

**EFFECTS OF SUPPLEMENTING NATIVE PASTURE HAY WITH
PUNCTURE VINE (*Tribulus terrestris*) ON THE INTAKE, WEIGHT CHANGE,
NITROGEN BALANCE AND EXCRETION OF PURINE DERIVATIVES OF
SHEEP**

[EFECTO DE LA SUPLEMENTACIÓN DE PASTO NATIVO CON *Tribulus terrestris* SOBRE EL CONSUMO, CAMBIO DE PESO, BALANCE DE NITRÓGENO Y EXCRECIÓN DE DERIVADOS DE PURINA EN OVINOS]

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SUMMARY

Eight male sheep (average 34.17 ± 3.33 kg LW) were used in a nitrogen balance trial to measure dry matter intake, weight change, nitrogen balance and microbial protein yield. A completely randomized design was used and the animals were randomly allocated to the two treatment diets. The sheep were given a) native pasture hay (NPH) + 100 g crushed maize (CM) (control diet) or b) NPH + 100 g CM + 160 g *Tribulus terrestris*. The native pasture hay comprised of a mixture of grasses with *Heteropogon contortus*, *Eragrostis rigidour* and *E. superba* being predominant and had a crude protein content of 37.5 g/kg DM. The puncture vine was harvested at the onset of flowering, dried and graded to remove large stems and woody fruits. The total feed intake (918.14 g/d) of sheep given *T. terrestris* supplement was significantly ($P < 0.05$) higher than that of the control diet (553.70 g/d). *T. terrestris* supplementation also resulted in an average weight gain of 38.13 g/d while on the control diet an average loss of 130.43 g/d was recorded. Puncture vine supplementation also resulted in higher nitrogen retention (2.30 g/d) as compared to the control diet (0.43 g/d). Microbial protein yield was 16.48 g/d with puncture vine supplementation and 6.39 g/d for the control diet. The results show that the inclusion of *T. terrestris* as a protein supplement to low quality natural pasture hay diets increases dry matter intake, nitrogen balance and microbial protein yield.

Key words: *Tribulus terrestris*, microbial protein yield, allantoin, nitrogen balance, native pasture hay

RESUMEN

Ocho borregos con un peso vivo de 34.17 ± 3.33 kg fueron empleados para medir consumo, cambio de peso, balance de nitrógeno y producción de proteína microbial. Los tratamientos fueron; A) heno de pasto nativo (NPH) + 100 g maíz quebrado (dieta control) y B) NPH + 100 g maíz quebrado + 160 g *Tribulus terrestris*. El heno de pasto nativo fue compuesto principalmente de una mezcla de pasto con *Heteropogon contortus*, *Eragrostis rigidour* y *E. superba* con un contenido de proteína cruda de 37.5 g/kg MS. *T. terrestris* fue cosechado a la floración, secado y seleccionado para remover tallos gruesos y frutos leñosos, el maíz se empleó como fuente de energía fermentable. El consumo total (918.14 g/d) de los borregos suplementado con *T. terrestris* fue mayor ($P < 0.05$) que la dieta control (553.70 g/d). El empleo de *T. terrestris* resultó también en una ganancia de peso de 38.13 g/d mientras que la dieta control registró pérdida de peso de 130.43 g/d. La suplementación con *T. terrestris* permitió una mayor retención de N (2.30 g/d) en comparación con la dieta control (0.43 g/d). La producción de proteína microbial was fue de 16.48 g/d con *T. terrestres* y 6.39 g/d para la dieta control. Los resultados muestran que la inclusión de *T. terrestris* como suplemento proteínico a henos de pastos nativos de baja calidad permite incrementar el consumo de materia seca, balance de nitrógeno y producción de proteína microbial.

Palabras clave: *Tribulus terrestris*, producción de proteína microbial, alantoína, balance de N, pasto nativo.

