

Condensed Tannins Enhance the Erythron Function and Deter the Development of Anaemia in Pregnant Goats Browsing in an East African Natural Range Land

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Key words

Browse feed, Condensed tannins, Erythron Function, Anaemia, Goats

1 Introduction

Mechanisms underlying the effects of nutritional stress in small ruminants are important because of the interrelationships among their nutritional, reproductive and productive characteristics (ABASSA, 1995). The erythron is a major component of the physiological machinery responsible for oxygen transport in livestock and deficiencies in its function are associated with reduced ruminant foraging activity (BLOOD and RADOSTITS, 1989). This lowers the productivity of affected animals. Feeding in relation to the erythron must be proper to ensure adequate supply of vitamins, minerals and proteins (COLES, 1986). Factors that influence dietary nutrient intake and utilisation are thus, important in foraging designs of small ruminants particularly in sub-Saharan Africa where nutritional factors have been implicated in the high reproductive losses of goats and sheep (ABASSA, 1995). Condensed tannins (CT), known to complex biopolymers such as proteins are abundant in many fodder trees and shrubs in the traditional grazing grounds of sub-Saharan Africa (LE HOUEROU, 1980). These phenolic compounds are not toxic to ruminants (WAGHORN *et al.*, 1994) and during selective grazing, intake of these compounds at levels less than 5% CT (COOPER and OWEN-SMITH, 1985) results into improved protein and amino acid utilisation in ruminants (WAGHORN *et al.*, 1994; KABASA *et al.*, 1999). This study examined the effect of browse CT ingested during selective grazing on the erythron function and development of anaemia in pregnant goats managed under a traditional free range feeding system.

2 Materials and Methods

2.1 Study area

The study was conducted in the Ankole range land, Mbarara district, southern Uganda, 1250 m above sea level. The relevant ecological and climatic characteristics of the area have been described by SCHWARTZ *et al.*, (1996) and KABASA *et al.*, (1999). The vegetation is typically Acacia shrub / wood-land. Dominant vegetation species include

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among others: *Acacia* ssp., *Grewia* ssp., *Cadaba* ssp., *Carissa* ssp., *Rhus* ssp., *Bracharia* ssp., *Themeda triandra*, *Cynodon dactylon* and *Chloris gayana*. The area is seasonally wet, with a long dry season from May to August. Rainfall is bimodal, April to May and September to November. Forage quality and production fluctuate seasonally and grazing browse species constitutes 60 – 85% of the goat's feeding time (J.D. Kabasa and J. Opuda-Asibo, 'personal communication').

2.2 Experimental Design

Details of the experimental design are already published (KABASA *et al.*, 1999). Briefly, 30 yearling F1 Anglo-Nubian x Mubende does, of a local stock and averaging 21 ± 0.45 kg body weight, were screened for health and nutritional status, effectively treated against helminth parasites with a broad spectrum combination of levamisole hydrochloride and oxcyclozanide (Nilzan Plus^R, Wellcome Ltd, Nairobi, Kenya), mated and randomly divided into 2 equal groups during a 3 months preparatory phase. Deworming was done at the peak of the first wet season to ensure a similar infection status in all the goats at the start of the experiment. Does that tested pregnant within the last 2 weeks of the preparatory phase were selected so that gestation was monitored throughout the long dry season.

During the 6 month long experimental phase, one group received a daily oral dose of 50 g / goat of PEG (Merck, Germany), and the other acted as the control (no PEG). The nutritional status of the goats, as well as the pasture helminth larvae contamination were ascertained (Table 1). Similarly, live weight changes of goats in the two groups were assessed (Table 2). A night shade of 1.2 m² / doe was provided. Grazing in a 1 sq. mile range area started at 9.00 h, followed by watering (13.00 h) and a midday rest until 15.00 h, when goats went back to the pasture. Return to the kraal was at 18.00 h. Spraying against external parasites was done once a month.

