Influence of concentrate level with or without N- carbamylglutamate on rumen bacteria and fermentation characteristics of Shami goats

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Abstract

This study was conducted to evaluate the effect of three levels of concentrate 2%, 3%, 4% with or without N-carbamylglutamate (NCG) on rumen bacteria and fermentation characteristics in Shami goats. Twenty-four male goats aged 8-10 months with initial weight 32,685 ± 1.52kg divided into six treatments in a 2×3 factorial experiment/ Completely Randomized Design. Individual feeding was used for 70 days. Rumen fluid was sampled at 0, 3, 6h after feeding while jugular blood was sampled after 70 days before morning feeding. Results showed decreasing (P<0.01) of rumen pH and increasing volatile fatty acids (P<0.01) after 3h and 6h of feeding for 3% level of concentrate with NCG. Rumen ammonia increased (P<0.01) after 3h of feeding with NCG. Total rumen bacteria increased insignificantly with superiority 3% level of feeding without NCG and 4% concentrate with NCG. A linear decrease (P<0.01) of blood urea with increasing feeding level with NCG and GPT decreased (P<0.05) with 3% concentrate. In conclusion, it is preferred to feed 3% concentrate and use NCG as additive with urea.

Keywords: Ruminant, ammonia, volatile fatty acids, blood urea.

Introduction

Iraq has been affected by drought and resulting contraction feed supply that led to decline small ruminant numbers. So, desertification combat could provide feed for animals. Goat is an important domesticated ruminant in agricultural sector due to ability to survive under harsh conditions, especially Shami goats which highly production of meat and milk [1]. To meet goat requirements for maintenance and production, replacing feed ingredients with available cheap source of crude protein like urea must be achieved. Roughage feeds and non-protein nitrogen sources (NPN) can be utilized in feeding ruminants to produce meat and milk [2], Urea isn't used more than 2% of concentrate [3] and high carbohydrate rations must be fed. Feeding urea at high levels lead to toxicity. In simple stomach animal, N-carbamylglutamate

(NCG) is used to treat hyperammonaemia by enhances N-acetylglutamate (NAG), for conversion ammonia to urea [4]. Although ruminants can synthesize amino acids, the needs of arginine are essential [5]. Therefore, arginine supplements were used, but due to rapid degraded in the rumen without reaching intestine in sufficient quantities, N-carbamylglutamate (NCG) supplements for ruminants as precursor of arginine [6] and polyamine and lower degraded in the rumen [5]. The physiological functions of NCG aren't toxic and free from side effects [7] without nutritional complications against other amino acids, especially Lysine, Tryptophan and Histidine. It is considered as an alternative, effective, and cheap supplement that is available in large commercial quantities instead of Arginine [8]. Therefore, this study aimed to study the effect of different levels of concentrate with or without N-carbamylglutamate on total rumen bacteria, fermentation characteristics and some blood parameters of Shami goats.

Materials and Methods

All ingredients of concentrated feed were provided from local market and included: 10% corn, 31.5% wheat bran, 55% barley, 1.5% urea, 2% mineral and vitamins. The chemical composition of concentrate and alfalfa hay (Table 1) as AOAC [9]. Concentrate was fed at three levels, 2%, 3% and 4% of body weight as DM basis, while alfalfa hay was provided ad libitum with remaining.

Table (1): Proximate analysis of concentrate feed and alfalfa hay (%) as DM basis.

Ingredients	Concentrate	Alfalfa hay
Dry matter (DM) %	92.02	85.17
Organic matter (OM) %	94.93	91.41
Crude protein (CP) %	14.90	15.54
Ether extract (EE) %	3.23	1.85
Crude fiber (CF) %	6.95	24.83
Inorganic matter (ash) %	5.07	8.59
Nitrogen free extract (NFE) %	69.85	49.18
*Metabolic energy (Me) (MJ/kg DM)	12.91	10.57
pH value	6.35	6.41

*Me (MJ/kg DM) = 0.012 × crude protein + 0.031 × ether extract + 0.005 × crude fiber + 0.014 × nitrogen free extract [10] .

Experimental animals and management

Twenty-four Shami male goats, aged 8-10 months and weighed 32,685 ± 1.52kg were randomly distributed to six treatments of three feeding levels of concentrate 2%, 3% and 4% of live body weight as DM basis with or without 6gm NCG/ head/ day. Individual feeding was conducted for 70 days preceded by 14 days as an adaptation

period. Vaccines and clean water were provided with continuous veterinary supervision. Alfalfa hay offered ad libitum with remaining.

