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ORIGINAL ARTICLE

Feed intake, digestibility, rumen fermentation pattern and blood biochemical profile of growing crossbred calves fed lime treated Jatropha (*Jatropha curcas*) cake

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Abstract

A study was conducted to observe the feed intake, digestibility, rumen fermentation pattern and blood biochemical profile of growing crossbred calves fed lime treated Jatropha (*Jatropha curcas*) cake (LTJC). Fourteen crossbred growing calves of 11-17 months of age were randomly divided in two groups i.e. T1 (Control) and T2 (LTJC at 4% of the concentrate mixture) following Completely Randomized Design. A digestion trial of 7 d duration was conducted towards the end of experiment of 80 days to assess the digestibility of nutrients. The intake of dry matter (DM) and organic matter (OM) in both the groups was similar and digestibility was higher in T2 during experiment. However, the digestibility of CP was lower ($P<0.01$) in T2 group. Animals in control group gain body weight while those in group T2 loose body weight. A peak ammonia nitrogen level in strained rumen liquor (SRL) was observed at 2 h post feeding and then declined up to 8 h post feeding with the same pattern in both groups. Maximum non protein nitrogen (NPN) concentration was recorded at 2 h and then decline up to 6 h and again rose at 8 h post feeding with the same pattern in both groups. The maximum total volatile fatty acids (TVFA) concentration was found at 4 h and declined up to 8 h of post feeding with the same pattern in both groups. The mean RBC and WBC count in T2 group was found to be lower ($P<0.05$). It is concluded that inclusion of LTJC at 4% level in the concentrate mixture for growing cattle resulted in adverse effect on growth performance of growing crossbred calves as weight loss is observed in present study. Lime treatment to detoxify JC was unable to remove all anti-nutritional factors especially phorbol ester.

Keywords: *Jatropha curcas*, lime treatment, feeding, rumen fermentation, serum metabolites

Introduction

Feed constitutes the major input cost in livestock sector. There is a bigger gap in demand and supply of fodders in most of the developing countries. In India, a calculated deficit of 47, 45, million tonnes (MT) of green forage and dry fodder, respectively is reported (Ramachandra *et al.*, 2001). The shortage of concentrate in tune of 18 MT of DCP and 94 MT of TDN is reported (Birthal *et al.*, 2005). Thus, the supply and demand of concentrates is of much concern to animal nutritionists and thus is in constant search of new feed resources for livestock. There is always need to search for non-conventional feed resources which have potential to be used as alternate feed resource. Unconventional oil cakes are major protein and energy source to fulfill the deficit of protein and energy feeds. In most of the countries different oil cakes are used as main feed ingredients in formulations of rations for the ruminants. Though rich in protein content, most of the unconventional oil cakes consist of one or more anti-nutritional factors, which limit their use as such. Wide varieties of usable oil cakes are produced in India including more than 35 non-conventional oilcakes. Non-conventional oilseeds like castor bean (*Ricinus Communis*), salseed (*Shorea robusta*), mahua (*Madhuca longifolia*), neem (*Azadirachta indica*), karanja (*Pongamia glabra*), Jatropha (*Jatropha curcas*) etc. are produced in substantial amounts in particular geographic areas. Jatropha (*Jatropha curcas*) cake (JC) is one such non-conventional feed which can be utilized as substitute for replacing costly and scare conventional protein source in animal diets.

Jatropha curcas belongs to the *Euphorbiaceae* family. It is a multipurpose tree of significant economic and nutritional importance. Jatropha is widely distributed in Central and South America, Africa, India and South East Asia (Cano-Asseleih, 1986; Cano-Asseleih *et al.*, 1989). About 2.5 MT hectares of Jatropha was planted in India and China which might rose to about 23 million acres in near future (Fairless, 2007). The production of Jatropha meal is estimated to be around 23 MT by the year 2015 (Ramchandran *et al.*, 2007). In tropics, Jatropha is traditionally used for medicinal purpose and as a hedge (Jones and Miller, 1992). Its use as green manure to rice grown on loamy acid soil was reported (Scherchan *et al.*, 1989). The plant (a shrub or small tree) grows readily in swamp or shade and is quick growing (Ishii *et al.*, 1987) survives in poor stony soils and is drought resistant (Munch and Kiefer, 1989). It reaches a height of 3-5 m and 5 tones/ha annual seed yield is reported (Raina and Gaikwad, 1987; Heller, 1996). The seed weighs about 0.75 g, contains 30-32% protein and 66% lipid (Liberalino *et al.*, 1988) indicating potential protein and energy rich source for livestock.

