# *Value of Ramon (*Brosimum alicastrum *Swartz) Foliage for Milk Production with Dual Purpose Cows*

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*The objective of the present study was to assess the value of ramon (Brosimum alicastrum Swartz) foliage as a N-source for replacing soybean-N in the diets of lactating, dual-purpose cows (Bos indicus x B. taurus). Four lactating cows were used in a latin rectangle design. Ramón was included in the diet to replace 0, 33, 66 and 100 per cent of the dietary soybean-N. Basal diet was fresh Taiwan (Pennisetum purpureum) grass fed ad libitum. Basal and total dry matter intake, digestibility (total fecal collection), saleable and calf suckled milk yields, milk composition (fat, protein and lactose) and microbial-N synthesis (purine derivatives method) were measured over 15 d periods. Ramon increased the yield of milk constituents ($P<0.05$) over those obtained with Taiwan grass alone, but production was inferior ($P<0.05$) than when soybean was the N source. Total milk yield was reduced 18 per cent in Ramón supplemented cows compared with those supplemented with soybean. Digestibility of dry matter, organic matter and fiber was reduced by ramón inclusion probably due to its higher fiber and ash content compared with soybean meal. No differences were found amongst diets in microbial-N synthesis ($P>0.05$). It was concluded that, although animal performance with Ramon foliage was compromised when compared with soybean meal, supplementing dual purpose cows with it could be a better strategy than relying on grass alone.*

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# INTRODUCTION

Ramón (*Brosimum alicastrum*) is a tropical forage tree, native to Southern Mexico and Central America. It is commonly used in South México to supplement lactating cows, partially replacing the grain based commercial concentrates which are more expensive. Although indigenous to moist forest, ramón is extremely tolerant of drought conditions. It keeps its leaves throughout the year and, therefore, is an important source of forage for any tropical area that suffers feed shortages during the dry season (N.A.S., 1975). Foliage yields from ramón may reach 2 t DM/ha/year. Despite the fact that ramon has been traditionally used by small-scale dairy farmers there is, currently, little knowledge regarding its nutritional value for milk production; although it is known that cattle prefer this forage amongst various fodder trees (Lizarraga *et al*., 2001, Nieto *et al*., 2001). The objective of the experiment described here was to assess the potential replacement value of soyabean meal by ramón in lactating dual purpose cows. A partial summary of this work has been presented elsewhere (Sandoval Castro *et al*., 2001)

# MATERIALS AND METHODS

Four *Bos indicus* x *B. taurus* cows were used in a 4 x 5 latin rectangle (5 treatments and four periods). Each period consisted of 10-days adaptation to the diet and 5-days data collection. At the start of the trial, the experimental animals were, on average, at the 86th day of lactation (s.d. = 22.8), a mean liveweight (LW) of 453 kg (s.d. 22.2) and were producing a saleable milk yield of 4.6 kg (s.d. 1.42). The animals were fed *ad libitum* with chopped Taiwán (*Pennisetum purpureum)* grass, and at milking time were offered 2.0 ± 0.1 kg DM of one of the following supplements (treatments):
A - Sorghum / soybean (14 *per cent* CP);
B - Diet A, plus Ramón providing 33 *per cent* of the supplement N;
C - Diet A, plus Ramón as 66 *per cent* of the supplement N;
D - Ramón 100 *per cent* of the amount of N given by diet A;
E - A negative control, representing a diet without supplements, but with 0.5 kg sorghum to homogenize management at milking time.
Cows were allocated individually in metabolism pens that allowed measurements of basal diet and supplement intake, as well as total collection of faeces. These were used to estimate total diet dry matter (DM), organic matter (OM), neutral detergent (NDF) and acid detergent fibre (ADF) digestibility (D).
Every morning, cows were machine milked in the presence of the calf. Immediately after milking, calves were allowed to suckle residual milk. In the afternoon, cows were not milked, but they were suckled to avoid a depression in milk production due to milk accumulation in the udder.
Total milk yield (TMY) was calculated as the sum of saleable milk (SM), recorded at milking time, and calf-suckled milk (CS); CS was measured (weigh-suckle-weigh) at least three times in each period after the morning milking, and three times after the afternoon suckling.
Saleable milk samples were taken over a three-day period and analyzed for fat (Gerber method, ILCA, 1988), protein and lactose (A.O.A.C., 1980). Basal (grass), supplement and total diet intake was recorded every day throughout the trial, and a pooled sample was taken and analyzed for DM, ash, ether extract (AOAC, 1980), NDF, ADF (Van Soest *et al*., 1991).
Urine spot samples were taken at milking time during the experimental period, and analyzed for allantoin (IAEA, 1997), uric acid and creatinine content (Guerci, 1979) to estimate microbial-N supply to the intestine according to the following equations,:

