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# Effects of *Leucaena leucocephala* supplementation to basal hay on Energy and Protein metabolism in West African Dwarf sheep

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## Abstract

*Energy and protein utilization and quantitative retention of protein, fat and energy was investigated with twelve castrated West African Dwarf (WAD) sheep averaging (23.0 ± 2.4 kg BW) in nitrogen and energy balance trials. Dried leaves of Leucaena leucocephala were offered as supplement at two levels 25% (diet 2) and 50% (diet 3) of dry matter intake (DMI), replacing hay in the basal hay diet. The basal hay diet without supplementation was the control. Measurements were performed by means of nitrogen and carbon balances and with the use of indirect calorimetry. The digestibility and utilization of protein were influenced (P<0.05) by supplementation. Metabolisability of energy (ME/GE) was on the average 42.9 (SEM 4.3)% being significantly (P<0.05) different among treatments. Diet 2 had a higher (P<0.05) retained protein (9.6 g/d) compared with the control diet and hence a superior (P<0.05) protein utilization than the control and diet 3, respectively. It was concluded that Leucaena leuccocephala improved protein utilization and retention in WAD sheep.*

**Key words:** *Leucaena leucocephala*, Protein, Energy, Fat utilization and retention, WAD sheep.

## Introduction

Low nutrients content of tropical grasses is the main limitation affecting performance of ruminant animals, consequently resulting in their low productivity. When the nitrogen content of feed is less than 1% the ruminants' appetite will be depressed and the voluntary intake reduced (Minson, 1990). Ruminants when fed with low nitrogen forages of straw-based diets with continuous supplementation of urea show an increase in feed intake and digestibility of protein (Preston and Leng, 1987). However, true protein supplementation was found to be more effective than non-protein supplementation.

The majority of the world's ruminants depend over their lifetime on forages that can be described only as poor quality, in which the limitations to production are a low protein supply from the microbial ecosystem and a virtual absence of dietary bypass protein. Under production systems, nutrient availability to the rumen microbes often sets the upper limit of production through a low microbial growth efficiency in the rumen (which directly results in a low protein to energy ratio in the nutrients absorbed). The other limitation is the amount of dietary protein that escapes rumen digestion, but which is digested in the intestines. Ruminants require more protein generally (particularly in tropical climates) than arises from microbial growth in the rumen whether the latter is efficient or inefficient (Leng 1990).

*Leucaena leucocephala* has been shown to have great potential as a source of high quality feed for ruminant. This legume has in its leaves a high protein content (>20% CP) and is able to retain feeding quality than tropical grasses, especially during dry periods (Clavero and Razz, 1997).

Similarly, ruminants when fed with *Panicum maximum* grass (Reynolds and Adediran, 1987) or *Agrostis* hay (Osakwe, 1999) supplemented with *Leucaena leucocephala* show an increase in dry matter intake resulting in higher nitrogen and energy intake and volatile fatty acid concentration in the rumen liquor. However, the effect of anti-nutritional factors (condensed tannins and mimosine) in *Leucaena leucocephala* on quantitative energy and protein retention in WAD sheep is scanty in literature. The objective of this study was therefore to determine the effects of anti-nutritional factors in *Leucaena leucocephala* on energy and protein metabolism of WAD sheep fed *L. leucocephala* as supplement to a hay diet.

## Materials and Methods

*Leucaena leucocephala* (Lam.) de Wit

Fresh leaves from *Leucaena leucocephala* plant were collected from the sub-humid zone of Cotonou (Republic of Benin). The *Leucaena* had been established 12 months previously in continuous rows with 2 meter spacing between rows in the experimental station of "Direction de la Recheche Agronomique", Cotonou. The leaves were harvested in the months of November/December and sun dried on raised wooden platform at the experimental station. The dried leaf samples were then packed in plastic containers and transported to the University of Hohenheim, Germany for analysis and feeding trial.

## Hay

The hay consisted primarily of cool-season grass (foxtail barley-*Hordeum jabatum*) harvested in mid-October at the Hohenheim University.

*Experimental diet and animal trial*

Dried leaves of *Leucaena leucocephala* were offered as supplement at two levels 25% (diet 2) and 50% (diet 3) of dry matter intake (DMI), replacing hay in the basal diet (control).

The experimental diets were as follows:

Diet 1 (100% hay =Control)

Diet 2 (25% *Leucaena* + 75% hay)

Diet 3 (50% *Leucaena* + 50% hay)

Twelve castrated WAD sheep (average 23.0 ± 2.4 kg BW) were used in a completely randomised design to investigate protein and energy utilization and quantitative retention of protein, fat and energy, in sheep fed *Leucaena* leaves.