INTRODUCTION

Poor quality native pasture grasses during the dry season depress ruminant animal productivity in the tropics. In Zimbabwe the dry season is from the end of April to the end of October. It is characterized by insufficient and poor quality feed for animals grazing in natural pasture (Topps and Oliver, 1993). The protein content of veld grasses declines from more than ten per cent in the early growing season to less than three per cent during the dry season, with neutral detergent fibre content increasing progressively to above sixty-five per cent (Elliott and Folkertsen, 1961). The net effect of these changes is that digestibility and voluntary intake of herbage decline, resulting in a drop in total nutrient intake (Sibanda *et al.*, 1992). Ngongoni and Manyuchi (1993) noted that ruminants that relied solely on natural grazing often failed to consume enough feed during the dry season to meet their total nutrient requirements with the consequence that both growth and reproductive performance were adversely affected. Protein supplements in the form of oilseed cakes and animal by-product meals are normally given to alleviate this problem. However, the majority of smallholder farmers in Zimbabwe cannot afford these supplements.

Herbaceous forage legumes such as *Stylosanthes guianensis*_var *intermedia* cv. Oxley (Fine stem stylo) and *Cassia rotundifolia* cv. Wynn have been promoted widely in the higher rainfall areas (> 550 mm annual rainfall) of Zimbabwe (Mupangwa, 2000). These forage legumes are, however, not suited to areas receiving annual rainfall of less than 450 mm. Livestock production is the major source of livelihood to smallholder farmers in the dry parts of Zimbabwe. Low animal productivity thus negatively affects the farmers. The search for low cost dry season protein supplements continues with several evaluations of tree legume seedpods having been undertaken (Sebata, 2002). There is also need to explore other herbaceous locally adapted plants as sources of cheap protein. This study sought to evaluate one such herbaceous plant, puncture vine (*Tribulus terrestris*) reported by Drummond (1984) to be regarded in some parts of South Africa and Zimbabwe as a life-saving fodder for sheep and goats. Puncture vine is moderately degradable in the rumen with a protein content of 156.25 g/kg dry matter (Sebata, 2005). However, when consumed in large quantities in excess of 80 percent of diet it has been reported to be toxic to livestock, especially sheep, (Sahelian, 2003).

The objective of this study was to determine feed intake, rumen microbial protein synthesis as estimated from purine (allantoin) derivatives, body weight changes and the nitrogen balance in growing sheep given a basal diet of native pasture hay supplemented with *T. terrestris* and crushed maize.

MATERIAL AND METHODS

Animals and management

Eight castrated sheep 24 months of age with an initial body mass of 34.17 ± 3.33 kg were used in a nitrogen balance trial. The sheep were vaccinated against pulpy kidney and drenched against internal parasites a week before the start of the experiment. The sheep were put into metabolism crates that facilitated separate collection of urine and faeces for a 14 days adaptation and 7 days total collection period.

Experimental design and treatments

The animals were randomly assigned to two treatment diets in a completely randomised design. The two treatments were native pasture hay (NPH) given *ad libitum* plus 100 g crushed maize (crude protein content of 87.5 g/kg dry matter) (control diet) and NPH given *ad libitum* plus 100 g crushed maize plus 160 g *T. terrestris*. The native pasture hay comprised of a mixture of grasses with *Heteropogon contortus*, *Eragrostis rigidior* and *E. superba* being predominant and had a crude protein content of 37.5 g/kg dry matter (Sebata, 2002). The *T. terrestris* provided approximately 23 g crude protein per day. Crushed maize provided readily fermentable energy.

T. terrestris was harvested just before flowering air dried, graded to remove large stems and woody fruits and then fed without being milled. Native pasture hay and the supplements were offered separately. Crushed maize was offered as the first feed at 06:30, the protein supplement at 07:30 and finally the NPH at 08:30. Native pasture hay allowances were adjusted daily according to the previous days consumption. Fresh drinking water was available at all times from drinking troughs.

Measurements and collections

The amount of NPH refusals was collected and weighted daily in the morning to calculate intake. The sheep consumed all the crushed maize and *T. terrestris* offered. The sheep were weighed before the start and at the end of the experiment to calculate weight change. After an adaptation period of 14 days total collection of faeces and urine was carried out for 7 days. The total urine produced daily was collected in plastic buckets with 25 mls of 10% v/v sulphuric acid as a preservative. A 10 % sample of homogenised faeces and of the urine excreted each day was taken from each animal and bulked over the days of collection and kept frozen (-20 °C) until required for laboratory analysis. A second sample of urine, 10% of the daily output, was collected daily from each animal and diluted five times with distilled water and stored frozen for use in the analysis of total purine

derivatives. A 100g sample of faecal pellets was taken daily from each animal for dry matter determination.