Microbial-N flow ($g.d^{-1}$) = Purine flow * 70 / 0.116 (Chen *et al.,* 1990)

Where : N content in purine derivatives = 70 mgN/mmol; Ratio Purine-N:Microbial-N = 0.116:1
Purine flow was estimated as:

A:C = 1.03 + 0.00438 (Purine flow) (Vagnoni *et al*., 1997)

Where: A:C = Allantoin:Creatinine in urine (mmol/mmol); Purine flow = purine flow in urine (mmol/d)
Data were analyzed using the general linear model procedure of Minitab release 12 (Minitab, 1997).

# RESULTS AND DISCUSSION

## Intake and digestibility

Chemical compositions of supplements and the basal diet (Taiwan grass) are presented in Tables 1 and 2. It can be seen that, the quality of the supplement, expressed in terms of fibre content (i.e. ADF), varies in relation to the amount of Ramon foliage in the mixture; the content of the remaining components were similar among the treatments. The chemical composition of the basal diet was similar throughout the experimental periods.

**Table 1**

*Chemical composition of the supplements ($g.kg^{-1}$ DM, except were stated).*

| Supplement | DM* | CP | EE | Ash | NDF | ADF |
|---|---|---|---|---|---|---|
| A | 882 (3.1) | 139 (4.4) | 24 (3.0) | 23 (3.5) | 403 (12.1) | 75 (46.1) |
| B | 883 (2.1) | 139 (5.9) | 21 (3.2) | 61 (7.6) | 412 (50.2) | 180 (46.3) |
| C | 887 (2.1) | 129 (4.3) | 24 (1.0) | 92 (12.7) | 435 (27.1) | 224 (25.9) |
| D | 887 (5.6) | 138 (5.6) | 22 (3.0) | 143 (11.2) | 467 (42.1) | 323 (60.9) |
| E | 873 (1.1) | 89 (1.3) | 24 (3.2) | 34 (1.3) | 331 (40.8) | 58 (8.9) |

* $g.kg^{-1}$ Fresh base; ( ): Standard error; A) Sorghum and soybean; B) 33% Ramon; C) 66% Ramon; D) 100% Ramon; (E) Sorghum grain alone.

**Table 2**

*Chemical composition of the Taiwan grass (*P. purpureum*) during experimental periods (g.kg$^{-1}$ DM except were stated).*

| Period | DM* | CP | EE | Ash | NDF | ADF |
|---|---|---|---|---|---|---|
| 1 | 246 (24.7) | 60 (4.0) | 8.9 (2.5) | 73 (8.5) | 793 (6.2) | 466 (18.9) |
| 2 | 262 (28.4) | 61 (3.2) | 9.1 (1.9) | 74 (8.5) | 787 (7.8) | 458 (26.1) |
| 3 | 262 (28.4) | 59 (2.3) | 9.8 (2.0) | 76 (7.2) | 788 (9.7) | 464 (23.4) |
| 4 | 249 (30.1) | 60 (4.0) | 9.9 (1.9) | 76 (7.6) | 789 (10.2) | 453 (21.1) |
| 5 | 256 (33.7) | 60 (4.1) | 9.8 (1.9) | 71 (4.4) | 791 (9.8) | 455 (23.9) |

* g.kg$^{-1}$ Fresh base; ( ): Standard error

Basal diet intake, DMD and OMD of the diets was reduced by Ramon inclusion, probably due to its high fibre and ash contents (*c*. 467 g NDF, 323 g ADF and 142 g Ash/kg DM), compared with the sorghum/soybean diet, which caused a small substitution effect. Total DMI was highest with soybean (Table 3). The lack of statistical difference between grass alone (E) and Ramón supplemented diets was probably due to the higher basal diet intake in treatment E, which allowed for a partial compensation of the low nutrient content of the grass (*c*. 60 g CP, 450 g ADF and 788 g NDF/kg DM).