Though rich in protein and superior amino acid profile, presence of anti-nutritional factors limits its use as an animal feed. Research on mice (Adam, 1974), rats (Liberalino *et al.*, 1988; Awasthy *et al.*, 2010), calves, sheep and goats (Ahmed and Adam, 1979 a and b), humans (Mampane *et al.*, 1987) and chickens (Samia *et al.*, 1992) showed that Jatropha is potentially toxic to the animals. However, it can be processed using chemical as well as physical methods to reduce the incriminating factors and may be useful as feed supplements (Katole *et al.*, 2011). Thus, without proper detoxification, Jatropha cannot be used as potential protein source. Further chemical processing of Jatropha yielded promising results in fish, rat, sheep and goat (Makkar and Becker, 1999; Aderibigbe *et al.*, 1997; Katole *et al.*, 2011; Katole *et al.*, 2013). Information regarding toxic constituents present in Jatropha is available (Ahmed and Adam, 1979; Adolf *et al.*, 1984; Makkar and Becker, 1999; Rakshit *et al.*, 2008). However, information regarding feeding detoxified Jatropha to livestock is limited (Aderibigbe *et al.*, 1997; Katole *et al.*, 2011; Katole *et al.*, 2013). Therefore, the present experiment was carried out to evaluate feed intake,

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digestibility, rumen fermentation pattern and blood biochemical profile of growing crossbred calves fed LTJC.

Materials and Methods

Experimental site: The present experiment was conducted at Animal Nutrition farm of Animal Nutrition Research Station, Anand Agricultural University, Anand – 388 110, Gujarat, India.

Processing of JC: The JC for the experiment was procured from experimental bio-diesel plant, Department of Bio-energy, Faculty of Food Processing and Bio-energy, Anand Agricultural University, Anand. This JC was detoxified using 4% lime treatment followed by extrusion cooking at Bhartiya Agro Industries Foundation, Urulikanchan, Pune, India.

Feeds, feeding and housing management: Fourteen crossbred growing calves (152-242kg BW and 11-17 months of age) were randomly divided in two groups i.e. T1 (Control) and T2 [lime treated Jatropha cake (LTJC)] following Completely Randomized Design. Two rations were prepared both comprising of concentrate mixture formulated as per BIS Type II standard with mature pasture hay (*Dicanthium annulatum*) and limited green NB-21.

In addition, the concentrate mixture comprised of LTDC in treatment group (T2) beginning with 2.5% for 20 days, 3.5% for 20 days and finally 4% level throughout the experimental period of 80 days. The experimental calves were individually fed to meet their protein and energy requirement as per NRC (2001). The concentrate mixture was offered at 9.00 h and green fodder at 12.00 h. Mature pasture grass (*Dicanthium annulatum*) was offered *ad libitum* at 15.00 h throughout the experimental period. Clean, fresh water was made available to all the experimental animals two times a day. Prior to the experiment all the animals were dewormed. All the experimental animals were housed under hygienic conditions and were let loose for exercise in the attached open yard daily for two hours in the morning and one hour in the afternoon under controlled conditions, during which they had free access to clean and fresh drinking water.

Digestibility study: Digestion trial of 7 d duration was conducted from d 73 of feeding trial to determine digestibility of nutrients. During digestion trial, feed, refusals and faecal samples of individual animals were collected daily, composited and stored for analysis. The feed intake and faecal outgo were recorded. Body measurements and body weights of all the animals were recorded fortnightly during experimental period.

Chemical analysis: The composite dried feed, refusals and faeces were pooled, ground to pass through a 1 mm screen and subjected for chemical analysis (AOAC, 2005).

