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RESEARCH ARTICLE

Effect of N- carbamylglutamate and feeding level on amino acids and biochemical parameters in goats

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ABSTRACT

The effect of feed additive N- carbamylglutamate (NCG) with different feeding levels on some biochemical parameters and amino acids in the serum of goats was investigated. Thirty male goats aged 10-11 months were distributed into three levels of concentrate 70%, 50%, and 30% with or without N- carbamylglutamate (NCG) in a 2 × 3 factorial experiment/Completely Randomized Design (CRD). After 77 days of individual feeding, jugular blood was sampled before morning feeding. The results showed that additive NCG with high concentrate (70%) led to a decrease ($P<0.01$) in total blood protein, triglycerides, and globulins, and a decrease ($P<0.05$) for creatinine, uric acid, and VLDL. Blood magnesium increased ($P<0.01$) with NCG of low concentrate (30%) treatment, while phosphor increased with NCG of 50% concentrate treatment. Calcium increased with all NCG treatments. The results of adding N- carbamylglutamate with high roughage led to a negative effect on blood amino acids. In conclusion, increasing roughage feeding without NCG is the best for blood homeostasis, and don't use N- carbamylglutamate without non-protein nitrogen additives.

Keywords: Amino acids, blood traits, N-carbamylglutamate, feeding level.

INTRODUCTION

The importance of feeding level or roughage to concentrate ratio is to keep the best possible conditions for the growth and reproduction of microorganisms in the herbivores (Cavallini et al., 2022; Raspa et al., 2022) including ruminants and non-ruminants, which is reflected in feed efficiency and animal production. In ruminants, urea is commonly used in feeds to reduce costs, but it may cause problems for the fetus, because of urea hydrolysis to ammonia by rumen microorganism's within 2h. after feeding and leads to a higher rate of NH3 (Ibrahim et al., 2021). The levels of blood ammonia are regulated by converting to urea in the liver and then excreted with urine and some of the blood urea participated in the saliva and re-enters the rumen as a source of crude protein in the form of non-protein nitrogen. N- carbamylglutamate (carbaglu), is used for ammonia detoxification and enhances N-acetylglutamate (NAG) that converts ammonia to urea (Tuchman et al., 2008) by obligatory stimulating carbamoyl phosphate synthetase 1 (CPS1) reaction, the first step of the urea cycle (Häberle et al., 2018). So, the use of N- carbamylglutamate with urea feeding improves the antioxidant capacity of the mother and promotes the growth of the fetus and placenta, and prevents hyperammonemia (Zhang et al., 2016), increasing productive performance by controlling blood urea (Mahdi et al., 2021) and enhances nitric oxide (NO) as vasodilation and relaxation of smooth muscle within the vessel walls (Wang et al., 2018). Wu et al. 2012 stated to NCG is a promoter for arginine and arginine family amino acids synthesis. The negative side effects of urea with emphasis on the use of NCG as amino acids promoters led to an investigation of the effect of N- carbamylglutamate on amino acids, minerals concentration, and blood biochemistry in goats fed different levels of concentrate.

MATERIALS AND METHODS

Experimental animal and management

Thirty male Shami goats aged 10-11 months were fed individually restricted feeding 3% of live body weight as DM basis, distributed to six treatments: T1 (30% roughage: 70% concentrate) 6 (g/day) NCG); T2 (50% roughage: 50% concentrate, 6 (g/day) NCG); T3 (70% roughage: 30%

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concentrate, 6 (g/day) NCG); T4 (30% roughage: 70% concentrate, 0 NCG); T5 (50% roughage: 50% concentrate, 0 NCG); T6 (70% roughage: 30% concentrate, 0 NCG). The clean water, vaccines, and veterinary supervision were submitted. Experimental procedures were carried out according to animal protection laws (Directive 2010/63/EU). All feeds were offered at 7.00 am, and after 77 days of the experiment, a 10ml heparin syringe was used to collect blood samples from the jugular vein before morning feeding, the samples were centrifuged immediately to separate the plasma, and stored at −20°C for further analysis, no hemolysis was found.

