

## Effect of Graded Levels of Cottonseed Cake Supplementation on Intake, Nutrient Digestibility, Microbial N Yield of Growing Native (*Bos Indicus*) Bulls Fed Rice Straw

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**ABSTRACT :** On a urea-molasses-straw (3:15:82; UMS) based diet effect of graded levels of cottonseed cake (CSC) supplementation on the performance of native (*Bos indicus*) bulls has been studied for 167 days. Eighteen growing bulls of  $129 \pm 13.4$  kg weight and about 14 months old were randomly allocated to three dietary treatments designed in a completely randomized design, having six animals in each treatment. Three dietary treatments were 0, 0.5 and 1.0 kg CSC per head/d. In addition, each animal also received *ad lib.* UMS, 4 kg Napier (*Pennisetum purpureum*) grass, 500 g of each of rice and wheat bran and 60 g mineral mix daily. For unit increase in CSC, total DM intake was increased by  $1 \text{ g/kg } W^{0.75}/\text{d}$  but the straw DM intake decreased by  $0.54 \text{ g/kg } W^{0.75}/\text{d}$ . Whole gut digestibility of DM and OM was not effected but N and ADF digestibility increased with incremental increase in dietary CSC. For unit (1kg) increase in dietary CSC intake N and ADF digestibility increased by 10 ( $\pm 1.155$ ) and 3 ( $\pm 1.732$ ) unit respectively. Microbial N yield for the 0, 0.5 and 1.0 kg CSC were 5.63, 5.28 and 5.16 g/kg OM apparently fermented in the rumen respectively. For each gram increase in CSC, N intake and N balance increased by  $0.626 (\pm 0.015)$  and  $0.625 (\pm 0.0814) \text{ mg/kg } W^{0.75}/\text{d}$ . High apparent N balance was contrasted with low live weight gain, e.g., for 1 kg increase in CSC supplementation, live weight gain increased by only  $0.077 (\pm 0.00288) \text{ kg/d}$  ( $r^2=0.99$ ;  $p<0.01$ ). The conversion efficiency was 12.98 kg CSC per kg of live weight gain. It was concluded that unless the protein is being protected from the rumen degradation, addition of CSC to UMS diet would have little nutritional or economic advantages. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 3 : 326-332)

**Key Words :** Cotton Seed Cake, Rice Straw, Microbial N, Live Weight Gain

### INTRODUCTION

Nutritional constraints for low quality crop residue like rice straw includes lower content of readily fermentable energy, N, minerals and vitamins. When they are fed alone or constitute a higher proportion of a diet, rumen fermentation does not provide the nutrients required by animals for productive purposes (Liu, 1995; Chowdhury et al., 1995). Different supplementation strategies have been proposed for the correction of these nutrition limits to improve animal productivity (Leng and Devendra, 1995; Huque and Chowdhury, 1995; McLennan et al. 1995; Poppi and McLennan, 1995). Recently, protein supplements are being used extensively in the beef industry and benefit in terms of improved animal performance is generally accepted (Leng, 1995; Dolberg and Finlayson 1995). However, effect of protein supplement is highly dependent on the quality of basal diet. For low to medium intake of protein meal, a linear relationship can be fitted to data for low quality roughage (McLennan et al. 1995). Over the full range of protein intake the response (growth) curve is curvilinear with a plateau at about 900 g/d for cattle (McLennan et al., 1995; Leng, 1995). In China, Dolberg and Finlayson (1995) supplemented urea treated straw with

cottonseed cake ranging from 0 up to 4 kg/head/d and recorded a curvilinear growth response ranging from 236 to 861 g/d. Despite utmost effort, urea treatment technology was largely been unaccepted by the local farmers in Bangladesh (Devendra, 1993; Huque et al., 1998). However, enriching straw with 3% urea and 15% molasses (UMS) was found to be largely acceptable (Huque and Chowdhury, 1995). Cottonseed cake (CSC) has a relatively low rumen degradability and is therefore a good source of by-pass protein (Göhl, 1993). Supplementation of urea treated rice straw with  $1.5 \pm 0.5$  kg CSC reported to have dramatic improvement in feed efficiency, bring total feed dry-matter requirements down from  $\geq 20$  kg per kilogram of live weight gain to 10-12 kg (Dolberg and Finlayson, 1995). present research program has therefore been designed to determine the effect of supplementation of graded levels of CSC to UMS based diet on the performance of native growing bulls (*Bos indicus*).

