VETERINARSKI ARHIV 75 (6), 521-530, 2005

Effect of urea molasses mineral granules (UMMG) on rumen fermentation pattern and blood biochemical constituents in goat kids fed sola (*Aeschonomene indica* Linn) grass-based diet

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JAIN, N., S. P. TIWARI, P. SINGH: Effect of urea molasses mineral granules (UMMG) on rumen fermentation pattern and blood biochemical constituents in goat kids fed sola (*Aeschonomene indica* Linn) grass-based diet. Vet. arhiv 75, 521-530, 2005.

ABSTRACT

A study was conducted on twenty graded jamunapari goat kids fed on four different groups. Group I was fed solely on roughage, i.e. sola (*Aeschonomene indica* Linn) grass hay and rice straw (70:30). Groups II, III and IV were fed on de-oiled rice bran (100 g/d), urea molasses mineral granules (50 g/d) and urea molasses mineral granules (50 g/d) plus fish meal (25 g/d). The experiment lasted for 105 days. The effect of supplementing urea molasses mineral granules and fishmeal was studied on rumen parameters, viz. microbial protein, bacterial and protozoal biomass and blood biochemical constituents, viz. BUN, plasma creatinine, glucose, total protein, Ca and P. Enzymatic activity for alkaline phosphatase, aspartate transaminase and alanine transaminase were also measured. The microbial protein synthesis was higher in group III (42.94 g/100 ml) and IV (57.04 g/100 ml) as compared to control (I) and II. There was a significant improvement of bacterial biomass (0.057 g/100 ml) and (0.082 g/100 ml). Similarly, bacterial and protozoal populations also become affected due to supplementation of fermentable-N and bypass protein, like fishmeal in the diet. The BUN was significantly ($P<0.05$) higher in group III (28.88 mg/100 ml) and IV (30.35 mg/100 ml) compared to control (I) and group II. Similarly, the activity of alkaline phosphatase was found to be higher in group III (29.77KA units) and IV (44.26 KA units), reflecting the better growth of kids in these groups. Other enzymatic activities were found to be unaffected. Concentration of plasma calcium and inorganic phosphorus were significantly ($P<0.05$) increased in groups III (9.85 and 9.93 mg/ 100 ml) and IV (7.57 and 8.31 mg/100 ml) compared to other groups. It is inferred that supplementation of fermentable-N and fishmeal in a catalytic amount increases the activity of bacterial and protozoal populations, some blood components and enzymatic activity which could support the growth of kids.

Key words: goat kids, *Capra hircus*, blood parameters, urea, sola, *Aeschonomene indica*, rumen fermentation

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ISSN 0372-5480
Printed in Croatia

Introduction

Urea has been used extensively for the feeding of ruminants under experimental conditions. When it is fed as such to a ruminant, it is hydrolyzed instantly in the rumen and the ammonia released is not utilized efficiently for microbial protein synthesis. In order to slow down the release of various complexes of urea with starch, molasses or as ammoniated crop residues have been reported (DEYCEC et al., 1968; NOLAN and LENG, 1972; CHOPRA et al., 1974; GUPTA et al., 1988). However, adequate supply of nitrogen, energy and minerals in the diet of ruminants is essential for maintaining optimum ruminal activity. Diets which are deficient in nitrogen when fed to animals not only cause depression in voluntary intake but also cause high production without adequate formation of ATP due to energy uncoupling. A consistent and continuous supply of both nitrogen and energy has been necessary for maintaining optimum microbial activity in rumen. There are reports (BRIGGS, 1967) that supplementation of readily available carbohydrates with NPN to the basal diets increased the level of blood glucose; blood urea nitrogen and rumen propionic acid. However, no effect was observed in the mean value of blood cell volume, haemoglobin, erythrocytes, leucocytes, calcium and phosphorus (SAWHNEY, 1963; BEAMES, 1959). TIWARI et al. (1990) and SAHOO et al. (1992) also reported that feeding of NPN with soluble carbohydrate affect the BUN as compared to those animals without an urea supplemented diet.

The effect of supplementing UMMG on some rumen parameters and certain blood biochemical constituents has been studied in this article in order to determine its adverse effect, if any, in goat kids maintained on sola hay and chaffed rice straw.