The substitution effect found when high quality forages (i.e. *Leucaena leucocephala*) or protein or energy rich sources (i.e. cotton seed and molasses) are used for supplementing dual purpose cows under tropical conditions are inconsistent. Ranges from 0.2 to 0.5 kg DM.kg$^{-1}$ supplemental DM have been reported (Combellas and Martinez, 1982, Muinga *et al*., 1995, Osuji *et al*., 1995) depending on the nature of the supplement. Khalili (1993), Khalili et al., (1993) and Osuji *et al*. (1995) found increases as well as reductions in basal diet intake. However, no effect on basal diet intake was found by Muinga *et al*. (1992). The responses, including those from the present experiment, might be explained in terms of the quality of the supplement (i.e. digestibility and chemical composition), which could improve rumen environment, changing total diet digestibility, thus modifying the rumen fill effect.

## Milk production

Diets C and D and E had similar DOMI (4.1, 3.9 and 4.1 kg.d$^{-1}$) (which could also be expressed as energy intake; 679, 650 and 676 kJ.kgW$^{0.75}$d$^{-1}$) but differed in CPI (0.81, 0.80 and 0.63 kg.d$^{-1}$), providing non-confounded evidence of a protein response in milk yield (7.0 and 6.8 vs. 5.5 kg.d$^{-1}$). This shows that supplementation was needed due to the low quality of the basal diet (c. 60 g CP.kg$^{-1}$ DM), which was almost N deficient. Thus, animals fed on tropical grass alone may not achieve their milk yield potential; and although Ramon is fibre rich, its N content offers clear benefits when this kind of diet is fed.

### Table 3

*Basal (BDMI) and total DM intake (DMI), Digestible organic matter intake (DOMI), protein intake (kg.d$^{-1}$)(CPI), ME intake (kJ.kgW$^{0.75}$d$^{-1}$)(MEI), DM, OM, NDF and ADF digestibility of cows fed Taiwan grass* ad libitum *and supplemented with: (A) Sorghum/soybean; (B) A+ 33% Ramon;( C) A + 66% Ramon; (D) 100 Ramon; (E) Taiwan grass alone.*

| | A | B | C | D | E | sed |
|---|---|---|---|---|---|---|
| BDMI | 9.8a | 9.4ab | 8.5b | 8.4b | 9.6a | 0.48 |
| TDMI | 11.8a | 11.4ab | 10.5bc | 10.4bc | 10.1c | 0.48 |
| DOMI | 5.2a | 5.0ab | 4.1bc | 3.9c | 4.1bc | 0.27 |
| MEI# | 864a | 823a | 679b | 650b | 676b | 46 |
| CPI | 0.90a | 0.87a | 0.81b | 0.80b | 0.63c | 0.022 |
| DMD | 0.55a | 0.53ab | 0.51bc | 0.51bc | 0.53ac | 0.017 |
| OMD | 0.47a | 0.45ab | 0.41c | 0.40c | 0.43bc | 0.014 |
| NDFD | 0.45ab | 0.46b | 0.41[a] | 0.41a | 0.45ab | 0.018 |
| ADFD | 0.38ab | 0.37a | 0.31c | 0.34c | 0.40b | 0.017 |

# ME calculated as 15.4 Mj.kg$^{-1}$ DOM

Means within a row followed by the same letter do not differ at the 0.05 level of probability by LSD multiple range test.