2.3 Goat Diet

Traditional herding was the mode of feeding. The dietary preference of the goats was observed and the composition of their diet monitored by the direct observation rapid-survey technique of DICKO-TOURE (1980) with slight modifications. This method was acceptable to the owner of the goats and farm. Observations were done 10 days / month, every third day. Three selected goats, previously conditioned to handling were watched from 2-5 m away. The plant visited, the part eaten, the number of bites taken, the size of a single bite and the feeding time at the plant were noted. A similar forage sample was immediately hand-picked from the area of plants being grazed during the observation, taken to mimic the grazing habits of the goats. Similar observations were made on each of the three goats for periods ranging between 3 h and whole day. Local persons with a sound knowledge of the flora in the area were involved. Verification of the data was complemented by cross-checking with the opinions of experienced herdsman. Each day's samples were taken and processed for laboratory analyses. The nutrient content was analysed using standard methods (NEUMANN-NUEDAMM, 1983), while the methods of SWAIN and HILLS (1959) and REED *et al.*, (1982) were adopted for

tannin assays. Absorbency readings for CT were converted into g/kg DM using a purified standard preparation of Quebracho CT provided by Dr. Ann E. Hagerman (Miami University, USA).

Table 1: Mean dietary composition, grazing time (h) and pasture helminth contamination (L_3 /kg) during the study.

Parameter/ dietary nutrient	Dry Season Mean (\pm s.e.m)	Wet Season Mean (\pm s.e.m)
Iron (ppm)	111.41 \pm 4.02	117.50 \pm 3.08
Copper (ppm)	10.22 \pm 2.0	112.31 \pm 2.38
Zinc (ppm)	24.91 \pm 1.1	125.76 \pm 1.68
Crude protein (g/kg DM)	110.00 \pm 3.50 a	184.00 \pm 4.20 b
Insoluble condensed tannin (g/kg DM)	32.75 \pm 1.08 a	19.35 \pm 0.53 b
Soluble condensed tannin (g/kg DM)	4.35 \pm 0.39 a	3.67 \pm 0.17 b
CP : TCT ratio	2.96 \pm 0.19 a	7.99 \pm 0.21 b
Grazing time	7.62 \pm 0.31 a	6.50 \pm 0.46 b
Pasture helminth larvae ($\times 10^3$)	0.50 \pm 0.03 a	2.51 \pm 0.02 b

TCT = soluble and insoluble condensed tannins combined; CP = crude protein; L_3 = helminth larval stage 3; DM = dry matter; Mean values in the same row and with different superscripts (a, b) are significantly ($p < 0.05$) different.

Table 2: Live weight (kg) and percent loss in live weight of pregnant range goats grazed on browse containing condensed tannin and treated with polyethylene glycol (PEG).

Category	June	July	August	September	October
PEG group live weight*	21.45 \pm 0.21	22.36 \pm 0.26	25.03 \pm 0.23	27.46 \pm 0.33	27.39 \pm 0.24
No PEG group live weight*	23.42 \pm 0.24	27.22 \pm 0.24	30.25 \pm 0.26	33.47 \pm 0.23	33.69 \pm 0.21
Percent loss; PEG relative to No PEG group	8.4 \pm 0.16	17.9 \pm 0.22	17.3 \pm 0.25	18.01 \pm 0.25	18.71 \pm 0.12

* Initial live weight of does was 21.0 \pm 0.45

2.4 Erythron indices

Whole blood samples were taken monthly from the jugular veins of goats previously conditioned to the handling procedures. Five ml per goat was aseptically collected between 6.00 and 9.00 h into sterile 6 ml vacutainer caps containing drops of 10% EDTA (ethylenediaminetetraacetic acid) anticoagulant, mixed well, clearly labelled and maintained on ice (0 - 4°C) until analysed within 24 h. An automated electronic counter (Becton-Dickinson, USA) calibrated to the size of animal cells was employed for the determination of packed cellular volume (PCV), haemoglobin (Hb) and red blood cells (RBC). The PCV values were ascertained with the micro-haematocrit method. The mean capsular volumes (MCV), mean capsular haemoglobin (MCH), and mean capsular haemoglobin concentrations (MCHC) were determined using the procedures of COLES (1986). Total plasma protein was measured by a refractometer (Hereaus, Germany) after centrifugation of the blood in a microhaematocrit tube and a plasma drop from just above the RBC line taken on the refractometer prism for reading. Plasma fibrinogen content was determined by difference between total plasma protein content and total fibrinogen free plasma protein value. Plasma was freed of fibrinogen by precipitation at 57°C for 3 minutes and re-centrifugation was done thereafter.