### MATERIALS AND METHODS

#### Experimental design, animals and diet

Eighteen indigenous (*Bos indicus*) growing bulls of  $129 \pm 13.4$  kg body weight and 14 months old were randomly allocated to three treatments designed in a completely randomized design, having six animals in each treatments. Three treatments were 0, 0.5 and 1.0 kg CSC per head/d. Three diets and chemical

composition of the feed ingredients are shown in table 1. The feeding trial continued for 167 days. Animals were housed in a face-out stanchion barn. They were offered *ad libitum* fresh UMS by mixing dried rice straw, molasses and urea at the rate of 82, 15 and 3 per cent respectively (Huque and Chowdhury, 1995). Concentrate was offered separately from that of roughage to ensure minimum waste in two equal halves at 08.00 and 16.00 h daily.

### Experimental techniques

**Digestibility trial:** After 120 days of feeding, experimental animals were transferred into metabolic crates, where faeces and urine were collected separately for five days. Records were kept on amount of feed offered, residue left, faeces and urine excreted. During the collection period, composite samples of feed, residue and faeces of individual animal were stored at  $-20^{\circ}\text{C}$ . Urine from individual animal was collected in 100 ml of concentrated commercial grade  $\text{H}_2\text{SO}_4$  to maintain urinary pH below 4. About 100 ml of representative urine sample from individual animal was stored daily at  $-20^{\circ}\text{C}$ .

### Chemical analysis

Samples of feeds, orts and faeces were analyzed for dry matter (DM), organic matter (OM), nitrogen (N) according to AOAC (1984). The acid detergent fibre (ADF) was determined according to Goering and van Soest (1970). Urinary N content was also

measured according to Kjeldhal N procedure of AOAC (1984). Urinary allantoin concentration was measured according to Chen and Gomes (1992). Urinary total purine excretion was estimated as allantoin + 15% correction for the uric acid (Chen and Gomes, 1992). Microbial N absorbed in the intestine was then estimated from the knowledge of purine-N : protein-N in the microbial bio-mass. Dietary metabolizable energy (ME) concentration was estimated from the digestible organic matter (DOM) intake as  $\text{DOM kg} \times 5.56$  (ARC, 1980).

### Statistical analysis

The response to dietary treatments on intake, digestibility, nutritive value, microbial N yield and growth rate were analysed by an ANOVA of randomized block design. A linear regression model of the form  $y=a+bx$  was used where appropriate. Statistical methods of Snedecor and Cochran (1967) used for the analysis.

## RESULTS AND DISCUSSION

### Intake

Increasing levels of CSC significantly ( $p<0.01$ ) increased the total DM intake (table 2). For unit increase in CSC, total DM intake increased by 1 g/kg  $\text{W}^{0.75}/\text{d}$  ( $r=0.999$ ,  $p<0.05$ ) but the straw DM intake decreased by 0.54 g/kg  $\text{W}^{0.75}/\text{d}$  ( $r=0.999$ ,  $p<0.05$ ). Using the Tolcamp and Ketelaars (1992) model, McLennan et al. (1995) suggested that with better quality feeds, intake of basal diet reduced even when low level of supplementation is fed. By contrast, with lower quality feeds and low levels of supplementation with nutrients which are limiting, intake of basal diet is increased. Further dietary crude protein content below 7% is known to decrease the DM intake (Poppi and McLennan, 1995). Basal diet in this trial was UMS, which had CP content of over 8% and also reported to maintain rumen  $\text{NH}_3\text{-N}$  concentration of over 150 mg/l (Huque and Chowdhury, 1995). Therefore, increasing level of CSC supplementation to UMS can not be expected to increase, but rather can decrease the straw DM intake. This is similar to my earlier observation with mustard oil cake supplementation to a UMS diet (Chowdhury, 1999). Dolberg and Finlayson (1995) showed that supplementation of CSC up to 13% of diet had no effect on DM intake from ammoniated straw, but straw DM intake decreased with subsequent levels. From the model of Tolcamp and Ketelaars (1992), this means that UMS is better quality basal diet than ammoniated rice straw.

