IN VITRO, *IN SITU* AND *IN VIVO* EVALUATION OF STRAW BASED DIETS SUPPLEMENTED WITH BYPASS FAT AS CONCENTRATED ENERGY SOURCE IN MURRAH BUFFALOES

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ABSTRACT

Four complete iso-nitrogenous rations containing crude protein 12.6% with 0 (T_1), 5 (T_2), 7.5 (T_3) and 10 (T_4) percent calcium soap of red palm oil (protected fat) were formulated and evaluated using *in vitro* and *in sacco* digestibility/ degradability techniques. The rations with 0 to 10 percent of calcium soap were further evaluated *in vivo* using four Murrah buffaloes (221.3±3.60 kg body weight) in a Latin Square Design (LSD) to find out optimum level of inclusion of protected fat in complete diets based on intake and nutrient utilization. The average *in vitro* DMD values were lowered (P>0.05) by 2.91, 4.84 and 7.04% due to incorporation of protected fat at 5, 7.5 and 10% level, respectively compared to control ration (T_1). The dry matter (DM) intake (kg/d or g/kg $w^{0.75}$) and the digestibility of proximate principles and cell wall constituents except ether extract (EE) were not significantly (P>0.05) affected by the level of protected fat in the diet. The EE digestibility was improved significantly (P<0.05) by 23.0 to 26.1% with protected fat (5, 7.5 and 10%) supplementation compared to control diet. Nitrogen retention (g/d, percent intake or percent absorbed) in buffaloes was not affected (P>0.05) with dietary supplementation of protected fat. The DCP values of rations with varying levels of calcium soap of red palm oil were not different from each other.

The TDN value of the rations with 5, 7.5 and 10% calcium soap was significantly (P<0.01) higher by 3.18, 4.84 and 6.80 percentage units, respectively as compared to ration without calcium soap. Further, the DCP and TDN intakes were non-significantly (P>0.05) higher in buffaloes fed rations supplemented with protected fat. Based on results, it is concluded from the present study that, calcium soap prepared from red palm oil can be used as an energy supplement up to 10% level for Murrah buffalo animals without affecting DMI and nutrient utilization for improved production.

Keywords: buffaloes, *Bubalus Bubalis*, bypass fat, supplementation, *in vitro, in sacco* evaluation, nutrient utilization, Murrah buffaloes

INTRODUCTION

India is currently the largest producer of milk (124.85 million tons) in the world with 115.4 and 218 million buffaloes and cattle, respectively (FAO, 2012). Further, the buffalo population in rural India continued to grow in the near future as they thrive well on fibrous crop residues. However, eenergy and protein are the two major limiting nutrients that affect the production potential of

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both small and large ruminants in India, owing to shortage of 45, 44 and 38% of dry roughage, concentrates and green fodder, respectively, which led to the deficiency of energy and protein to the extent of 37 and 34%, respectively, for the existing ruminant population (Ramachandra *et al.*, 2007).

Deficiency of energy, especially during critical physiological stages such as early lactation, advanced stage of pregnancy and faster rate of growth, adversely affects milk production, growth and subsequent breeding performance (Horton *et al*., 1992) causing heavy economic loss to the milk and meat producers.

Productivity of ruminants can be enhanced by strategic supplementation with energy and protein rich feedstuffs (Preston and Leng, 1987). Interest in the use of fats as source of energy for ruminants is increasing in the tropics owing to their high energy density and low heat increment.

Devendra and Lewis (1974) reported that at dry matter intake (DMI) of 18 to 22%, if contributed by the fibre component in the ration, the supply of energy by fat becomes more important for meeting the increased demand for production. Feeding of fat to ruminants at 5 to 6% level in the ration would maximize the efficiency of nutrient utilization (Coppock and Wilks, 1991). However, feeding of free or unprotected fats in excess of 3 to 4% results in reduction in microbial activity in the rumen and depresses the digestion of cellulose (Czerkawski *et al.*, 1996; Henderson, 1973). Jenkins and Palmquist (1984) and Schauff and Clark (1989) reported that protected fat in the form of calcium soap allows normal rumen fermentation and digestibility of nutrients. The present experiment aimed at to evaluate the rations supplemented with varying levels of by-pass fat by *in vitro* and *in sacco* techniques and effect on digestibility and utilization of nutrients in Murrah buffaloes.

