EFFECTS OF FEEDING ACACIA (*Samanea saman* (Jacq.) Merr.) POD MEAL ON GROWTH PERFORMANCE OF HEIFERS AND IN VITRO RUMEN FLUID METHANE AND CARBON DIOXIDE PRODUCTION IN RUMEN-CANNULATED CATTLE

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ABSTRACT

The objectives of this study were to evaluate the effects of feeding acacia pod meal (APM) on the growth performance of heifers and in vitro carbon dioxide (CO_2) and methane (CH_4) production in rumen-cannulated cattle. In Exp. 1, 18 growing heifers (initial BW: 220 ± 20 kg) were randomly distributed to 3 dietary treatments: 1) 100% Rice bran-copra meal combination (RBC), 2) 50% Rice bran-copra meal-50% APM combination (RCA), and 3) 100% APM. Animals were provided their respective rations for 60 d based on a feeding rate equivalent to 3.5% of the animal's BW (on a DM basis). Dietary treatments were offered at 70:30 roughage to concentrate ratio with the concentrate containing varying levels of APM. Inclusion of APM did not affect ADFI but negatively affected (P<0.05) both ADG and F:G. In Exp. 2, 3 rumen-cannulated cattle were used to measure the in vitro total gas, CO_2 and CH_4 production using either APM, napier grass, and rice bran D1-copra meal as incubation substrate. There were no differences in the quantities of total gas, CH_4, CO_2, and CO^2:CH^4 ratio measured between the treatments. It can be concluded that feeding acacia pod meal to growing cattle negatively affected ADG and F:G. In vitro gas production alone cannot explain the poorer growth performance observed in cattle fed with acacia pod meal.

Keywords: acacia pod meal, heifers, growth performance, carbon dioxide, methane, in vitro gas production,

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INTRODUCTION

The decreasing pasture areas for ruminant production has led to research on inexpensive, non-traditional, and indigenous feed ingredients. Among these feed ingredients are fodder trees. Leguminous trees, being nitrogen-fixers, are naturally rich in proteins. Some tree legume pods are able to store highly soluble carbohydrates making them a potential nutrient source for livestock. In addition, tree legumes are known to contain compounds that facilitate propagation of beneficial microorganisms to support high rates of fermentative digestion. Enhancing levels of productivity by improving diet quality decreases the obligatory methane emissions from fermentation of feed associated with meeting the animal's maintenance requirement. Modifying rumen metabolism also have performance-regulating effects in ruminant animals. For example, changes in bacterial diversity may favor growth of economically-important cellulolytic microbial groups that results in better utilization of dietary cellulosic materials.

However, tree legumes also contain secondary metabolites that have been implicated in limiting the use of trees and shrubs. Compounds in tree pods such as tannins, saponins, and lignin can negatively affect growth of methanogenic bacteria in the rumen which can potentially lower green-house gas emissions (Van Soest, 1992). They can inhibit digestion, have toxic effects, inhibit some enzymes and/or metabolic processes, or act as precursors of anti-nutritional compounds (Palo, 1987). Secondary compounds can also be toxic to animals or cause reduction in their productivity by reducing feed intake.

Samanea saman Jacq. Merr. or locally known as acacia is a leguminous tree planted as shade whose leaves are utilized as a good dry matter reserve for summer months. Previously, adding acacia pods to a rice straw diet enabled buffaloes to maintain their BW throughout the dry season (Seetakoses *et al.*, 1988). *Samanea saman* were also shown to inhibit growth of ruminal protozoa leading to better ruminant performance (Teferedegne, 2000). Therefore, evaluating the feeding value of acacia pod meal in growing cattle and determining its rumen-modifying properties in terms of in vitro methane and carbon dioxide production is worth investigating.

This study aimed to determine the effects of feeding acacia pod meal (APM) on the growth performance of heifers, in vitro carbon dioxide (CO_2) and methane (CH_4) production in rumen-cannulated cattle.

MATERIALS AND METHODS

The study was conducted from July 2011 to January 2012 at the Animal Nutrition Analytical Service Laboratory (ANASL), and Animal Biotechnology Laboratory, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna.

Sample preparation and chemical analysis

Fallen ripe acacia pods were collected from different areas of Laguna, Philippines and chopped into smaller pieces (2.54-3.81 cm) prior to oven-drying at 105°C for 8 h. Samples were subjected to proximate analyses using AOAC methods (AOAC, 1995).

