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RESEARCH ARTICLE

Effect of N- carbamylglutamate on amino acids and blood biochemistry in young male goats

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ABSTRACT

To study the effect of feed additive N- carbamylglutamate (NCG) with different levels of feeding on amino acids profile and some analytes in serum of goats, thirty male goats aged 7-8 months were distributed into three levels of concentrate 2%, 3%, 4% with or without NCG in a 2×3 factorial experiment/Completely Randomized Design (CRD). After 72 days of individual feeding, the blood from jugular vein was obtained before feeding in the morning. Results showed that additive NCG with 3% concentrate led to an increase ($P<0.01$) in total protein and albumin ($P<0.05$), globulins increased ($P<0.01$) with increasing concentrate without NCG. Creatinine decreased with increasing concentrate without NCG. Additive NCG led to a linear decrease in uric acid with increasing feed intake ($P<0.05$) and a linear increase without NCG. Blood magnesium, zinc, and copper increased with NCG. No difference in calcium between treatments. The level of feeding without NCG didn't affect fat parameters, while LDL, HDL, and cholesterol increased with NCG and 4% concentrate. Additive N- carbamylglutamate affected negatively blood amino acids, especially with 4% concentrate. In conclusion, 3% concentrate was the best for blood homeostasis with or without NCG, and preferred to use NCG with urea.

Keywords: Blood amino acid; Blood traits; Concentrate; N-carbamylglutamate; Goats

INTRODUCTION

The white powder N- carbamylglutamate sold under different brand names carbaglu, ucedane, carglumic acid, and (S)-2-ureidopentanedioic acid, used as ammonia detoxification or treatment of acute or chronic hyperammonemia because it enhances rapidly N-acetylglutamate (NAG), an essential cofactor for converting ammonia to urea in the liver (Tuchman et al., 2008) by obligatory stimulating carbamoyl phosphate synthetase 1 (CPS1) reaction, the first step of the urea cycle (Häberle et al., 2018). The deficiency of NAG causes hyperammonemia. In ruminants, NCG could be used with urea feeding without negative effects to improve the antioxidant capacity of the mother, fetus, placenta and to promote the growth of fetus and placenta during pregnancy (Zhang et al., 2016) growth and maintenance (Feng et al., 2018), controlling on blood urea, increasing feed efficiency to improve productive performance (Mahdi et al., 2021) and increases nitric oxide (NO) which works as a widening blood vessels, vasodilation and relaxation smooth muscle cells within the vessel walls (Wang et al., 2018). Researchers referred that NCG increases the endogenous synthesis of arginine (Wu et al., 2012) and arginine family amino acids like glutamine, glutamate, proline, ornithine, and citrulline. A common use of urea as a source of non-protein nitrogen to reduce feed costs in ruminants and activate rumen bacteria, is accompanied by many negative side effects for the host animal, like excess urea and ammonia circulated in the blood, causing poisoning or acute hyperammonemia, for these reasons, the objective of this study was to investigate the effects of N- carbamylglutamate and different levels of feeding on amino acids and blood biomarkers in goats.

MATERIALS AND METHODS

Experimental animal and management

Thirty male goats aged 7-8 months were distributed to six treatments, feeding three levels of concentrate 2%, 3%, and 4% of the live body weight as DM basis, with and without 6g/head/day of N-carbamylglutamate. Individual feeding was conducted with ad libitum of alfalfa hay. Clean water, vaccines, and veterinary supervision were submitted. Concentrate feeds were offered at 7.00 am., on the last

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day of the experiment after 72 days, blood samples were collected from the jugular vein using a 10ml heparin syringe before morning feeding, blood samples were centrifuged immediately to separate the plasma, which was stored at −20℃ for further analysis, no hemolysis was found.

Estimation of blood parameters

A. Blood biochemistry

A blood biomarker included protein test, some macro and trace minerals, cholesterol, and fat parameters were implemented on plasma with an automated blood chemistry analyzer, Spin200- SPINREACT with kits.

B. Blood amino acids

Preparation of sample

Plasma samples were prepared by adding 250μl of sample and methanol (MERCK) 500μl, thorough mixing then incubated at laboratory temperature for 5 min, centrifugated at 5000 rpm for 5 min, 250μl of supernatant were collected and mixed with 100 μl of borate buffer. For derivatization of amino acids, 50μl of o-phthalaldehyde (OPA) solution was added to the above solution and then incubation was performed for two minutes at room temperature.

