QUANTITATIVE PROTEIN AND FAT METABOLISM IN WEST AFRICAN DWARF SHEEP FED MARGARITARIA DISCOIDEA AS SUPPLEMENT

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ABSTRACT

Protein and energy utilization and quantitative retention of protein, fat and energy was investigated with twelve castrated Djallonke sheep averaging (20.0 ± 2.2kg BW) in nitrogen and energy balance trials. Dried leaves of Margaritaria discoidea were offered as supplement at two levels (25% (diet 2) and 50% (diet 3) of DMI), replacing hay in a basal hay diet. The basal hay diet without supplementation was the control. Measurements were performed by means of nitrogen and carbon balances with the use of indirect calorimetry. The digestibility of protein was not influenced by supplementation, while utilization of protein was influenced (P<0.05). Metabolisability of energy (ME/GE) was on the average 46.7 (SEM 1.6) % being not significantly (P>0.05) different between treatment. Diet 3 had a higher (P<0.05) total amount of energy retained in protein and fat (0.28 MJ/d) compared with the control diet. It was concluded that Margaritaria discoidea improved protein utilization and retention in Djallonke sheep.

Key words: Margaritaria discoidea, Protein, Energy, Fat utilization and retention, Sheep

INTRODUCTION

Multipurpose trees provide a cheap source of protein supplement during the dry season, when both the quantity and quality of pasture herbage is limited. They are becoming particularly important in more humid, agriculturally productive areas where the increasing human population has necessitated the cultivation of grazing land. These trees can be integrated into these high potential crop-livestock production systems as live fences, feed gardens, fodder banks, alley farms, wind breaks and multi-strata systems as sources of homegrown supplements for low-quality crop residues during dry season.

Inspite of considerable attention that has been focused on the use of multipurpose trees as feed supplement for small ruminant during dry season (Larbi *et al*., 1993; Osakwe *et al*., 1999; Rittner, 1992), less attention has been paid to the influence of anti-nutritional factors and in particular-condensed tannins on the quantitative protein, fat and energy metabolism. Few experiments with nitrogen and energy balances have been performed with poultry (Steenfeldt *et al*., 1998), pigs (Jorgensen, 1998) and sheep (Osakwe *et al*., 2000, 2003), but no results based on nitrogen and carbon balances with Djallonke sheep can be found in literature. Jackson and Barry (1996) reported that forages with low concentration of condensed tannins could improve the efficiency of nitrogen digestion.

The effect of condensed tannins in *Margaritaria discoidea*, on the utilization and retention of protein, fat and energy has been investigated in the present experiments with Djallonke sheep.

MATERIALS AND METHODS

Sample Collection: Leaves from mature *Margaritaria discoidea* (also called *Phyllanthus discoideus*), Family Euphorbiaceae from the humid/sub-humid zone of Cotonou (Benin) Republic were collected during the dry season, sun dried on a raised wooden platform at the experimental station of "Direction de la Recheche Agronomique", Cotonou. 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 grasses harvested in mid-October at the Hohenheim University. Grass species composition was predominantly redtop bend grass (*Agrotis stolonifers*). The experiment included twelve Djallonke castrated sheep (BW 20 kg) investigated in balance experiments including measurements of the gas exchange in a respiration chamber. *Margaritaria discoidea* leaves were offered as supplement at two levels (25% (diet 2) and 50% (diet 3) of DMI) replacing hay in the basal diet. The basal hay diet without supplement was used as the control diet.

Four animals each were randomly assigned to the control, diets 2 and 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. After the nitrogen balance trial, the animals were transferred to respiration chambers for another trial 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 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 received the experimental diet and water. 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 standardized 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 samples were ground in a hammer mill to pass a 1mm mesh sieve for chemical analysis. Nitrogen content was determined by the Kjeldahl method and ash by burning at 550°C (AOAC, 1990), Crude protein was calculated from N x 6.25. 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 hemicellulose, and between ADF and ADL as cellulose. Samples of faeces were dried at 65 °C for 48 h, ground through a 1 mm diameter screen and together with urine were analysed for N (AOAC, 1990). Gross energy of feed and faeces were measured by bomb calorimetry 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 Singleton and Rossi (1965). 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 treatments were differentiated using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

The chemical composition and gross energy content of the experimental diets and *Margaritaria discoidea* is presented in Table 1. *Margaritaria discoidea* has a high CP (156 g/kg) and a relatively high GE content (19.3 kJ/kg DM).

