



Mohammad Naser Nazem¹,
Nasrin Askari^{*2}

1) Department of Basic Sciences,
Faculty of Veterinary Medicine,
Shahid Bahonar University of
Kerman, Kerman, Iran.

2) Student of Veterinary
Medicine, Faculty of Veterinary
Medicine, Shahid Bahonar
University of Kerman, Kerman,
Iran.

EFFECT OF SUPPLEMENTING RUMEN-PROTECTED METHIONINE ON SERUM BIOCHEMISTRY OF IRANIAN RAYENI CASHMERE GOAT

ABSTRACT

Objective: This study was set to investigate whether the administration of ruminally protected methionine as a supplement on lactating goats could have been some toxic effects on serum biochemistry.

Methods: Thirty adult (3-4 years old) female Rayeni goats were randomly divided into two groups as follow: control and treatment groups each contain thirty goats. Goats in both groups were fed on the pasture. Goats of the treatment group received Mepron® (Degussa Corporation, Germany) at 5 g (i.e. 3 g methionine) per head per day. On Days 30 and 60, blood samples were collected by jugular venipuncture and centrifuged at 3000 rpm for 15 min. Samples were analyzed for Total Bilirubin (TBIL), Direct Bilirubin (DBIL), Total Serum Protein (TP), Blood Urea Nitrogen (BUN), Alkaline Phosphatase (ALP), Aspartateaminotransferase (AST), Alanine aminotransferase (ALT) and Serum concentration of Albumin (Alb) using a CX7/CX7 serum chemistry analyzer.

Results: There was no significant difference between TBIL, DBIL, TP, BUN, ALP, AST, ALT and Alb in treatment groups in comparison to control groups on days 30 and 60 ($P > 0.05$).

Conclusions: Results showed that administration of methionine supplementation in lactating goats doesn't have any blood and hepatotoxic effects. Considering nontoxic and safe nature of methionine, it can be one of the suitable choices to improve milk yield postpartum, cashmere yield and quality without any adverse effects.

Key words: Methionine, Serum factors, Rayeni Goat, Toxic effect, Cashmere

INTRODUCTION

Goats are economically important producers of meat, cashmere, and milk in many countries. Rayeni goat is an Iranian cashmere goat which is raised in large numbers in Kerman province of Iran where goat production contributes significantly to the agricultural economy. Rayeni goat is one of the famous cashmere breed in Iran [1].

Methionine is the first limiting amino acid in a variety of ruminant diets. The metabolic requirement for methionine is high in dairy cows, because of its role as a methyl donor in transmethylation reactions in the synthesis of milk fat [2]. Previous studies have indicated that methionine is also first limiting for wool growth and body weight gains of sheep [3].

Literature dealing with the use of ruminally protected methionine in dairy cows is extensive. There are some reports on effects on milk performance and physiological measures [4], reports on the effect on rumen microorganisms [5, 6] as well as reports on effects during the periparturient period and in the course of lactation [7]. Moreover, methionine supplementation in the last weeks of pregnancy may spare body reserves of the dam and positively influences protein metabolism and milk yield postpartum [8]. According to the authors' knowledge, there isn't enough information about the effect of supplementing rumen-protected methionine on goats. Therefore, this study was set to investigate whether the administration of ruminally protected methionine as a supplement on lactating goats could have been some toxic effects on serum biochemistry.

Materials and Methods:

Animals and Diets

The use of the animals was approved by the Animal Care Committee of Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran. Thirty adult (3-4 years old), female Rayeni cashmere goats were randomly divided into two groups as follow: control and treatment groups each contain fifteen goats. Goats in both groups were fed on the pasture daily. Goats of the treatment group received Mepron® at 5 g (i.e. 3 g methionine) per head per day. Mepron® (Degussa AG, Germany) contains 85% of methionine, 3% of crude fibre and 2% of crude fat.

Blood sampling

On Days 30 and 60 thereafter, blood samples were collected by jugular venipuncture into plain Vacutainer tubes (Becton Dickinson) and centrifuged at 3000 rpm for 15 min. Only clear non-hemolyzed sera were obtained and kept frozen until further analysis. Samples were analyzed for Total bilirubin (TBIL), Direct Bilirubin (DBIL), Total Serum Protein (TP), Blood Urea Nitrogen (BUN), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and serum concentration of Albumin (Alb) using a CX7/CX7 serum chemistry analyzer.