Trial 1: Four animals were randomly assigned to the control (Diet 1), diet 2 and Diet 3, respectively. The animals were housed in individual metabolism crates and adapted for 10 days to the experimental diets. This was followed by a 7 days nitrogen balance trial during which feed, faeces and urine were collected daily

Trial 2. Four animals from each treatment were transferred into the respiration chambers during which 24 hr measurement of gas exchange of carbon dioxide, methane and oxygen was carried out.

The gas exchange measurement was carried using an open circuit respiration system with four chambers. The chamber volume, which is 5,000 cubic litres each is equipped with air conditioners that maintain a constant humidity of 60-70% (±2%) and a temperature of 20°C (±0.3°C) within the chambers. The animals were put in the chambers for 4x24 hr, for the measurement of their gas exchange and they were offered the experimental diets and water in the respiratory chamber. About 35,000 litres/day of air is pumped in and out of each chamber. During a measuring period of 24 hr, aliquot sample of the spent/waste air is collected in gas receptors for the analysis of carbon dioxide, methane and oxygen. In addition the air intake was also analysed. The gas analysis was carried out under a standardised condition of 0 °C, 0 % humidity and 760 mm Hg. Carbon dioxide and methane were measured with Uras 10 using the principle of infrared-absorption-gas-analyser. Oxygen was measured with Magnos 2T using the principle of paramagnetic oxygen-analyser.

In addition to the experimental diet, animals received a mineral premix supplement (10 g/d). Feed was offered twice a day at 0800 and 1600 hr, and water provided *ad libitum*.

*Analytical methods*

Feed and faeces samples were dried and ground in a hammer mill to pass a 1mm mesh sieve for chemical analysis. The nitrogen content of these samples plus that of urine was determined by the Kjeldahl method according to AOAC, (1990) procedure while Neutral detergent fibre (NDF), Acid detergent fibre ((ADF), and Acid detergent lignin (ADL) were determined as described by Goering and Van Soest (1970). The difference between NDF and ADF was designated as hemi-cellulose, and between ADF and ADL as cellulose. Gross energy of feed and faeces were measured by bomb calorimeter using benzoic acid as a standard (26437 J/g). Analyses of extractable condensed tannins were carried out by the method described by Markkar *et al.*, (1993). Total extractable phenol and tannin phenol were analysed by the method described by Osakwe *et al.* (2000). Carbon content in feed, faeces and urine was determined according to the principle of electric conductivity by means of a carmograph apparatus (Schiemann *et al.*, 1971). Retention of protein (RP), fat (RF) and energy (RE) were calculated by means of carbon and nitrogen balances with the set of constants and factors described by Brouwer (1965):

RP, g = Retained Nitrogen x 6.25

RF, g = (carbon balance – carbon in RP)/0.767

RE, kJ = RP, g x 23.86 + RF, g x 39.76

Analysis of Variance (ANOVA) was used to analyse the data using the General Linear Modelling Procedure (SAS, 1985). Significant treatment means were separated using Duncan's Multiple Range Test (Duncan,1955).

## Results

The chemical composition and gross energy content of *Leucaena leucocephala* and the experimental diets is presented in Table 1. The high CP content (301 g/kg) and gross energy (GE) content (21.4 kJ/g DM), of *Leucaena leucocephala* would seem to suggest its suitability as a fodder tree.

*Intake of metabolisable energy, digestible protein and utilization in sheep*

The results obtained for intake of metabolisable energy (ME) and digestible protein (DP) of sheep supplemented with *Leucaena leucocephala* is summarised in Table 2.

There were significant differences ($P<0.05$) in the daily intake of ME and DP of sheep in the different treatments. The daily intake of ME was lower ($P<0.05$) in diet 3 while intake of DP was higher ($P<0.05$) in diet 3.

The digestibility and utilization (RP/DP) of protein was measured in individual nitrogen balance experiments. Digestibility and utilization of protein were strongly ($P<0.05$) influenced by supplementation. Diet 3 had a lower ($P<0.05$) protein digestibility than the control while diet 2 had higher ($P<0.05$) protein utilization than both the control and diet 3, respectively.

Fat retention (RF) and protein retention (RP) were calculated from carbon balances, and the mean values of RF and RF are shown in Figure 1. The RP values were higher in the supplemented groups compared with the control. The metabolisability of energy (ME/GE) was significantly ($P<0.05$) different among treatments, with the control being more superior to diets 2 and 3, respectively. The metabolisability was on the average 42.9 (SEM 4.3)%.