Materials and methods

This study was carried out on twenty Jamunapari goat kids (aged 4-8 months, average. weight 12.43 kg), divided into four groups of 5 kids in each group. All animals were offered sola hay and chaffed rice straw *ad libitum*. Kids in group II were given de-oiled rice bran (100 g). Kids in group III were given UMMG (50 g) and kids in group IV were given fish meal (25 g) plus UMMG (50 g). Animals in group I served as control group. The UMMG contained 40% molasses, 33% de-oiled rice bran, 8% urea, 10% mustard cake, 6% Ambadi cake, 2% mineral mixture, 1% salt and Vitablend 20 g/quintal. Prior to the start of the experiment all kids were fed a common dietary regimen for 4 weeks. Thereafter a feeding trial of 105 days was conducted. For rumen fermentation studies the rumen liquor was collected from individual kids at the end of digestion trial before feeding and watering, with the help of a stomach tube. The strained rumen liquor was analyzed for total volatile fatty acids (TVFA) (BERNETT and REID, 1957) ammonia-N, total nitrogen, non-protein nitrogen (NPN), trichloro acedic acid (TCA), perceptible nitrogen-N (AOAC 1975: Official methods of analysis of AOAC International, Association of Official Analytical Chemists,13th ed.). Washingon, D.C.) total bacterial and protozoal count, differential protozoal count, bacterial

and protozoal biomass (as per method described in laboratory manual, PATHAK et al., 1996) and microbial protein (AOAC, 1975). The pH was determined with a digital pH meter, and for blood biochemical studies samples were collected at the middle of growth trial by jugular puncture in heparinised tubes. Blood was mixed with anticoagulant by gentle rotation between the palms, blood samples were centrifuged at 3000-rpm for 10 to 15 minutes and deep frozen at -20 °C for determination of calcium (CLARK and COLIP, 1925); alkaline phosphatase (KIND and KINGS, 1954); creatinine (BONANES and TAUSSKY, 1945); total protein (ANNINO, 1976); urea nitrogen, phosphorus, ALT and AST.

Results and discussion

The pH in the rumen of kids under four different groups is presented in Table 1. The pH of the rumen was affected by the level of protein intake (BRIGGS, 1967) and NPN in the diet. In the present study, pH was slightly higher in groups III and IV, where diets were supplemented with UMMG and/or fishmeal, compared to control group. This ranged

Table 1. Rumen microbial protein and biomass and bacterial and protozoal counts in kids under various diets (Mean ± SE)

	Feeding groups			
	I No Supplements (Control)	II DORB (100 g)	III UMMG (50 g)	IV UMMG (50 g) plus Fish meal (25 g)
Parameters	Each group received sola hay + rice straw			
pH	6.9 ± 0.10^{b}	6.88 ± 0.08^{b}	7.22 ± 0.15^{a}	7.24 ± 0.15^{a}
TVFA meq/100 ml	9.44 ± 0.38^{b}	9.52 ± 0.33^{b}	10.55 ± 0.23^{a}	10.28 ± 0.30^{a}
Ammonia-N mg/100 ml	7.89 ± 0.78^{c}	9.08 ± 0.72^{b}	10.64 ± 0.91^{a}	10.76 ± 0.67^{a}
TCA-ppt-N mg/100 ml	48.98 ± 0.95^{ab}	48.60 ± 2.36^{b}	52.96 ± 4.99^{a}	52.44 ± 2.10^{ab}
Total-N mg/100 ml	82.65 ± 2.73^{b}	75.66 ± 0.71^{c}	88.97 ± 3.15^{a}	85.45 ± 3.33^{ab}
NPN mg/100 ml	33.69 ± 4.32^{ab}	26.99 ± 2.46^{c}	36.00 ± 7.44^{a}	33.01 ± 3.47^{abc}

Figures bearing different superscripts in a column differ significantly ($P<0.05$).

from 6.90 in group I to 7.24 in group IV. This demonstrated that in spite of maximum nitrogen intake in groups III and IV the pH was not much affected. This could not adversely affect the buffering capacity of rumen required for normal growth of rumen microbes and fermentation pattern.

Table 2. Rumen fermentation pattern in kids under various feeding groups (Mean ± SE)