Statistical Analysis

The T- Test was used to compare data of two groups. One-way ANOVA, followed by Tukey's test, was used to determine the significance of differences between two groups. Values were expressed as mean \pm S.E.M. (standard error of mean). The significance level considered was $P < 0.05$.

Result & Discussion:

The results of biochemical analysis of serum in experimental animals are presented in Table 1.

There was no significant difference between TBIL, DBIL, TP, BUN, ALP, AST, ALT and Alb in treatment groups in comparison to control groups on days 30,60 ($P > 0.05$).

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This study demonstrates that administration of methionine supplementation in lactating goats doesn't have any toxic effects on serum biochemistry. Albumin represents the most abundant serum protein. Many epidemiological studies have established an inverse relationship between serum albumin level and mortality risk ^[9,10].

Among the variety of biological mechanisms which have been proposed to explain the beneficial effects of higher albumin concentrations, a direct protective effect of the albumin molecule has been suggested. There is now ample evidence for a significant antioxidant activity of serum albumin. In fact, this molecule may represent the major and predominant circulating antioxidant in plasma which is known to be exposed to continuous oxidative stress. Antioxidant activity of albumin was principally related to its capacity to bind metal ions and to scavenge free radicals.

It has been recently suggested that oxidation of surface-exposed Methionine residues to methionine sulfoxide may represent an endogenous antioxidant defense that protects proteins from extensive and irreversible oxidative modification ^[11, 12]. Methionine accounted for 40% of total antioxidant activity of human serum albumin ^[13]. Methionine can also reduce the lipid peroxide content in blood ^[14].

It is reported that dietary methionine did affect the concentration of total serum protein and albumin of broilers ^[15]. Albumin is a blood transport protein binds many biomolecules and drugs including hormones, lipoproteins and amino acids ^[16]. In poultry, this hyperproteinemia is associated with an increase in vitellogenin and lipoproteins which is induced by estrogens because they are necessary for yolk production ^[17].

Sahlu, et al at 1992 showed that following administration of methionine, mohair yield and quality have been increased but jugular blood ammonia N, plasma glucose and total protein concentrations were not affected by treatment ^[18]. According to Zhang, et al (2010) methionine concentrations did not affect serum urea nitrogen, total protein, insulin and IGF-I concentration of growing meat rabbits ^[19].

The globulin concentration was obtained by subtracting albumin from the total protein. Heugten et al (1994) measured total serum immunoglobulin (IgM and IgG) to investigate the effect of aflatoxin and dietary methionine on immune responses of swine and reported that supplementation of methionine did not improve immune function in pigs [20].

Nevertheless, our obtained results are in agreement with these previous investigations which have clearly demonstrated dietary methionine didn't affect the concentration of total serum protein, total serum immunoglobulin, albumin and blood urea nitrogen.

In the pasture-fed dairy cows, more than 50% of the protein ingested is degraded in the rumen into ammonia and this extensive protein degradation may limit the amount of protein passing to the small intestine [21]. It has been postulated that identifying and increasing post-ruminal supply of the most limiting amino acids would be an effective way to improve milk protein yield and quality [22]. For this reason, in the present study, Mepron® was used. On the other hand, an excessive intake of methionine increases plasma homocysteine concentrations by enhancing the synthesis of homocysteine. Elevated plasma total homocysteine (tHcy, both bound and free) is an independent risk factor for atherosclerotic vascular disease. There is convincing evidence to suggest that high plasma concentration of this sulfur amino acid is an indicator of increased risk of cardiovascular morbidity and mortality [23]. While the mechanism of homocysteine's involvement in the development of premature cardiovascular disease is not understood, homocysteine has been shown to cause damage to endothelial cells, probably by the generation of reactive oxygen species such as hydrogen peroxide with the formation of oxidized low density lipoprotein. Endothelial lesions induced by oxidized LDL may contribute to atherogenesis [24].

It was found that varying dietary vitamin E levels resulted in marked changes in LDL oxidation parameters, specifically the lag time and total diene production. The hypocholesterolemic effect of vitamin E was accompanied by a redistribution of plasma lipoprotein cholesterol profile.