Estimation of blood parameters

Amino acids

All plasma samples were prepared by adding 250µl of sample and 500µl methanol (MERCK), thoroughly mixing and then incubating at laboratory temperature for 5 min, centrifuging at 5000rpm for 5 min, mixing 250µl of the supernatant with 100µl of borate buffer. Added 50µl of o-phthalaldehyde (OPA) and incubated for 2 min at room temperature. HPLC method for separation and quantification of amino acids (Aspartate, Threonine, Serene, Glutamate, Proline, Glycine, Alanine, Cysteine, Methionine, Leucine, Tyrosine, Lysine, Arginine) in plasma samples was conducted using an amino acid analyzer, model YL9100 Korea, acetonitrile mobile phase: buffer: DW (60: 10: 30), flow rate (1ml/min), column separation C18-NH2 (25cm × 4.6 mm), the detector = florescence Ex = 365 nm, Em = 445 nm (Mohammad et al., 2016).

Blood chemistry

A blood biomarker included, Cholesterol, Triglycerides, LDL, VLDL, HDL, minerals like P, Mg, Ca++, Zn, Cu, and included protein tests like Total protein, Albumin, Globulins, Creatinine, Uric acid were implemented on plasma samples with an automated blood chemistry analyzer, Spin200- SPINREACT with kits.

Proximate analyses

Three levels of roughage feed (alfalfa hay) was fed 30%, 50% and 70% with or without NCG. The proximate analyses of alfalfa hay and concentrate (Table 1) were implemented as AOAC (2012). Concentrate feed included: 32.5% bran, 1.5% urea, 54% barley, 10% maize, 1% minerals and 1% vitamins. The pH value of feeds was measured by the weight of 1g of feed, adding 10ml distilled water, after 10min, filtered with cheesecloth, and measured with a pH meter from HANNA Instrument (Tawfeeq and Al-Attar, 2014).

Statistical analysis

The effect of three levels of concentrate feed 70%, 50%, and 30% with or without NCG were statistically studied in a 2×3 factorial experiment/Completely Randomized Design (CRD). All data were analyzed using Completely Randomized Design (CRD), 2×3, factorial experiment. One-way ANOVA analysis was performed using a statistical program (SAS, 2012). Significant differences among treatments were determined using Duncan's multiple range test (Duncan, 1955) using the following formula: Bijk = µ+ Ti + Nj + TN(ij) + eijk; Where:

Bijk = The observed value of NCG and concentrate feed, µ = The overall mean, Ti = The main effect of NCG (0 or 6gm), Nj= The main effect of concentrate feed level (70%,50%, 30%) and roughage feed level (30%,50%, 70%), TN(ij) = The interaction effect between NCG and concentrate feed, eijk = The random residual effect.

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

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

*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)

RESULTS AND DISCUSSION

The effect of NCG and roughage to concentrate ratio on blood cholesterol and fat parameters in goats showed non-significant effects on cholesterol and LDL (Table 2). Triglycerides, VLDL, and HDL decreased with increasing concentrate with NCG, while the heist levels were without NCG (50% roughage: 50% concentrate). Triglycerides were within the physiological range for this species as reported by Tudisco et al. (2019). More concentration of HDL means more excretion of cholesterol, and our results led to an increase in HDL with high roughages. Chacher et al. (2012) referred to a low proportion of rumen degradation of NCG (17.8%) after 24h. of in-vitro incubation compared with 100% rumen degradation of arginine and led to more acetate in contrast without arginine and NCG. Increasing acetic acid means increasing the level of blood triglyceride because acetic acid is converted to fatty acids which combine with blood glycerol to produce triglycerides and participated in milk or stored in adipose tissues. The results of in vivo experiment in the table 2 referred to low triglycerides with NCG treatments, because of the role of NCG as diuretic material with more excretion of blood metabolites. A decrease in cholesterol and triglycerides with

NCG and a low level of feeding agreed with Zhang et al. (2021). Recent studies stated to increase fat parameters with NCG and high levels of concentrate feeding, these findings recommended the possibility of providing NCG with good quality nutrition.