Rumen fermentation study: Change in the pH and total volatile fatty acid (TVFA) and nitrogenous constituents of rumen liquor were studied by collecting 250 ml of rumen liquor from each animal, using a stomach tube employing suction (Lane *et al.*, 1968). The rumen liquor was immediately brought to the laboratory and strained through four layers of cheese cloth. The pH was determined immediately using a digital pH analyzer (ELICO LI 614). Samples of rumen liquor were analyzed for total-N (Kjeldahl's method), ammonia-N (Pearson and Smith, 1943) and TVFA (Barnett and Reid, 1957). Soluble-N in rumen liquor was estimated by Kjeldahl's method and non protein-N (NPN) was estimated by trichloro-acetic acid precipitation of rumen liquor and estimating the N content of supernatant by Kjeldahl's method.

Blood biochemical, haematology and enzymatic profile: At the end of experiment, 4 ml blood was collected before feeding in vacuum tubes (Biovacuum, Labtech Disposables, Ahmedabad, Gujarat, India)-

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containing appropriate quantity of EDTA for haematological parameters viz. red blood cells (RBC), haemoglobin, haematocrit, mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), platelets (PLT) and white blood cells (WBC) using fully automated heamatology analyzer (Medonica CA620/530 Vet.).

For blood biochemical and enzymatic profile 10 ml of blood was collected in cleaned, dried sterilized test tubes. Serum was separated and stored at -20°C for further analysis of total protein, albumin, globulin and glucose, creatinine, sodium (Na), potassium (K), calcium (Ca) and phosphorus (P), and alanine aminotransferases (ALT), aspartate aminotransferases (AST) and lactate dehydrogenase (LDH) using commercial diagnostic kits (CREST Biosystems Ltd., Goa, India).

Statistical analysis: Data obtained were analyzed using student's "t" test (Snedecor and Cochran, 1989) and treatment means were tested by applying Tukey's test by using SPSS software package, version 13 (SPSS, Chicago, IL, USA).

Results and Discussion

Chemical compositions of concentrate mixtures and roughages: The CP content of LTJC was lower and that of CF, NDF and ADF was higher than reported values (Anonymous, 2005). This might be due to the addition of husk at 15%, to kernels for the maximum recovery of oil (up to 85.13%). However, the values for proximate constituents reported by Anonymous (2005) are more or less in agreement with the present finding (Table 1).

Feed intake and digestibility of nutrients: The intake of DM and OM in both the groups was similar during experimental period (Table 2). Similar to the present findings treated Jatropha based diets did not depress feed intake in goat (Belewu et al., 2010) and in sheep and dairy cows (Deshpande, 2012). The digestibility of DM and OM was higher in T2 as compared to T1 group. However, the digestibility of CP was lower ($P<0.01$) in T2 group. Digestibility of EE and CF was found to be similar among both the groups. Contrary to this, Katole *et al.* (2011) and Deshpande (2012) observed the comparable digestibility of DM, OM and CP as compared to the control group in sheep.

Growth performance: The lower ($P<0.05$) body weight was observed in group T2. The average total gain in body weight (kg) of animals in group T1 was 41.36 during experimental period of 80 days. However, there was loss of 7.33 kg of body weight in T2. The average daily gain (g/d) was 517.02 and the loss (g/d) was 91.67 in T1 and T2, respectively (Table 2). This loss in body weight is attributed to the diet containing LTJC, being less palatable than diet of group T1 even after detoxification or might be due to other biochemical and metabolic alterations. Similarly, Deshpande (2012) also observed reduced body weight in sheep and dairy cows. Reduction in body weight of sheep and goat fed on processed Jatropha meal was also reported (Katole, 2007).

Rumen fermentation study: The average pH in strained rumen liquor (SRL) of animals in T1and T2 was 6.72 and 6.70, respectively and was statistically similar and did not reflect any treatment effect (Table 3). The average total nitrogen in SRL was similar in both the groups. The peak concentration was found at 2 h post feeding and then decline up to 8 h post feeding. The result revealed that average ammonia nitrogen in SRL did not differed significantly.