Exp. 1: Feeding trial for growing animals

Eighteen growing heifers (initial BW: 220±20 kg) were randomly distributed to 3 treatments: 1) 100% rice bran-copra meal mix (RBC), 2) 50% rice bran-copra meal- 50% Acacia pod meal combination (RCA) and 3) 100% Acacia pod meal (APM). There were 6 replicates per treatment. Animals were provided their respective diets for 60 d based on a feeding rate equivalent to 3.5% of the animal's BW (on a DM basis). Dietary treatments were supplemented with concentrate provided at 70:30 roughage to concentrate ratio with varying APM inclusion. Animals were individually weighed at d 0, 30, and 60 (end of experiment). Daily feed consumption was monitored by recording the amount of feed offered and feed refused. Periodic and cumulative ADG, ADFI, and F:G (on a DM basis) were calculated and compared among the treatments.

Table 1. Nutrient composition (DM basis) of feedstuffs included in the experimental diets.

Nutrient, %	Napier grass[1]	Rice bran-copra concentrate mix[2]	Acacia pod meal[3]
DM	30.60	88.40	88.70
CP (N × 6.25)	7.97	17.48	17.33
TDN	65.00	68.86	67.51[4]
NDF	61.07	43.60	43.16
ADF	46.00	21.56	39.45
Acid detergent lignin	7.20	9.21	22.79
Ash	13.10	8.43	3.77
Cellulose	24.00	16.20	16.66
Hemicellulose	29.80	22.04	3.71
Lignin	5.87	0.78	19.02

[1]Feed composition table for the Philippines, PCAARRD (1984).
[2]Contain Rice bran D1 (41.4%) and Copra meal (58.56%). Nutrient contents are calculated from the nutrient content of the ingredients from National Research Council for Dairy (2001) and Beef (2000).
[3]As analyzed at the Animal Nutrition Analytical Laboratory, ADSC-CA, UPLB.
[4]Hosamani *et al.* (2005)

In vitro rumen fluid relative gas production analyses

The efficiency of rumen fermentation is measured by the volume of gas evolved. Gas production potential of heifers fed with different dietary treatments was measured using the in vitro gas production based on the modified methods of Halliwell (1954) and Menke and Steingass (1988). Modifications include type of syringe used, buffer components and incubation period. Rumen fluid samples were collected through gastric tube from each animal 2 h after morning feeding on d 0, 15, 30 and 60. The procedure followed the steps in Figure 1 using concentrate feed as substrate. Since the purpose is simply to compare gas production potential, the incubation period lasted only for 24 h rather than the 40 h in the procedure by Menke and Steingass (1988). At the end of the incubation period, total volume of gas (in ml) produced was recorded.

Exp. 2: In vitro production analysis using on rumen-cannulated cattle

Three (3) rumen-cannulated cattle housed in individual elevated metabolism stalls were fed at 3% of their BW (on a DM basis) with 3 treatments using a replicated 3 X 3 Latin square design. Dietary treatments were: 1) All napier grass (NG), 2) Napier grass + concentrate feed (NC), and 3) Napier grass + Acacia pod meal (NA). Treatments 2 and 3 were added to the ration to achieve roughage to concentrate ratio of 70:30.

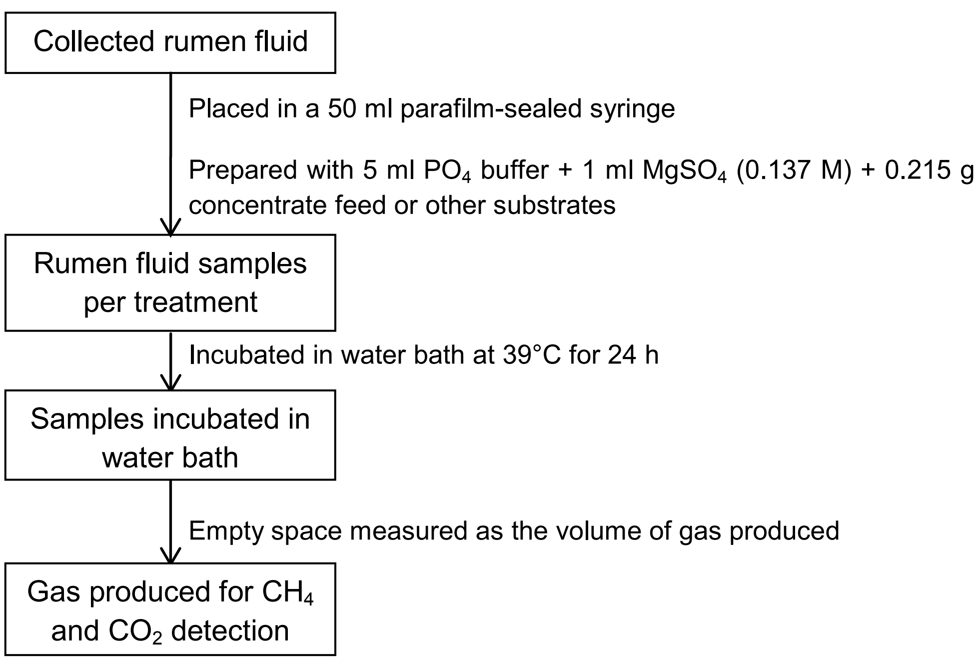

Figure 1. Diagrammatic procedure of in vitro gas production

In vitro gas production analyses

At the end of each round of collection, samples of rumen fluid were obtained and were subjected to in vitro gas production assay following the modified methods of Halliwell (1954) and Menke and Steingass (1988). In experiment 2, napier grass, concentrate feed and acacia pod meal were used as substrates.