2.5 Statistical analysis

Group comparison tests were done using the Sigmastart statistical package (Jandel Corp. USA., 1995). Differences between haematological indices of the goats in the two treatment groups were tested for significance by analysis of variance (ANOVA). Turkey test was used for all pair-wise comparisons of the mean responses across months of gestation within a treatment group. A probability of < 0.05 was considered significant and results were presented as mean \pm standard error of the mean (s.e.m.).

3 Results

3.1 Erythrocytic indices

Figures 1, 2 and 3, and Table 3 summarise the results of the study. Erythrocyte populations (millions / μ l) fluctuated between 7 and 12 in goats of the PEG group. RBC counts in this group dropped below the physiological limit during the fifth month of gestation (Fig. 1). Control goats sustained a normal RBC count throughout gestation, ranging from 10.7 to 15.92 million / μ l. Mean RBC counts were 9.07 ± 0.82 (PEG group) and 12.54 ± 0.94 (control group). RBC counts of the 2 groups were significantly ($p < 0.05$) different. Blood haemoglobin content (Fig. 2) significantly ($p < 0.05$) dropped below the physiological limit in the 4th and 5th months of gestation. Hb content in the control goats remained within the physiological range. Hb levels in the PEG group averaged 8.55 ± 0.61 g/dl, and ranged from 6.9 to 10.5 g/dl. Mean PCV (%) values of the PEG group ranged between 18.50 and 30.33 with a mean of 23.99 ± 2.21 . This was significantly lower than that of the control group (mean = 29.54 ± 0.81). PCV in the PEG group dropped below the physiological limit (Fig. 3) during the last phase of gestation. The PCV of the control group ranged between 27 and 32.5%. There were no significant differences between the MCV, MCH, MCHC values of the 2 groups throughout the experiment (Table 3). All experimental goats maintained the physiological values of these parameters throughout the study. Within the control

group, monthly fluctuations in all the parameters measured did not differ significantly ($p>0.05$) across gestation. However, PCV, Hb and RBC values of goats in the PEG group during the 5th month of gestation were significantly different ($p< 0.01$) from those observed during other months in the same group.

3.2 Plasma Protein

Table 4 summarises the results of plasma protein changes during the study. The plasma protein in the PEG group dropped ($p<0.05$) below the physiological value by 400 mg during the last two months of gestation. In addition, the plasma protein : fibrinogen ratios of 11.6 during the fourth month of gestation and 10.8 during the fifth month were below the critical levels for absolute lack of hypoproteinemia (Table 4). Plasma protein concentration of the PEG group ranged between 5.6 and 6.6 g/dl with a mean of 6.06 g/dl, while in the control group, it ranged from 6.2 to 6.7 g/dl with a mean of 6.36 g/dl. The ratio of total protein to fibrinogen in the plasma of goats in the PEG group dropped below the critical margin for absolute normal blood protein concentration (Table 4), and ranged between 10 and 12 g/dl in the last 2 months of gestation.

Table 3: Mean capsular volume (MCV), mean capsular haemoglobin (MCH) and mean capsular haemoglobin concentration (MCHC) of pregnant range goats grazed on browse containing condensed tannins and treated with polyethylene glycol (PEG).

