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Assessment on the Replacement Value of the Banana (*Musa paradisiaca*) Plant By-Products for Their Fodder Potential in Complete Diet of Ruminants

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Abstract: In an effort to assess the replacement value of banana by-products viz., stem, psedostem and leaves over conventional roughage ten iso-nitrogenous and isocalorofic rations were formulated. Conventional roughage at 60% in the complete diet (control) replaced by any of the banana by-products viz., stem, psedostem and leaves either individually or in combination at two levels (30 or 60%) was evaluated in four measurements at 24 h through Hoheneim gas production test on digestibility and rumen fermentation pattern. The percent molar proportion of acetate: butyrate: propionate across the diets ranges from 63.8-86:1.7-6.5:11.5-30.66. The level of banana leaves in the complete diet proportionately influenced acetate to propionate ratio. Consequently Non Glucogenic Ratio (NGR) was also influenced and the impact was observed wherever leaves are included in the diet. The values of true digestibility indicated that leaves and psedostem at 30% in complete diet were comparable to control. It was observed that as the level of inclusion of leaves or pseudostem increased from 30 to 60%, depression ($p<0.05$) in digestibility was noticed, where as such trend was not observed in stem and were comparable to 60% of combination of any two banana by-products. The ATP yield for the ten different complete diets did not show any significant difference. However, the efficiency of microbial biomass production revealed that only banana leaves at 30% was comparable to control and the rest were significantly ($p<0.05$) lower. The ^{Y}ATP was highest ($p<0.05$) in control followed by leaves at 30%. The data on ^{Y}ATP, volatile fatty acids, gas produced, microbial mass generated in relation to degraded substrate of the ten complete diets indicate that control diet had the most preferred fermentable characteristics and comparable to that was leaves at 30%, where as rest diets were inferior. Hence it is concluded that banana leaves could be used in complete diet up to 30% inclusion level.

Key words: Banana, by products, stem, leaves, pseudostem, fermentation

INTRODUCTION

Animal agriculture is one of the most important components of global agriculture. However, a major constraint of livestock rearing in tropical countries is chronic feed shortage. Tapping the available fodder resources is one of the options to bridge the gap. Crop residues are the best resources to be explored in this context; as such attempts do not require additional cultivable land. However, very few of fodder have been screened for their use in animal feed. Many more are yet to be evaluated before being used as animal feed. Banana by-products are one among them.

Banana (*Musa paradisiaca*) is a traditional plant cultivated widely for its fruits. After harvesting of the fruit, the various other parts of the plant (by products) are not effectively utilised. It has been

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estimated that a residual biomass (pseudostem and leaves) of 13-20 tonnes dry matter ha^{-1} $year^{-1}$ is available. Gupta *et al.* (2001) suggested that feeding of whole banana plants (stem and leaves) will meet the maintenance requirement of cattle. Hembade and Patel (2004) concluded that banana leaves can be incorporated in the diet of kids. However, at present they are thrown out as waste on roadsides, or allowed to rot away in the fields or some times burnt in the field.

Amarnath and Balakrishnan (2007) studied the efficacy of banana by products to serve as a potential source of roughage to ruminants and observed that the microbial biomass production, energetic efficiency of volatile fatty acids and ^{Y}ATP were significantly ($p<0.05$) highest in leaves compared to stem and pseudostem at T½ of c.

They concluded that among by-products evaluated for their fodder potential to cattle, banana leaves rank first with low partition factor, high ^{Y}ATP and high microbial biomass followed by pseudostem and banana stem. However, the use of banana stem, pseudostem and leaves as a roughage component in the complete diet of ruminants is not yet assessed. Hence a study was conducted in this direction to assess the optimal level of inclusion in the complete diet of ruminants.