Rumen fluid in vitro carbon and methane production analyses

Quantification of the type of gas produced was carried out by using a dual portable gas analyzer (Figure 2) that has detectors for methane (CH_4) and carbon dioxide (CO_2). Ten (10 ml) of total gas produced in each syringe was collected using an improvised gas container consisting of a 10 ml syringe, 'Corning' gas cylinder, and a needle permanently attached to a portable gas analyser. The syringe was pulled to the required volume of gas (1ml-10 ml) to be injected from the 50 ml syringe of rumen sample. The 10 ml and 50 ml syringes were pumped in and out 3 times simultaneously to properly distribute the gas sample inside the chamber. Reading of methane and carbon dioxide concentration (in ppt) was recorded after 30 sec.

Figure 2. Non-dispersive infrared gas detector used to quantify methane and carbon dioxide from the in vitro gas production of rumen fluid from cattle with different diets. Sensitivity: parts per thousand

Statistical analyses

Data were analyzed using ANOVA in SAS (SAS Inst. Inc., Cary, NC). Pairwise comparisons of treatment means were performed using the Duncan's Multiple Range Test. Statistical significance were set at $P \leq 0.05$ for all statistical tests.

RESULTS AND DISCUSSION

Exp. 1: Feeding trial in growing heifers

Inclusion of APM on rations of growing heifers did not affect ADFI and cumulative feed intake as % of BW; however, heifers fed the APM diet had reduced (P<0.05) ADG and poorer (P<0.01) F:G compared with those fed the RBC diet after 30 and 60 d of feeding (Table 2). Despite the diets being formulated close to levels in the concentrate mix, APM-fed cattle exhibited poorer growth rate which may be attributed to compounds that hinder optimal growth performance of growing heifers. This suggests the possible presence of secondary compounds in APM, that despite high voluntary feed intake, growth performance was negatively affected.

Table 2. Growth performance (mean ± SD) of growing heifers fed acacia pod meal, Exp. 1.

Item	Treatment[1]			CV, %
	RBC	RCA	APM	
n	6	6	6	--
d 0 to 30				
ADFI, kg DM	7.62±0.14	7.57±0.35	7.67±0.34	3.85
Cumulative FI as %BW, DM basis	3.21±0.12	3.23±0.13	3.33±0.21	4.79
ADG, kg	1.03±0.13[a]	0.75±0.23[ab]	0.67±0.20[b]	23.69
F:G	7.52±1.05[a]	11.21±2.87[ab]	12.16±3.27[b]	19.44
d 31 to 60				
ADFI, kg DM	8.84±0.12	8.82±0.34	8.94±0.33	3.05
Cumulative FI as %BW, DM basis	3.49±0.07	3.59±0.14	3.70±0.19	3.62
ADG, kg	0.67±0.07[a]	0.39±0.11[ab]	0.25±0.12[b]	22.78
F:G	13.41±1.59[a]	21.48±2.94[b]	30.53±21.8[c]	32.07

[1]Dietary treatments were: 1) 100% Rice bran-copra meal combination (RBC), 2) 50% Rice bran-copra meal-50% APM combination (RCA), and 3) 100% APM to contain roughage to concentrate ratio of 70:30 (APM).

[a,b,c]Within a row, means without a common superscript differ (P<0.05)

In vitro total gas production

No differences in in vitro total gas production were observed at d 0 and 15. At d 30, APM had the least (*P*<0.05) gas produced among the treatments while at d 60, the same treatment had the highest (*P*<0.05) gas production.

Table 3. In vitro total gas production (ml) in rumen fluid from growing heifers incubated for 24 h at 39°C with concentrate feed as substrate at d 0 (initial), 15, 30 and 60.

Item	Treatment[1]			CV, %
	RBC	RCA	APM	
Day of incubation				
0 d	8.57	11.29	11.19	25.92
15 d	10.13	10.59	9.29	5.61
30 d	7.44[a]	9.57[a]	5.10[b]	18.77
60 d	7.67[a]	6.92[a]	10.26[b]	11.74

[1]Dietary treatments were: 1) 100% Rice bran-copra meal combination (RBC), 2) 50% Rice bran-copra meal-50% APM combination (RCA), and 3) 100% APM to contain roughage to concentrate ratio of 70:30 (APM).
[a,b]Within a row, means without a common superscript differ (*P*<0.05)

The quantity of gas and VFA produced during fermentation reflects both the amount of substrate digested and the microbial metabolic pathways. Traditionally, feeding ruminants at high roughage to concentrate ratio leads to greater production of ketogenic VFA (acetic and butyric acid) whereas increasing the proportion of the concentrate leads to greater production of glucogenic VFA (propionic acid). Generating more glucogenic VFA results in a reduction of noxious gas production.