Parameter	Group	May	June	July	August	Sept.	Oct.	Nov.
MCV* (\pm s.e.m) in femto litre (fl)	PEG group	25.11 ± 1.00	24.33 ± 0.59	23.97 ± 1.00	22.75 ± 0.65	20.00 ± 0.71	21.60 ± 1.03	22.32 ± 0.54
	Control Group	24.80 ± 0.85	24.50 ± 0.66	22.20 ± 0.42	23.60 ± 0.92	22.22 ± 1.14	23.00 ± 0.82	23.20 ± 0.65
	Physiological range	16-25	16-25	16-25	16-25	16-25	16-25	16-25
MCH * (\pm s.e.m) in picogram (pg)	PEG group	6.10 ± 0.40	6.24 ± 0.29	6.34 ± 0.39	5.90 ± 0.46	6.61 ± 0.53	5.39 ± 0.30	6.10 ± 0.34
	Control (no PEG)	7.12 ± 0.31	7.38 ± 0.23	7.43 ± 0.43	6.02 ± 0.20	7.00 ± 0.43	6.79 ± 0.23	7.10 ± 0.41
	Physiological range	5-8	5-8	5-8	5-8	5-8	5-8	5-8
MCHC* (\pm s.e.m) in g / dl	PEG group	28.30 ± 0.45	29.51 ± 0.52	29.90 ± 0.87	29.13 ± 0.91	29.01 ± 0.51	29.83 ± 0.64	28.81 ± 0.52
	Control (no PEG)	29.11 ± 0.61	31.62 ± 0.70	33.12 ± 1.00	32.70 ± 0.71	31.15 ± 0.86	33.74 ± 0.83	32.80 ± 0.74
	Physiological range	28-34	28-34	28-34	28-34	28-34	28-34	28-34

* = MCV, MCH, MCHC values of the 2 groups throughout the experiment are not significantly ($p>0.05$) different.

Table 4: Plasma protein (mg/dl) and total plasma protein : fibrinogen ratio in pregnant range goats grazed on browse containing condensed tannin and treated with polyethylene glycol (PEG).

Group	Parameter	June	July	August	Sept *	October *
PEG group	Total plasma protein (x 10 ³)	6.0	6.6	6.3	5.8	5.6
	Plasma fibrinogen	370.00	410.00	400.00	450.00	520.00
	PP:F**	16.20	16.10	15.80	11.60	10.80
No PEG group (control)	Total plasma protein (x 10 ³)	6.20	6.50	6.20	6.70	6.20
	Plasma fibrinogen	380.00	400.00	390.00	404.00	410.00
	PP:F	16.30	16.30	15.90	15.60	15.10

PP = total plasma protein; F = plasma fibrinogen; PP:F = plasma protein : fibrinogen ratio;

* = PP values between groups in September and October were significantly ($p < 0.05$) different.

** = PP:F ratio for moderate hypoproteinemia is 10 £ 15 and for severe hypoproteinemia is < 10 (COLES, 1986)

4 Discussion

Nutritional factors have been advanced as one of the major causes of the low productive performance of small ruminants in sub-Saharan Africa (Abassa, 1995). Therefore, plausible explanations of the possible mechanisms underlying the effects of nutritional stress in these livestock are important. This study has demonstrated a pre-parturient alteration in haematological indices and a subsequent anaemia that is associated with the deactivation of browse CT ingested by pregnant range does grazed freely in a natural range ecosystem. A reduced blood Hb, PCV and RBC level, coupled with a moderate alteration in plasma protein concentration of the goats drenched with a CT deactivator (PEG) was observed during late pregnancy (Figs. 1 - 3; Table 4). There were no abnormal changes in the MCV, MCH and MCHC values of the does (Table 3). Therefore, affected goats exhibited a normocytic normochromic anaemia. The PEG group blood Hb, PCV and RBC levels went low in the late stage of gestation. On the contrary, control goats (no PEG) sustained normal haematological indices throughout gestation (Figs. 1 - 3; Table 3 and 4).

There could be several reasons to explain these observations. However, besides a potential deficiency in protein utilisation, the rapid foetal development demands and the accompanying competition for nutrients between the foetal and maternal organisms during the critical period of gestation (normally 90-120 days), combined with the rising level of helminth re-infections (Table 1) during the wet season may be implicated. Nutritional deficiencies resulting from the presence of PEG in the gut may predispose goats to the blood sucking nematode parasites leading to leakage and loss of plasma

Figure 1-3: Blood erythrocyte counts, haemoglobin levels and packed cellular volumes of pregnant range goats grazed on browse containing condensed tannin and drenched with polyethylene glycol (PEG).