HPLC condition

A high-performance liquid chromatography method with pre-column derivatization for separation and quantification of amino acids in plasma samples were conducted using an amino acid analyzer, model YL9100 Korea, the mobile phase was acetonitrile: buffer: DW (60: 10: 30), a Flow rate (1ml/min) and column separation was C18-NH2 (25 cm * 4.6 mm), the Detector = Florescence Ex =365 nm, Em = 445 nm (Mohammad et al., 2016).

Chemical analyses and pH values

The chemical composition of concentrated feeds and alfalfa hay (Table 1) was implemented as AOAC (2012). Concentrate feed included: 55% barley, 10% corn, 31.5% bran, 1.5% urea, 1% mineral and 1% vitamins. pH value of feed was measured by the weight of 1g of feed, added 10ml of distilled water, after 10 minutes, filtered with cheesecloth and measured with a portable pH meter from HANNA Instrument (Tawfeeq and Al-Attar, 2014).

Table 1: Chemical composition of concentrate feed and alfalfa hay (%) as DM basis

Approximate analysis	Alfalfa hay	Concentrate
Dry matter (DM) %	87.37	92.80
Organic matter (OM) %	92.76	93.99
Crude protein (CP) %	17.89	15.76
Ether extract (EE) %	2.33	5.14
Crude fiber (CF) %	22.31	7.32
Inorganic matter (ash) %	7.24	6.01
Nitrogen free extract (NFE)	50.23	65.77
*Metabolic energy (MJ/kg DM)	11.02	13.06
pH value	6.58	7.39

* Metabolic energy (MJ/kg dry matter) = 0.012×crude protein + 0.031× ether extract + 0.005×crude fiber + 0.014 ×nitrogen free extract (MAFF,1975)

Statistical analysis

Data were statistically analyzed using a factorial experiment, 2×3 Completely Randomized Design (CRD), One-way ANOVA analysis was performed using a statistical program (SAS, 2012). Duncan's multiple range test was used to determine the significant differences among treatments (Duncan, 1955) using the following formula: $Yijk = \mu + Ai + Bj + AB(ij) + eijk$; Where:

Yijk = The observed value of NCG and level of concentrate, μ = The overall mean, Ai = The main effect of NCG (0 or 6gm), Bj= The main effect of concentrate level (2% or 3% or 4%), AB(ij) = The interaction effect between NCG and concentrate level, eijk = The random residual effect.

RESULTS AND DISCUSSION

The effect of additive NCG with different levels of concentrate showed increasing ($P<0.01$) total protein with NCG treatments in contrast without NCG at the level of concentrate 2% and 3% (Table 2) with non significance between treatments of NCG feed additives. Albumin increased ($P<0.05$) with 3% concentrate with NCG, globulins increased ($P<0.01$) with increasing concentrate without NCG. Increasing albumin gives us a monitor as good function for liver, kidney, body nourishment and no inflammation (Garcovich et al., 2009). The same thing for globulins which includes enzymes, antibodies, and other blood proteins. The increase in globulins mean a healthy body. Creatinine increased ($P<0.01$) with 2% and 4% concentrate without NCG and decreased with 4% concentrate with NCG. The breakdown of creatine produces creatinine in muscles and increased creatinine may indicate diabetes, damage to kidneys, or high blood pressure. Additive NCG led to a linear decrease of uric acid with increasing feed intake ($P<0.05$) especially 4% feeding concentrate. While without NCG, led to a linear increase of uric acid with increasing concentrate level. The homeostasis of protein is very important for maintenance and production. Our results agreed with Mahdi (2020), who stated that no effects for adding NCG on total blood proteins in lambs. Zhang et al. (2021) referred to decrease total protein and globulins with the low level of feeding and 20gm of arginine or 5gm/day of NCG for Hu ewes.

The effects of concentrate level and NCG on some blood minerals in goats showed increases in blood phosphor ($P<0.05$) with and without NCG and a decrease with 4% concentrate with NCG (Table 3). Phosphate levels are important for growth and nerve function (Serna and Bergwitz, 2020). The decreased level of phosphate is associated with malnutrition. Magnesium increased (P<0.05) for all feeding levels with NCG in contrast with without NCG. Magnesium (Mg) is found inside body cells, a low level of Mg indicates severe malnutrition, diarrhea, and diuretics. Zinc increased (P<0.01) with 3% and 4% concentrate and decreased with 2% with or without NCG, while Copper (CU) increased (P<0.01) with NCG treatments in contrast without NCG. No difference in calcium between treatments. In ruminant, the bioavailability of minerals doesn't like simple stomach animals and appear to be affected by rumen and many dietary factors. The low absorption of copper due to modification in the rumen environment (Jerry, 2003). Generally, organic sources are the best for minerals bioavailability than inorganic sources. Diet change had positive effect on serum levels of phosphor and magnesium (Miller et al., 2010).