MATERIALS AND METHODS

The experiment was conducted at the Department of Animal Nutrition, College of Veterinary Science of Sri Venkateswara Veterinary University Rajendranagar, Hyderabad (17^{o} 12'N. 78^{o} 18'E, 518 m above sea level) in India. The ambient temperature and relative humidity values during the period of study were in the range of 28 to 42^{o}C and 64 to 88%, respectively.

Procurement of crude oil and preparation of calcium soaps

In the present experiment, based on the yield and cost of different oils prevailing in the market, red palm oil was chosen as it was relatively cheaper oil source for the preparation of protected fats. Crude red palm oil was obtained from M/s. Andhra Pradesh Cooperative Oilseeds Growers Federation Ltd., Hyderabad.

Calcium soap from red palm oil was prepared by the 'double decomposition method' originally proposed by Duell (1951) and standardized in this laboratory by Ramana *et al*. (1999) using commercial-grade calcium chloride and sodium hydroxide. The procedure used to prepare calcium soap was as follows. Calcium salts of long-chain fatty acids were prepared by heating a mixture of 25 kg of crude red palm oil and 38.75 L of a 0.1 (w/v) sodium hydroxide solution in a metallic drum. The mixture was heated on a hotplate and stirred until the fatty acids were completely dissolved. Calcium chloride (5.875 kg) dissolved in 11.75 L of water was added slowly to the water-soluble soaps while stirring for to aid precipitation of calcium soaps. Excess solid calcium chloride

was added to salt out the insoluble soaps. Excess water was removed by squeezing the soap through muslin cloth. Calcium salts were then dried in a tray drier at 105°C overnight.

Ration formulation

Four iso-nitrogenous rations with 12.6% CP using sorghum stover as roughage base and groundnut extract, maize, deoiled rice bran, sunflower extract as concentrate sources were formulated with 0 (T1), 5 (T2), 7.5 (T3) and 10 (T4) percent levels of protected fat for further evaluation.

***In vitro* and *in sacco* evaluation**

To determine the effective level of inclusion of protected fat of red palm oil, rations with varying levels of protected fats were evaluated using two stage *in vitro* dry matter digestibility (IVDMD) technique (Tilly and Terry, 1963) and *in sacco* method (Kempton, 1980).

Three grams of ground (2 mm) samples were weighed into each of five bags and incubated in the ventral sac of the rumen of each buffalo steer with the aid of 60 cm nylon string secured to each bag to allow free movement of bags with in the rumen. The canulated buffalo steers were maintained on a ration to have 50:50 roughage to concentrate ratio throughout the study to maintain constant rumen out flow rate. At the end of 12, 24, 36, 48 and 72 h incubation, the bags were retrieved from the rumen and washed under tap and dried to a constant weight at 70°C for 48 h in a forced draft oven and percent DM disappearance was determined. The constants a, b and c were derived using computer formula (Mc Donald, 1981) and the effective degradable dry matter (EDDM) of different samples were calculated using an assumed out flow rate (k) value of 0.05/h.

Nutrient utilization and nitrogen balance studies

The experimental rations were evaluated in a 4 x 4 latin square design using buffalo steers with average body weight of 221.3±3.60 kg. The buffalo steers were dewormed before the start of the experiment. A 14-day preliminary period and 7 days collection period were followed during the metabolism trial. The rations were offered twice daily at 8.30 and 14.00 h in equal proportions. The live weight of the animals was recorded before the start and at the end of the each metabolism trial consecutively for 3 days.

During the metabolism trial, samples of feed offered and of leftovers were collected daily in separate polythene bags for each animal for chemical analysis. Total faeces voided during 24 h were collected and an aliquot of 10% was composited in polythene bags and frozen. Daily urine output was measured and an aliquot of 5% was added to a glass bottle with few drops of HCl and preserved at 4°C. After completion of the collection period, frozen samples were thawed and mixed thoroughly for nitrogen (N) and dry matter (DM) determination. For further chemical analysis, faecal materials were dried, ground through a Wiley mill and processed in airtight bottles. Feed samples were also prepared similarly for analysis.