Intake of excess dietary methionine (a precursor of homocysteine) can also raise the requirements for folate, vitamin B12 and/or vitamin B6, which is involved in the metabolism of homocysteine [25]. The amount of supplementing rumen-protected methionine in our experiment, was according to the previous study [26].

CONCLUSION

Considering nontoxic and safe nature of methionine, it can be one of the suitable choices to improve milk yield postpartum, cashmere yield and quality. More research work is needed to reveal the exact mechanism of Met on hair follicles of suckling kids.

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REFERENCES

1. Mozaffari AA, Shahriarzadeh MH, Ja'fari H. Analysis of serum and cerebrospinal fluid in clinically normal adult Iranian Cashmere (Rayeni) goats. *Comp Clin Pathol* 2011; 20:85–88.
2. Lee C, Hristov AN, Cassidy TW, Heyler KS, Lapierre H, Varga GA, et al. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J Dairy Sci* 2012; 95(10):6042-56.
3. Khan MJ, Abbas A, Ayaz M, Naeem M, Akhter MS, Soomro MH. Factors affecting wool quality and quantity in sheep African. *Journal of Biotechnology* 2012; 11(73): 13761-13766.
4. Brake DW, Titgemeyer EC, Brouk MJ, Macgregor CA, Smith JF, Bradford BJ. Availability to lactating dairy cows of methionine added to soy lecithins and mixed with a mechanically extracted soybean meal. *J Dairy Sci* 2013; 96(5):3064-74.
5. Karnati SKR, Sylvester JT, Noftsger SM, Yu Z, St-Pierre NR, Firkins JL. Assessment of ruminal bacterial populations and protozoal generation time in cows fed different methionine sources. *J Dairy Sci* 2007; 90: 798–809.
6. Noftsger S, St-Pierre NR, Sylvester JT. Determination of rumen degradability and ruminal effects of three sources of methionine in lactating cows. *J Dairy Sci* 2005; 88: 223–237.
7. Patton RA. Effect of rumen protected methionine on feed intake, milk production, true milk protein concentration, and true milk protein yield, and the factors that influence these effect: A metaanalysis. *J Dairy Sci* 2010; 93:2105-2118.
8. Hadrová S, Křížová L, Richter M, Trínáctý J, Dračková M. The effect of duodenal infusion of histidine on milk yield, milk composition, and plasma amino acids in dairy cows. *Journal of Animal and Feed Sciences* 2012; 21: 555–565.
9. Gattani A, Gupta KK, Joshi G, Gupta SR. Metabolic profile of foot and mouth disease stressed sheep in semi arid region. *Journal of Stress Physiology & Biochemistry* 2011; 7(2): 148-153.
10. Zuheir IAI, Abdaldayem M. The effect of intramuscular injection of spiramycin at therapeutic dose on some blood biochemical and hematological parameters in Assaf sheep. *Scientific Journal of Veterinary Advances* 2012; 1(6): 142-146
11. Taverna M, Marie A-L, Mira J-P, Guidet B. Specific antioxidant properties of human serum albumin. *Ann Intensive Care* 2013; 3: 4.
12. Shen N, Yan F, Guo Y, Guo Y, LÜ Sh-W, Gong P-S, Xu Y-W, et al. Imprinted Human Serum Albumin with Antioxidant Activity. *Chem res Chinese university* 2011; 27(2); 258—263
13. Okazaki T, Okudaira N, Ishii N, Yotsuyanagi H, Nagai T, Tokudome Sh, et al. Comparison of the Antioxidant Activity of Albumin from Various Animal Species. *Zoological Science* 2008; 25(2):172-177.
14. Toborek M, Kopieczna-Grzebieniak E, Drózd M, Wiczorek M. Increased lipid peroxidation and antioxidant activity in methionine-induced hepatitis in rabbits. *Nutrition* 1996 ; 12(7-8):534-7.
15. Adeyemo G.O, Ologhobo A.D, Adebisi O.A. The Effect of Graded Levels of Dietary Methionine on the Haematology and Serum Biochemistry of Broilers. *International Journal of Poultry Science* 2010; 9 (2): 158-161.
16. E. Maroufyan E, Kasim A, Ebrahimi M, Loh T.C, Hair-Bejo M, Soleimani A.F . Dietary methionine and n-6/n-3 polyunsaturated fatty acid ratio reduce adverse effects of infectious bursal disease in broilers. *Poult Sci* 2012; 91(9).2173-2182.
17. Thrall MA. *Veterinary Hematology and Clinical Chemistry*. Lippincott Williams and Wilkins. Philadelphia; 2004, pp. 518.