The effect of NCG with different levels of feeding at 2%, 3%, and 4% on blood cholesterol and fat parameters showed no differences between levels of concentrate without NCG (Table 4). While feeding NCG led to a decrease (P<0.01) in cholesterol, LDL (bad cholesterol), and decrease HDL (good cholesterol) (P<0.05) with 2% concentrate and a decrease in VLDL (P<0.01) with a 3% level of feeding. Cholesterol is an essential blood fat, but high level associated with a higher risk of heart diseases and clogged blood vessels, total cholesterol in the blood includes LDL and HDL. LDL or bad cholesterol because of deposit cholesterol in arterial walls and restricting blood flow then causing atherosclerosis and other complications. While HDL or good cholesterol carrying cholesterol away from the arteries to the liver for excretion. Zhang et al. (2021) stated a decrease in triglycerides with the low level of feeding and increased with NCG, while cholesterol decreased with low levels of feeding with arginine and with NCG.

The results referred to increase amino acids with 3% concentrate with and without NCG in contrast with 2% and 4% (Table 5). The results of feeding N- carbamylglutamate affected negatively plasma amino acids, there was decreasing (P<0.01) of all amino acids with NCG especially with 4% concentrate. Our results disagree with Chacher et al. (2016) and Huang et al. (2019), they referred that NCG plays as a precursor and enhances arginine without degraded in the rumen. The additive NCG to fattening

Table 2: Effects of feeding levels with or without NCG on blood protein parameters (mg/dL) in goats (Mean±Standard error)

Treat.	T1	T2	T3	T4	T5	T6	Sign.
Total protein	69.35±1.58[b]	70.45±1.41[ba]	68.05±1.06[bc]	62.63±0.33[d]	65.8±0.51[c]	72.95±0.37[a]	**
Albumin	31.15±0.14[b]	33.45±0.14[a]	32.3±0.17[ba]	33.1±0.69[a]	32.5±0.63[ba]	32.25±0.49[ba]	*
Globulins	38.2±1.44[ba]	37.00±1.27[b]	35.3±0.98[bc]	29.70±0.40[d]	33.30±0.11[c]	40.70±0.86[a]	**
Creatinine	0.74±0.04[bc]	0.70±0.02[dc]	0.57±0.01 [d]	0.92±0.06[a]	0.71±0.05[dc]	0.87±0.06[ba]	**
Uric acid	0.27±0.01[a]	0.22±0.01[ba]	0.17±0.01 [b]	0.215±0.01[ba]	0.26±0.01[a]	0.37±0.03[a]	*

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

Table 3: Effects of feeding levels with or without NCG on some blood minerals (mg/dL) in goats (Mean±Standard error)

Treat.	T1	T2	T3	T4	T5	T6	Sign.
calcium	8.20±0.02	8.15±0.08	7.95±0.20	7.8±0.28	8.4±0.23	8.15±0.08	NS
phosphor	6.65±0.60[ba]	6.60±0.17[ba]	5.10±0.98[b]	6.40±0.05[ba]	5.85±0.60[ba]	7.05±0.02[a]	*
Magnesium	2.55±0.04[ba]	2.62±0.10[ba]	2.69±0.01[a]	2.47±0.05[bc]	2.29±0.09[c]	2.40±0.01[bc]	*
Zinc	26±1.73[c]	34.5[b]±0.86[a]	38±4.04[ba]	31±0.57[bc]	38.5±2.59[ba]	39±2.3[a]	**
Copper	73±0.57[a]	71.5±8.37[a]	58.5±6.06[ba]	53.5±7.21[ba]	53.5±2.59[ba]	50.5±6.06[b]	**

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

Table 4: Effects of feeding levels with or without NCG on blood cholesterol and fat parameters (mg/dL) in goats (Mean±Standard error)

