Salivary amino acid concentrations in zebus (Bos indicus) and zebu hybrids (Bos indicus × Bos taurus) fed a tannin-rich diet

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Abstract: Many animals show adaptation to tannins in the form of tannin-binding salivary proteins (1). Among ruminants, such proteins have been demonstrated in saliva of several species (usually browsers and intermediate feeders) (2, 3, 4, 13). There is some circumstantial evidence to suggest that zebu cattle (Bos indicus) are different from temperate cattle breeds with respect to their salivary and digestive physiology. Apart from differences in susceptibility to heat and tropical disease (5), a difference in salivary anti-tannin defenses (and a resulting difference in rumen physiology) could be another reason zebu cattle are particularly suited for agricultural systems in the tropics, where available forages often contain high levels of tannins (6, 7). Although non-proline-rich proteins exist that also have affinity for tannins (1, 8), it is interesting to compare the proline content of different cattle breeds. Here, we report such a screening for a comparison of zebu cattle and zebu-Holstein- Friesian in the Jimma area located at 7°40 N and 36°50 E at 1760 masl in southwest Ethiopia.

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Salivary amino acid concentrations in zebus (*Bos indicus)* and zebu hybrids (*Bos indicus* × *Bos taurus*) fed a tannin-rich diet

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Many animals show adaptation to tannins in the form of tannin-binding salivary proteins (1). Among ruminants, such proteins have been demonstrated in saliva of several species (usually browsers and intermediate feeders) (2, 3, 4, 13). There is some circumstantial evidence to suggest that zebu cattle (*Bos indicus*) are different from temperate cattle breeds with respect to their salivary and digestive physiology. Apart from differences in susceptibility to heat and tropical disease (5), a difference in salivary anti-tannin defenses (and a resulting difference in rumen physiology) could be another reason zebu cattle are particularly suited for agricultural systems in the tropics, where available forages often contain high levels of tannins (6, 7). Although non-proline-rich proteins exist that also have affinity for tannins (1, 8), it is interesting to compare the proline content of different cattle breeds. Here, we report such a screening for a comparison of zebu cattle and zebu-Holstein-Friesian in the Jimma area located at 7°40′N and 36°50′E at 1760 masl in southwest Ethiopia. For the study eight heifers were used: four were zebu (100% *Bos indicus*) and four were zebu × Holstein Friesian (HF) crosses. The blood level of crossbreed heifers (*Bos indicus* × *Bos taurus*) were composed of 70% HF+30% zebu, heifer 1; 66% HF+ 34% zebu, heifer 2; 68% HF+ 32% zebu, heifer 3 and 70% HF+ 30% zebu, heifer 4. The animals were 2.5 years old with comparable body weight and similar body condition scores. The body condition score was evaluated based on 1-9 point score scale (9). They were fed on a diet that included the tannin-rich plant *Albizia gummifera* for 28 days. The animals were fed on a local hay mixture as a basal diet and experimental diet of leaves of *A. gummifera*. The diets were composed weekly to ensure that cattle would consume *A.gummifera* at a rate of 10% of total dry matter (DM) requirement, estimated as 2.5% of live body weight. To minimize selectivity by the animals, the *A. gummifera* forage was provided in the morning (8:00) whereas hay mixture was offered only later at 10:30. After 21 days, saliva samples were collected from the animals' mouths using a sponge. When the sponge was saturated with saliva, it was squeezed manually (with the investigator wearing fresh latex gloves), allowing the collection of a minimum of 10 ml saliva into a plastic cup with screw top. The saliva was then passed through a tea sieve to remove feed particles, and stored at -43°C. When the samples were thawed for analysis, they were passed through a 0.3µm syringe filter to remove bacteria. Amino acids were determined according to Hendriks et al. (2002). From these data, the proportion of proline in the total amount of measured amino acids was calculated. Differences between genotypes were evaluated by means of a Student's t-test. Significant differences were considered at $P < 0.05$.

The chemical composition of a test diet (*A. gummifera)* and the hay mixture included in the study (g/kg DM) are presented in Table 1. Saliva from pure zebu always had either similar or numerically higher concentrations of

TABLE 1

Chemical composition of *Albizia gummifera* and the hay mixture applied in the study (g/kg DM).
DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; CF: crude fibre: NFE: nitrogen free extract (16); NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; HC: Hemicellulose (17); CT: condensed tannins as measured by the butanol-HCl-iron method (18).