### Digestibility

The levels of supplementation did not effect

**Table 1.** Feed ingredients and their chemical compositions used for different treatments (amounts in g/head/d)

Ingredients	Levels of cotton seed cake (kg/head/d)			
	0.0	0.5	1.0	
UMS*	<i>ad libitum ad libitum ad libitum</i>			
Wheat bran (g)	500	500	500	
Rice mill feed (g)	500	500	500	
Cottonseed cake (g)	0	500	1000	
Salt (g)	40	40	40	
Oyster shell (g)	20	20	20	
Napier grass (g)	4000	4000	4000	
Chemical composition	Dry matter (g/100 g fresh)	Organic matter (g/100g DM)	Crude protein (g/100g DM)	ADF (g/100g DM)
UMS*	85.7	82.2	5.06	47.7
Wheat bran	87.7	94.5	20.3	20.0
Rice mill feed	91.7	88.8	11.5	43.5
Cottonseed cake	91.7	94.2	21.4	46.7
Green grass	22.9	87.4	7.7	29.9

\* UMS: 82:15:3 of Rice straw: cane molasses : urea.

**Table 2.** Effect of supplementing graded levels of cotton seed cake (CSC) on intake of growing bulls fed urea-molasses straw as the basal diet

Parameter	Level of CSC (kg/head/d)			SED (Residual df=14)	Significance
	0 CSC	0.5 CSC	1.0 CSC		
Total DM intake (kg/d)	7.41	7.81	8.32	0.023	p<0.01
Total DM intake (g/kg W <sup>0.75</sup> /d)	168	169	178		p<0.01
Straw DM intake (kg/d)	6.06	6.00	6.05	0.024	NS
Straw DM intake (g/kg W <sup>0.75</sup> /d)	133	130	127	2.87	NS
Dig. OM intake (g/kg W <sup>0.75</sup> /d)	96	102	113	5.71	p<0.05
CSC as % of total DMI	0	5.86	11.00	-	-
Substitution rate (%)	-	6.54	6.54	-	-

NS: Not significantly different at p<0.05.

digestibilities of DM and OM (table 3). However, N and ADF digestibilities increased significantly (p<0.05) at 1 kg dietary CSC intake. This is probably due to increase in the ratio of higher digestible N from CSC than to lower digestible straw N. Similarly, higher ADF digestibility at 1 kg CSC supplementation could be due to increase in the ratio of higher digestible ADF from CSC than to lower digestible straw ADF. Absence of any effect of increasing levels of CSC on digestibility of DM and OM is also similar to my previous (Chowdhury, 1999) observation on mustard oil cake. Lack of response could be due to adequacy of UMS of limiting nutrients required for maximizing rumen fermentation. In other words, CSC had no positive effect on the improvement of rumen fermentation that has been reflected on the efficiency of microbial N yield.

#### Nitrogen utilization

Nitrogen utilization by different groups of animals is shown in table 4. An obvious effect of increasing levels of CSC supplementation was a linear increase in dietary N intake. For each gram increase in CSC intake, N intake increased by 0.626 ( $\pm 0.015$ ) mg/kg W<sup>0.75</sup> ( $r^2=0.99$ ; p<0.05). Level of dietary CSC had little or no effect on faecal and urinary N excretion of

animal. However, N balance increased linearly by 0.625 ( $\pm 0.0814$ ) mg/kg W<sup>0.75</sup> ( $r^2 = 0.98$ ; p = 0.082) with increasing levels of CSC. When N balance data was regressed against the N intake, the relationship became as follows:

$$Y=0.934X-1211 \quad (r^2=0.704; t=5.96; n=17; p<0.01)$$

This equation suggests that zero N balance will be achieved at the N intake of 1211mg N/kg W<sup>0.75</sup>. However, similar values for same types of animals and diet but supplemented with mustard oil cake, basal N excretion was 246 mg N/kg W<sup>0.75</sup> (Chowdhury 1999).