Figure 1: Blood erythrocyte (RBC) counts

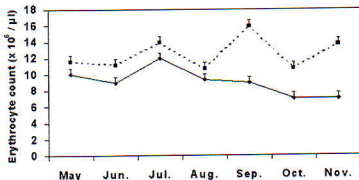
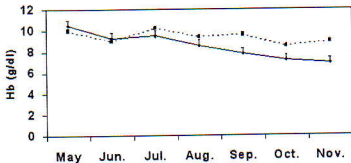
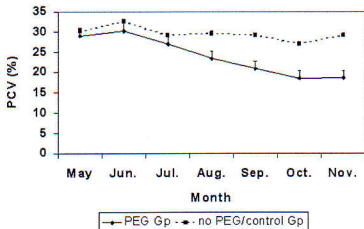


Figure 2: Blood haemoglobin (Hb) level



proteins into the gut. During the study, *Haemochus* nematodes were observed in the experimental goats (KABASA *et al.*, 1999) and are a common cause of reduced albumin in domestic ruminants (URQUHART *et al.* 1988). However, control goats may have derived benefit from the low CT levels ingested during grazing (Table I) to overcome the harmful effects of the internal parasites. Dietary CT is directly implicated in the present findings because no CT adsorbent (PEG) was provided to the control group of goats and all other experimental factors remained constant for all groups.

Figure 3: Blood packed cellular volumes (PCV)



CT at low dietary concentrations (<5% of DM) improve the nutritive value of herbage by binding to plant proteins and protecting them from excessive degradation in the rumen. Bypass protein is increased and essential amino acids utilisation enhanced. Besides, the efficiency of utilisation of blood urea nitrogen in the formation of bacterial protein is improved. Thus, pregnant goats with reduced protein supply may still overcome a marginal protein deficiency through the beneficial effects of CT.

The finding of deficient haematological indices in the last 2 months of gestation (Figs. 1 - 3) in goats drenched with PEG (the CT deactivator), is suggestive of the overall significance of fed browse CT in the functional state of the goat erythron particularly during periods of nutritional deficiency in the natural grazing range lands. This may as well, have strategic implications in the management of goat feeding and foraging designs under different production systems. Pregnant goats that have no access to fresh browse CT in the free range system are potential victims of malnutrition and become predisposed to helminth infections and anaemia. This, may particularly complicate the physiological status of pregnant does. Affected animals have reduced foraging time, are weak and lose weight, thereby lowering the quality of the foetus including its survival. Wentzel *et al.*, (1974) observed that the incidence of abortion was reduced in flocks when does were fed for proper size and development prior to the breeding season and during gestation.

On the other hand, the observed alterations in plasma proteins during the late stage of gestation among goats in the PEG group could indicate either a pathologic, physiologic or other inducing factor in the animal's function. It is known that protein lack or

deficiency has its most marked effect on plasma gamma globulin and albumin levels (Coles, 1986), and a decrease in gamma globulin could lead to a slow build-up or non-response of immunological defences among affected goats resulting into impaired resistance to disease. However, this study did not focus on examining the various plasma protein fractions.

It has long been assumed that browse feed influences range goat performance by simply acting as a major standing feed reserve that supplies essential nutrients particularly during periods of critical shortfall such as during drought. However, this study has examined another possibility, the mechanism of condensed tannin in the browse to enhance the animal's nutrition and thereby enhance their erythron function to resist the development of anaemia. The demonstration of browse CT benefits in goats under selective feeding in the Ankole range land has considerable nutritional and practical significance especially in Uganda where feeding practices under the traditional feeding systems (tethering and free range) are often badly managed. Access to optimal quantities of diverse CT-containing browse may not only minimise nutritional stress among goats, but also improve physiological (erythron) function to enhance productivity. The study has demonstrated that selective feeding in the natural range ecosystem may expose goats to optimal concentrations of dietary CT with resultant beneficial effects. Thus, feed and feeding management strategies adapted or developed for improving goat production, should consider feeding goats on a variety of fresh CT-containing browse forages at levels that would elicit the beneficial effects.