Chemical analysis

The feed, refusals and faecal samples were analysed for dry matter (DM) and nitrogen, while urine was analysed for nitrogen according to standard procedures (AOAC, 1990). Purine derivatives (allantoin) were determined according to the method of Chen and Gomes (1992). The amount of absorbed microbial purine and the microbial protein yield were estimated from the daily excretion of purine derivatives based on the model described by Chen and Gomes (1992).

Statistical analysis

Analysis of variance was carried out to determine the treatment effects using the Proc GLM procedure of SAS (SAS, 1998). The following model was used:

$$Y_{ijk} = \mu + P_i + e_{ijk},$$

Where:

Y_{ijk} = response variable (e.g. intake),

μ = mean,

P_i = protein source and

e_{ijk} = residual error.

A comparison of means was done using the Tukey's studentized range test of SAS (1998) to compare the two treatments.

Microbial protein yield

Microbial protein yield (MPY) was calculated using the formula:

$$\text{MPY (g/d)} = [(X \text{ mmol}) \times 70] / (0.83 \times 0.116 \times 1000)$$

Where:

$$X = D/0.84$$

D = allantoin excretion (mmol/day)

Intake

Intake was calculated as the difference between offers and refusals corrected for dry matter contents of feeds and refusals on a daily basis.

Nitrogen balance

Nitrogen balance (g/d) (NB) was calculated as the amount of average daily nitrogen intake (g/d) (NI) not excreted in faeces (g/d) (FN) and urine (g/d) (UN).

$$\text{NB} = \text{NI} - (\text{FN} + \text{UN})$$

Rumen fermentable organic matter

Rumen fermentable organic matter (RFOM) was calculated using the formula (ARC, 1984):

$$\text{RFOM} = 0.65 \times \text{DOMI (g/day)}$$

Where: DOMI = digestible organic matter intake

RESULTS

Feed intake and weight changes

The intake of native pasture hay + crushed maize (control diet) and control diet + *T. terrestris* as well as live weight change of the sheep are given in Table 1. *T. terrestris* supplementation resulted in a higher ($P < 0.05$) dry matter intake and positive live weight changes.

Table 1: Feed intake and live weight changes of sheep given native pasture hay (NPH) + crushed maize (control diet) and control diet + *Tribulus terrestris*.

	NPH + crushed maize	NPH + crushed maize + <i>T. terrestris</i>	s.e
DM intake (g/d)			
Grass hay	465.15 ^a	680.53 ^b	49.34
Crushed maize	88.55	88.55	--
Supplement	---	149.06	--
Total intake	553.70 ^a	918.14 ^b	53.31
% supplement	--	16.25	
Live weight change			
Initial wt (kg)	35.00	35.17	--
Final wt (kg)	32.00	36.07	--
Total wt change (kg)	-3.0	0.90	--
Daily wt change (g/d)	-130.43 ^a	38.13 ^b	32.66

^{ab}Means in the same row with different superscripts differ significantly ($P < 0.05$)

s.e. = standard error of the means

Nitrogen balance

The nitrogen balance values are given in Table 2. The total nitrogen intake and nitrogen retention were higher ($P < 0.05$) in sheep given *T. terrestris* supplements than those on the control diet giving higher nitrogen retention with supplementation.

Microbial protein yield

The purine derivative excretion values and estimated microbial protein yield are given in Table 3. The daily purine derivative excretion in sheep given *T. terrestris* supplement was significantly ($P < 0.05$) higher than in the control diet giving the microbial protein yield with supplementation of 16.48 g/d and 6.39 g/d in the control diet.