FEED STUFF	DM	OM	Ash	CP	EE	CF	NFE	NDF	ADF	ADL	HC	CT
A. gummifera	904	955	45	294	13.3	380	268	740	575	116	165	72
Hay mixture	944	886	114	227	4.9	249	405	892	687	201	205	-

TABLE 2

Salivary amino acid concentrations (mg/l) in pure zebu cows and zebu x Holstein crossbreeds fed a tannin-rich diet.

	ZEBU (N=4)		CROSSBREED (N=4)		*P*	%DIFFERENCE
	MEAN	SD	MEAN	SD		
proline	1.8	0.4	1.0	0.2	0.014	80
threonine	2.8	0.8	1.6	0.2	0.030	75
serine	2.2	0.6	1.4	0.1	0.039	57
glycine	1.9	0.6	1.2	0.2	0.112	58
arginine	1.6	0.4	1.2	0.1	0.149	33
alanine	2.2	0.7	1.7	0.3	0.210	29
histidine	1.2	0.3	0.9	0.1	0.231	33
valine	2.4	0.7	1.9	0.3	0.280	26
isoleucine	1.5	0.5	1.2	0.2	0.364	25
leucine	2.8	0.8	2.3	0.3	0.356	22
glutamate	4.5	1.3	3.8	0.5	0.372	18
tyrosine	1.1	0.4	1.0	0.1	0.746	10
aspartate	3.1	0.9	3.0	0.4	0.862	3
lysine	2.0	0.6	2.0	0.3	0.996	0
phenylalanine	1.3	0.4	1.3	0.2	0.929	0
Sum	32.4	9.4	25.5	3.5	0.24	27

individual or total amino acids than did saliva from crossbreeds (Table 2). The difference was significant for proline, threonine and serine ($P<0.05$). Correspondingly, when the concentration of proline was expressed as a proportion of all amino acids, pure zebu had a significantly higher proportion of proline in saliva than did crossbreeds ($P<0.05$; Fig. 1).

These findings suggest that differences exist between zebu and Holstein-Friesian breeds with respect to salivary amino acid composition; the differences are strongly suggestive of a difference in salivary anti-tannin defense, although this needs to be further substantiated by studies on tannin-binding capacities (2, 3). Natural browsers such as goats or deer are better adapted to tannin-rich diets than are domestic cattle, which are grazers, because of a lack of tannin-binding proteins in the cattle (2, 6, 12, 13). Threonine (14) and serine (15) are two of the amino acids least affected by tannin; however, in our finding zebu cattle heifers which had a long exposure to a tannin-rich diet had a higher concentration of these amino acids in their saliva as compared to crossbreeds.

In this study, the experimental animals consistently ingested a diet that contained a certain amount of condensed tannins. In view of previous reports on ruminants, this precaution may not have been necessary – in all cases where the presence of tannin-binding salivary proteins has been investigated in ruminants so far, their occurrence did not vary with previous exposure to dietary tannins (2, 4, 7). In this respect, ruminants seem to differ from other herbivores in which tannin-binding salivary proteins can be induced by the diet (1). Whether the diet used in this study had an effect on the amino acid composition of the saliva would have to be tested in experiments with different diets.

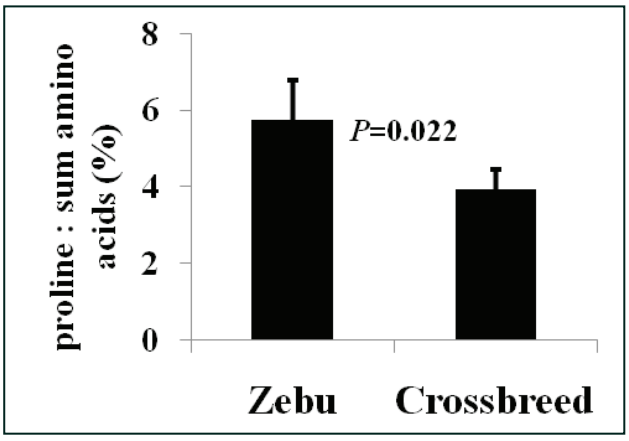

Fig. 1. – The percentage of proline in the total of measured amino acids (weight:weight) in saliva of pure zebu cattle and zebu x Holstein crossbreeds fed a tannin-rich diet.