The effect of NCG and roughage to concentrate ratio on blood protein parameters of goats (Table 3) showed a linear decrease ($P<0.01$) of total protein, albumin, and globulins ($P<0.05$) with increasing the level of concentrate with NCG, and non-significant without NCG. Creatinine and uric acid increased ($P<0.05$) with NCG of the high level of roughages treatments, In general, creatinine increases in association with a decrease in renal function but, in this case, the registered values were not outside the physiological range reported for this species (Musco et al., 2022). The body needs different amino acids for maintenance and growth, so, poor feeding leads to activation deamination and transamination (amino acid metabolism) to provide necessary amino acids, accompanied by an increase in creatinine (Crn). So, creatinine is a byproduct of amino acid metabolism (an endogenous byproduct of muscle metabolism), and NCG is used as a diuretic, for this reason, NCG must use with feeds containing non-protein nitrogen like urea. Elmahdy et al. (2021) referred to an increase in the level of creatinine with the uptake of NCG for a long time. Globulins increased with increasing roughages that referred to healthy conditions because globulins include antibodies, enzymes, and other different blood proteins. High concentrate led to a decrease in total blood protein while increasing alfalfa as a source of roughage led to a linear increase of total blood protein, these results disagree with Zhang et al. (2021) who referred to decrease total protein and globulins with the low level of feeding, these differences of results may because of we used alfalfa hay which supports the increasing of blood proteins and overall health. Albumin increased with high alfalfa which means good health and no inflammation (Garcovich et al., 2009).

The effect of NCG and roughage to concentrate ratio on blood minerals in goats referred to increase ($P<0.01$) phosphor with treatment of NCG and high roughage (alfalfa), and decreased without NCG treatments. Magnesium increased ($P<0.01$) with high roughage with

Table 2: Effect of N- carbamylglutamate and roughage to concentrate ratio on blood cholesterol and fat parameters (mg/dL) in goats (Mean ± Standard error)

Treat.	T1	T2	T3	T4	T5	T6	Sign.
Cholesterol	51.0±0.0	51.0±6.3	53.5±2.0	54.0±5.1	57.0±0.5	54.5±0.8	NS
Triglycerides	12.0±0.0^{d}	12.5±0.8^{cd}	17.5±1.4^{b}	16.5±0.8^{bc}	26.0±0.0^{a}	19.5±2.5^{b}	**
LDL	20.0±1.1	20.0±3.4	19.5±2.0	18.0±3.4	18.5±0.2	20.5±0.2	NS
VLDL	2.0±0.0^{d}	2.5±0.2^{cd}	3.5±0.2^{bc}	3.5±0.2^{bc}	5.0±0.0^{a}	4.0±0.5^{b}	*
HDL	29.0±1.1^{b}	28.5±2.6^{b}	30.5±0.2^{ab}	32.5±1.4^{ab}	33.5±0.2^{a}	30.0±0.5^{ab}	*

*Significant differences 0.05, **Significant differences 0.01; NS=Non-significant; T1=30% roughage: 70% concentrate, 6 (g/day) NCG; T2=50% roughage: 50% concentrate, 6 (g/day) NCG; T3=70% roughage: 30% concentrate, 6 (g/day) NCG; T4=30% roughage: 70% concentrate, 0 NCG; T5=50% roughage: 50% concentrate, 0 NCG; T6=70% roughage: 30% concentrate, 0 NCG

Table 3: Effect of N- carbamylglutamate and roughage to concentrate ratio on blood protein parameters (mg/dL) in goats (Mean ± Standard error)