Diets A and B had similar intakes of protein (0.90 and 0.87 $kg.d^{-1}$) and ME (864 and 823 $kJ.kgW^{0.75}d^{-1}$) (Table 3) although they differed ($P<0.05$) in total milk yields (Table 4). This provided clear evidence of a milk yield response due to protein quality. The superiority of the sorghum and soybean supplement may rest in the nature and site of digestion of its protein. Ramon is highly degradable in the rumen, and it is not as good a source of by-pass protein as soybean. It has been found that the *in situ* rumen degradation parameters for Ramon are A= 0.374, a= 0.199, b= 0.759 and c= 0.063 (Sandoval *et al.,* 1999) which implies that is will be rapidly available in the rumen, thus reducing the optimal use of its nitrogen, since it is used for microbial growth and then digested in the gut and intestine.

The substitution of Ramon-N for soybean-N caused a reduction in milk yield ($P<0.05$), which was 1.4 $kg.d^{-1}$ with total substitution (18 *per cent* TMY and 25 *per cent* SM) (Table 4). This reduction in milk yield might be associated with the reduced energy content of the supplement as a consequence of Ramon inclusion, which might have precluded total utilization of the supplemental N, which was similar in all supplements. Nevertheless, there was a possible indication (significant at 0.10 level of probability) that TMY with Ramón was higher (by 22 *per cent*) than with no supplementation (E).

Although in tropical forages, energy is considered the primary limiting factor (Stobbs and Thompson, 1975), results found in literature also show a response due to an increased flow of protein to the lower tract. However, benefits of this effect are not clear as the increased milk yields may not always support (economically) the high cost of the supplement (Combellas and Mata, 1992). In addition, if protein flow to the intestine is increased, it becomes a stimulus for the mobilization of body fat reserves (Orskov and Dolberg, 1985). Thus, the higher milk yield achieved with the soybean diet might also result in higher body weight loss. On the other hand, the milk production with Ramon diets might also have been supported by body reserves if demand of nutrient was enough to trigger mobilization. Therefore, longer-term studies need to be done to assess changes in live weight in order to provide clear recommendations.

There were no clear effect ($P>0.05$) on milk composition of the addition of Ramón. However, when expressed as yields of fat, protein and lactose, then yields were greater ($P<0.05$) with sorghum and soybean diet than with Ramón supplemented cows, which in turn were greater ($P<0.05$) than with the unsupplemented cows. Similarly, Muinga *et al*. (1992, 1995) did not find an effect on milk composition of supplementing dual purpose cattle with *L. leucocephala* foliage. There are few additional reports on milk composition responses due to supplementation despite the economic advantages it might represent for milk pricing, probably as a result of the local markets and economic scenarios that do not impose any kind of pressure for this pricing scheme.

**Table 4**

Saleable (SM), calf suckled (CS) and total (TMY) milk yield ($kg.d^{-1}$), composition ($g.kg^{-1}$) and yield of milk constituents ($g.d^{-1}$) of cows fed Taiwan grass *ad libitum* and supplemented with : (A) Sorghum/soybean; (B) A+ 33% Ramon;( C) A + 66% Ramon; (D) 100 Ramon; (E) Taiwan grass alone.

| | A | B | C | D | E | sed |
|---|---|---|---|---|---|---|
| SM | 4.4a | 3.4b | 3.3b | 3.3b | 2.9b | 0.35 |
| CS a.m. | 0.8ab | 0.6a | 1.0b | 0.9ab | 0.8ab | 0.17 |
| CS p.m. | 3.0a | 2.5ab | 2.7a | 2.6ab | 1.8b | 0.35 |
| TMY | 8.2a | 6.5bc | 7.0ab | 6.8bc | 5.5c | 0.67 |
| Milk composition<br>Fat | 31.7ab | 31.4ab | 30.6b | 32.0ab | 32.7a | 0.68 |
| Protein | 26.5 | 24.8 | 25.3 | 24.9 | 24.4 | 1.11 |
| Lactose | 56.0 | 55.4 | 55.8 | 56.1 | 54.3 | 1.12 |
| Yield of constituents | | | | | | |
| Fat | 250a | 203bc | 210b | 208bc | 174c | 16.7 |
| Protein | 212a | 162b | 176b | 165b | 133c | 10.8 |
| Lactose | 460a | 361b | 390a | 378a | 297b | 42.9 |

Means within a row followed by the same letters do not differ at the 0.05 level of probability by LSD multiple range test.