The results of this study must be considered preliminary. However, they could stimulate a series of experiments with zebu cattle, in which their readiness to consume tannin-containing forages in cafeteria trials was compared to other domestic cattle breeds, and in which their digestive efficiency and food conversion rate on such forages were determined. Although *Bos taurus* and *Bos indicus* diverged 0.3-0.8 million years ago and are the two most closely related bovine species (11), we still may detect relevant physiological differences that could allow us to make differentiated use of these species in various environments.

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REFERENCES

1. Shimada T (2006) Salivary proteins as a defense against dietary tannins. Journal of Chemical Ecology 32:1149-1163.
2. Austin PJ, Suchar LA, Robbins CT, & Hagermann AE (1989) Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. Journal of Chemical Ecology 15:1335-1347.

3. Fickel J, Goritz F, Joest BA, Hildebrandt T, Hofmann RR, & Breves G (1998) Analysis of parotid and mixed saliva in roe deer (*Capreolus capreolus*). Journal of Comparative Physiology B 168:257-264.
4. Clauss M, Lason K, Gehrke J, Lechner-Doll M, Fickel J, Grune T, & Streich WJ (2003) Captive roe deer (*Capreolus capreolus*) select for low amounts of tannic acid but not quebracho: fluctuation of preferences and potential benefits. Comparative Biochemistry and Physiology B 136:369-382.
5. Turner JW (1980) Genetic and biological aspects of zebu adaptability. Journal of Animal Science 50:1201-1205.
6. Kumar R, & Singh M (1984) Tannins: their adverse role in ruminant nutrition. Journal of Agricultural Food Chemistry 32:447-453.
7. Makkar HPS (2003) Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Research 49:241-256.
8. Makkar HPS, & Becker K (1998) Adaptation of cattle to tannins: Role of proline-rich proteins in oak-fed cattle. Animal Science 67:277-281.
9. Nicholson, M J, Butterworth, M H (1986) A guide to body condition score of Zebu cattle, ILCA Addis Ababa.
10. Hendriks WH, Butts CA, Thomas DV, James KAC, Morel PCA, & Verstegen MWA (2002). Nutritional quality and variation of meat and bone meal. Asian-Australian Journal of Animal Science 15:1507-1516.
11. Ritz LR, Glowatzki-Mullis ML, Machugh DE, & Gaillard C (2000) Phylogenetic analysis of the tribe Bovini using microsatellites. Animal Genetics 31:178-185
12. Mehansho H (1992) Tannin mediated induction of proline-rich protein synthesis. Journal of Agricultural and Food Chemistry 40:93-97.
13. Lamy E, Rawel H, Schweigert FJ, Capela e Silva F, Ferreira A, Rodrigues Costa, Antunes C, Martinho Almeida A, Varela Coelho A & Sales-Baptista E (2011) The Effect of Tannins on Mediterranean Ruminant Ingestive Behavior: The Role of the Oral Cavity. Molecules. 16:2766-2784. www.mdpi.com/journal/molecules
14. Mansoori B & Acamovic T (2009) Influence of tannic acid and polyethylene glycol on the excretion and digestibility of amino acids in gelatin-fed broilers. British Poultry Science 50(2):199-2006.
15. Mansoori B & Acamovic T (2007) The effect of tannic acid on the excretion of endogenous methionine, histidine and lysine with broilers. Animal Feed Science and Technology 134(3-4): 198-210.
16. AOAC (1997) Official methods of analysis. Association of Official Analytical Chemists, Arlington, VA, USA.
17. Van Soest PJ, Robertson JB, & Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583-3597.
18. Makkar HPS (2001) Chemical protein precipitation and bioassays for tannins, effects and fate of tannins and strategies to overcome detrimental effects of feeding tannins-rich feeds. Proceedings of the FAO-CIHEAM Meeting on Sheep and Goat Nutrition, Hammamet, Tunisia 9:40-41.

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