An ammonia nitrogen level showed peak at 2 h post feeding and then declined up to 8 h post feeding with the same pattern in both the groups. However, the ammonia nitrogen concentration differed significantly during different periods. Katole *et al.* (2010) reported the lower ($P<0.05$) NH_3N concentration on raw, NaCl treated Jatropha meal containing concentrate mixture which

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Table 1: Chemical composition of concentrate mixture and lime treated Jatropha cake offered to the growing crossbred calves (%DM basis)

Nutrient composition	T1	T2	MPG	Green NB-21	LTJC
OM	88.12	88.08	92.91	88.62	89.42
CP	21.73	22.19	1.83	6.91	22.86
Ash	11.88	11.92	7.09	11.38	10.58
NDF	39.91	38.26	77.95	69.18	65.51
ADF	19.39	17.90	55.07	47.13	49.57
CF	9.68	10.67	43.93	33.28	29.86
NFE	53.44	51.78	46.55	45.47	31.18

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, CF: Crude fibre, NFE: Nitrogen Free Extract, MPG: mature pasture grass, LTJC: lime treated Jatropha cake

Table 2: Effect of feeding lime treated Jatropha cake on feed consumption, nutrient intake and diet digestibility in growing crossbred calves

Parameters	Groups [#]		
	T1	**T2**	**P-value**
Feed Consumption (g/kg $BW^{0.75}$)			
Concentrate	38.01±0.68	41.82±3.17	0.267
Roughages	56.91±1.92	57.76±2.91	0.813
Nutrient intake (g/kg $BW^{0.75}$)			
Dry matter	96.04±1.51	100.79±5.26	0.406
Organic matter	55.14±1.05	59.50±3.06	0.208
Crude protein	9.93±0.10	11.43±0.62	0.039
Digestibility of nutrients (%)			
Dry matter*	59.80±0.72	61.97±0.41	0.026
Organic matter*	63.16±0.50	65.17±0.62	0.030
Crude protein**	66.44±0.46	62.37±0.34	0.001
Crude fibre	53.89±0.43	55.99±1.52	0.213
Nitrogen free extract	69.40±0.78	70.36±1.12	0.498
Ether extract	75.13±1.03	73.82±2.00	0.576
Nutrient content of diet (%)			
Digestible crude protein	6.91±0.10	7.08±0.17	0.413
Total digestible nitrogen	60.56±0.44	61.69±0.54	0.136
Body weight (kg)			
Initial	177.25±9.01	171.44±4.18	0.572
Final **	218.61±9.08	164.11±5.14	0.001
Average daily gain/loss (g/d)**	517.02±24.09	91.67±34.90	0.001

The parameters mark as *(P<0.05) and **(P<0.01) differ significantly.
[#]T1: Control and T2: Lime treated Jatropha cake fed groups

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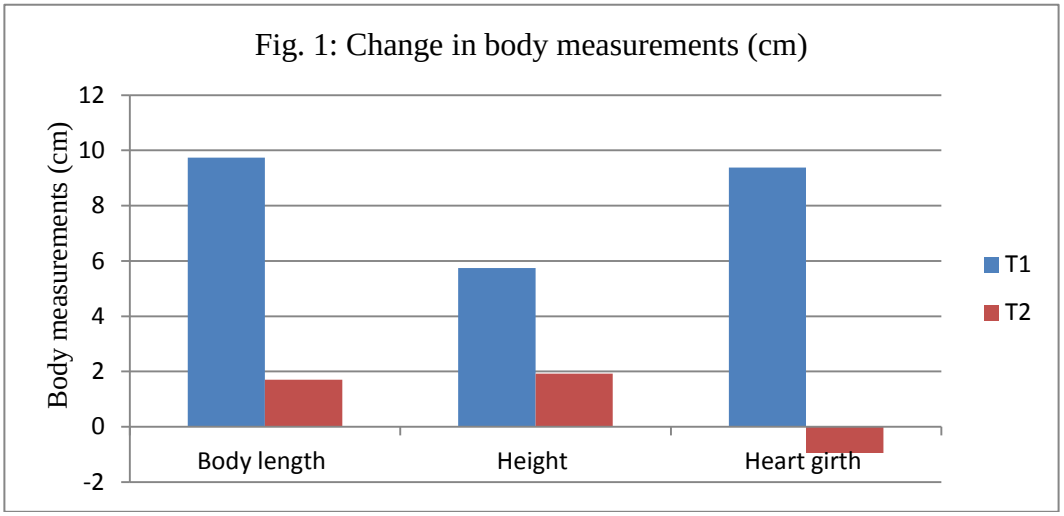