## Purine derivatives excretion

The excretion of purine derivatives and estimated synthesis of microbial-N are presented in Table 5. In spite of the numerical differences amongst diets, no significant (P>0.05) differences were found, probably due to the large variation inherent in the spot sampling method, or possibly reflecting similar efficiencies of N-usage at the ruminal level in all diets. If the latter is the case then it helps to explain the differences in milk yield (Table 4) as caused by the quality and/or site of digestion of the protein contained in soybean and Ramon.

**Table 5**

*Purine derivatives and creatinine excretion (mmol.d$^{-1}$) in spot urinary sample and estimated synthesis of microbial-N.*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Diet | Allantoin | Uric acid | Creatinine | A:C* | Microbial-N** | | |
| | | | | | g.d$^{-1}$ | g.kg$^{-1}$OMI | g.kg$^{-1}$DOMI |
| A | 10.4 | 3.38 | 3.99 | 2.78 | 240.8 | 23.2 | 51.7 |
| B | 10.2 | 3.16 | 5.41 | 2.27 | 170.6 | 16.4 | 38.4 |
| C | 10.6 | 3.49 | 3.86 | 2.76 | 238.6 | 26.4 | 70.5 |
| D | 8.74 | 3.59 | 4.05 | 2.20 | 160.6 | 18.8 | 51.4 |
| E | 8.77 | 3.08 | 3.32 | 2.39 | 220.5 | 23.6 | 55.6 |
| s.e.d. | 1.683 | 0.949 | 1.317 | 0.543 | 74.92 | 8.21 | 21.03 |

* Allantoin:creatinine (mmol/mmol); ** OMI: Organic matter intake; DOMI: Digestible organic matter intake.

It was noticeable that, the efficiency of microbial-N synthesis was higher than the 32 g.kg$^{-1}$ DOMR reported by A.R.C. (1984), suggesting that the methodology of urinary spot sample might have over estimated the real value. This overestimation might arise due to differences among animals (i.e. breed and size) used by Vagnoni *et al*. (1997) and those in the present experiment. However, the purine derivative methodology still allowed a comparison amongst diets and to obtain insight in the N-usage.

# CONCLUSIONS

The replacement of a sorghum and soybean meal supplement with Ramon foliage resulted in a slight milk yield reduction. However, milk quality was not affected by the inclusion of Ramon. Supplementing cows fed on tropical grass alone had a pronounced effect on yield of milk and its components (fat and protein). Intake and digestibility was not affected when soybean meal and sorghum diet was replaced with 33 *per cent* of Ramon. However, total and 66 *per cent* replacement caused a reduction on both intake and digestibility.

Rumen available-N from Ramon seems to be similarly utilized for microbial-N synthesis as soybean rumen available-N. However, due to the short experimental periods used in the present experiment (15 d), it was not possible to assess the effects of treatments on cows' LW change. Thus, additional long-term trials (full lactation) need to be carried out to fully assess energy balance and its implications for animal performance before final conclusions are drawn.

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# REFERENCES

**A.O.A.C.** 1980. Official methods of analysis. 13$^{th}$ Edition. Washington, D.C. USA.

**A.R.C.** 1984. The nutrient requirement of ruminant lovestock, supplement 1. Report of the protein group of the ARC working party. Commonwealth Agricultural Bureau. Farnham Royal. UK.

