A RESPONSE OF *IN VITRO*, *IN SACCO* AND *IN VIVO* DIGESTIBILITY AND RUMEN PARAMETERS OF SWAMP BUFFALOES SUPPLEMENTED *SESBANIA GRANDIFLORA* LEAVES

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ABSTRACT

The ruminal parameters, *in vivo*, *in vitro, in sacco* digestibility, and water extractable dry matter (DM), were measured in buffaloes fed rice straw and elephant grass with supplementation of fresh leaves of *Sesbania grandiflora.* The design was a 2x2 factorial arrangement with three replications. The first factor of the experiment was feed (rice straw or elephant grass); the second factor was supplementation of 4 kg/day of fresh leaves of *Sesbania grandiflora.* The buffaloes received the forage at 80% of their requirement and the sesbania leaves were supplemented once in the morning. Ruminal ammonia concentration, bacteria population and volatile fatty acids were significantly higher for the diets with *Sesbania grandiflora* supplementation. Supplementation also significantly improved *in vivo* DM and NDF digestibility but had no effect on *in vitro* measurement of digestibility nor on "a", "b" and "c" parameters in the *in sacco* test. However, the effective DM degradability value as measured by the *in sacco* method was significantly increased by supplementation compared to the control. *In vivo* DM digestibility was more closely correlated with water extractable DM values than with any of the *in vitro* analyses. It is concluded that farmers could supply a supplement of *Sesbania grandiflora* leaves to buffaloes fed low quality forages to improve their rumen function and production.

Keywords: ruminant, legume leaves, rumen microbes, feed digestion, supplement, rumen environment, water extractable DM

INTRODUCTION

Due to the demand for land for crop production in Vietnam, the grassland areas for ruminants has been reduced. This is one of the factors that has contributed to the reduction of the buffalo population reduction in the Mekong delta (Nguyen, 1988). However, buffalo meat has an important role for people in this region. Recently, buffalo production has been favourably considered by farmers as a means of raising their income (Nguyen, 2012), but low quality of buffalo feeds, which are mainly crop residues, has dominated in their diets and has limited the buffalo performance. Therefore some supplements to diets were used to improve cattle and buffaloes performance such as cotton seed cake, soybean extraction meal, coco nut cake, multi nutrient cake, etc (Nguyen and Nguyen, 2015). Beside them, plant protein sources as alternative sources of supplements in buffalo diets have been abundant and available in this region such as Sesbania, Leuceana and duckweeds. Estimation of feed digestibility by the *in vivo*

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technique in ruminants has been useful; however, the high cost and time required have been limiting factors to its common use. Alternatively, *in vitro* and *in sacco* digestibility methods have been used effectively due to low cost and many more feed samples can be evaluated (Lopez *et al*., 2000). Therefore, this study aimed to investigate whether the rumen environment and feed digestibility could be improved by supplementation of buffalo diets with leaves of *Sebania grandiflora*, using *in vitro* and *in sacco* digestibility procedures.

MATERIALS AND METHODS

The study was carried out at the experimental farms of Cantho University. It was a 2x2 factorial design experiment with 3 replications. The first factor was feed (rice straw or elephant grass) and the second factor was supplementation (with and without leaves of *Sesbania grandiflora*). The experimental animals were swamp male buffaloes (420±25 kg live weightfitted with rumen cannulae. The experimental period was 3 weeks including one week for diet adaptation. The fresh *Sesbania grandiflora* leave was supplemented once at a level of 4 kg per day in the morning. The experimental animals were fed at 7.00 am and 2.00 pm.

Samples of rumen contents were collected at 3 h post-feeding to measure rumen pH, ammonia N (NH_3-N), protozoa and bacteria populations. Rumen pH was measured by pH meter and NH_3-N was analyzed by the micro Kjeldahl method. For counting protozoa the preparation of rumen content samples followed the procedure of Dehority (1984) and a 0.2 mm deep chamber under 100 x magnification was used. Total bacteria populations were counted in a Neubauer chamber under 1200 x magnification after the preparation of rumen content samples following the procedure of Warner (1962). Total VFA were measured by steam distillation following the procedure described by Barnett and Reid (1957).