Treat.	T1	T2	T3	T4	T5	T6	Sign.
Total protein	63.4±1.3^{b}	74.0±2.3^{a}	75.35±0.8^{a}	69.3±3.7^{a}	73.2±0.0^{a}	72.75±0.4^{a}	**
Albumin	32.45±0.2^{b}	32.20±0.1^{b}	34.75±0.3^{a}	32.50±0.2^{b}	32.6±0.5^{b}	32.80±0.1^{b}	**
Globulins	30.95±1.1^{b}	41.30±2.2^{a}	40.60±1.2^{a}	36.80±3.4^{a}	40.6±0.5^{a}	39.95±0.4^{a}	*
Creatinine	0.785±0.0^{b}	1.065±0.0^{ab}	0.87±0.0^{ab}	0.955±0.0^{ab}	0.73±0.0^{b}	1.19±0.2^{a}	*
Uric acid	0.24±0.0^{c}	0.37±0.0^{a}	0.30±0.0^{bc}	0.25±0.0^{c}	0.34±0.0^{ab}	0.26±0.0^{c}	*

*Significant differences 0.05, **Significant differences 0.01; T1=30% roughage: 70% concentrate, 6 (g/day) NCG; T2=50% roughage: 50% concentrate, 6 (g/day) NCG; T3=70% roughage: 30% concentrate, 6 (g/day) NCG; T4=30% roughage: 70% concentrate, 0 NCG; T5=50% roughage: 50% concentrate, 0 NCG; T6=70% roughage: 30% concentrate, 0 NCG

Table 4: Effect of N- carbamylglutamate and roughage to concentrate ratio on some blood minerals (mg/dL) in goats (Mean ± Standard error)

Treat.	T1	T2	T3	T4	T5	T6	Sign.
p	6.40±0.1^{c}	7.55±0.2^{a}	7.05±0.0^{b}	6.50±0.1^{c}	5.90±0.1^{d}	6.05±0.2^{cd}	**
Mg	2.33±0.0^{d}	2.56±0.0^{bc}	2.81±0.0^{a}	2.55±0.0^{c}	2.66±0.1^{abc}	2.74±0.0^{ab}	**
Ca++	8.20±0.1^{ab}	8.35±0.1^{a}	8.30±0.0^{ab}	8.35±0.2^{a}	8.55±0.2^{a}	7.85±0.0^{b}	*
Zn	34.50±0.8^{a}	35.5±2.6^{a}	40.0±2.3^{a}	34.50±4.9^{a}	33.0±0.5^{a}	24.0±1.1^{b}	*
Cu	65.0±1.1	76.0±2.8	65.5±4.9	71.0±8.0	75.5±9.5	66.0±5.1	NS

*Significant differences 0.05, **Significant differences 0.01; NS=Non-significant; T1=30% roughage: 70% concentrate, 6 (g/day) NCG; T2=50% roughage: 50% concentrate, 6 (g/day) NCG; T3=70% roughage: 30% concentrate, 6 (g/day) NCG; T4=30% roughage: 70% concentrate, 0 NCG; T5=50% roughage: 50% concentrate, 0 NCG; T6=70% roughage: 30% concentrate, 0 NCG

Table 5: Effect of N- carbamylglutamate and roughage to concentrate ratio on blood amino acids (ppm) in goats (Mean ± Standard error)