Rumen fermentation

Rumen fermentation contain pH, volatile fatty acids (VFA) and ammonia nitrogen. Oral stomach tube was used to collect rumen fluid at different times after feeding as 0, 3, 6h., pH values were directly measured with portable pH meter (HANNA Instrument), then, some of rumen fluid used to determine bacterial count by kept at 4ºC and 0.1N HCl was added to another part of rumen fluid and kept in deep freeze to determine total volatile fatty acids and ammonia nitrogen cementations. Ammonia nitrogen was measured according to AOAC [9] as follows: After thawing frozen rumen fluid, 5ml was taken into a Kjeldahl digestion tube, added 0.5g MgO and 0.5 ml $CaCl_2$ 25% and 10 ml of distilled water, then measured with Kjeldahl apparatus. Ammonia was received with 5 ml of receiving solution, 2% boric acid and drops of the mixture (methyl red 0.099g and 0.066 bromocresol green dissolved in 100 ml ethyl alcohol), then titrated with 0.05 HCL to calculate ammonia concentration according to the equation:

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

Total rumen volatile fatty acids were measured after thawing frozen rumen fluid by taking 5ml rumen fluid into Kjeldahl digestion tube, added 1ml orthophosphoric acid, The tube was washed with a little distilled water, receiving flask contain 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 0.1M sodium hydroxide [11]. The concentration of VFA's calculated as following: Total VFA's (mmol/100ml) = 0.1M × (titration volume of NaOH sample – titration volume for blank) × 100/5ml.

Rumen bacteria count

Fresh rumen fluid (1ml) was taken into 9ml of physiological saline. All dilutions were completed in test tubes containing 9ml of dilution solution to appropriate numbers between 25-300 colonies of microorganisms. Then, 1ml of appropriate dilution of each sample was transferred to empty Petri dishes using a sterile pipette, and poured out Chocolate Agar or Nutrient agar. After gently mixing, the medium was placed inside the anaerobic containers in an inverted form with several anaerobic conditions (Gas pack) to make anaerobic conditions then incubated at 37 °C for 48 hours. After development, the numbers of colonies were calculated for each petri dishes as Roberts and Greenwood [12].

Blood sampling

After 70 days of experimental feeding, blood samples were collected from the jugular vein before morning feeding by using a 10ml syringe needle. Glutamic-oxaloacetic

transaminase (GOT), glutamic-pyruvic transaminase (GPT) and urea were analyzed in the laboratory by using Biosystem, design BTS-300 Germany origin.

Statistical analysis

Three levels of feeding concentrate with or without NCG were statistically analyzed using a 2 × 3 factorial experiment, completely randomized design (CRD). One-way ANOVA analysis was performed using a statistical program [13]. Duncan's multiple range test was used to determine significant differences ($p<0.05$) and ($p<0.01$) among treatments [14] using following formula:

$Yijk = \mu + Ai + + Bj + AB(ij) + eijk$.

Results and Discussion

Rumen fermentation

The effect of concentrate level with or without N-carbamylglutamate on rumen pH at different times after morning feeding indicated to significant decrease ($P<0.01$) of rumen pH after 3h and 6h of morning feeding for feeding level 3% with and without NCG (Table 2). At zero time, there were no significant differences between treatments. Rumen pH increased at level 2% of concentrate due to low concentrate intake, that insufficient feed leads to intake more roughages which may cause an increase rumen pH. The results agreed with Carro et al.[15], they noticed higher pH value with increasing roughages to concentrate 80:20%. The decrease pH with NCG due to pH value of NCG (2.6) [16]. The effect of concentrate feeding level with or without N-carbamylglutamate on rumen ammonia nitrogen referred to insignificant variations for rumen ammonia between different levels of concentrate at zero time (Table 3). While there were high increased ($p<0.01$) after 3h of feeding with superiority to NCG treatments. Rumen ammonia was 22.58 mg/100ml for T4 in contrast with 17.07, 15.75, 14.27, 13.13, 11.20 mg/100ml for treatments T6, T1, T5, T3, T2 respectively. Results of activity ruminal microorganisms appear in the form of degraded products, such as ammonia, volatile fatty acids and pH of the rumen liquor. So, these results due to degraded NCG in the rumen that led to increase ammonia nitrogen or to enhance rumen degradability with additive NCG. Increase fermentation products means an effective fermentation medium and enhance microbial growth, that leads to an increase feed intake, daily gain, feed efficiency, rumen out flow rate and indicates a good quality feed [17]. About 80% of rumen microbial protein composed of rumen ammonia nitrogen [18]. Geron et al. [19] indicated that the peak of rumen ammonia nitrogen elevation after 3-5h after feeding true protein, and after 1-2h. for NPN [1]. Agle et al. [20] referred to less ammonia emissions from manure with low level intake of protein or low rumen degradable protein (RDP) compared to high levels and high rumen degradable protein (RDP) without affecting performance. Mahdi [16] stated that 4% concentrate without NCG led to significant increase ($p<0.05$) of rumen ammonia after 3h of feeding and insignificant results with NCG.