MATERIALS AND METHODS

The use of banana stem, pseudostem and leaves as a roughage component in the complete diet of ruminants was investigated through Hohenheim gas production test (Menke and Steingass, 1988). Ten iso-nitrogenous and isocalorofic rations were formulated. The crude protein content and TDN values of banana by-products (Amarnath, 2002) were taken into consideration while formulating the complete diets. The experiment was conducted at Department of Animal nutrition, Madras Veterinary College, Chennai during summer. The stem, pseudostem and leaves were incorporated at two levels in the rations as roughage component, replacing the conventional roughage by 50 or 100% either alone or in combination. Percent ingredient compositions of ten complete diets are furnished in Table 1. The ingredients of the complete diets were individually weighed and incubated in the respective syringes and four measurements were made for each of ten diets tested.

Proximate Composition

Four samples of each complete diets were analysed for their proximate principle, viz., total dry matter, crude protein, ether extract, crude fibre and total ash as per the methods of AOAC (2000). Nitrogen, ether extract and crude fiber were estimated using kjeltec system (Model No. 1002 Tecator, Sweden), Soxtec system (Model No. 1043, Tecator, Sweden) and Fibretec system (Model No. 1020, Tecator, Sweden), respectively. Nitrogen free extract was calculated by difference.

In vitro Gas Production Studies

The rumen liquor was obtained from three Jersey crossbred cattle maintained on grazing alone to ensure that cellulolysis was optimum. All the laboratory handling of rumen fluid was carried out under

Table 1: Percent ingredient composition of complete diet having 12% crude protein* and 58% TDN*

Ingredients	Control	L30	L60	PS30	PS60	S30	S60	L30PS30	L30S30	PS30S30
Test mix**	35	35	35	35.0	35	35.0	35	35.0	35	35.0
Wheat bran	5	5	5	-	5	-	-	-	-	-
Maize	-	-	-	2.5	-	2.5	-	2.5	2	2.5
GNC	-	-	-	2.5	-	2.5	5	2.5	3	2.5
Napier hybrid grass	60	30	0	30.0	0	30.0	0	0.0	0	0.0
Banana leaves	0	30	60	0.0	0	0.0	0	30.0	30	0.0
Banana Pseudostem	0	0	0	30.0	60	0.0	0	30.0	0	30.0
Banana stem	0	0	0	0.0	0	30.0	60	0.0	30	30.0

*Calculated values, ** Test mix (g/100 g) contains maize 35, pearl millet 35, GNC 20, wheat bran 7, salt 1, etc. Mineral mix guaranteed to contain calcium-28%, phosphorus-9%, iron-0.06%, iodine-0.1% and manganese-0.12%, marked by Bell Devx, 95 Max Min, L = Banana leaves; PS = Banana Pseudostem; S = Banana stem

continuous flushing with CO_2. Accurately weighed 500 mg samples and 40 mL rumen buffer volume was taken per 100 mL glass syringe for incubation as described by Blummel and Becker (1997) to minimize the analytical error in gravimetric determinations of apparent and truly degraded substances. The syringe nozzle was fastened with butyl rubber cap designed to fit airtight.

Three blanks containing rumen buffer alone were included for each incubation period. The syringes were rotated at one rotation per minute as described by Menke *et al*. (1979). The piston of the syringes was reset to 10 mL mark by releasing the gas production whenever it reached the level of 60 mL mark. The release of gas was done by withdrawing the gas with the help of another syringe by piercing through the butyl rubber cap of the 100 mL syringe with 23G needle. This step prevents the atmospheric air equilibrating the fermented gas in the process of releasing the excess gas. Net gas volume at each incubation hour was calculated by subtracting the mean gas volume of blank from mean gas volume of syringe with sample. The volume of gas produced (mL) was studied at 24 h of incubation.

At the end of 24 h of incubation, the whole content of the syringes was transferred into 45 mL capped centrifuge tubes. Residues were centrifuged in an ultracentrifuge (HIMAC, model SCR 20BA, Hitachi) at 20,000 g for 30 min at 4°C. About 2.5 mL of supernatant was collected for VFA and added into glass tubes, which already contained 0.5 mL metaphosphoric acid (25%). Thetotal and individual short chain fatty acids concentrations were measured by gas chromatograph method as per the procedure of Chase (1990). Netel make of gas chromatograph model omega QC was used in this study. The internal standard used in analysis was ethyl butyric acid. Stainless steel column of 6 inches x 2 mm internal diameter packed with 10% SP 1200/1% phosphoric acid and on 30/100 chromosorb W(AW), was used in the present study of separating C_2 to C_5 volatile fatty acids in the sample. Column temperature was maintained at 130°C, inlet temperature and Detector temperature were maintained at 170 and 210°C, respectively. The flow of carrier nitrogen was kept at 40 mL min^{-1}. The flame ionization detector was used. The concentrations of volatile fatty acids were calibrated from the standards.

