3-methylhistidine as an Indicator for Protein Beakdown: An Experimental Model in Male *Capra hircu*

by ***A.M. Almeida[1,*], L.M. Schwalbach[2], H.O. de Waal[2], J.P.C. Greyling[2] & L.A. Cardoso[1]***
[1]IICT – Centro de Veterinária e Zootecnia, Faculdade Medicina Veterinária, Lisboa, Portugal
[2]FNAS – Dep. of Animal Science, University of the Free State, Bloemfontein, South Africa

Summary
The role of the amino acid 3-methylhistidine as an indicator of protein breakdown and weight loss is often suggested. Despite existing information for other animal species, little is known about the actual levels of 3-methyhistidine in the serum of less studied domestic species such as the goat. We have evaluated the 3-methyhistidine serum concentrations in young Boer goat bucks subjected to two distinct feeding regimens: winter-grass hay with or without supplementation. Non-supplemented animals had a negative nitrogen balance and experienced weight loss throughout the experiment and significantly higher concentrations of 3-methyhistidine than supplemented animals that had a slight increase in live weight. This amino acid can be considered a valid indicator of protein breakdown and weight decrease in male goats. Serum 3-methylhistidine concentrations in adequately fed male goats were similar throughout the assay (20-40 µmol/l) whereas in weight-losing animals, concentrations of up to 170µmol/l can be expected.

Introduction
Although not extensively used in laboratory animal science, goats (*Capra hircus*) are gaining international recognition in several fields of biomedical research (*Czerniak, 2001*). However, many specific aspects of goat physiology are still relatively unknown and are often assumed to be equal or similar to the other small ruminant species widely used in both farming and laboratory research, the sheep (*Ovis aries*). Although both species share a reasonable number of features, there are certain aspects of digestive physiology that cannot be assumed to be analogous. There is hence a substantial need to conduct research in several aspects of goat nutritional physiology in order to obtain more accurate and comparable results when using these animals as laboratory animals. The amino acid 3-methylhistidine is considered a component of several structural proteins and its presence in the urine in large quantities is considered an indicator of protein breakdown (*Plaizier et al., 2000*) as recorded in laboratory rats (*Kim and Lee, 1990*). Although well described in other less conventional species like dairy cattle (*Blum et al., 1984*) or the dog (*Neumann et al., 2008*) under a variety of physiological conditions, little information regarding 3-methylhistidine in goats seems to be available. In fact, its role as an indicator of protein breakdown in this species is the subject of some controversy as data based on 3-methylhistidine excretion in the urine have not led to conclusive results (*Brown et al., 1987*). No additional data on 3-methylhistidine levels in goats have been published since this last reference and serum concentrations have not, to the best of our knowledge, been described yet.

**Correspondence:* A.M. Almeida
IICT – Centro de Veterinária e Zootecnia, Faculdade Medicina Veterinária, Rua Prof. Cid dos Santos, 1300-477 Lisboa, Portugal
E-mail amalmeid@itqb.unl.pt

In this paper, we aim to study 3-methylhistidine serum concentrations in the goat buck under weight loss. The role of this amino acid as an indicator of protein breakdown is also discussed.

Materials and Methods

Fifteen intact bucks (6-8 months; 28.0 ± 0.2 kg) of the Boer Goat breed were purchased from a registered breeder in South Africa and divided in two groups (WH and WH+S). The animals were housed in individual metabolic cages that allowed intake monitoring and faeces and urine collections as illustrated in Figure 1.

WH (n=8) was fed 500 g winter veld hay. WH+S (n=7) was fed 600 g winter veld hay plus supplement: 170 g of maize meal, 44 g of molasses meal and 15 g of feed-grade urea. Feed composition is presented in table 1.

Animals were weighed once a week and nitrogen (N) balance measures were performed on the same days. Faeces and urine were collected at days 2, 7, 14, 21 and 28 of the observation period for the determination of N content by Kjeldahl digestion (Buchi 315, Schweiz, Germany). Apparent N balances were determined by subtracting urinary (Nu) and faecal (Nf) N from ingested N. Methodologies used in N balances have been described earlier (*Almeida et al., 2006*).

Blood was extracted on the same days using standard heparin-lithium vacuum tubes and needles, prior to daily feeding. Serum was separated by standard centrifugation using a refrigerated Hettich Rotanta centrifuge at 2500 rpm. Serum (300 μl) was deproteinised with sulphosalicylic acid, followed by freezing at – 20ºC, centrifugation at 45000g and pH reduction to 2.2 using lithium hydroxide.

Figure 1. Experimental animals in metabolic cages

Table 1. Feed composition

	Maize	Urea	Molasses	Hay
Dry Matter (%)	90.0	99.0	74	91.6
Crude protein (%DM)	9.1	285.7	4.9	3.8
Crude Fiber (% DM)	7.8	-	-	41.0
Ash (%DM)	1.6	-	8.6	10.3
Ether Extract (%DM)	2.5	-	-	1.6
NFE (%DM)	79.0	-	87.9	43.3
Gross Energy (kJ/100g)	1583	897	1309	1508

DM: Dry Matter; NFE: Nitrogen Free Extractives

Concentrations of 3-methylhistidine were determined using a HPLC and Beckman Coulter Amino Acid Analyser 7300 (Fullerton, CA, USA), with a lithium buffer system.