DISCUSSION

The native pasture hay used as the basal diet was of low quality as shown by the low crude protein (37.5 g/kg DM) and high neutral detergent fibre content (645 g/kg DM) (Sebata, 2002). Supplementation with *T. terrestris* increased total dry matter intake. This was in agreement with results reported in literature for tropical legumes (Umunna *et al.*, 1995b). The increase in intake was due to an improved supply of rumen

ammonia nitrogen to the rumen microbes by the *T. terrestris*. Supplementation with the source of rumen fermentable nitrogen resulted in a sixty-six per cent increase in dry matter intake. The inclusion of a source of fermentable energy helped to reduce the weight loss to an average 130 g per day and to maintain a positive nitrogen balance. The maize had a crude protein content of 87.5 g/kg dry matter which was enough to meet the maintenance requirements of the sheep but could not meet the minimal requirements for growth of 113 g/kg dry matter (ARC, 1984).

The high dry matter intake could be attributed to the availability of both a readily fermentable source of energy and nitrogen that are important in the utilization of poor quality roughages. This assisted in increasing the efficiency (P/E ratio) of microbial nitrogen synthesis (Osuji *et al.*, 1993; Nsahlai and Umunna, 1996). The rumen microbes were able to multiply and quickly digest the fibrous material allowing for a quick passage rate through the gastrointestinal tract. Protein supplementation of grass diets containing less than 70 g crude protein per kg dry matter has been reported to increase dry matter intake, DM digestibility and animal performance (Osuji, *et al.*, 1993; Muinga *et al.*, 1995; Umunna *et al.*, 1995a). Nitrogen retention also increased with *T. terrestris* supplementation, leading to a higher nitrogen balance.

Table 2. Nitrogen metabolism in sheep given native pasture hay (NPH) + crushed maize (control diet) and control diet + *Tribulus terrestris*.

	NPH + crushed maize	NPH + crushed maize + <i>T. terrestris</i>	s.e.
N intake (g/d)	4.07 ^a	9.12 ^b	0.30
N excretion (g/d)			
Faecal	2.78 ^a	5.76 ^a	0.99
Urinary	0.86 ^a	1.05 ^a	1.05 ^a
N retention (g/d)	0.43 ^a	2.30 ^b	0.73

^{ab}Means in the same row with different superscripts differ significantly ($P < 0.05$)
s.e. = standard error of the means

Table 3. The excretion of purine derivatives and estimated microbial protein yield by sheep given native pasture hay (NPH) + crushed maize (control diet) and control diet + *Tribulus terrestris*.

	NPH + crushed maize	NPH + crushed maize + <i>T. terrestris</i>	s.e.
Purine derivatives (mmol/day)			
Allantoin excreted	3.97 ^a	10.24 ^b	3.29
Total purines excreted	5.28	13.62	
Microbial purines absorbed	8.80	22.70	
Microbial protein yield (g/d)	6.39 ^a	16.48 ^b	4.61
Microbial protein yield (g/kg RFOM ⁺)	16.58 ^a	47.35 ^b	5.72

^{ab}Means in the same row with different superscripts differ significantly ($P < 0.05$)

⁺ RFOM = rumen fermentable organic matter; RFOM = 0.65 x DOMI (g/day) (ARC, 1984).

The measurement of microbial protein yield in the rumen is critical in the evaluation of amino acids available for absorption and utilization by ruminants (Mupangwa, 2000). Microbial proteins make up the main part of amino acids that flow to the small intestines in ruminants. As a result of *T. terrestris* supplementation, there was a significant ($P < 0.05$) increase in microbial protein yield. The increase in microbial protein yield with *T. terrestris* supplementation of low quality diets is in agreement with results of studies in legume forages (Abdulrazak *et al.*, 1996). The microbial protein yield of 47.35 g/kg of rumen fermentable organic matter (RFOM) was within the range of 14 to 49 g of organic matter apparently digested in the rumen reported (ARC, 1984).

This study showed that supplementing sheep basal diets with *T. terrestris* results in the animals retaining a positive nitrogen balance and thus keeping positive weight changes during the dry season.

CONCLUSION

It can be concluded from this study that inclusion of *T. terrestris* in poor quality native pasture hay diets improves ruminant intake of dry matter through increased rumen supply of nitrogen which stimulates microbial fermentation of the roughages. Further studies need to be carried out to ascertain at what level of inclusion the *T. terrestris* becomes toxic to livestock and characterize the components responsible for toxicity.

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