Stoichiometry Derivations

The following stoichiometrical derivations were considered in this study.

- Calculation of CO_2 and CH_4 produced from VFA (Wolin, 1960):

CO_2 (moles) = a/2 + p/4 + 1.5 x b
CH_4 (moles) = a + 2b − CO_2

Where, a = acetate, b = butyrate and p = propionate.

In *in vitro* gas production, additionally 1 mmol of SCFA produces 1 mmol of CO_2

Volumes (v) were calculated as:

V = mmol x gas constant x temperature (K)/pressure (p)

- The relationship between SCFA production and ATP production (1997a)

1 mmol acetate = 2 mmol ATP
1 mmol propionate = 3 mmol ATP
1 mmol butyrate = 3 mmol ATP
1 mmol CH_4 = 1 mmol ATP

- $\text{A/P ratio} = \frac{\text{Acetate}}{\text{Propionate}}$

- Energy efficiency of VFA = 0.622 A + 1.092 P + 1.56 B/A + P + 2B (where, A, P and B represent acetic, propionic and butyric acids (Mol percent) (Orskov *et al*., 1968)

***In vitro* Degradability Studies**

The degradability studies on banana by-products were studied at 24 h of incubation.

The apparent digestibility was determined as per Blummel *et al*. (1997b). The *in vitro* true degradabiliy was determined by refluxing the pellet contained in the centrifuge tubes to separate the microbial mass from the undegraded substrate. To achieve this, the pellet was transferred from the tubes into 600 mL spoutless beakers and the centrifuge tubes were thoroughly washed with about 70 mL neutral detergent solution and refluxed for 1 h (Van Soest and Robertson, 1985) which was added to the beakers. True degradability was calculated as the weight of substrate incubated minus the weight of the residue after NDS treatment.

Partition Factor

Partition factor was calculated using the following formula (Blummel *et al*., 1994 and 1997a)

$$\text{Partition factor (PF) mg mL}^{-1} = \frac{\textit{In vitro}\text{ turly degraded substrate}}{\text{Volume of gas produced}}$$

Microbial Biomass

The microbial biomass was calculated from the equation quoted by Blummel *et al*. (1997a).

Microbial biomass = Substrate truly degraded-Substrate apparently degraded

Efficiency of Microbial Production

The efficiency of microbial production was calculated using the following formula:

$$\text{Efficiency of microbial production} = \frac{\text{Microbial biomass}}{\text{Substrate turly degraded}}$$

^{Y}ATP

^{Y}ATP was calculated using the following formula:

$$^{Y}\text{ATP} = \frac{\text{Efficiency of microbial production}}{\text{ATP produced}}$$

RESULTS AND DISCUSSION

Proximate Composition

The results on the proximate principles (Table 2) indicated that the analytical values for crude protein are in agreement to the calculated values. The percent crude protein, crude fiber and ether extract of ten complete diets were comparable with one another. The results further indicated that there exists a significant ($p<0.05$) difference in the total ash content and consequently in the nitrogen free extract of the ten complete diets tested. Highest ($p<0.05$) total ash was observed at 60% inclusion of banana stem. The diet containing 30% banana stem either alone or in combination was comparable to control diets. The rest of the diets were significantly ($p<0.05$) lower in total ash. The higher total ash content (27.90%) of banana stem reported by Amarnath (2002) contributed to the higher total ash content of banana stem included complete diets either alone or in combination. Similar higher level of total ash (29.2%) has been reported by Pieltain *et al*. (1999) in Banana stem.