Treat.	T1	T2	T3	T4	T5	T6	Sign.
Cholesterol	51.5±2.59[b]	60.5±0.86[ba]	77±9.81[a]	57±9.81[ba]	59±4.61[ba]	58.5±4.33[ba]	**
Triglycerides	25.5±1.44	13.5±3.57	16±2.3	21.5±7.21	13.5±3.17	17±0.57	NS
LDL	20.5±0.86[b]	28±0.57[ba]	36.5±7.21[a]	23.5±4.9[ba]	26±4.61[ba]	27±3.46[ba]	**
VLDL	5.5±0.28[a]	2.5±0.86[b]	3±0.57[ba]	4.5±1.44[ba]	3±0.57[ba]	3.5±0.28 [ba]	**
HDL	25.5±1.44[b]	30±1.15[b]	37.5±3.17[a]	29±3.46[b]	30±0.57[b]	28±0.57[b]	*

LDL=low density lipoprotein, VLDL= Very-low-density lipoprotein, HDL=high density lipoprotein, Different litters in the same row means significant differences; NS=non-significant, * Significant at level 0.05, ** Significant at level 0.01; T1 = 6 (g/day) NCG, 2% concentrate; T2 = 6 (g/day) NCG, 3% concentrate; T3 = 6 (g/day) NCG, 4% concentrate; T4 = 0 NCG, 2% concentrate; T5 = 0 NCG, 3% concentrate; T6 = 0 NCG, 4% concentrate

Table 5: Effects of feeding levels with or without NCG on blood amino acids (ppm) in goats (Mean±Standard error)

Treat.	T1	T2	T3	T4	T5	T6	Sign.
Aspartic acid	65.06±0.8[c]	70.2±0.75[b]	54.4±0.61[d]	69.96±0.86[b]	76.73±0.71[a]	72.1±1.0[b]	**
Threonine	42.26±0.29[c]	46.46±0.33[b]	32.83±0.36[d]	51.86±0.98[a]	53.93±0.88[a]	41.83±0.86[c]	**
Serene	43.66±0.46[d]	47.20±0.30[c]	36.83±0.34[e]	54.56±0.68[a]	52.03±0.02[b]	42.83±0.33[d]	**
Glutamic acid	235.53±0.39[d]	241.53±0.60[c]	223.13±0.91[f]	246.5±1.08[b]	255.2±0.30[a]	230.93±0.29[e]	**
Proline	10.7±0.40[c]	12.86±0.06[b]	8.6±0.15[d]	13.36±0.29[b]	15.63±0.67[a]	10.46±0.14[c]	**
Glycine	9.63±0.13[d]	12.2±0.41[c]	7.53±0.17[e]	13.13±0.35[b]	16.36±0.24[a]	9.3±0.1[d]	**
Alanine	23.73±0.52[c]	26.6±0.43[b]	23.16±0.38[c]	26.56±0.28[b]	33.2±0.35[a]	27.5±0.70[b]	*
Cysteine	8.5±0.23[c]	10.83±0.23[b]	8.36±0.26[c]	10.86±0.20[b]	18.36±0.31[a]	10.36±0.08[b]	*
Methionine	13.26±0.17[d]	16.66±0.24[b]	12.8±0.05[d]	16.00±0.20[b]	22.23±0.87[a]	14.66±0.16[c]	**
leucine	91.56±0.49[d]	93.53±0.52[c]	74.76±0.40[f]	97.20±0.20[b]	99.16±0.51[a]	77.3±0.05[e]	**
Tyrosine	30.66±0.12[ed]	34.36±0.42[c]	29.93±0.55[e]	46.03±0.44[a]	42.53±0.6[b]	31.6±0.10[d]	**
Lysine	75.06±0.42[d]	78.53±0.31[c]	65.7±0.15[f]	82.33±0.56[b]	85.03±0.72[a]	67.16±0.31[e]	**
Arginine	66.83±0.54[e]	68.5±0.21[d]	61.7±0.43[f]	75.2±0.57[b]	81.46±0.63[a]	71.26±0.35[c]	**

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

balls led to increasing arginine, essential, and non-essential amino acids with increasing NCG doses and increasing daily gain, feed efficiency, crude protein digestibility, N retention, N utilization, and decreasing fecal N and urinary nitrogen (Yang et al., 2021). The additive of NCG led to a change in Jersey dairy cow amino acid metabolism (Liu et al., 2022).

CONCLUSIONS

Feeding goats 3% concentrate as DM basis of live body weight preferable for blood hemostasis, while feeding 4% concentrate led to an increased blood uric acid and creatinine. Feeding NCG and 4% concentrate led to a decrease in blood creatinine, uric acid, phosphorous and amino acids pool. The level of 3% of concentrate was the most proper for blood protein parameters, minerals, fat, and amino acids in goats as ruminant animals.

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Contributions of authors

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

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