-4328.

Roberts, D. and M. Greenwood. 2003. Practical Food Microbiology. 3rd ed. Blackwell Publishing Inc., Malden, Massachusetts, USA.

SAS. 2012. User's Guide. Statistical, Version 9. 11th ed. Statistical Analysis System, Cary, NC, USA.

Satter, L D. and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. Br. J. Nutr. 32: 199-208.

Tawfeeq, J. A. and S. A. Hassan. 2014. Handbook of Nutrition Science. 1st ed. University of Baghdad, Academic Press, Iraq.

Tilley, J. M. A. and R. A. Terry. 1963. A two-stage technique for *in vitro* digestion of forage crops. J. Br. Grassl. Sci. 18: 104-111.

Wanapat, M., K. Phesatcha and S. Kang. 2016. Rumen adaptation of swamp buffaloes (*Bubalus bubalis*) by high level of urea supplementation when fed on rice straw-based diet. Trop. Anim. Health Prod. 48: 1135-1140.

Wang, P., S. Zhao, X. Nan, D. Jin and J. Wang. 2018. Influence of hydrolysis rate of urea on ruminal bacterial diversity level and cellulolytic bacteria abundance *in vitro*. Peer J. 6: e5475.

Warner, A. C. I. 1964. Production of volatile fatty acid in the rumen methods of measurements. Nutr. Abstr. Rev. 34: 339-343.

Xu, Y., Z. Li, L. E. Moraes, J. Shen, Z. Yu and W. Zhu. 2019. Effects of incremental urea supplementation on rumen fermentation, nutrient digestion, plasma metabolites, and growth performance in fattening lambs. Animals. 9: E652.

Zhang, S., L. Cheng, X. Guo, C. Ma, A. Guo and Y. Moonsan. 2016. Effects of urea supplementation on rumen fermentation characteristics and protozoa population *in vitro*. J. Appl. Anim. Res. 44: 1-4.