Intake of metabolizable energy (ME), digestible protein (DP), digestibility and utilization of protein of sheep supplemented with *Margaritaria discoidea* are summarized in Table 2. The daily intake of ME was lowest in the control diet while intake of DP was lowest in diet 3, but the differences were not significant ($P>0.05$). The digestibility and utilization (RP/DP) of protein was measured in individual nitrogen balance experiments. The digestibility of protein was not influenced ($P>0.05$) by supplementation. The utilization of protein was strongly ($P<0.05$) influenced at the higher level

Table 1: Composition of experimental diets and *Margaritaria discoidea* (% of DM)[a]

Item	Control	Diet 2	Diet 3	*Margaritaria discoidea*
CP	11.5	12.6	13.6	15.6
Ash	9.3	8.8	8.3	7.4
Ether extract	1.5	2.3	3.0	4.6
Crude fibre	30.2	27.2	24.1	18.6
NFE	40.7	44.5	43.2	45.7
NDF	58.9	54.2	49.5	40.2
ADF	34.6	31.8	29.0	23.3
ADL	3.2	3.5	3.8	4.4
Cellulose	31.4	28.3	25.2	18.9
Hemicellulose	24.2	22.4	20.5	16.8
Total phenols [1]	-	1.6	2.13	4.52
Tannin phenol [1]	-	0.3	0.7	1.33
Condensed tannins [2]	n.a.	0.32	0.64	1.28
GE (kJg $^{-1}$ DM)	18.04	18.34	18.65	19.25
Mineral premix [3]	10.0	10.0	10.0	n.a.

[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 triplicate assays per sample

Table 2: Intake of ME, digestible protein, digestibility and protein utilization of sheep supplemented with *Margaritaria discoidea*

Item	Control	Diet 2	Diet 3	SEM
Metabolizable energy [MJ/d]	4.54	4.84	4.62	0.16
Digested protein (DP), (g/d)	46.4	47.1	38.4	2.91
Digestibility (%)	60.8	61.6	62.3	0.36
Utilization (RP/DP), %	23.3	12.7	38.5	3.4

Table 3: Effect of supplementation with *Margaritaria discoidea* on retained protein, fat and total amount of energy retained in protein and fat

Item	Control	Diet 2	Diet 3	SEM
Retained protein (g/d)	10.8	6.0	14.8	2.6
Retained fat (g/d)	-2.6	3.2	2.1	1.6
Energy retained in protein [MJ/d]	0.26	0.14	0.35	0.08
Energy retained in fat [MJ/d]	-0.1	0.13	0.08	0.03
Total Energy retained [MJ/d]	0.156	0.27	0.44	0.09

of supplementation. Supplementation of *Margaritaria discoidea* in diet 3 caused an increment ($P<0.05$) in RP of 4 g/d compared with the control.

The retention of fat (RF) was calculated from the carbon balances, and the mean values of RF and total amount of energy retained in protein and fat are shown in Table 3. The RF values were higher in the supplemented groups compared with the control that had a negative RF value. The metabolizability of energy (ME/GE) was on the average 46.7 (SEM 1.6) % being not significantly different ($P>0.05$) between treatments. The total amount of energy in retained protein and fat showed an increase ($P<0.05$) of 0.28 MJ/d in diet 3 compared with the control. As the ME intake varied with supplementation, the energy retained in protein and fat was compared with the ME intake (Fig. 1). The RE in relation to ME showed no difference ($P>0.05$) with supplementation.