Results of both groups were compared by ANOVA Repeated Measures for the 3-methylhistidine concentrations and ANOVA Single Factor for the nitrogen balances and live weights.

Experimental procedures were authorized by the Animal Welfare and Ethics Committee of the University of the Free State, following European Union and South African regulations. Authors A.M. Almeida and L.A. Cardoso are holders of a Grade C FELASA diploma.

Results and Discussion

Results regarding evolution of live weight are presented in Figure 2. Animals fed winter veld hay had a final decrease in live weight of 20% whereas supplemented animals experienced an increase of 10%. Nitrogen balance data is presented in Table 2. Ingested N for the WH group was always significantly lower than for the supplemented animals (about 20%). Similar results were observed in faecal N (Nf). Nf of WH animals was only about 20 to 35% of that observed in the supplemented animals. However, N excreted in urine was very similar for both groups and no significant differences were recorded. These results affected the N balances, which were always higher in the WH+S group than in the WH group that had negative balances from day seven onwards. The WH group can therefore be regarded as typical of animals subjected to mild undernutrition, reducing

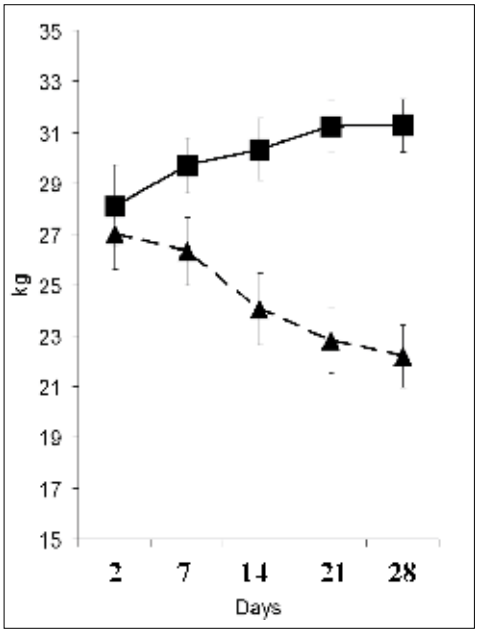

Figure 2. Evolution of live weight of WH+S (■) and WH (▲) Boer goat bucks. Results were significantly different between groups except on day 2.

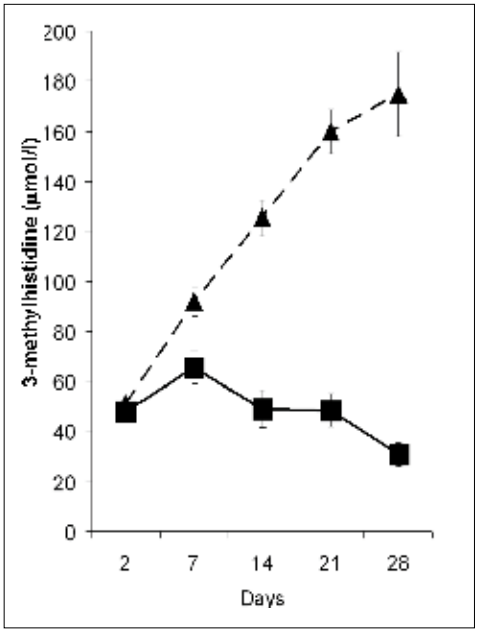

Figure 3. Evolution of 3-methyhistidine serum concentrations of WH+S (■) and WH (▲). Results were similar for both groups at Day 2 and significantly different between experimental groups for all other dates. Significant differences were recorded for all dates in the WH animals whereas WH+S animals showed constant values throughout the experiment.

Table 2. Nitrogen Balances of Boer bucks

	Day 2		Day 7		Day 14		Day 21		Day 28	
	WH+S	WH	WH+S	WH	WH+S	WH	WH+S	WH	WH+S	WH
Ni	9.80*	2.30	9.35*	1.92	11.7*	2.20	9.49*	1.73	11.4*	1.80
(g)	(.71)	(.38)	(.73)	(.13)	(.53)	(.14)	(.30)	(.12)	(.49)	(.09)
Nf	.77	.59	3.55*	1.53	6.84*	1.88	4.41*	1.42	6.35*	1.42
(g)	(.18)	(.03)	(.21)	(.24)	(.59)	(.18)	(.17)	(.11)	(.56)	(.28)
Nu	1.18	1.59	1.28	1.27	2.19	1.64	1.75	1.57	1.86	1.85
(g)	(.31)	(.24)	(.23)	(.21)	(.33)	(.21)	(.18)	(.17)	(.22)	(.29)
NB	8.11*	.09	4.52*	-.88	2.69*	-1.36	3.22*	-1.01	3.12*	-1.47
(g)	(.91)	(.55)	(.62)	(.48)	(.35)	(.36)	(.88)	(.26)	(.67)	(.38)

Ni – Ingested Nitrogen; Nf – Nitrogen excreted in faeces; Nu – Nitrogen excreted in the urine; NB – Nitrogen Balance. *Significant difference for $p<0.001$;Standard Error of Mean values in parenthesis

nitrogen excretion in a preserving effort to reduce the negative effects on the nitrogen metabolism.