Feeds and refusals were collected daily and pooled weekly for analysis of DM to calculate feed intake. Feeds and samples for rumen incubation were analyzed for DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and ash following the procedure of AOAC (1990) and Van Soest *et al.* (1991). Animals were weighed on two consecutive days at the beginning of the experiment and at the end of each period to calculate the live weight change.

Feed samples were dried and ground to pass a 1mm sieve for the rumen incubations. *In sacco* incubation was made at 12, 24, 48, 72 and 96 h in duplicate to measure feed degradability following the method of Ørskov *et al*. (1980). Their values were also fitted to the non-linear model DMD = a+b $(1-e^{-ct})$ following Ørskov and McDonald (1979). DMD is the dry matter disappeared after time (t), “a” is the intercept of the degradation curve at time zero, “b” is the fraction which degrades with time at rate “c” and “a+b” represent potential degradability. The effective dry matter digestibility (ED) was calculated following Ørskov and McDonald (1979) by ED = $a+bc[1-e^{-(c+k)t}]/(c+k)$, where k = 0,0246 in the case of buffalos (Bartocci *et al*., 1997) and “a”, “b” and “c” values fit to the DMD = a+b $(1-e^{-ct})$ as above.Feed samples were also used for measuring OM degradability *in vitro* at 12, 24, 48, 72 and 96 h by using rumen fluid as described by Goering and Van Soest (1970). *In vivo* DM, OM and NDF digestibility were determined by faecal collection for 7 days (Mc Donald, 1998). The water extractable DM

(WEDM) was determined in duplicate for three representative samples of each of the leaves following the procedure described by Ly and Preston (1997). The samples (1 g) were put in bags (50 x 150 mm) made from nylon filter cloth with a pore size of 45 to 55 microns and thereafter washed at random in one, two, three or four consecutive cycles of 30 minutes each. The volume of water used in every cycle was in the ratio of 3 litres per bag. After washing, the dry matter in the residue was estimated by microwave radiation to constant weight.

Data were analyzed by the General Linear Model using the software of Minitab (1998) Comparisons between feeds, and supplementation, were made by the Tukey test.

RESULTS AND DISCUSSION

Feed composition

Rice straw and elephant grass were low in crude protein but a high fiber content, while Sesbania leaves were high in crude protein but low fiber. Thus supplementing Sesbania leaves to the rice straw and elephant grass improved the crude protein content of the diets. Rice straw had a higher lignin content compared to elephant grass.

Rumen parameter, VFA production, bacteria and protozoa populations

Ruminal pH was not effected by supplementation (Table 2) and was in the range suitable for the growth and activities of bacteria (Maeng, 1998).

Ammonia concentration in the rumen was higher when the buffaloes were fed elephant grass, rather than rice straw, and when the diets were supplemented with Sesbania leaves. The rumen ammonia concentration reflected the crude protein levels in the diets and was associated with higher numbers of bacteria. VFA concentration was higher on the supplemented diet; however there was no difference between the grass and rice straw diets. These results are similar to the findings of Bitende and Ledin (1996); Kaitho *et al.* (1998).

Water extractable DM values and *in vivo* digestibility

Loss of DM after washing and digestibility coefficients were higher for the elephant grass diet compared with the rice straw diet and for the diets supplemented with sesbania leaves (Table 3). There was a close relationship ($R^2 = 0.91$) between the DM loss by washing and the DM digestibility.

The coefficients in the *in sacco* model of digestibility mostly favoured the grass versus the rice straw and sesbania leaf supplementation versus no supplementation (Tables 4 and 5). However the relationships between digestibility coefficients derived from this model and *in vivo* digestibility were much lower than when the water extractable DM was the predictor.Nguyen Van Thu and Preston (1999) found that supplement of *Sesbania grandiflora* leaves to a basal diet of rice straw brought about improvements in the rumen environment and the feed intake of swamp buffaloes. Similarly, Thongsuk *et al*. (2011) in their results also concluded that a way of using local protein rich trees for a suitable and worthwhile method improved the quality of buffalo feeding systems in the tropics.

CONCLUSIONS AND IMPLICATIONS

Sesbania grandiflora leaf supplementation improved rumen function parameters and

Table 1. Chemical composition of the experimental feeds (as % of DM, except for DM which is on fresh basis).