The efficiency of N utilization (estimated as : (N balance + basal N excretion)/N intake; assuming basal N excretion of 246 mg/kg W<sup>0.75</sup>/d) were 0.47, 0.51 and 0.59 respectively for 0, 0.5 and 1.0 kg CSC, which are higher than our earlier observations (Chowdhury, 1998, 1999). Very high N utilization efficiency has not been reflected on the growth rate of animals (table 8). Understandably, higher N utilization efficiency is an artifact of overestimated N economy by the N balance. This means that despite increasing levels of CSC supplementation probably had little effect on overall N economy of the animals.

#### Microbial N production

The excretion of purine derivative and the estimated microbial N yield are shown in table 5. Urinary allantoin excretions were 31.3, 31.6 and 33.7 mM/d respectively for the 0, 0.5 and 1.0 kg CSC supplementation. The estimated microbial N yield per kg digestible organic matter were 5.6, 6.3 and 5.2 g respectively for the 0, 0.5 and 1.0 kg CSC supplementation. Absence of any additive effect of CSC supplementation is similar to our earlier observation in growing bull fed UMS as the basal diet and supplemented with graded levels of mustard oil cake (Chowdhury, 1999). Increasing levels of CSC expected to increase the microbial N yield by providing additional readily fermentable energy (Ben-Ghedalia et al., 1978), peptides and amino acids (Wallece et al.

**Table 3.** Effect of supplementing graded levels of cotton seed cake (CSC) on whole gut digestibility of growing bulls fed urea-molasses straw as the basal diet

Parameter	Level of CSC (kg/head/d)			SED (Residual df=14)	Significance
	0	0.5	1.0		
Dry matter	67	69	72	1.09	NS
Organic matter	70	72	75	1.07	NS
Nitrogen	59 <sup>b</sup>	63 <sup>b</sup>	69 <sup>a</sup>	1.92	p<0.05
ADF	79 <sup>b</sup>	79 <sup>b</sup>	82 <sup>a</sup>	1.32	p<0.05

<sup>a,b</sup> Values with different superscripts differ significantly.

NS: Not significantly different at p<0.05.

**Table 4.** Nitrogen utilization by different groups of animals fed graded levels of cotton seed cake (CSC)

Parameter	Level of CSC (kg/head/d)			SED (Residual df=14)	Significance
	0	0.5	1.0		
N intake (mg/kg W <sup>0.75</sup> /d)	2058	2358	2684	41.1	p<0.01
Faecal N (mg/kg W <sup>0.75</sup> /d)	846	900	832	128.4	NS
Urinary N (mg/kg W <sup>0.75</sup> /d)	500	504	519	47.6	NS
N balance (mg/kg W <sup>0.75</sup> /d)	712	954	1337	78.6	p<0.05
Efficiency of N utilization*	0.47	0.51	0.59	0.0065	NS

\* Efficiency of N utilization=(N retention+basal N excretion)/N intake; here basal N excretion is taken to be 246 mg/kg W<sup>0.75</sup>/d.

NS: Not significantly different at p<0.05.

**Table 5.** Excretion of purine derivatives and estimated microbial N yield by different groups of animals fed graded levels of cotton seed cake (CSC)

Parameter	Level of CSC (kg/head/d)			SED (Residual df=14)	Significance
	0	0.5	1.0		
Allantoin excretion (mmol/d)	31.31	31.63	33.74	1.33	NS
Total purine absorption (mmol/d)	21.62	22.22	24.42	1.599	NS
Microbial N (g/d)	15.71	16.15	17.75	1.169	NS
Microbial N g/kg DOMR <sup>a</sup>	5.63	5.28	5.16	0.356	NS

<sup>a</sup> DOMR=Digestible organic matter apparently fermented in the rumen.

NS: Not significantly different at (p<0.05).