Table (2): Effect of concentrate level with or without N-carbamylglutamate on rumen pH (mean ± SE)

Tret.	pH/ 0h.	pH/ After 3h	pH/ After 6h
T1	7.00 ±0.14	6.65 ±0.06 a	6.52 ±0.11 a
T2	6.85 ±0.17	6.07 ±0.02 c	5.77 ±0.06 b
T3	6.80 ±0.10	6.40 ±0.17 ab	6.35 ±0.06 a
T4	6.67 ±0.25	6.37 ±0.04 abc	6.35 ±0.21 a
T5	6.60 ±0.30	6.25 ±0.08 bc	5.72 ±0.02 c
T6	6.87 ±0.11	6.37 ±0.11 abc	6.15 ±0.15 a
Sign.	NS	**	**

Different litters in the same column means significant differences; NS= non-significant differences; ** Significant differences at level 0.01; T1 = 0 NCG, 2% concentrate; T2 = 0 NCG, 3% concentrate; T3 = 0 NCG, 4% concentrate. T4 = 6 (g/day) NCG, 2% concentrate; T5 = 6 (g/day) NCG, 3% concentrate; T6 = 6 (g/day) NCG, 4% concentrate.

Table (3): Effect of concentrate level with or without N-carbamylglutamate on rumen ammonia nitrogen (mg/100 ml) (mean ± SE)

Tret	NH3-N (mg/100ml) zero time	NH3-N (mg/100ml) After 3h	NH3-N (mg/100ml) After 6h
T1	10.50 ±1.42	15.75 ±1.23 bc	20.13 ±3.23
T2	14.88 ±2.08	11.20 ±1.68 c	17.94 ±2.30
T3	13.13 ±0.87	13.13 ±0.97 bc	19.17 ±2.40
T4	13.83 ±1.16	22.58 ±2.05 a	20.57 ±1.31
T5	12.95 ±1.83	14.27 ±0.76 bc	17.07 ±2.80
T6	14.44 ±0.83	17.07 ±2.97 b	20.83 ±1.16
Sign	NS	**	NS

Different litters in the same column means significant differences; NS= non-significant differences; **Significant differences at level 0.01; T1 = 0 NCG, 2% concentrate; T2 = 0 NCG, 3% concentrate; T3 = 0 NCG, 4% concentrate. T4 = 6 (g/day) NCG, 2% concentrate; T5 =6 (g/day) NCG, 3% concentrate; **T6 = 6 (g/day) NCG, 4% concentrate.**

The results rumen volatile fatty acids showed increasing (P<0.01) with NCG after 3h and 6h of feeding and superiority 3% concentrate level with NCG (Table 4). No significant effects at zero time or before feeding and numerical increasing for 3% level of concentrate. The superiority of 3% level even after 24h of feeding referred to regular feed intake and availability of nutrients. Rumen microorganisms activity produce volatile fatty acids to enhance microbial protein when synchronization with ammonia, or its absorbed to blood as a source of energy and fatty acids [21]. High availability of rumen VFA's leads to decrease rumen pH and prevent animals to consumed highly fermented roughages or highly concentrate [15]. The necessity of

adaptation period prevents the accumulation of volatile fatty acids by gradual feeding for 10-14 days.

Table (4): Effect of concentrate level with or without N-carbamylglutamate on rumen volatile fatty acids (Mmol/100ml) (mean ± SE)

Tret.	VFA (Mmol/100ml) zero time	VFA (Mmol/100ml) After 3h	VFA (Mmol/100ml) After 6h
T1	2.18 ±0.44	4.68±0.34abc	4.91±0.88 b
T2	3.06 ±0.35	3.87 ±0.26 c	5.06±0.18ab
T3	2.81 ±0.67	4.62 ±0.86 bc	3.87 ±0.16 b
T4	2.22 ±0.43	5.68 ±0.69 ab	4.32 ±0.26 b
T5	3.53 ±0.24	6.43 ±0.44 a	6.56 ±0.83 a
T6	2.72 ±0.29	5.18±0.40abc	3.43 ±0.18 b
Sign.	NS	**	**

Different litters in the same column means significant differences; NS= non-significant differences; ** Significant differences at level 0.01; T1 = 0 NCG, 2% concentrate; T2= 0 NCG, 3% concentrate; T3 =0 NCG, 4%concentrate. T4 = 6 (g/day) NCG, 2% concentrate; T5 = 6 (g/day) NCG, 3% concentrate; T6 = 6 (g/day) NCG, 4% concentrate.