Total amount of energy retained (RE), in protein (RP) and fat (RF) in relation to ME intake (Figure 2), showed a decrease of –34.9% in diet 3 compared with the control.

As the ME intake varied significantly with supplementation, the energy retained in protein and fat when compared with the ME intake showed a significant decrease with supplementation.

## Discussion

Many of the browse legumes, e.g. *Acacia* and *Erythrina spp*, have characteristics such as

**Table 1.** *Chemical composition of Leucaena leucocephala and experimental diets (% of DM) a*

| Item | *Leucaena leucocephala* | Diet 1 | Diet 2 | Diet 3 |
|---|---|---|---|---|
| CP | 30.1 | 13.1 | 17.4 | 21.6 |
| Ash | 8.5 | 10.5 | 10.0 | 9.5 |
| Ether extract | 4.6 | 2.0 | 2.7 | 3.3 |
| NDF | 49.9 | 61.0 | 58.2 | 55.5 |
| ADF | 30.6 | 35.4 | 34.2 | 33.0 |
| ADL | 15.9 | 3.7 | 6.8 | 9.8 |
| Cellulose | 14.7 | 31.7 | 27.5 | 23.2 |
| Hemicellulose | 19.3 | 25.6 | 24.0 | 28.9 |
| Total Phenol[1] | 1.7 | - | 0.43 | 0.85 |
| Tannin Phenol[1] | 0.5 | - | 0.13 | 0.3 |
| Condensed tannins [2] | 1.2 | n.a. | 0.3 | 0.6 |
| GE ($kJg^{-1}$ DM) | 21.4 | 17.9 | 18.8 | 19.7 |
| Mineral premix [3] | n.a. | 10.0 | 10.0 | 10.0 |

[1]As tannic acid equivalent [2]As leucocyanidin equivalent; n.a.: Not applicable; [3]Composition/kg: vit A 600,000 IU, vit D3 75,000 IU, vit E 300 mg, Zn 3,000 mg, Mn 480 mg, Co 12 mg, Se 10 mg.
a The values in each column represent duplicate assays per sample
Diet 1 = hay only (control); Diet 2 = hay + 25% *Leucaena*; Diet 3 = hay + 50% *Leucaena*

**Table 2** *Intake of ME, digestible protein and utilization of sheep supplemented with Leucaena leucocephala. (n = 4)*

| Item | Control | Diet 2 | Diet 3 | SEM |
|---|---|---|---|---|
| Dry matter intake (g/d) | 490.8[a] | 519.1[a] | 540.8[a] | 19.3 |
| Dry matter digestibility | 0.703[a] | 0.653[ab] | 0.524[b] | 2.95 |
| Metabolizable energy [MJ/d] | 5.17[a] | 4.25[b] | 3.46[c] | 0.4 |
| Digested protein (DP), (g/d) | 45.7[b] | 56.1[ab] | 67.4[a] | 5.4 |
| Digestibility (%) | 0.709[a] | 0.616[ab] | 0.504[b] | 0.05 |
| Utilization (RP/DP), % | 16.0[b] | 30.1[a] | 13.2[b] | 4.5 |

[a,b,c] Means in a row with different superscripts differ significantly ($P<0.05$)

variable content of condensed tannins which elicit less degradation in the rumen and increase the availability of by-pass protein. Feng and Leng (1991), for example, have shown that relatively low concentrations of condensed tannins from *Acacia floribunda* provided protection of proteins in lupins and stimulated growth and wool production in sheep. Osakwe *et al.* (2000) observed an improvement in nitrogen digestibility and retention in sheep when *Phyllanthus discoideus* was offered. There was a trend of increasing dry matter intake with supplementation (Table 2) and this led to an increase in the digested protein. This observation is in line with the reports of Mtenga and Shoo (1990) who reported that increased protein intake is often associated with high dry matter intake resulting in faster rates of passage of diets through the gastro-intestinal tract.