	DM	OM	Ash	CP	CF	EE	NDF	ADF	Lignin
Rice straw	79,9	83,8	16,2	4,43	32,0	2,07	69,6	41,4	13,1
Elephant grass	11,5	86,3	13,7	9,86	32,1	3,76	71,5	38,3	9,09
Sesbania leaves	22,4	90,5	9,50	21,8	-	-	37,3	22,5	-
Rice straw + Sesbania leaves	65,8	84,2	15,8	8,82	33,6	4,08	56,1	37,2	10,8
E. grass + Sesbania leaves	12,6	85,6	14,4	12,1	31,4	4,52	60,3	36,6	10,3

DM = dry matter, OM = organic matter, CP = crude protein, NDF = Neutral detergent fiber, ADF = acid detergent fiber, CF = crude fiber and EE = Ether extract.

Table 2. Effect of Sesbania leaves supplementation on Rumen parameters, VFAs production, bacteria and protozoa population of the experimental buffaloes.

	Feed(F)		Supplementation (S)		Significance level		
	Ele.grass	Rice straw	Suppl.	No suppl.	F	S	F x S
pH	6,62	6,84	6,69	6,77	*	ns	ns
Ammonia, mg/100 ml	18,1	8,11	16,7	9,48	***	***	ns
Bacteria count, x 10^9/ml	2,83	2,02	2,76	2,09	**	*	ns
Protozoa count, x 10^5/ml	9,94	7,43	9,84	7,54	ns	ns	ns
VFAs, mM	113	101	116	98,5	ns	*	ns

ns non significant difference, *Significant difference (P<0.05).
**Significant difference (P<0.01); ***Significant difference (P<0.001).

Table 3. Effect of the supplement on the washing loss values and digestibility *in vivo.*

	Feed (F)		Sesbania (S)		Significance level		
	Elephant grass	Rice straw	Sesbania	No sesbania	F	S	F x S
Wash loss value at 45 min, %	25,5	17,0	22,8	19,7	***	*	ns
Wash loss value at 90 min, %	28,2	19,7	26,4	21,6	***	*	ns
DM digestibility, %	66,6	51,1	60,5	57,2	***	*	ns
NDF digestibility, %	69,4	57,1	66,2	60,3	***	*	ns
ADF digestibility, %	65,6	48,6	58,9	55,3	***	ns	ns

ns non significant difference, * Significant at P<0.05, ** Significant at P<0.01; *** Significant at P<0.001.

Table 4. Effect of sesbania supplementation on coefficients of the *in sacco* rumen degradation of OM.

	Feed (F)		**Sesbania (S)**		**Significance level**		
	Elephant grass	**Rice straw**	**Sesbania**	**No sesbania**	**F**	**S**	**F x S**
a, %	15,9	5,36	10,7	10,6	ns	ns	ns
b, %	54,7	57,9	54,3	58,2	ns	ns	ns
a+b, %	70,6	63,2	65,0	68,9	*	ns	ns
c, %/h	3,95	2,98	3,00	3,94	ns	ns	ns

A intercept of degradation curve at time zero, b fraction which degrades with time at rate c and a+b represent potential degradability of $y = a+b(1-e^{-ct})$. ns non significant difference, * Significant at P<0.05.

Table 5. Effect of sesbania supplementation on DM digestibility *in sacco* at 48 h.

	Feed (F)		**Supplementation (S)**		**Significance level**		
	Ele.grass	**Rice straw**	**Suppl.**	**No suppl.**	**F**	**S**	**F x S**
a, %	23,0	15,2	19,0	19,2	**	ns	Ns
b, %	51,7	60,2	59,5	52,4	*	ns	*
a+b, %	74,7	75,4	78,6	71,5	ns	ns	Ns
c, %/h	2,82	1,75	2,86	1,71	*	*	Ns
ED, %	50,0	38,1	46,6	41,5	***	**	Ns

A intercept of degradation curve at time zero, b fraction which degrades with time at rate c and a+b represent potential degradability of $y = a+b(1-e^{-ct})$; ED effective digestibility; ns non significant difference, *Significant difference (P<0.05). **Significant difference (P<0.01); ***Significant difference (P<0.001).

coefficients of digestion in swamp buffaloes fed elephant grass or rice straw as the basal diet. The water extractable DM was a better predictor of *in vivo* DM digestibility. Farmers could use sesbania leaves to improve performance of their buffaloes due to the supplement being available in their gardens.

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