1999). On a basal diet like UMS, rumen microbes expected to be predominantly of cellulolytic bacteria, which uses ammonia as the main source of N, while, non-cellulolytic species mostly uses peptides and amino acids (Russel et al., 1992). As UMS diet provide sufficiently high level of NH<sub>3</sub>-N in the (>150 mg NH<sub>3</sub>-N/l; Chowdhury, 1999) rumen, additional fermentable N from increasing levels of CSC can not be expected to increase the microbial N yield. In fact, additional fermentable N from CSC must have further increased the rumen NH<sub>3</sub>-N level. Wanapat and Pimpa (1999) have showed that increases in the rumen NH<sub>3</sub>-N concentration from 176 mg/l to 344 mg/l decreases the urinary purine derivative excretion from 44 mM/d to 31.5 mM/d in swamp buffalo. Thus on a UMS based diet addition of CSC had little or negative effect on the microbial N yield of cattle.

### Energy Utilization

Estimated (as DOM kg×15.56; ARC, 1980) metabolizable energy (ME) at different levels of CSC intake are shown in table 6. For unit (1 kg) increase in CSC intake ME intake increased by 271 kJ/kg W<sup>0.75</sup> (r<sup>2</sup>=0.97; n=3; p<0.01). Incremental increase in dietary CSC also linearly increased the dietary energy concentration, which were 9.16, 9.38 and 9.85 MJ/kg DM for 0, 0.5 and 1 kg CSC, respectively. However, increasing levels of estimated energy had no profound

effect either on microbial N production or on the growth rate of animal. Although we have not measured rumen and plasma ammonia concentration in this trial but previous trial with UMS diet showed that rumen ammonia levels remains high (around 200 mg/l; Chowdhury, 1999) throughout the day. This high ruminal and plasma NH<sub>3</sub> concentration may decrease the energy utilization efficiency by: i) interfering the energy metabolism and ii) energy expenditure for detoxification of excess rumen NH<sub>3</sub> as urea.

At high rumen pH (>7), NH<sub>4</sub><sup>+</sup> ↔ NH<sub>3</sub> + H<sup>+</sup> balance shifts towards the right (Visek, 1968). This unionized ammonia preferentially absorbed and induces hyperglycemia, under utilization of glucose, insulin resistant and increases in plasma non-esterified fatty acids (Visek, 1984; Fernandez et al., 1988; Di-Marco, et al., 1998). A commonly expressed hypothesis is that increased glutamate formation (from NH<sub>3</sub> and α-ketoglutarate) depletes the TCA cycle of α-ketoglutarate and interrupts energy metabolism and ATP synthesis (Visek, 1968). UMS diet generally maintains high rumen pH (6.8-7.3, Chowdhury, 1999). Thus high rumen pH and NH<sub>3</sub> may induce such hyperammonemia, which might have further aggravated by additional RDN of largely rumen degradable CSC. (table 7). The estimated requirement of RDN relative to the fermentable energy supply (1.25 g/MJ ME, ARC, 1980) were 85, 92 and 135 g/head/d, while the

**Table 6.** Energy utilization by different groups of animals fed graded levels of cotton seed cake (CSC)

Parameter	Level of CSC (kg/head/d)			SED (Residual df=14)	Significance
	0	0.5	1.0		
Dig. OM intake (kg/d)	4.36 <sup>b</sup>	4.71 <sup>b</sup>	5.27 <sup>a</sup>	0.209	p<0.05
ME intake (MJ/D)	67.91 <sup>c</sup>	73.30 <sup>b</sup>	81.98 <sup>a</sup>	1.075	p<0.05
ME intake (kJ/kg W <sup>0.75</sup> /d)	1488 <sup>c</sup>	1586 <sup>b</sup>	1759 <sup>a</sup>	141.96	p<0.05
Energy concentration (MJ/kg DM)	9.16	9.38	9.85	-	-

<sup>a,b,c</sup> Values with different superscripts differ significantly.

estimated RDN supply were 135, 144 and 157 g/head/d respectively for 0, 0.5 and 1.0 kg CSC (table 7). The estimated amount of energy required to excrete the excess RDN as urea (about 1.213, 1.27 and 1.21 mol urea; assuming 1 mole of urea for 2 moles of ammonia) were 403, 422 and 403 kJ ME (assuming : 4 moles of ATP/mole of urea synthesized

ME intake), which is similar to observation that in grazing steers, energy cost associated with detoxification of excess ruminal ammonia is of minor importance (Di Marco et al., 1998).