DISCUSSION

Naturally occurring polyphenols, particularly condensed tannins inhibit utilization of protein and energy from multipurpose trees. In the present investigation, though the mean intake of DP was lowest in diet 3, while intake of ME was lowest in the control diet, the differences were not significant, indicating no depressive effect of

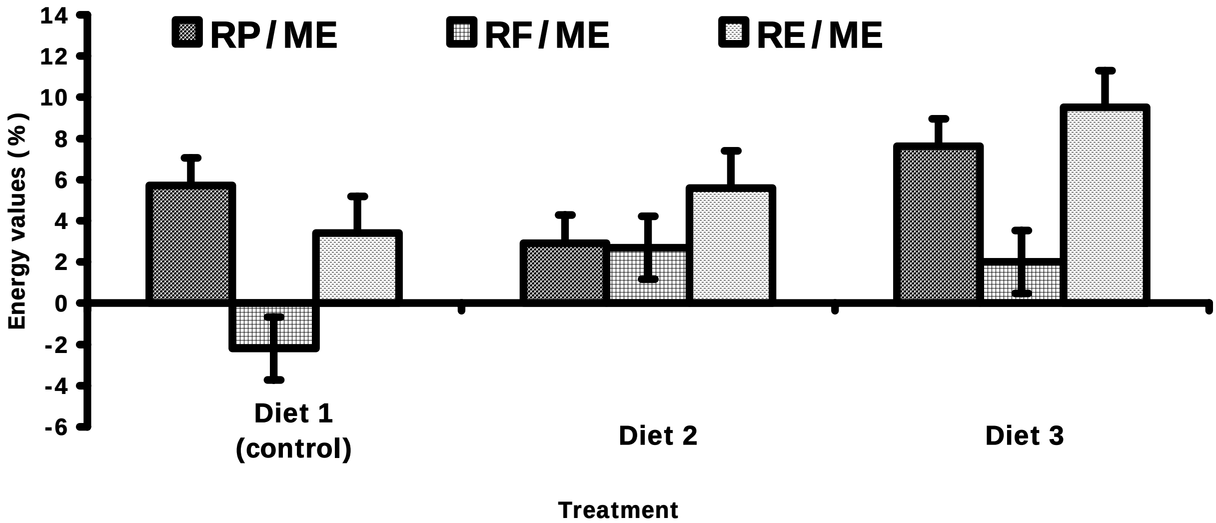

Figure 1: Energy retained in protein (RP), fat (RF) and total (RE) in relationship to ME intake of sheep.

condensed tannins on nutrient and energy consumption. In spite of similar protein digestibility between treatment groups, there were significant (P<0.05) differences in protein utilization with diet 3 having the highest value. Improved protein utilization has also been observed as a reduction of urinary nitrogen excretion (ILCA, 1988, Osakwe *et al*., 2003, Rittner, 1987). It is interesting to note that the higher supplementation level improved utilization in diet 3, consequently, resulting in the highest protein retention in diet 3. On the average RP increased by 27% in diet 3 compared with the control animal. 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 finding of this study is in agreement with their reports.

The present study showed that there were no differences (P>0.05) in protein digestibility but the utilization of protein was increased (P<0.05). Thus at higher level of supplementation, the animals were able to retain more protein in the body not by an increase in digestible protein (DP) but by the improved utilization of the absorbed protein, i.e. by using more protein for anabolism and less for oxidation (Chwalibog *et al*,. 1994). There were no differences in the total energy retention between the control and diet 2 animals, but a shift in energy retention from fat to protein. When comparing the control and diet 2 animals, it is difficult to answer whether the reduction of retained fat (RF) and energy retained (RE) was caused by changes in the intermediary metabolism or caused by the tendency for lower metabolisable energy (ME) intake in the control. However, when expressing energy retention in relation to ME the pattern was the same for absolute values.

CONCLUSION

In conclusion, these findings may indicate that increased protein retention is stimulated by lower concentration of condensed tannins than the reduction of fat retention. It would appear lower concentration of condensed tannins protected the feed protein and made it available at the hind gut where improvement in utilization was observed. The implication of this study to livestock farmers is that farmers engaged in ruminant production can utilize 25% to 50% of *Margaritaria discoidea* leaves as supplement to hay diet during dry season feeding.

ACKNOWLEDGEMENT

The author thanks Oekumenisches Studienwerk eV. (OSW) Bochum, Germany for sponsorship, Dr. W. Eggert for technical assistance and Dr. J. Greiling for the supply of research materials from West Africa.

REFERENCES

AOAC (1990). *Official Methods of Analysis 15th edition*. Association of Official Analytical Chemists, Washington, DC, 2044.