Parameters	Feeding groups			
	I No Supplements (Control)	II DORB (100 g)	III UMMG (50 g)	IV UMMG (50 g) plus Fish meal (25 g)
	Each group received sola hay + rice straw			
Microbial protein (%)	34.96 ± 8.44 ^{bc}	42.03 ± 10.21 ^{abc}	42.94 ± 16.92 ^{ab}	57.04 ± 14.48^{a}
Bacterial biomass g/100 ml SRL	0.0412 ± 0.0115 ^{a}	0.0474 ± 0.0249 ^{a}	0.0568 ± 0.028^{b}	0.082 ± 0.0314 ^{b}
Protozoal biomass g/100 ml SRL	0.1549 ± 0.0610 ^{a}	0.1234 ± 0.0277 ^{a}	0.1183 ± 0.05223 ^{b}	0.1169 ± 0.067 ^{b}
Total bacterial count $\times 10^9$/ml SRL	7.8 ± 0.05 ^{ab}	7.43 ± 0.21^{a}	8.28^{b}	11.23 ± 0.98^{c}
Total protozoal count $\times 10^5$/ml SRL	3.45 ± 0.91^{a}	3.89 ± 0.64 ^{a}	3.79 ^{b}	3.65 ± 0.83 ^{b}
Protozoal count % Holotricha	67.1^{a}	70.1^{a}	16.1 ^{b}	24.1 ^{c}
Entodinium	24.8 ^{a}	27.0	73.6 ^{b}	70.6 ^{b}
Diplodinium	3.0 ^{a}	2.0 ^{a}	1.6 ^{a}	3.3 ^{a}
Epidinium	1.5 ^{a}	2.1 ^{a}	1.5 ^{a}	3.1 ^{a}
Ophryoscolex	1.4 ^{a}	1.0 ^{a}	2.2 ^{a}	3.9 ^{a}

Figures bearing different superscripts in a column differ significantly ($P<0.05$).

The TVFA concentration was found to be affected by different levels of protein intake and cellulolytic activity of rumen microbes. The TVFA concentration was significantly ($P<0.05$) higher in groups III and IV compared to group I. It is evident that proteolysis is a major process in the rumen-produced peptide and amino acids used for energy and other biosynthesis processes. The amino acids produced, either incorporated into bacterial cells or further deaminated to form ammonia, carbon dioxide and volatile fatty acids (HUNGATE, 1966). Molasses, a component of UMMG also served as a source of VFA in the rumen.

Table 3. Blood biochemical constituents in kids of various feeding groups (Mean ± SE)

Parameters	Feeding groups			
	I No Supplements (Control)	II DORB (100 g)	III UMMG (50 g)	IV UMMG (50 g) plus Fish meal (25 g)
	Each group received sola hay + rice straw			
Blood urea nitrogen (mg/100 ml)	21.14 ± 2.53^{c}	25.44 ± 2.91^{b}	28.88 ± 1.41^{a}	30.35 ± 1.83^{a}
Plasma creatinine (mg/100 ml)	0.95 ± 0.33^{a}	1.05 ± 0.33^{a}	1.00 ± 0.31^{a}	0.90 ± 0.14^{a}
Alkaline phosphatase (KA units)	26.77 ± 4.69^{b}	27.10 ± 5.52^{b}	29.77 ± 6.86^{b}	44.26 ± 10.52^{a}
AST activity (U/ml)	334.00 ± 48.87^{a}	339.00 ± 45.13^{a}	343.60 ± 51.68^{a}	349.00 ± 46.67^{a}
ALT activity (U/ml)	38.80 ± 3.63^{a}	38.40 ± 4.34^{a}	39.20 ± 6.42^{a}	34.80 ± 6.42^{a}
Blood glucose (mg/100 ml)	40.52 ± 3.36^{b}	45.32 ± 4.22^{b}	53.87 ± 3.21^{a}	53.72 ± 6.18^{a}
Calcium (mg/100 ml)	8.87 ± 1.12^{b}	8.66 ± 0.42^{b}	9.85 ± 0.45^{a}	9.93 ± 0.43^{a}
Inorganic Phosphorus (mg/100 ml)	5.89 ± 0.58^{b}	5.77 ± 0.36^{b}	7.57 ± 1.09^{a}	8.31 ± 1.67^{a}
Total protein (gm/100 ml)	5.35 ± 0.52^{b}	5.55 ± 0.45^{b}	6.52 ± 0.44^{a}	6.45 ± 0.32^{a}
Albumin (gm/100 ml)	2.86 ± 0.81^{a}	2.28 ± 0.63^{a}	2.83 ± 0.64^{a}	3.02 ± 0.43^{a}
Globulin (gm/100 ml)	2.37 ± 0.98^{b}	3.27 ± 1.01^{ab}	3.68 ± 0.50^{a}	3.43 ± 0.36^{a}
Albumin/Globulin ratio	1.41 ± 0.77^{a}	0.81 ± 0.52^{a}	0.79 ± 0.29^{a}	0.89 ± 0.22^{a}

Figures bearing different superscripts in a column differ significantly ($P<0.05$).

Higher ($P<0.05$) ammonia nitrogen, total nitrogen, NPN and TCA perceptible nitrogen in strained rumen liquor (SRL) of kids fed UMMG than in those fed basal roughage ration might be due to high solubility of urea and its rapid hydrolysis by rumen micro organisms.