Chemical analysis

Feed, feces, and urine samples were analyzed for N using "Terbotherm" and "Vapodest" (Gerhard, Germany) analysed on micro-Kjeldal method (AOAC, 1997). DM and total ash were determined according to the procedures described by AOAC (1997). Ether extract was estimated after acid hydroly-sis (Wiseman *et al.*, 1992). Cell wall constituents in feeds, feces, and residues were performed as per the method described by Van Soest *et al*. (1991). Digestible energy (DE) and

metabolizable energy (ME) values were calculated using the NRC (1989) formulae: 1 kg TDN=17.45 MJ DE; ME=DE × 0.82, where TDN=total digestible nutrients.

Statistical analysis

The data collected during the experiment was subjected to least square analysis of variance. The differences between the means were tested by significance using Duncan's multiple range test (Duncan 1955). All the statistical procedures were carried out as per the procedures of Snedecor and Cochran (1980) by programming and processing in computer.

RESULTS AND DISCUSSION

Ingredient and chemical compositions

Ingredient and chemical compositions of rations are shown in Tables 1 and 2. The level of groundnut cake was increased by 60, 90 and 110 g/kg, respectively, as the level of protected fat increased in the supplemental component of the ration from 0 to 50, 75 and 100 g, to maintain the isonitrogenous (12.6% CP) status of the rations (Table 1). Correspondingly the proportion of sunflower cake, maize, deoiled rice bran (DORB) was decreased in the rations. The EE content increased progressively as the level of protected fat increased from 0 to 5%, 7.5% and 10% in the rations, which was attributed to the addition of fat in the ration at graded level (Table 2).

Whereas, the CF and NFE content of rations T2, T3 and T4 were lowered by 0.81, 3.13; 2.04, 4.41 and 3.01 and 5.44 percentage units, respectively, as compared to ration T1 which was due to the dilution effect of added protected fat at varying levels in rations T2, T3 and T4. The organic matter (OM) was also slightly decreased and the ash content increased as the level of fat increased in the diets, which may be due to added chemical reagents and residual water-soluble salts left in the protected fat after drying. Cell wall fractionations progressively were also decreased as the level of protected fat increased from 0 to 5, 7.5 and 10% in the rations, which may be due to dilution effect of added protect fat.

Table 1. Ingredient composition (g/kg) of different experimental rations.

Ingredient	Ration			
	T1	T2	T3	T4
Sorghum straw	400	400	400	400
Maize	125	115	90	70
Groundnut extract	140	200	230	250
Sunflower extract	150	100	70	55
Deoiled rice bran	100	50	50	40
Protected fat	0	50	75	100
Molasses	70	70	70	70
Mineral mixture	10	10	10	10
Salt	5	5	5	5

Vitamin AD_3 was added 10 g/100 kg

***In vitro* and *in sacco* evaluation**

The average *in vitro* DMD values were lowered (P>0.05) by 2.91, 4.84 and 7.04% (Table 2) due to incorporation of protected fat at 5, 7.5 and 10% level, respectively compared to control ration (T_1). Our results were in consistent with the findings of Raman Malik *et al.* (1999) who observed no adverse effect on *in vitro* DM and OM digestibility of ration having up to 7.5% calcium soap of soybean and mustard oil. Similarly, Ramana *et al*. (2001) and Kumar *et al.* (2006) also reported that the inclusion of calcium soap of palm oil up to 15% level in rations of sheep had no adverse effect on *in vitro* dry matter digestibility.

The average *in sacco* DM disappearance was non-significantly decreased with increase in the level of calcium soap inclusion as the period of incubation increased from 12 to 72 h. Among the rations evaluated, higher (P>0.05) average DM disappearance values were observed for T1 ration with 0% calcium soap irrespective of period of incubation (Table 3). The average *in sacco* DM disappearance was lower (P>0.05) by 2.13, 4.41 and 6.40% irrespective of time of incubation in rations with 5, 7.5 and 10% protected fat, respectively in comparison to ration without protected fat. However, the average DM disappearance values of rations with varying levels of calcium soap did not differ significantly (P>0.05) irrespective of time of incubation in the rumen. Whereas, effective dry matter degradability was comparable among the dietary treatments (Table 4). Similar to our results, Ramana *et al*. (2001) reported that supplementation of protected fat up to 15% level in straw based diet

Table 2. Chemical composition (%) and *in vitro* dry matter digestibility (IVDMD) of experimental rations with varying levels of calcium soap of red palm oil.