In the present investigation, the low crude protein digestibility with level of

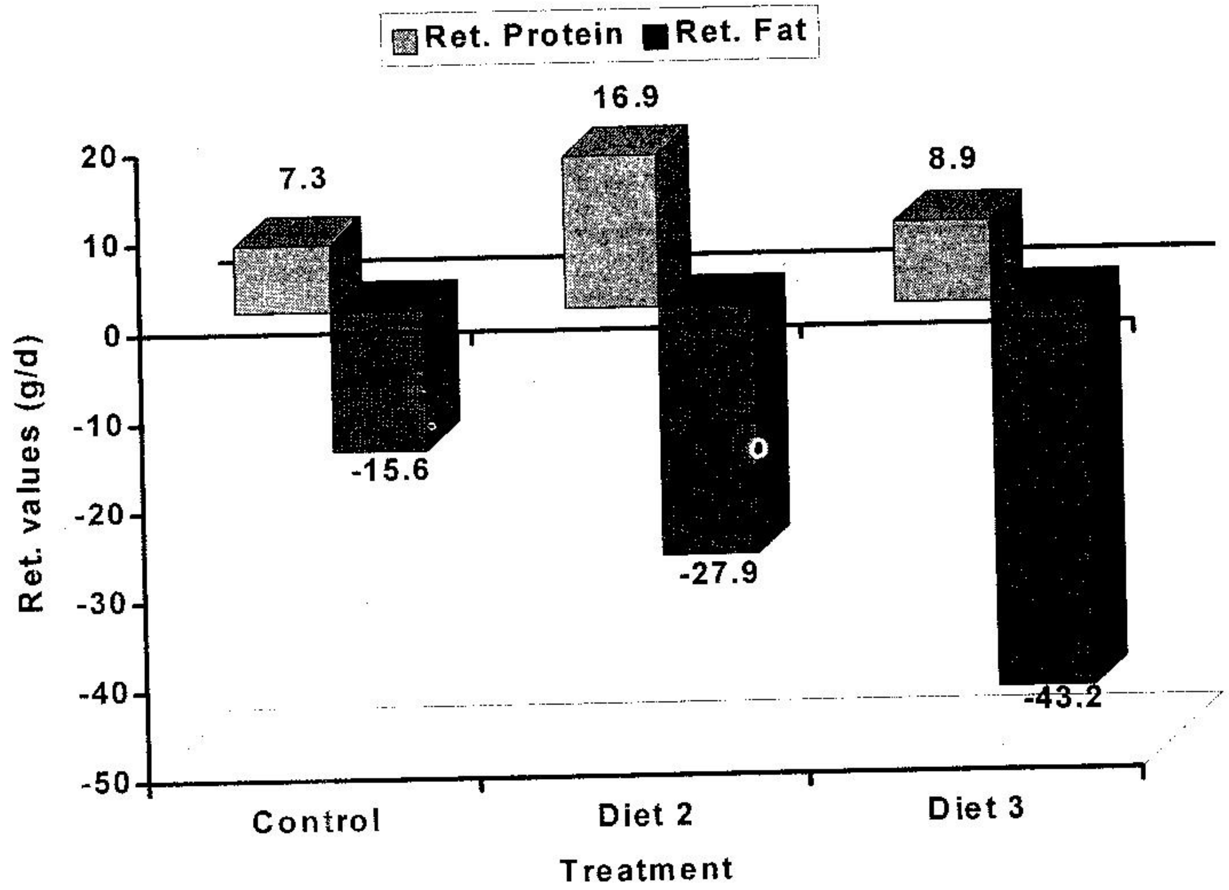


Figure 1 **Effect of *Leucaena leucocephala* supplementation on retained protein and fat**

supplementation could be du· :o inhibition of protein degradation in the ru. :n as a result of condensed tannins and mim· ne.(Kumar and Singh 1984, Osakwe *et al.*, ·99). Improved protein utilization was obse··ed in diet 2. It would appear that beyond 25% ·upplementation level the retention of p ·tein dropped significantly. Supplementat· n of *Leucaena leucocephala* led to an increa· of 9.6 g/day of retained protein in diet 2, c· ·pared with the control. The increased retain· l protein in the body was not only by an incre· e in the digested protein but also by the impro· ed utilization of the absorbed proteins i.e. usin· more protein for anabolism and less for oxidat· n (Chwalibog *et al.*, 1994).

Although there were no differences in the total energy retention among treatments, there was a clear trend of increase in the loss of energy retained as a result of increase in the total heat loss with supplementation level. There was a shift in energy retention from fat to protein among the treatments. It is interesting to note that the higher level of supplementation (diet 3) did not improve the utilization but resulted in a reduced RP/DP in comparison with diet 2. Although diet 2 showed no difference with diet 3 in the amount of digested protein, it had significantly higher protein utilization, consequently its protein retention was higher. On the average RP increased by 57% in diet 2, but only 18% in diet 3 compared with the control animals. The inhibitory effect of higher level of supplementation of tannin containing browse plant on protein utilization correlates the depressive effect of tannin on energy retention. This observation is in agreement with the findings