#### Growth rate

Daily live weight gain of cattle at 0, 0.5 and 1.0

**Table 7.** The estimated<sup>a</sup> rumen degradable N (RDN) and undegradable N (UDN) supply from different dietary ingredients and also the corresponding estimated<sup>b</sup> RDN requirement of animal in different dietary group (amounts in g/d/animal)

Ingredient	0 kg CSC		0.5 kg CSC		1.0 kg CSC	
	RDN	UDN	RDN	UDN	RDN	UDN
Rice straw	24.36	6.48	24.12	6.42	24.32	6.47
Molasses	4.32	-	4.32	-	4.32	-
Urea	86.63	-	83.63	-	83.63	-
Wheat bran	10.66	3.95	10.66	3.95	10.66	3.95
Rice bran	3.72	4.72	3.72	4.72	3.72	4.72
CSC	-	-	12.24	3.445	24.49	6.90
Green grass	5.40	5.85	5.40	5.48	5.40	5.48
Total supply	135.09	21.00	144.09	24.02	156.54	27.52
RDN requirement	84.88		91.63		102.47	

<sup>a</sup> Using RDN and UDN values of Sampath et al. (1993).

<sup>b</sup> RDN requirement calculated as 1.25 × metabolizable energy intake MJ/d/animal (ARC, 1980).

**Table 8.** Effect of graded levels of cotton seed cake (CSC) supplementation on live weight gain of growing bulls fed urea-molasses straw as the basal diet

Parameter	Level of CSC (kg/head/d)			SED (Residual df=14)	Significance
	0	0.5	1.0		
Initial weight (kg)	128	128	123	13.99	NS
Final weight (kg)	177	183	185	19.17	NS
Growth rate (g/d)	296	332	373	87.8	NS
Feed conversion ratio (g DOME/g gain <sup>a</sup> )	14.74	14.19	14.12	-	-

<sup>a</sup> DOMI=Digestible organic matter intake.

and 1 mole of ATP  $\approx$  83 KJ ME; Blaxter, 1989) in 0, 0.5 and 1.0 kg CSC group, respectively. However, energetic loss due to urogenesis is very low compared to that of the ME intake (only 0.49-0.57% of total the

kg CSC supplementation were 296, 332 and 373 g/d respectively (table 8). The response relationship between daily weight gain and CSC intake shows that for 1 kg increase in CSC supplementation, live weight

gain increased ( $p < 0.01$ ) by only 0.077 kg/d. The conversion ratio estimated as reciprocal of the slope of the regression line relating these parameters was about 12.98 kg CSC per kg of live weight gain. This is very inefficient compared to 1.5-2.5 kg protein meal per kg live weight gains that reported by McLennan et al. (1995). Chowdhury et al. (1995) supplemented UMS with daily 2 kg wheat bran and either with 0.5 kg sesame oil cake (SOC) or with ad libitum algal suspension (mixture of *Chlorella* & *Scenedesmus*). Despite higher digestible protein (14 vs. 10 g/kg  $W^{0.75}/d$ ) and ME (1.38 vs. 1.36 MJ/kg  $W^{0.75}/d$ ) intake, oil cake supplemented heifers had lower live weight gain (400 vs. 458 g/d) and feed conversion efficiency (10.3 vs. 8.6 g DOM/g weight gain) than the algae fed animals.

This inefficient utilization of oil cake protein is probably related to the fact that increasing levels of CSC or SOC have not increased the intestinal protein supply per unit of OM intake or protein/energy ratio of absorbed products (table 5). The asynchrony of energy and N in the rumen leads to an inefficient incorporation of N into microbial cells and leads to increase ammonia production. It has already been discussed that hyperammonemia reduces the energetic efficiency of absorbed products. Thus, incremental increase in CSC intake can not be expected to increase the growth rate of cattle.

## CONCLUSION

It can be concluded that on a UMS based diet, supplementation of CSC had little beneficial effect on the overall productivity of the animal. Unless the protein is being protected from the rumen degradation, addition of CSC to UMS diet will have little nutritional or economic advantages.

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