Parameter	Ration			
	T1	T2	T3	T4
Dry matter	89.44	89.72	88.38	89.07
Organic matter	91.19	90.80	90.19	90.03
Crude protein	12.65	12.65	12.67	12.64
Ether extract	1.93	5.48	7.36	9.23
Crude fiber	26.70	25.89	24.66	23.69
Nitrogen free extract	49.91	46.78	45.50	44.47
Total ash	8.81	9.20	9.81	9.97
Neutral detergent fiber	49.15	46.37	43.73	41.71
Acid detergent fiber	30.86	28.98	27.31	26.09
Hemicelluloses	18.29	17.39	16.42	15.62
Cellulose	24.85	23.30	21.87	20.83
Lignin	6.01	5.68	5.44	5.26
IVDMD*	42.32	41.09	40.27	39.34

Each value is an average of duplicate analysis

*Each value is an average of four observations and mean values did not differ significantly (P>0.05)

did not influence the effective DM degradability.

In vitro and *in sacco* evaluation studies revealed that supplementation of calcium soap of red palm oil up to 10% in the ration had no profound effect on DM digestibility though the values decreased as the level of calcium soap increased from 0 to 5, 7.5 or 10% level in the rations. Hence, all the four rations with varying level of calcium soap of red palm oil were further evaluated *in vivo*.

Voluntary intake and nutrient digestibility

The dry matter intake (DMI, g/kg $W^{0.75}$or per day) in buffalo steers was not affected (P>0.05) by gradual increase (0, 5, 7.5 and 10%) in dietary supplementation of bypass fat through complete ration (Table 5). Our results were in accordance with the findings of Ramana Reddy *et al*. (2003) and Kumar *et al*. (2006) who found no difference in DMI in sheep due to supplementation of protected fat at 10 to 12% level in the diet. Similarly, Thakur and Shelke (2010) stated that supplementation of Ca salts of soya acid oil fatty acids at 4% could not affect the DMI of Murrah buffaloes. Purushothaman *et al*. (2008) also noted no difference (P>0.05) in DMI of lactating crossbred cows with gradual increase in bypass fat (calcium soaps of palm oil fatty acids) supplementation in the diet up to 6%. In contrast, Ganjkhanlous *et al*. (2009) observed

Table 3. Effect of different levels of protected fat on *in situ* dry matter degradability in Murrah buffaloe steers fed straw based diet.

Incubation Interval	Complete ration				
	T1	T2	T3	T4	SEM
12	30.70	29.44	28.65	27.57	0.66
24	42.28	41.24	40.22	39.6	0.59
36	46.54	45.84	44.61	43.90	0.59
48	55.17	53.97	52.71	51.45	0.80
72	62.64	61.79	60.66	59.63	0.66

Each value is an average of four observations, Mean values did not differ significantly (P>0.05)

Table 4. Effect of supplementation of different levels of protected fat on rumen kinetics and effective rumen degradable dry matter (EDDM) in graded Murrah buffaloes steers.

Parameter	Complete ration			
	T1	T2	T3	T4
a	18.72	17.18	16.96	15.67
b	57.17	57.35	57.49	56.34
c	0.0203	0.0208	0.0198	0.0209
EDDM (%)	35.30	34.10	33.30	32.20

a (soluble), b (insoluble but degradable) and c (rate constant/h) are constants and EDDM represents effectively degradable dry matter (Orskov and McDonald, 1979) at an assumed outflow rate (k) of 0.05/h.

reduction (P<0.05) in DMI of lactating Holstein cows with supplementation of rumen protected fat (30 g/kg prilled protected or 35 g/kg Ca salts of protected fat). Whereas Kumar and Thakur (2007) observed higher (P<0.05) DMI in Murrah buffalo calves by addition of 2.5% by pass fat to the basal diet. Similarly, Shelke *et al*. (2012) reported that, DMI (P<0.05) was improved in Murrah buffaloes with inclusion of bypass protein and bypass fat (2.5%) to the basal diet.