5 Summary

To investigate the effect of selectively browsed CT on the erythron function of pregnant goats under free range grazing in a natural ecosystem, 30 yearling F1 Anglo-Nubian x Mubende goats (21 ± 0.45 kg), were screened, dewormed, mated and randomly divided into 2 equal groups during a 3 months preparatory phase. During the 6 months that followed, one group received 50 g per goat and day of a CT deactivator - polyethylene glycol (PEG) and the other treated as the control (no PEG). Goats were monitored for their nutritional status and erythron characteristics: packed cellular volume (PCV), blood haemoglobin (Hb), erythrocyte (RBC) counts, volume per RBC (MCV), Hb per RBC (MCH), Hb concentration per RBC (MCHC) and plasma protein. RBC counts, blood Hb and PCV level in goats treated with the CT deactivator (PEG) significantly ($p < 0.05$) dropped below the physiological limit in the 4th and 5th months of gestation. Plasma protein in the PEG group dropped ($p < 0.05$) below the physiological value by 400 mg during the last two months of gestation. These results suggest that under selective feeding in the natural Ankole range land ecosystem, fed browse condensed tannin enhance the erythron function of goats in gestation and reduce the development of anaemia. This has practical significance in the management of goat feeding, health and production.

Zusammenfassung

Kondensierte Tannine erhöhen die Erythrozytenfunktion und reduzieren die Ausbildung von Anämie in tragenden Ziegen, die auf ostafrikanischem, natürlichem Weideland grasen.

Um den Einfluß von selektiv aufgenommenen kondensierten Tanninen (CT) auf die Funktion der roten Blutkörperchen bei tragenden Ziegen unter natürlichen Weidebedingungen zu untersuchen, wurden über eine 3-monatige Vorbereitungsphase 30 Jährlinge der F1-Generation aus Anglo-Nubian x Mubende-Ziegen (21 ± 0.45 kg) untersucht, entwurmt, belegt und nach dem Zufallsprinzip in zwei gleiche Gruppen aufgeteilt. Während der darauffolgenden 3 Monate erhielt eine Gruppe (Versuchsgruppe) 50g pro Tier und Tag das CT-neutralisierende Polyethylenglycol (PEG), während die andere Gruppe als Kontrollgruppe unbehandelt blieb. Die Tiere wurden beobachtet im Hinblick auf ihren Ernährungszustand und ihre Erythrozyten-Charakteristik, wobei folgende Blutparameter ermittelt wurden: Hämatokrit(PCV), Blut-Hämoglobin (Hb), Erythrozytenzahl(RBC), Volumen pro RBC (MCV), Hämoglobin pro RBC (MCH), Hb-Konzentration pro RBC (MCHC) und Plasmaprotein. RBC-Zahl, Bluthämoglobin und Zellvolumenmenge sanken unter die physiologische Grenze während des 4. und 5. Trächtigkeitsmonats signifikant ($p < 0.05$) bei den Ziegen, die mit PEG behandelt wurden (Versuchsgruppe). Das Plasmaprotein in der Versuchsgruppe sank unterhalb des physiologischen Wertes auf 400 mg während der letzten 2 Monate der Trächtigkeit. Diese Ergebnisse weisen daraufhin, daß bei freier Futteraufnahme im unberührten Ankole Weidelandgebiet kondensierte Tannine der Nahrung die Erythrozytencharakteristika bei Ziegen während der Trächtigkeit verbessern und einer Anämie entgegenwirken. Dies hat praktische Bedeutung für das Management von Ziegen in Bezug auf Fütterung, Gesundheit und Produktion.

Schlüsselworte: Weidefutter, kondensierte Tannine, Erythrozytenfunktion, Anämie, Ziegen

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