Table 3: Effect of feeding lime treated Jatropha cake on rumen fermentation pattern in growing crossbred calves

Treatment	Hours post-feeding					
	0	2	4	6	8	Mean
Groups[#]			pH			
T1	7.21±0.13	6.82±0.06	6.54±0.09	6.60±0.09	6.43±0.23	6.72
T2	7.25±0.13	6.62±0.05	6.51±0.10	6.59±0.06	6.51±0.26	6.70
	Total Nitrogen (mg/dl)					
T1	39.25±1.62	61.04±1.16	58.57±1.43	55.48±1.85	43.44±1.74	51.56
T2	39.92±2.43	64.65±2.17	60.34±0.60	56.35±0.94	44.95±1.45	53.24
	Ammonia – N (mg/dl)					
T1	8.01±0.54	28.18±0.96	21.13±1.36	10.75±0.67	9.62±0.36	15.54
T2	7.64±0.59	29.80±0.60	21.63±0.94	11.97±0.85	9.49±0.43	16.11
	Non Protein Nitrogen (mg/dl)					
T1	20.84±0.88	47.86±1.24	38.57±1.48	24.72±1.61	26.45±0.97	31.69
T2	21.83±1.10	49.86±2.10	39.21±1.13	26.92±1.57	27.38±0.74	33.04
	Total volatile fatty acid (moles/dl)					
T1	7.84±0.21	8.85±0.57	9.50±0.72	7.58±0.42	6.20±0.04	7.99
T2	7.63±0.44	8.03±0.47	9.20±0.78	7.75±0.26	6.30±0.21	7.78

[#]T1: Control and T2: lime treated Jatropha cake fed groups

was attributed to lower degradation of dietary or slow NH_3N release by the rumen microbes and/or inhibition by the residual toxin. NPN in SRL in treatment groups and the interaction between treatment and intervals did not differ significantly. Maximum NPN nitrogen concentration was recorded at 2 h and then decline up to 6 h and again rose at 8 h post feeding with the same pattern in both groups, indicating that NPN concentration differed significantly ($P<0.05$) during different periods. The maximum TVFA concentration was found at 4 h and declined up to 8 h of post feeding with the same pattern in both groups. The average TVFA concentration in SRL was 7.99 and 7.78

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moles/dl in T1 and T2, respectively. The periodical changes in total volatile fatty acid were found to be significant (P<0.05). Katole *et al.* (2010) reported that TVFA concentration was higher (P<0.01) on control and lime treated Jatropha meal incorporated concentrate mixture as compared to raw and NaCl treated Jatropha meal containing concentrate mixture.

Blood haematological, biochemical and enzymatic profile: The mean RBC and WBC count of experimental animals was 8.02, 6.08 (10^6/µl) and 12.13, 5.81×10^3/µl in group T1 and T2, respectively, which was found to be lower (P<0.05) in T2 (Table 4). However, these values are within normal range reported for cross bred calves (Kaneko *et al.*, 1997). Similarly, Chivandi *et al.* (2006) also reported the reduced RBC and WBC count in pigs fed detoxified Jatropha meal. The mean haemoglobin concentration of experimental animals in T1 and T2 was 9.72 and 7.48 g/dl, respectively, which was similar among the groups. These values are lower than the normal values (Kaneko *et al.*, 1997). On the contrary, reduced concentration of haemoglobin was reported in pigs and sheep fed detoxified Jatropha meal (Chivandi *et al.*, 2006; Katole *et al.*, 2011).