BROUWER, E. (1965). Report of sub-committee on constants and factors. Proceedings 3rd Symposium on Energy Metabolism, Troon, Scotland. *European Association of Animal Production Publication, 11:* 441.

CHWALIBOG, A., JENSEN, K. and THORBEK, G. (1994). Effect of heat stress on the efficiency of utilization of metabolisable energy for lactation. *Proceedings 13th Symposium on Energy Metabolism. European Association of Animal Production Publication, 76*: 213

DUNCAN, D. G. (1955). Multiple range and multiple F-tests. *Biometrics, 11*: 1 - 42.

GOERING, H. K. and VAN SOEST, P. J. (1970). *Forage fibre analyses apparatus reagents, procedures and some applications.* Agricultural Handbook 379, ARS, USDA, Washington DC, pp. 1 - 20

ILCA (1988). Effects of polyphenolic compounds in forage from multipurpose fodder trees on growth, intake and digestion in sheep and goats. *International livestock Centre for Africa Annual Report 1987*, Addis Ababa, Ethiopia pp. 1 -11.

JACKSON, F. S. and BARRY, T. N. (1996). The extractable and bound condensed tannin content of leaves from tropical tree, shrub and forage legumes*. Journal of the Science of Food and Agriculture 71*: 103 - 110.

JORGENSEN, H. (1998). Energy utilization of diets with different sources of dietary fibre in growing pigs. *Proceedings of the 14th Symposium on Energy Metabolism. McCracken, Unsworth and Wylie (eds.)*. CAB International, pp. 367 - 370.

LARBI, A., OSAKWE, I. I. and LAMBOURNE, J. W. (1993) Variation in relative palatability to sheep among *Gliricidia sepium* provenances. *Agroforestry Systems, 22:* 221 - 224.

MAKKAR, H. P. S., BLÜMMEL, M., BOROWY, N. and BECKER, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of The Science of Food and Agriculture, 61*: 161 - 165.

MANGAN, J. L. (1988). Nutritional effect of tannins in animal feeds. *Nutrition Research Review, 1*: 209 - 231.

OSAKWE, I. I., STEINGASS, H. and DROCHNER, W. (1999). The feeding value of *Dialium guineense* as a supplement to West African dwarf sheep fed natural grass hay. *Animal Research and Development, 49*: 24-31.

OSAKWE, I. I., STEINGASS, H. and DROCHNER, W. (2000). The chemical composition of *Phyllanthus discoideus* and its effect on the ruminal ammonia and volatile fatty acid concentration when fed to WAD sheep. *Archive of Animal Nutrition, 53*: 191-205.

OSAKWE, I. I., STEINGASS, H. and DROCHNER, W. (2003). The feeding value of *Mangifera indica* and its effects on crude protein metabolism and energy partitioning when fed to Djallonke sheep. *Tropical Journal of Animal Science, 6 (1)* : 47-52.

RITTNER, U. (1987). *Polyphenolic compounds including tannins in Ethiopian browse species and their biological effects when fed to small ruminants*. M.Sc. thesis, University of Hohenheim, Stuttgart, Germany, pp. 64.

RITTNER, U. (1992). *Polyphenolics of African multipurpose trees and shrubs and their effects in ruminant nutrition*. Dissertation, University of Hohenheim, Stuttgart, Germany, 178 pp.

SAS, (1985). User's Guide 1985 Edition. Statistical Analysis Systems Institute Inc, Cary, NC, USA.

SCHIEMANN, R., NEHRING, K., HOFFMANN, L., JENTSCH, W. and CHUDY, A. (1971). *Energetische Futterbewertung und Energienormen.* VEB Deutscher Landwirtschaftsverlag Berlin. 344 pp.

SINGLETON, V. L. and ROSSI, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*: 144-158.

STEENFELDT, S., HAMMERSHOJ, M., MULLERTZ, A. and JENSEWN, J. F. (1998). Energy supplementation of wheat-based diets for broilers 2. Effect on apparent metabolisability. *Animal Feed Science and Technology, 75*: 45-64.

WAGHORN, G. C., ULYATT, M. J., JOHN, A. and FISCHER, M. T. (1987). The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed *Lotus corniculatus* L. *British Journal of Nutrition, 57*: 115-126.