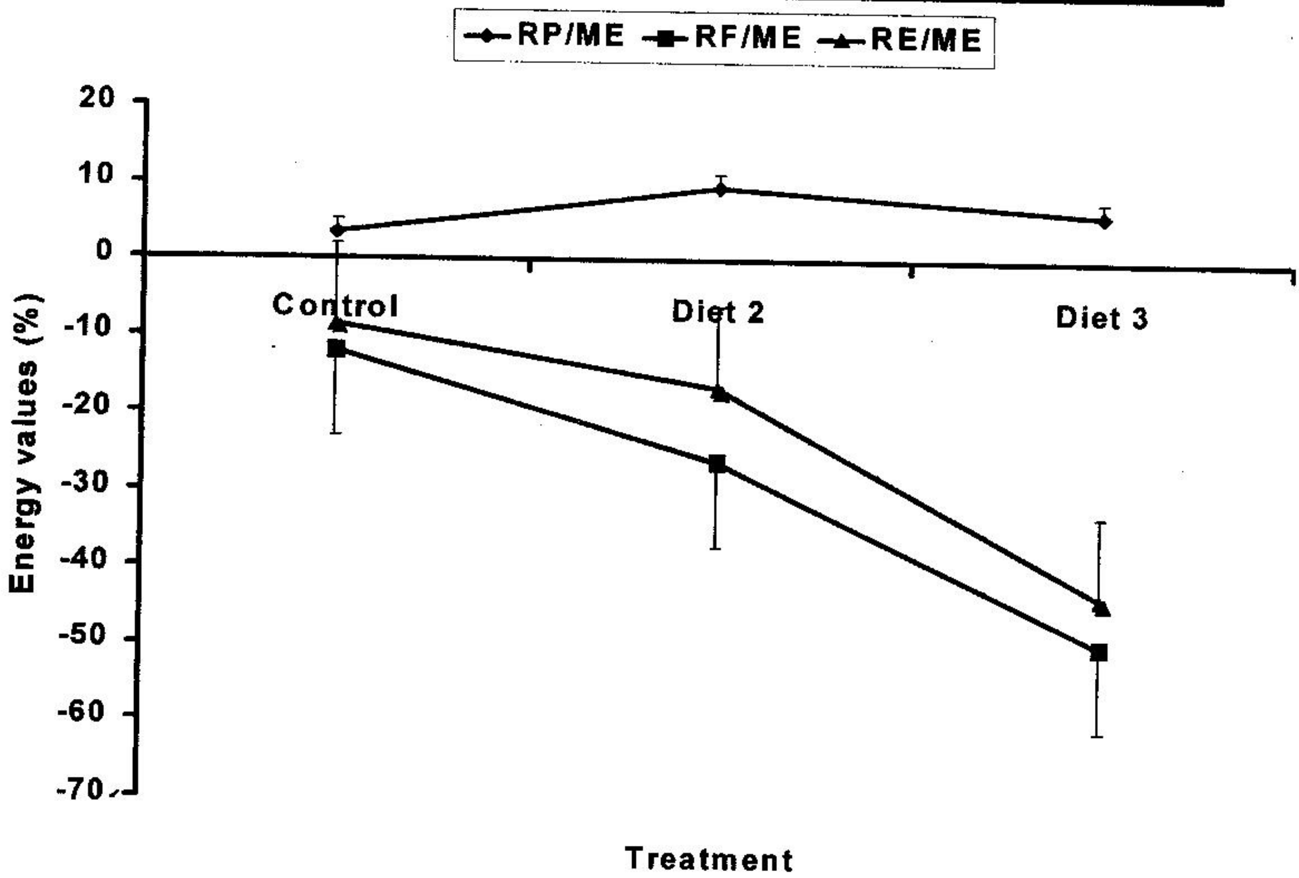


**Figure 2** Energy retained in protein (RP), fat (RF) and total (RE) in relation to ME intake of sheep

of (Osakwe, 2003; Osakwe *et al.* 2003; Kumar and Singh, 1984). The improved protein utilization and retention in the present investigation is in agreement with the reports of Jackson and Barry (1996) that forages with low concentration of condensed tannin could improve the efficiency of nitrogen digestion. Waghorn, *et al.* (1987) and Mangan (1988) reported the possibility of protein protection by condensed tannins leading to improved nitrogen utilization. The findings from this study were in agreement with these reports.

## Conclusion

In the conclusion, these findings support the observations made in other studies that lower concentration of condensed tannins could lead to improvement in nitrogen utilization, by protecting the protein in the rumen and making it available at the hindgut. The improved protein utilization observed in this study could have been stimulated by lower concentration of condensed tannins than the reduction of fat retention. The implication of this study to livestock farmers and nutritionists is that they can offer 25% of dried *Leucaena leucocephala* leaves as supplement to hay diet.


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