Treat.	T1	T2	T3	T4	T5	T6	Sign.
Aspartate	70.2±0.5^{d}	65.1±0.6^{e}	58.8±0.7^{f}	72.8±0.3^{c}	74.9±0.5^{b}	77.9±0.5^{a}	**
Threonine	50.8±0.2^{b}	41.1±0.5^{d}	39.9±1.0^{d}	54.8±0.6^{a}	50.7±0.2^{b}	45.4±0.3^{c}	**
Serene	54.0±0.4^{b}	42.8±0.3^{d}	42.1±1.0^{d}	58.8±0.9^{a}	52.8±0.3^{b}	49.9±0.6^{c}	*
Glutamate	249.9±0.1^{b}	233.2±0.3^{d}	233.3±1.5^{d}	254.2±0.6^{a}	252.9±0.3^{a}	246.3±0.7^{c}	**
Proline	14.9±0.1^{b}	10.6±0.2^{d}	10.7±0.2^{d}	18.7±0.4^{a}	12.3±0.1^{c}	14.9±0.2^{b}	**
Glycine	13.6±0.1^{c}	8.8±0.1^{e}	10.3±0.2^{d}	17.1±0.1^{a}	15.5±0.2^{b}	14.2±0.4^{c}	**
Alanine	27.8±0.4^{c}	21.5±0.7^{d}	30.3±0.7^{b}	31.5±0.6^{b}	30.1±0.1^{b}	39.8±0.3^{a}	**
Cysteine	13.6±0.1^{c}	8.7±0.1^{e}	10.7±0.2^{d}	18.4±0.2^{a}	18.7±0.1^{a}	16.4±0.3^{b}	**
Methionine	19.5±0.3^{c}	12.6±0.2^{e}	15.8±0.1^{d}	24.3±0.6^{a}	22.3±0.1^{b}	20.0±0.2^{c}	**
Leucine	98.3±0.7^{c}	89.6±0.3^{d}	80.5±0.4^{f}	107.4±1.0^{a}	102.3±1.2^{b}	84.8±0.6^{e}	**
Tyrosine	36.6±0.5^{d}	30.1±0.2^{e}	35.8±0.6^{d}	50.1±0.7^{a}	44.3±0.3^{b}	42.8±0.2^{c}	**
Lysine	78.2±1.1	71.3±0.6	71.1±0.4	88.1±0.6	86.2±0.3	70.5±13.8	NS
Arginine	72.9±0.4^{c}	69.6±0.5^{d}	62.9±0.3^{e}	81.6±0.6^{a}	79.7±0.5^{b}	72.3±0.4^{c}	**

*Significant differences 0.05, **Significant differences 0.01; NS=Non-significant; T1=30% roughage: 70% concentrate, 6 (g/day) NCG; T2=50% roughage: 50% concentrate, 6 (g/day) NCG; T3=70% roughage: 30% concentrate, 6 (g/day) NCG; T4=30% roughage: 70% concentrate, 0 NCG; T5=50% roughage: 50% concentrate, 0 NCG; T6=70% roughage: 30% concentrate, 0 NCG

or without NCG. Calcium and zinc decreased (P<0.05) without NCG with high roughage, and there were no differences between the treatments for blood copper content. All blood minerals increased with increasing alfalfa and NCG in contrast without NCG, it may because of diuretic effect of NCG that led to need more minerals to keep blood homeostasis. Minerals have many important functions in production, reproduction and all the vital processes in the body (Tawfeeq and Al-Attar, 2014). Alfalfa rich in mineral concentration. For this reason, it may be necessary to give alfalfa hay with strow to achieve the needs of minerals especially for young animals.

The results of amino acids concentrate in goats showed decreases (P<0.01) of blood amino acids with NCG comparing without NCG, and increasing amino acids with high concentrate comparing with high roughage excepted alanine, which decreased with high concentrate. N- carbamylglutamate used to enhance cofactor N-acetylglutamate (NAG) to convert ammonia to urea in the liver as a drug (Tuchman et al., 2008), that leads to lost more amino acids with urea and played negative roll. Chacher et al. (2012) referred to NCG as a precursor for arginine production, and enhances amino acids (Huang et al., 2019) and recently work referred to negative effect of NCG on plasma amino acids and stated to feed 4% concentrate with NCG led to more negative effect in contrast with 2% and 3% levels of concentrate feeding.

CONCLUSIONS

Blood amino acids concentrations decreased with NCG feeding. Plasma minerals levels and blood protein parameters of goats feeding high level of roughage (alfalfa) were higher in contrast with high concentrate feed. Blood creatinine, uric acid and fat parameters decreased with feeding NCG. The level of 50%:50% of roughage to concentrate feed ratio without NCG was the most proper for blood amino acids, protein parameters, fat and minerals in goats as ruminant. Using N- carbamylglutamate leads to increased urination, so,don't use NCG without non-protein nitrogen additives. We need to study the effect of NCG on milk production and components.

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CONTRIBUTIONS OF AUTHORS

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