Table 2: Percent proximate composition (Mean±SE) of the ten complete diet formulated to test the utility of banana by products (on DM basis)

Complete diets	Crude protein	Crude fibre	Ether extract	Total ash	Nitrogen free extract
Control	12.85±0.95	10.21±0.85	2.35±0.48	11.21±0.52^{b}	63.38±3.10^{b}
L30	12.83±1.00	11.01±1.13	2.41±0.52	9.26±0.67^{a}	64.49±4.01^{b}
L60	12.87±0.74	10.78±0.98	2.56±0.69	7.54±0.71^{a}	66.25±3.20^{b}
PS30	12.53±1.08	10.56±0.72	2.17±0.47	9.51±0.63^{a}	65.23±3.50^{b}
PS60	12.80±0.89	10.78±1.19	2.18±0.35	8.53±0.58^{a}	65.71±2.90^{b}
S30	12.51±0.82	10.91±1.50	2.16±0.81	14.68±0.68^{b}	59.74±4.60^{a}
S60	12.41±1.09	10.26±1.41	2.33±0.70	17.92±0.54^{c}	57.08±3.06^{a}
L30PS30	12.72±1.16	10.38±0.94	2.12±0.66	8.12±0.59^{a}	66.66±4.40^{b}
L30S30	12.97±0.90	11.01±1.17	2.13±0.80	11.75±0.62^{b}	62.14±2.80^{ab}
PS30S30	12.45±0.68	10.57±1.02	2.71±0.51	12.55±0.57^{b}	61.25±3.50^{ab}

Mean of four observations, Means bearing different superscript within column differs significantly ($p<0.05$), L= Banana leaves; PS = Banana Pseudostem; S = Banana stem

Table 3: The level of VFA and their derivations as effected by ten complete diet containing banana by products at different levels (Mean±SE) at 24 h of incubation

Complete diets	Acetate	Butyrate	Propionate	A:P	NGR	Energetic efficiency (%)	CO_2:CH_4
	---------- mmol L^{-1} ----------						
Control	38.16±2.01^{d}	2.56±0.34^{b}	11.80±1.05^{c}	3.23±0.19^{a}	16.89±2.04^{a}	44.41±2.41^{d}	1.49±0.51
L30	36.71±1.54^{d}	1.79±0.36^{b}	5.36±0.91^{b}	6.85±0.28^{b}	22.53±2.31^{b}	34.13±3.15^{c}	1.25±0.28
L60	22.28±1.09^{a}	0.44±0.41^{a}	2.97±0.64^{a}	7.51±0.22^{b}	52.41±1.94^{c}	18.45±2.18^{a}	1.18±0.34
PS30	20.38±1.26^{a}	2.06±0.39^{b}	9.20±0.59^{c}	2.22±0.31^{a}	11.90±1.57^{a}	29.05±2.31^{bc}	1.75±0.28
PS60	31.70±1.38^{c}	2.20±0.46^{b}	10.30±0.64^{c}	3.08±0.19^{a}	16.41±1.28^{a}	37.68±2.42^{c}	1.51±0.36
S30	18.27±1.67^{a}	1.69±0.32^{b}	6.44±0.81^{b}	2.84±0.17^{a}	12.81±1.38^{a}	23.61±2.16^{b}	1.59±0.45
S60	24.95±1.69^{b}	1.68±0.29^{b}	7.48±0.72^{b}	3.34±0.21^{a}	16.87±1.64^{a}	28.82±2.38^{b}	1.47±0.28
L30PS30	32.39±2.04^{c}	1.78±0.36^{b}	6.56±0.64^{b}	4.94±0.22^{ab}	20.18±2.05^{b}	32.74±2.46^{c}	1.33±0.34
L30S30	20.31±1.08^{a}	1.76±0.28^{b}	9.76±0.59^{c}	2.08±0.17^{a}	13.55±1.08^{a}	28.70±2.33^{b}	1.77±0.33
PS 30S30	36.76±1.28^{d}	1.83±0.31^{b}	8.22±0.67^{bc}	4.47±0.16^{a}	22.09±1.21^{b}	37.41±2.61^{cd}	1.34±0.41

Mean of four observations, Means bearing different superscript within column differs significantly ($p<0.05$), L = Banana leaves; PS = Banana Pseudostem; S = Banana stem

Fermentation End Products and Stoichiometric Derivations

The details on the VFA produced (mmol L^{-1}), acetate to propionate ratio, non glucogenic ratio, energetic efficiency and CO_2 to CH_4 ratio for the ten complete diet tested are furnished in the Table 3.