Table 5. Effect of supplementation of different levels of calcium soap of red palm oil on dry matter intake (DMI), nutrient digestibility, nutritive value and plane ofnutrition in Murrah buffalo steers fed straw based diet.

Parameter	Complete ration				
	T1	T2	T3	T4	SEM
Average body weight (kg)	221.25	227.00	32.75	235.75	3.21
DMI (g/day)	6.02	6.24	6.07	6.22	0.05
DMI (g/kg $W^{0.75}$ per day)	104.9	106.7	101.9	103.4	1.03
Digestibility					
Dry matter	60.53	59.64	60.47	61.01	0.28
Organic matter	62.32	61.39	61.25	62.23	0.28
Crude protein	70.74	72.03	73.63	73.46	0.68
Crude fibre	52.35	51.24	50.16	49.69	0.59
Ether extract	65.97[a]	81.17[b]	82.86[b]	83.19[b]	4.13
Nitrogen free extract	65.17	63.63	62.40	61.69	0.76
Neutral detergent fibre	56.28	54.32	51.46	50.14	1.39
Acid detergent fibre	47.58	45.35	43.93	42.36	1.11
Hemicellulose	68.35	65.75	63.99	62.63	1.23
Cellulose	62.22	59.67	57.14	56.25	1.35
Nutritive value					
DCP (g/kg DM)	8.95	9.11	9.33	9.29	0.09
TDN (g/kg DM)	58.97[a]	62.15[b]	63.81[bc]	65.77[c]	1.44
DE (MJ/kg DM)	10.88[a]	11.47[b]	11.77[bc]	12.13[c]	0.26
ME (MJ/kg DM)	8.92[a]	9.40[b]	9.65[bc]	9.95[c]	0.22
Nutritive ratio	1 : 5.59	1 : 5.82	1 : 5.84	1 : 6.08	
Plane of nutrition					
DCP intake (g/kg $w^{0.75}$)	9.39	9.72	9.50	9.60	0.07
TDN intake (g/kg $w^{0.75}$)	61.87	66.27	64.97	68.02	1.30

Each value is an average of four observations.

[abc]Mean value bearing different superscript letters in a row differ significantly (P<0.05).

DCP: digestible crude protein; TDN: total digestible nutrients; DE: digestible energy; ME: metabolizable energy.

The digestibility of proximate principles and cell wall constituents was not significantly (P>0.05) affected by the supplementation of protected fat at different levels except for ether extract (EE) (Table 5). The digestibility of EE was higher (P>0.05) by 15.20, 16.89 and 17.22, percentage units, respectively in buffaloes fed rations T2, T3 and T4 as compared to those fed ration T1 (Table 5). Whereas, no significant (P>0.05) differences were observed among rations T2, T3 and T4.

Our results were in consistent with the findings of Ramana *et al*. (2003) and Kumar *et al.* (2006) who reported that supplementation of calcium soap of fatty acids (up to 15%) in sheep ration enhanced the dietary lipid digestibility without affecting the digestibility of other nutrients. Similar findings were reported by Thakur and Shelke (2010), who observed no difference (P>0.05) in digestibility of DM, CP, CF, NDF and ADF with exception of EE, which digestibility coefficient was higher in Murrah buffaloes fed on diet supplemented with bypass fat (4% Ca salts of soya acid oil) compared to usupplemented buffaloes. Similarly, Sirohi *et al*. (2010) observed comparable digestibilities of nutrients except that of EE which was higher in bypass fat (300 g/d) supplemented lactating crossbred cows compared to control group (no bypass fat supplementation). Kumar and Thakur (2007) found higher (P<0.05) digestibility of EE with bypass fat supplementation (2.5% or 4%) in Murrah buffalo calves while no adverse effect was recorded on the digestibility of other nutrients. Purushothaman *et al*. (2008) found no significant difference in digestibility of DM, OM, CP and CF, however, EE digestibility in cows of bypass fat (2% and 4%) supplemented groups was significantly (P<0.05) higher than the control group (0% bypass fat). Whereas, Tyagi *et al*. (2009) noted better utilization of DM and CP in lactating crossbred cows with addition of 2.5% bypass fat to the basal diet.