In this study, APM in the diet at d 30 resulted in rumen fluid that has the least in vitro gas production potential. This may suggest that secondary compounds present in APM may have resulted in a reduction of fermentative microorganisms that can lead to poorer growth performance. Phytochemical screening of acacia revealed the presence of tannins, flavanoids, and saponins (Hosamani *et al.*, 2005). Condensed tannins are thought to directly inhibit methanogens, as well as indirectly limit methanogenesis through a reduction in hydrogen availability (Tavendale *et al.*, 2005). At greater levels (5–9%), tannins become highly detrimental (Barry, 1983) as they reduce digestibility of fiber in the rumen (Reed *et al.*, 1985) by inhibiting the activity of bacteria (Chesson *et al.*, 1982) and anaerobic fungi (Akin and Rigsby, 1985). However, feeding APM for 60 d resulted in rumen fluid with the highest in vitro gas production potential. This suggests that the animals already adapted, leading to the increase in in vitro gas production potential.

Exp 1. In vitro gas production using rumen-cannulated cattle

In vitro carbon dioxide and methane production

No differences were observed in the quantities of total gas, CH^4, CO^2, and CO^2:CH^4 ratio among the rumen-cannulated cattle fed with all the diets with their rumen fluid samples subjected to napier and concentrate substrates. This suggests that APM do not significantly alter the gas production potential of the animals and is comparable to cattle fed with all napier and concentrate supplemented rations. This is in contrast with Lovett *et al.* (2005), where greater proportion of concentrate in the diet results in a reduction in methane emission. Fermentation of cell wall fiber yield higher acetic:propionic acid and higher methane losses (Moe and Tyrrell, 1979; Beever *et al.*, 1989). Moe and Tyrrell (1979) found fermentation of soluble carbohydrate to be less methanogenic than cell wall carbohydrates. Since rice bran-copra meal in concentrate feed is classified as a cell wall carbohydrate, there should be greater reduction in methane.

Table 4. Total gas, carbon dioxide (CO_2), and methane (CH_4) production and CO_2:CH_4 ratio (mean±SD) of incubated rumen fluid with different substrates from rumen-cannulated cattle fed with all napier grass (NG), napier grass + concentrate feed (NC), and napier grass + APM (NA), Exp. 2.

Item	Treatment			CV, %
	NG	NC	NA	
Total gas production, ml				
Napier^{ns}	10.33±1.26	11.00±3.00	14.17±0.76	2.48
Concentrate^{ns}	10.50±3.04	11.67±2.89	14.17±2.02	2.20
Acacia pod meal^{ns}	8.50±1.00	8.33±2.75	10.67±0.76	3.11
CO_2 production, ml				
Napier^{ns}	0.91±0.32	1.15±0.45	1.61±0.30	3.16
Concentrate^{ns}	1.10±0.50	1.43±0.60	1.63±0.43	3.46
Acacia pod meal^{ns}	0.79±0.25	0.68±0.40	1.14±0.23	6.54
CH_4 production, ml				
Napier^{ns}	0.25±0.06	0.35±0.25	0.37±0.01	5.96
Concentrate^{ns}	0.21±0.08	0.31±0.20	0.31±0.07	5.28
Acacia pod meal^{ns}	0.19±0.02	0.23±0.16	0.32±0.13	8.02
CO_2:CH_4 ratio				
Napier^{ns}	3.64±0.44	3.77±1.04	4.38±0.91	2.63
Concentrate^{ns}	5.34±1.88	5.11±1.02	5.34±0.95	1.84
Acacia pod meal^{ns}	4.14±0.81	3.08±0.51	3.74±0.86	0.27

^{ns} non-significant

There may be a diurnal variation in the production of CH_4 and CO_2 (Pedersen *et al.*, 2008) which contributed to the variable results of the experiment. This may be caused by the variation in animal activity and fermentation rate depending on feed intake.

CONCLUSION

In conclusion, feeding acacia pod meal to growing heifers result in a reduction in growth rate and feed efficiency. The presence of secondary compounds in acacia pod meal may result in rumen-modifying properties; however, in vitro gas production in rumen fluid was unaffected when cattle was fed with different levels of acacia pod meal. This suggests that in vitro gas production alone cannot explain the poorer growth performance observed in cattle fed with acacia pod meal.

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