The molar proportion of acetate: butyrate: propionate across the diets ranges from 63.8-86:1.7-6.5:11.5-30.66. The acetate and butyrate production of control group was comparable to L30 and PS30S30 and significantly ($p<0.05$) differed with L60. In all other diets either acetate or butyrate differed ($p<0.05$). The propionate production was comparable to control in PS30, PS60, L30S30 and PS30S30 and significantly ($p<0.05$) lowered in the rest of the diets L30 recorded the lowest propionate production.

Consequent to the variation in the molar proportion of VFA in the ten different complete diets, the acetate to propionate ratio was significantly ($p<0.05$) higher in L30 and L60 diet than the rest of the eight diets which include control. The NGR was highest in L60 and was significantly ($p<0.05$) different from L30, L30PS30 and PS30S30. All the other diets had significantly ($p<0.05$) lower NGR than the above mentioned diets.

An in-depth analysis of their acetate to propionate ratio and NGR revealed that banana leaves plays an important role in shifting the molar concentration of VFA. The acetate to propionate ratio was proportionately influenced by the level of banana leaves. Consequently NGR was also influenced and the impact was observed wherever leaves are included in the diet. Amarnath and Balakrishnan (2007) recorded higher and consistent acetate to propionate ration in leaves compared to stem and pseudostem. Thus leaves exhibits it's influence in complete diet. Higher acetate to propionate ratio as well as elevated NGR reflects poor utilisation of VFA.

Table 4: Percent apparent of true DM digestibility, microbial biomass production (mg), cumulative gas produced (mL), ATP (mmol L^{-1}), percent efficiency of microbial production, ^{Y}ATP and partition factors at 24 h of incubation of the complete diets tested

Parameters	Control	L30	L60	PS30	PS60	S30
ADMD	40.60±3.45^{b}	42.33±3.87^{b}	40.32±3.29^{b}	48.66±4.19^{c}	35.33±3.48^{a}	49.33±4.61^{c}
TDMD	64.40±5.67^{c}	61.90±5.34^{c}	50.20±4.68^{b}	62.20±5.62^{c}	40.40±4.62^{a}	53.80±4.99^{b}
MBP	23.80±1.23^{c}	19.57±1.05^{c}	9.88±0.67^{b}	13.54±1.14^{bc}	5.07±0.58^{a}	4.47±0.41^{a}
GAS	70.50±6.21^{c}	60.00±5.63^{b}	50.00±4.50^{a}	61.00±5.72^{b}	52.00±4.38^{a}	63.00±4.68^{b}
ATP	2.60±0.23	2.57±0.13	2.55±0.25	2.64±0.16	2.61±0.22	2.62±0.16
EMP	37.00±0.05^{c}	32.00±0.02^{c}	20.00±0.02^{b}	22.00±0.01^{b}	13.00±0.01^{b}	8.00±0.01^{a}
^{Y}ATP	9.14±0.53^{d}	7.61±0.42^{c}	3.88±0.30^{a}	5.13±0.41^{b}	1.94±0.20^{a}	1.70±0.11^{a}
PF	4.57±0.19	5.16±0.48	5.02±0.46	5.10 ±0.38	3.88±0.36	4.20±0.48