Table 1. presents concentration of ammonia -N, TVFA, TCA perceptible nitrogen, total nitrogen and NPN in rumen of kids. It is clear from the data that the level of ammonia-N in the rumen depends upon the amount of granules consumed by kids.

Similar trends were obtained by other workers (LENG, 1984; MEHRA et al., 1991; SAHOO et al., 1992; TIWARI et al., 1990; NIRAL et al., 1999). It was suggested that total N concentration of rumen contents increased in response to increase in nitrogen intake in sheep (HUME et al., 1970).

Microbial protein synthesis was reflected by the availability of fermentable N and soluble carbohydrate in the rumen (PHILLIPSON, 1970). This was significantly higher in groups III and IV compared to group I. The fermentable nitrogen incorporated in the microbial cells, together with keto-acids available from soluble carbohydrate, may have increased the microbial protein synthesis.

Bacterial biomass was found to be higher in groups III and IV compared to group I, which can be explained on the basis that UMMG supplied fermentable –N and readily available carbohydrate for their incorporation into bacterial cells. The slow release of ammonia from NPN and availability of carbon skeleton from VFA favours the growth of bacteria (HUNGATE, 1966). Protozoal biomass was found to be higher in groups I and II compared to groups III and IV (Table 2).

Total bacterial count was significantly higher in group IV (Table 2), which could possibly be due to the ready availability of N to bacteria. A report of significant increase in total bacterial count existed as a result of long-term urea feeding in cattle (OGRA, 1980). Total protozoal count was significantly lower ($P<0.01$) in kids in group IV due to feeding of urea, and is in agreement with the existing reports (MATHUR et al., 1991; OGRA, 1980). The different types of protozoa encountered in SRL were similar to those reported earlier (MATHUR, 1963; MATHUR et al., 1991). Feeding of fishmeal as a source of protected protein increased the number of Holotrichs. On UMMG feeding the number of Holotrichs decreased, while that of Entodinium increased considerably. No difference was observed in the counts of Diplodinium, Epidinium and Ophryoscolex in any treatment group (Table 2). A report on an increase in percentage of other species due to an increase in rumen- N exists (NOUR et al., 1979).

The average values for blood urea nitrogen, creatinine, alkaline phosphatase, AST and ALT activity are shown in Table 3.

The BUN were found to be significantly ($P<0.05$) higher in groups (III and IV) where fermentable-N was supplemented. Urea being readily hydrolysable in the rumen might have increased the BUN. However, it had no adverse effect on buffering capacity either at the rumen level or the cellular level. The level of BUN was found to be lower than the tolerance limit of 80 mg/100 ml (LEHNINGER et al., 1993).

In present study it was observed that the level of dietary protein did not affect the creatinine level of plasma (GUYTON and HALL, 1988) (Table 3). It can change when animals are either in a stress condition or generating insufficient dietary energy to maintain a normal physiological condition (LEHNINGER et al., 1993). In muscle, energy is largely stored in the form of phosphocreatinine, which is a high energy phosphate bond. During stress, muscle catabolism takes place and phosphocreatinine becomes converted into pyrophosphate, which is later utilized by the body tissue as a source of energy, and creatine is excreted in the form of creatinine through urine.

Plasma alkaline phosphatase activity was found to be at its maximum in groups III (29.77 ± 6.86) and IV (44.26 ± 10.52) and at its minimum in group I (26.77 ± 4.69 KA units) (Table 3). This was probably due to deposition of protein and energy in muscular tissue of body, in addition to increased rate of deposition of calcium in bone tissue. Osteoblasts cells secrete a large quantity of alkaline phosphatase when they are actively depositing bone matrix (GUYTON and HALL, 1988). This enzyme is believed either to increase the local concentration of inorganic phosphate or to activate the collagen fibres in such a way that they cause deposition of calcium salts. Since some alkaline phosphatase diffuses into the blood stream, its blood level is increased. Thus this enzyme is usually a good indicator of the rate of bone tissue formation in growing animals.

The level of AST and ALT was found to be statistically non-significant among the groups (Table 3) and indicated that the animals were maintained in normal health condition without any cellular dysfunction, otherwise it would affect the cellular synthesis of protein and growth performance (LEHNINGER et al., 1993).