Serum concentrations of 3-methylhistidine were evaluated and are presented in Figure 3. Results were similar for both groups at day 2 and significantly different between experimental groups for all other days. Significant differences were recorded for all days in the WH animals where there was registered a clear increase in 3-methylhistidine serum concentrations (up to four-fold) whereas WH+S animals showed constant values throughout the experiment.

There is only one previous study on 3-methylhistidine levels in the goat species (*Brown et al., 1987*) and that research was conducted at the level of urinary excretion. The authors concluded that 3-methylhistidine is not a valid index of muscle protein degradation. Data from live weight evolution and nitrogen balances presented in this work demonstrate that there is clear nitrogen depletion as a natural consequence of the feeding regimens, and subsequent weight loss, that the experimental animals were subjected to. An analogous situation was reported in one of our previous studies that showed losses of nitrogen content at the carcass levels of mildly (*Almeida et al., 2006*) and severely (*Almeida et al., 2002*) underfed rats despite no alterations in specific myofibrillar proteins at the level of the gastrocnemius muscle itself. Consequently it can be assumed that in the underfed animals used in this study, body reserves, including structural proteins, are being degraded and used for maintenance purposes (*Belkhou et al., 1991*). The increase in 3-methylhistidine concentrations experienced by WH animals seems therefore to confirm its role as a consistent indicator of protein breakdown for small ruminants, and male goats in particular, as seen in other species like mice (*Vissers et al., 2007*) and confirming its important role as a method for measuring tissue protein breakdown in vivo as suggested earlier (*Chinkes, 2005*), contradicting earlier studies.

To the best of our knowledge, little or no information is available regarding 3-methylhistidine concentrations in serum of young male bucks. Results from this experiment indicate that under normal circumstances, 3-methylhistidine concentrations values are within the range of 20-40 μmol/l. An up to four-fold increase may be expected as a consequence of the level of undernutrition reaching as much as 170μmol/l. Data presented in this study concern only male young animals of the Boer breed. It would be interesting to conduct similar research in other goat breeds, with special reference to dwarf goats as this breed is more often used in biomedical research. Similarly studies on females, varied physiological conditions and ages could also be conducted.

Acknowledgements

Authors acknowledge financial support from Fundação para a Ciência e a Tecnologia (BM/17921/98 and BPD/17522/2004) and Mr. T. Müller, Miss D. duBruÿn and Mr. W. Combrink for valuable technical assistance.

References

Almeida, AM, S van Harten & LA. Cardoso. Serum amino acid and myofibrillar protein profiles of fed and underfed laboratory rats. Nutr. Research, 2002, *22*, 1453-1459.

Almeida, AM, S van Harten & LA Cardoso. Myofibrillar protein status of the Gastrocnemius in male rats, effects of mild undernutrition. Scand. J. Lab. Anim. Sci., 2006, *33*, 101-106.

Belkhou, R, Y Cherel, A Heitz, JP Robin & Y Le Maho Y. Energy contribution of proteins and lipids during prolonged fasting in the rat. Nutr. Res., 1991, *11*, 365-374.

Blum J, T Reding, F Jans, M Wanner, M Zemp & K Bachmann. Variations of 3-methylhistidine in blood of dairy cows. J. Dairy Sci., 1984, *68*, 2580-2587.

Brown DL, DM Barnes & CC Calvert. Delayed excretion of 3-methylhistidine in goats. J. Nutr., 1987, *117*, 2106-2108.

Chinkes DL. Methods for measuring tissue protein breakdown rate in vivo. Curr Opin Clin Nutr Metab Care, 2005, *8*, 534-537.

Czerniak R. Gender-based differences in pharmacokinetics in laboratory animal models. Int J Toxicol., 2001, *20*, 161-163.

Kim, Y & YB Lee. Effect of cimaterol on growth and 3-methylhistidine excretion in rats. Australas. J. Anim. Sci., 1990, *3*, 313-318.

Neumann S, H Wellin, T Bilzer & S Thuere. Myopathy and alterations in serum 3-methylhistidine in dogs with liver disease. Res Vet Sci., 2008, *84*, 178-184.

Plaizier JC, JP Walton, A Martin, T .Duffield, R Bagg, P Dick, & BW McBride. Effects of monensin on 3-methylhistidine excretion in transition dairy cows. J. Dairy Sci., 2000, *83*, 2810-2812.

Vissers YL, MF von Meyenfeldt JM Argilés, YC Luiking, CH Dejong & NE Deutz. Protein breakdown on whole-body and organ level in non-cachectic tumour-bearing mice undergoing surgery. Clin Nutr., 2007, *26*, 483-490.