Nutritive value and plane of nutrition

The data on digestible crude protein (DCP) values (%) of rations indicated that DCP values were not significantly (P>0.05) affected by varying levels of protected fat due to iso nitrogenous nature of rations (Table 5). Ramana Reddy *et al.* (2003) and Kumar *et al.* (2006) in sheep also did not find any significant difference in DCP value of ration up to 15% level of calcium soap supplementation. Total digestible nutrients (TDN) values of rations T2, T3 and T4 were significantly (P<0.01) increased by 3.18, 4.84 and 6.80 percentage units, respectively as compared to ration T1.

Because of higher EE intake and digestibility, the rations T2, T3 or T4 rations had higher TDN than that of control ration (T1). The nutritive ratio of rations T1, T2, T3 and T4 were 1 : 5.59, 1 : 5.82, 1 : 5.84 and 1 : 6.08, respectively. The narrow nutritive value for ration T2, T3 and T4 could be attributed to added energy through protected fat supplementation. Digestible energy and metabolizable energy values followed same trend as TDN. Supplementation of calcium soap in sheep diet increased (P<0.05) the TDN content of diet at 10% level (Alexander *et al*., 2002) or 15% level (Ramana *et al*., 2003). In contrast, Hill and West (1990) reported that supplementation of 4.5% of calcium soap of fatty acids did not significantly affect the digestibility of energy. The intake of DCP and TDN (g/d) in buffalo fed rations T2, T3 and T4 was non-significantly (P>0.05) higher than those fed ration T1 due to increased digestibility of CP and energy. Similar findings were also reported by Ramana *et al.* (2003). Sklan *et al.* (1990) reported that increasing the amount of CSFA up to 90 g/

kg diet enhanced energy (ME) intake by 14.22%. Thakur and Shelke (2010) found no difference in CP intake, whereas observed higher TDN intake in Murrah buffaloes fed on diets supplemented with bypass fat (4% Ca salts of soya acid oil) compared to no bypass fat supplemented buffaloes. The requirements for growing buffaloes weighing 240 kg body weight with an average daily gain of 450 g are DM 6.1 kg, DCP 375 g, TDN 3.3 kg (ICAR, 1998). The nutrient intake was higher in buffaloes fed ration T1, T2, T3 and T4 than the recommended level of ICAR (1998) for growing buffaloes.

Nitrogen balance

N intake (g/d) and N excretion (g/d) through faeces, urine or total was not significantly (P>0.05) affected by supplementation of protected fat at different levels (Table 6). There was no significant (P>0.05) difference observed in N balance among dietary treatments and irrespective of the diet, all the buffalo steers were on positive nitrogen balance. This might be due to comparable DMI of iso-nitrogenous complete rations by all experimental animals. Ngidi *et al.* (1990), Sklan *et al.* (1990) and Ramana *et al.* (2003) reported that addition of calcium soap did not alter N digestion/ retention of rations. In contrast, Ohajuruka *et al.* (1991) observed slight improvement in the digestibility of N in dairy cows supplemented with bypass fat.

CONCLUSIONS

In vitro and *in sacco* studies indicated that calcium soap of red palm oil can be added up to 10% level in straw based diets of buffaloes without any significant effect on DM digestibility. Inclusion of calcium soap of red palm oil at 10% level in the rations of Murrah buffaloes was found to be optimum without affecting the dry matter intake, nutrient digestibility, N balance with improved

Table 6. Effect of supplementation of different levels of calcium soap of red palm oil on nitrogen balance in Murrah buffalo steers fed straw based diet.

Parameter	Complete ration				
	T1	T2	T3	T4	SEM
N intake (g/d)	121.8	126.2	123.0	125.8	1.07
N output (g/d)					
In faeces	35.63	35.35	32.41	33.48	0.77
In urine	34.96	39.44	42.08	37.51	1.51
Total	70.59	74.78	74.49	70.98	1.12
Balance (g/d)	51.22	51.43	48.50	54.85	1.30
% of intake	42.05	40.75	39.44	43.59	0.89
% of absorbed	59.44	56.60	53.55	59.39	1.40

Each value is an average of four observations.

Mean values did not differ significantly (P>0.05).

nutrient intake.

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