18. Sahlou T, Fernandez JM. Effect of intraperitoneal administration of lysine and methionine on mohair yield and quality in Angora goats. *Journal of animal science* 1992; 70(10):3188-93.
19. Zhang YC, Li FC. Effect of dietary methionine on growth performance and insulin-like growth factor-I mRNA expression of growing meat rabbits. *J Anim Physiol Anim Nutr (Berl)* 2010; 94(6):803-9.
20. Heugten E van, Spears J W, Coffey M T, Kegley E B, Qureshi M A. The effect of methionine and aflatoxin on immune function in weanling pigs. *J ANIM SCI* 1994; 72:658-664.
21. Ardalan M, Banadaky D, Rezayazdi K, Hossein-Zadeh N.G. The effect of rumen-protected methionine and choline on plasma metabolites of Holstein dairy cows. *The Journal of Agricultural Science* 2011 ; 149 (05) : 639-646.
22. Wang J-L, Gao X-J, Li Q-Z, HUANG J-G, Lu L-M, Luo Ch-Ch, et al. Influence of Methionine on Subcellular Proteins of Dairy Cow Mammary Gland Epithelial Cell. *Acta veterinaria et zootechnica sinica* 2012; 39(4): 109-112.
23. Onomhaguan Ebesunun M, Odunayo Obajobi E. Elevated plasma homocysteine in type 2 diabetes mellitus: a risk factor for cardiovascular diseases. *The Pan African Medical Journal* 2012;12:48.
24. Nick Hidiogloua N, Gilania G.S, Longb L, Zhaob X, Maderea R, Cockella K, et al. The influence of dietary vitamin E, fat, and methionine on blood cholesterol profile, homocysteine levels, and oxidizability of low density lipoprotein in the gerbil. *Journal of Nutritional Biochemistry* 2004 ; 15: 730- 740.
25. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. *Lancet* 1999;354:407- 13.
26. Nazem MN, Rezaian M, Adib Moradi M, Asadi Fuzi M, Kyaei S M, Razavi Ebrahim P. The effect of oral administration of coated methionine on the female goats and suckling kids hair follicles: A histomorphometrical approach. *Journal of Veterinary Research* 2013; 8:1.

| Serum chemistry parameter | Day 30 | | Day 60 | |
|---------------------------|------------------|-----------------|------------------|--------------------|
| | Control (n = 10) | Treatment(n=10) | Control (n = 10) | Treatment (n = 10) |
| TBIL (mg/dl) | 0.35 ±0.09 | 0.52±0.07 | 0.25±0.01 | 0.30±0.01 |
| DBIL (mg/dl) | 0.15±0.04 | 0.17±0.03 | 0.14 ± 0.01 | 0.17± 0.01 |
| TP (g/ dl) | 7.59±0.17 | 7.59±0.11 | 7.51 ± 0.10 | 7.76±0.14 |
| Alb (g/dl) | 4.45±0.05 | 4.45±0.12 | 4.45±0.02 | 4.45 ± 0.03 |
| ALP (IU/L) | 2.21±77.9 | 3.04±52.48 | 1.02± 81.76 | 2.65±89.93 |
| AST (IU/L) | 93.6 ± 5.09 | 93.90±6.05 | 84.00 ± 5.08 | 85.80±5.97 |
| ALT (IU/L) | 18.10±2.35 | 18.10±2.06 | 17.10 ± 1.90 | 18.10±2.15 |
| BUN (mg/dl) | 13±0.66 | 13±0.62 | 13±0.42 | 13±0.25 |

There was no significant difference between TBIL, DBIL, TP, BUN, ALP, AST, ALT and Alb in treatment groups in comparison to control groups on days 30 and 60 ($P>0.05$).

Table 1. Comparative means (SE) of serum biochemistry parameters for control and treatment groups.