Table 4: Effect of feeding lime treated Jatropha cake on blood biochemical profile in growing crossbred calves

Parameters	Groups[#]		
	T1	T2	P-value
Haematology			
RBC (10^6/µl)*	7.86±0.42	6.26±0.57	0.048
WBC (10^3/µl)**	11.82±1.09	5.67±0.82	0.001
Hb (%)*	9.72±0.37	7.48±0.68	0.016
Haematocrit (%)**	27.43±1.25	19.73±1.95	0.008
Mean cell volume (fl)	35.20±1.92	31.42±1.27	0.132
Mean cell hemoglobin concentration (g/dl)**	35.65±0.49	38.23±0.50	0.004
Platelets (10^3/µl)	236.50±23.19	192.00±24.27	0.215
Blood biochemical			
Glucose (mg/dl)	49.66±1.78	47.21±1.55	0.324
Total protein (g/dl)	6.21±0.10	6.20±0.03	0.903
Albumin (g/dl)	3.31±0.11	3.37±0.04	0.613
Globulin (g/dl)	2.90±0.04	2.83±0.07	0.372
A:G ratio	1.14±0.04	1.20±0.04	0.380
Creatinine (mg/dl)	1.26±0.06	1.66±0.05	0.001
Serum mineral profile			
Ca (mg/dl)	10.55±0.94	9.12±1.13	0.354
P (mg/dl)*	6.38±0.57	9.40±1.16	0.042
Na (m mol/L)	131.83±3.02	142.50±6.98	0.191
K (m mol/L)	4.17±0.31	3.98±0.45	0.744
Serum enzyme profile (U/L)			
AST	61.59±4.94	53.61±0.91	0.144
ALT	24.03±2.01	20.09±1.23	0.126
LDH	1072.99±8.82	1084.08±17.04	0.576

The parameters mark as *(P<0.05) and **(P<0.01) differ significantly.

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The average haematocrit (HCT) value of experimental animals was 27.43 and 19.73% in T1 and T2, respectively, which was lower ($P<0.05$) in T2. Similar decreased in haematocrit concentrates were also reported in pigs and sheep (Chivandi *et al.*, 2000; Chivandi *et al.*, 2006; Katole *et al.*, 2011). Mean cell volume (MCV) of experimental animals in T1 and T2 groups was 35.20 and 31.42 fl, respectively.

Mean cell hemoglobin concentration (MCHC) of experimental animals in T1 and T2 groups was 35.54 ± 0.38 and 37.57 ± 0.74 g/dl, respectively. The two groups did not differ significantly. On the contrary, reduced platelets counts, MCV and MCHC were reported in pigs and sheep (Chivandi *et al.*, 2006, Deshpande, 2012). The mean platelets counts of experimental animals in T1 and T2 was 236.50 and 192×10^3/µl, respectively.

The average serum protein, albumin, globulin, glucose concentration and serum [#]T1 (Control) and T2 lime treated Jatropha cake fed groups mineral profile of experimental animals in T1 and T2 groups did not differ significantly. Similar results were also reported by Deshpande (2012) in sheep fed processed Jatropha meal. The average creatinine (mg/dl) concentration in the serum of experimental animals in T2 was significantly ($P<0.05$) higher. Similarly Belewu and Ogunsola (2010) also observed increased creatinine and urea content in African goats fed treated *Jatropha curcas* seeds.

Concentration of serum P was found to be increased in group T2. The average activities of serum AST, ALT and LDH were comparable among the both the group. However, Adam and Magzoub (1975) reported increased activity of AST in Anglo Nubian goats fed *Jatropha curcas* seeds. Increased activity of AST, concentration of urea, decreased TP and albumin in goats fed raw jatropha seed was reported (Gadir *et al.*, 2003). However, the levels and activities of enzymes were within the normal range (Kaneko *et al.*, 1997).

Conclusion

It is concluded that inclusion of LTJC at 4% level in the concentrate mixture for growing cattle resulted in adverse effect on growth performance of growing crossbred calves as weight loss is observed in present study. The lime treatment used to detoxify JC was unable to remove all anti-nutritional factors especially phorbol ester. It is imperative that an economical, effective and efficient detoxification method be developed and long term study be carried out to explore future possibility to utilize JC.

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