Table 4: Continued

Parameters	Control	S60	L30PS30	L30S30	PS30S30
ADMD	40.60±3.45^{b}	48.33±4.21^{c}	48.40±4.68^{c}	44.66±3.91^{b}	44.66±3.28^{b}
TDMD	64.40±5.67^{c}	52.30±4.26^{b}	53.70±4.26^{b}	49.20±4.28^{b}	49.50±4.61^{b}
MBP	23.80±1.23^{c}	3.97±0.38^{a}	5.3.00±0.37^{a}	4.54±0.34^{a}	4.84±0.51^{a}
GAS	70.50±6.21^{c}	48.00±5.37^{a}	52.00±6.48^{a}	51.00±5.13^{a}	49.50±5.38^{a}
ATP	2.60±0.23	2.60±0.24	2.58±0.16	2.63±0.24	2.58±0.22
EMP	37.00±0.05^{c}	8.00±0.01^{a}	10.00±0.01^{a}	9.00±0.01^{a}	10.00±0.01^{a}
^{Y}ATP	9.14±0.53^{d}	1.52±0.09^{a}	2.05±0.08^{a}	1.72±0.12^{a}	1.87±0.10^{a}
PF	4.57±0.19	5.45±0.39	5.00±0.37	4.82±0.45	5.10±0.47

Mean of four observations, Means bearing different superscript within column differs significantly ($p<0.05$), L= Banana leaves; PS = Banana Pseudostem; S = Banana stem

The energetic efficiency was lowest in L60 and highest in control. The CO_2:CH_4 did not show any significant difference among the ten complete diets. Apparent digestibility indicates that complete diets L30, L60, L30S30, PS30S30 have comparable digestibility with control, while the PS60 had significantly ($p<0.05$) lowest digestibility. The rest diets had significantly ($p<0.01$) better digestibility than control diet.

The excellent performance of diets containing leaves (L30, L60) had widest acetate to propionate ratio and high NGR. Diets containing stem (high total ash) in combination with other by-products were able to register better apparent digestibility inspite of their ash content.

However, when the values of true digestibility (Table 4) were examined, only L30 and PS30 were comparable to control and were significantly ($p<0.05$) higher than the rest. The true digestibility could be assessed according to the level of inclusion of test materials (banana stem, pseudostem and leaves). It was observed that as the level of inclusion of leaves increased from 30 to 60%, depression ($p<0.05$) in digestibility was noticed. Similarly, when the pseudostem was enhanced from 30 to 60%, depression ($p<0.05$) in digestibility was observed, where as such trend was not observed in stem and were comparable to L30PS30, L30S30 and PS30S30. It is quite possible that tannin could be attributed to this inversely proportional effect as quoted by Kumar and Vaithiyanathan (1990) who concludes that presence of tannins in many nutritionally important tree leaves reduces their utilization as ruminant feed. A tannin content of about 5% in browse was reported act as a feeding deterrent and influence digestibilities.

Percent apparent of true DM digestibility, microbial biomass production (mg), cumulative gas produced (mL), ATP (mmol L^{-1}), percent efficiency of microbial production, ^{Y}ATP and partition factors at 24 h of incubation of the complete diets tested are presented in Table 4. Microbial mass production data revealed L30 and PS30 were comparable to control and the rest had significantly ($p<0.05$) lower microbial biomass production. The PS60, S30, S60, L30PS30, L30S30 and PS30S30 were all very poor ($p<0.05$) in supporting the microbial mass production.

Highest cumulative gas (mL) production was observed in control. Significantly ($p<0.05$) lower gas production than control was observed in diets L30, P30 and S30, while the rest diets had significantly ($p<0.05$) lower ability to produce gas.

The ATP yield for the ten different complete diets did not show any significant difference. However, the efficiency of microbial biomass production revealed that only L30 was comparable to control and the rest were significantly ($p<0.05$) poorer, among which L60 and PS30 were having significantly ($p<0.05$) higher efficiency than the rest diets. Consequent to the performance of the above mentioned parameters, ^{Y}ATP was highest ($p<0.05$) in control followed by L30, which inturn was significantly higher ($p<0.05$) than PS30. All the other diets were significantly ($p<0.05$) lower in ^{Y}ATP (Table 4).

The data on ^{Y}ATP, SCFA and gas produced, microbial mass generated in relation to degraded substrate of the ten complete diets indicate that control diet had the most preferred fermentable characteristics and comparable to that was L30. All the rest diets were inferior. Hence it is concluded that banana leaves could be used in complete diet upto 30% inclusion level.

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