**Chen X.B., Hovell, F.D.DeB., Orskov, E.R., and Brown, D.S.**1990. Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep. *British Journal of Nutrition*. 63. 131-142.
**Combellas, J. and Mata, D.** 1992. Suplementación estratégica en bovinos de doble propósito. In: Avances en la producción de leche y carne en el trópico americano. Fernández Baca, S. (Ed.). FAO. Santiago, Chile. pp. 99-130.
**Guerci, A.A.** 1979. Métodos de análisis clínicos y su interpretación. 2ª. ed. El Ateneo, Buenos Aires, Argentina.
**I.A.E.A.** 1997. Estimation of rumen microbial protein production from purine derivatives in urine. IAEA-TECDOC-945. IAEA, Vienna, Austria.
**I.L.C.A.** 1988. Rural dairy technology, experiences in Ethiopia. ILCA Manual No. 4. F. O'Mahony (ed).
**Khalili, H.** 1993. Supplementation of grass hay with molasses in crossbred (*B. taurus x B. indicus*) non lactating cows. Effect of level of molasses supplemented on feed intake, digestion, rumen fermentation and rumen digesta pool size. *Animal Feed Science and Technology*. 41: 23-38.
**Khalili, H., Varvikko, T. and Osuji, P.O.** 1993. Supplementation of grass hay with molasses in crossbred (*B. taurus x B. indicus*) non lactating cows: Effect of level of timing molasses supplements on feed intake, digestion, DM degradation and rumen fermentation. *Animal Feed Science and Technology*. 41: 39-50.
**Lizarraga Sánchez, H.L, Solorio and Sandoval Castro C.A.** 2001. Voluntary intake of grass and a forage tree when offered simultaneously. *Proceedings of the British Society of Animal Science*. p: 107.
**Minitab.** 1997. Minitab user's guide 2: Data analysis and quality tools. State College, PA. USA.
**Muinga, R.W., Thorpe, W. and Topps, J.H.** 1992. Voluntary food intake, live weight change and lactation performance of crossbred dairy cows given *ad libitum Pennisetum purpureum* (napier grass var. Bana) supplemented with leucaena forage in the lowland semi-humid tropics. *Animal Production*. 55: 331-337.
**Muinga, R.W., Topps, J.H., Rooke, J.A. and Thorpe, W.** 1995. The effect of supplementation with *Leucaena leucocephala* and maize bran on voluntary food intake, digestibility, live weight and milk yield of *Bos indicus x Bos* taurus dairy cows and rumen fermentation in steers offered *Pennisetum purpureum ad libitum* in the semi-humid tropics. Animal Science: 60: 13-23.
**N.A.S.** 1975. Underexploited tropical plants with promising economic value. National Academy of Science. Washington, D.C. USA. pp: 114-118.
**Nieto-Marin, C., Monforte-Braga, G., Ayala-Burgos, A., Rios-Arjona, G., Sandoval-Castro, C.A., Ramirez-Aviles, L. and Hovell, F.D.DeB** 2001. Short term intake of six local forage trees in Yucatan, Mexico to local Zebu cattle (*Bos indicus*). *Proceedings of the Nutrition Society*. 60: 29A.
**Orskov, E.R. and Dolberg, F.** 1985. Recent advances in ruminant nutrition and their relevance to milk production in developing countries. In. Milk production in developing countries. Smith, A.J. (Ed.). CTVM-University of Edimburgh. UK. pp. 177-192.
**Osuji, P.O., Khalili, H. and Umunna, N.N.** 1995. The effects of cottonseed cake with or without molasses on feed utilization, purine excretion, and milk production of Boran (*Bos indicus*) cows fed a mixture of wheat and oat straw. *Tropical Agriculture (Trinidad)*. 72: 63-69.
**Sandoval Castro, C.A., Anderson, S., and Leaver, J.D.** 1999. Influence of milking and restricted suckling regimes on milk production and calf growth in temperate and tropical environments. *Animal Science*. 69: 287-296.
**Sandoval Castro, C.A., Cetina, R., Pérez, I. and Ramírez Avilés, L.** 2001. Ramon (*Brosimum alicastrum*) foliage as supplement for lactating dual purpose cows. Proceedings of the XIX International Grassland Congress. 11-21 February 2001, Sao Paulo, Brazil. pp: 409-410.
**Stobbs, T.H. and Thompson, P.A.C.** 1975. Milk production from tropical pastures. *World Animal Review*. 13: 27-31.
**Vagnoni, B.D., Broderick, G.A., Clayton, M.K. and Hatfield, R.D.** 1997. Excretion of purine derivatives by Holstein cows abomasally infused with incremental amounts of purines. *Journal of Dairy Science*. 80: 1695-1702.
**Van Soest, P.J., Robertson, J.B. and Lewis, B.A.** 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Jounal of Dairy Science*. 74: 3583-3597.