The complex environment of rumen characteristics and the availability of synchronization led to increase rumen total bacteria (Table 5). Rumen bacteria increased insignificantly with superiority 3% level of concentrate without NCG and 4% concentrate with NCG. Mahdi [16] and Al-Shefea [22] referred to increase rumen bacteria at 4% feeding level with NCG in lambs. Increasing microbial growth led to increase feed intake, feed efficiency, rumen out flow rate and referred to good quality of feed [17]. The effect of concentrate level with or without N-carbamylglutamate on liver functions enzymes and blood urea (Table 6) referred to a linear decrease of urea ($P<0.01$) with increasing feeding level with NCG treatments (T4, T5, T6), 32.27, 31.72, 28.28 mg/ 100ml for 2%, 3% and 4% respectively. Glutamic-oxaloacetic transaminase (GOT) decreased with 3% concentrate with NCG. Similar results for glutamic-pyruvic transaminase (GPT) and decreased with additive NCG at 3% concentrate. The results agreed with Al-Shefea [22], he referred to insignificant increased for GPT when fed 3gm/ day NCG while insignificant decrease for GOT and feeding 1.5% urea led to increase blood urea and GPT enzyme. Mammalia produce urea out of body to detoxify blood ammonia from catabolism of nitrogen sources. Because of concentrate contain 1.5% urea, that led to increasing blood urea without NCG with increasing concentrate intake. Gradual adaptation must be achieved to a non-protein nitrogen and urea supplementation does not exceed 1-2% of total dry matter in the diet [23]. Mahdi [16] referred to increase blood urea when fed 1% urea with 4% concentrate, while GOT and GPT didn't affect.

Table (5):Effect of concentrate level with or without N-carbamylglutamate on rumen bacterial count (Cfu/ml) (mean ± SE)

Tretments	Bact. Count, zero time $\times 10^9$ Cfu/ml	Bact. Count, After 3h $\times 10^9$ Cfu/ml	Bact. Count, After 6h $\times 10^9$ Cfu/ml
T1	88.00 ±19.93	123.00 ±21.00	137.50 ±20.88
T2	135.50 ±45.28	173.50 ±40.35	190.00 ±43.64
T3	110.00 ±4.76	147.00 ±5.25	166.00 ±11.97
T4	91.00 ±20,48	124.50 ±20.98	142.00 ±38.51
T5	142.00 ±30.87	162.500±28.39	181.00 ±28.66
T6	177.00 ±28.40	191.00 ±27.23	202.00 ±26.26
Sign.	NS	NS	NS

Different litters in the same column means significant differences; NS= non-significant differences; T1 = 0 NCG, 2% concentrate; T2 =0 NCG, 3% concentrate; T3 = 0 NCG, 4% concentrate. T4 = 6 (g/day) NCG, 2% concentrate; T5 = 6 (g/day) NCG, 3% concentrate; T6 = 6 (g/day) NCG, 4% concentrate.

Table (6): Effect of concentrate level with or without N-carbamylglutamate on blood urea and liver functions enzymes (mg/100ml) (mean ± SE)

Tret.	Urea (mg/100ml)	GOT (mg/100ml)	GPT (mg/100ml)
T1	38.50 ±2.84 ab	80.00 ±3.67	24.00 ±1.82 ab
T2	41.03 ±3.22 a	78.50 ±5.20	23.00 ±1.08 ab
T3	35.78 ±2.02 ab	81.50 ±3.27	22.75 ±2.05 ab
T4	32.27 ±1.33 ab	81.25 ±1.88	25.02 ±2.03 ab
T5	31.72 ±5.10 ab	75.75 ±4.71	22.00 ±1.00 b
T6	28.28 ±3.48 b	112.75 ±30.32	34.00 ±7.60 a
Sign.	**	NS	*

Different litters in the same column means significant differences; NS=non-significant differences; * Significant differences at level 0.05; **Significant differences at level 0.01; T1 = 0 NCG, 2% concentrate; T2 = 0 NCG, 3% concentrate; T3 = 0 NCG, 4% concentrate. T4 = 6 (g/day) NCG, 2% concentrate; T5 = 6 (g/day) NCG, 3% concentrate; T6 = 6 (g/day) NCG, 4% concentrate.

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