The maximum level of blood glucose was obtained in groups supplemented with UMMG and/or fish meal (Table 3). The level of glucose in blood may be correlated with the level of volatile fatty acid by the in vitro experiments of BELASCO (1954) in that urea would stimulate concentration of propionic acid from cellulose. However, the rumen VFA is more likely to be affected by the type of carbohydrate, which is fermented. In vitro fermentation studies by different workers (STEWART and SCHULTZ, 1958) appear to support this view. In our experiment also, concentration of TVFA was higher in the groups receiving UMMG plus fish meal, which might have contributed more propionic acid than any other VFAs. Since propionic acid is glucogenic in nature it could have been converted to glucose through the process of gluconeogenesis (LEHNINGER et al., 1993) and a comparatively higher glucose level was obtained in groups III and IV.

The plasma calcium level was normal in groups receiving urea molasses-based supplemental granules (III) and UMMG supplement plus fish meal (IV) (Table3). However, it was slightly lower in the control group, as UMMG contained 3.11% calcium and that of fishmeal 5.19% calcium, which could have resulted in a higher calcium level in groups III and IV compared to control group. This indicates that animals in groups III and IV were

obtaining adequate dietary calcium for depositing in bone tissue and maintaining normal blood calcium level. However, it was lower in the control group.

The availability of phosphorus in UMMG supplemented group with or without fish meal was greater, which was due to the ingredient composition of UMMG. From the growth performance of animals in groups III and IV, it appeared that kids received an adequate calcium and phosphorus ratio. These findings were in accordance with earlier workers (SAADULLAH et al., 1983; MEHRA et al., 1991; SINGH et al., 1999)

No significant difference could be obtained amongst the groups with regard to albumin and albumin and globulin ratio. However, there was a marginal increase in the total protein and globulin concentration in plasma in group III (Table 3), which might have favoured the defensive mechanism against certain unidentified diseases.

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Received: 28 March 2002
Accepted: 3 November 2005

JAIN, N., S. P. TIWARI, P. SINGH: Učinak granulirane mineralne melase ureje na vrenje u buragu i biokemijske pokazatelje u krvi jaradi hranjene s *Aeschonomene indica* Linn. Vet. arhiv 75, 521-530, 2005.

SAŽETAK

Istraživanje je provedeno na 20 jaradi jamunapari pasmine. Prema načinu hranidbe, jarad je razvrstana u 4 skupine. Prva skupina hranjena je isključivo voluminoznom krmom - sijenom *Aeschonomene indica* Linn i rižinom slamom (70:30). Obrok 2., 3. i 4. skupine sastojao se od odmašćenih rižinih posija (100 g/d), granulirane mineralne melase ureje (50 g/d) i granulirane mineralne melase ureje (50 g/d) s dodatkom ribljeg brašna (25 g/d). Pokus je trajao 105 dana. Utjecaj dodavanja granulirane mineralne melase ureje i ribljeg brašna istraživan je na pokazateljima buraga odnosno mikrobnih bjelančevina, bakterijske i protozoalne biomase i biokemijskih pokazatelja u krvi, tj. BUN, kreatinina plazme, glukoze, ukupnog proteina, kalcija i fosfora. Također je određivana i aktivnost enzima alkalne fosfataze, aspartat transaminaze i alanin transaminaze. Sinteza mikrobnih proteina bila je veća u skupinama 3 (42,94 g/100 ml) i 4 (57,04 g/100 ml) u usporedbi s kontrolnom skupinom i skupinom 2. Značajno je povećana i bakterijska biomasa (0,057 g/100 ml odnosno 0,082 g/100 ml). Slično, bakterijska i protozoalna populacija su bile pod utjecajem dodatka «bypass» bjelančevina koje u obroku potječu od ribljeg brašna. BUN je bio statistički značajno ($P<0,05$) veći u skupini 3 (28,88 mg/100 ml) i 4 (30,35 mg/100 ml), a u usporedbi s kontrolnom skupinom (1) i skupinom 2. Također, utvrđena je veća aktivnost alkalne fosfataze u skupini 3 (29,77 KA jedinica) i 4 (44,26 KA jedinica), što je imalo utjecaja na bolji rast jaradi u tim skupinama. Aktivnosti ostalih enzima nisu bile promijenjene. Koncentracije kalcija u plazmi i fosfora bile su statistički značajno ($P<0,05$) povećane u skupinama 3 (9,85 i 9,93 mg/100 ml) i 4 (7,57 i 8,31 mg/100 ml) u usporedbi s drugim skupinama. Za pretpostaviti je da dodavanje fermentirajućeg-N i ribljeg brašna, u količinama koje djeluju katalitički, povećava aktivnost bakterijske i protozoalne populacije, podiže razinu nekih pokazatelja u krvi i aktivnost enzima. Navedeno može poboljšati rast jaradi.

Klučne riječi: jarad, *Capra hircus*, pokazatelji u krvi, urea, *Aeschonomene indica,* fermentacija u buragu