

## **PLASMA HORMONES AND MILK PRODUCTION PERFORMANCES IN EARLY LACTATION BUFFALOES SUPPLEMENTED WITH A MIXTURE OF PRILLED FAT, SWEETENER AND TOXIN BINDER**

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**Abstract:** The effect of combined feed supplements (prilled fat, sweetener and toxin binder) was studied on 24 apparently healthy early lactating rural and urban maintained Murrah buffaloes. The feeding of combined feed supplement was carried out for a period of 90 days. DMI, BCS, body weight were recorded at fortnightly intervals and milk composition was analyzed at weekly intervals. Blood samples were analyzed for hormones, plasma metabolites and lipid profile. The supplementation increased ( $p < 0.01$ ) milk yield by 13.6 and 17.0% in urban and rural Murrah buffaloes with respective increases of 20.14 and 14.98% in milk fat ( $p < 0.01$ ). BCS and DMI varied non-significantly ( $P > 0.05$ ) between the groups. Body weight increased in rural buffaloes in comparison to urban buffaloes. Plasma GH was higher ( $p < 0.05$ ) before supplementation and fluctuated non-significantly ( $P > 0.05$ ) during supplementation period. Mean leptin levels decreased ( $p < 0.05$ ) while plasma estradiol and IgG level increased during the supplementation period. Plasma progesterone and ghrelin level varied non-significantly before and during supplementation. Plasma IGF-1 and glucose levels was more and NEFA level was lower ( $p < 0.05$ ) during the experiment. Mean HDL, triglyceride and cholesterol concentration increased ( $P < 0.05$ ) during supplementation than before supplementation. Blood urea nitrogen and plasma urea level was lower before feeding and increased during the experiment. The conception rate was more and service period was less ( $P < 0.05$ ) in urban buffaloes as compared to rural buffaloes. The complete feed supplementation was highly economical and generated an additional income of Rs. 114.45/day/buffalo with cost benefit ratio of 1:5. It was concluded that complete feed comprising of prilled fat, sweetener and toxin binder augment overall productive performance of rural and urban buffaloes.

**Key words:** Prilled fat, lipid profile, hormone, milk yield, milk composition, buffaloes

## Introduction

Most of the animals in developing countries including India are being fed on agriculture by-products and low quality crop residues, which have inherent low nutritive value and digestibility. The requirement of energy is very high during early lactation but increased nutrient demand and limited feed intake due to physiological stage affects production potential of animal (*Sirohi et al., 2010*). Due to this reason animals are often forced to draw on body reserves to satisfy energy requirements (negative energy balance) leading to substantial loss in body weight and lower milk yield (*Kim et al., 2003*). Cereal grains and fats play an important role as source of energy in the ration of high yielding dairy animals for optimum productivity. However use of cereals for human consumption and monogastric animals, the alternate source of energy in the form of bypass fat in dairy ration has been reported (*Saijpaal et al., 2010; Singh et al., 2015*). Inclusion of unprotected fat in dairy ration up to 3% of dry matter (DM) intake, reduces digestibility of fibre (NRC,2001) and depresses rumen cellulolytic microbial activity (*Ranjan et al., 2012*). Supplementation of bypass fat increases energy intake and unsaturated fatty acid content of buffalo milk and provide more economic returns to dairy farmers (*Parmerkar et al., 2010*). The experiment on effect of prilled fat containing vegetable palm oil have shown significant impact on milk yield, fat percent and reproductive performance in cows and buffaloes of organized herd (*Rajesh et al., 2014; Yadav et al., 2015; Singh et al., 2015a*). Since prilled fat feeding does not affect digestibility of feed and improves the reproductive performance, its effect on milk production and reproductive performance needs to be investigated in buffaloes maintained in tropical condition. In present investigation the effect of a supplementation mixture containing prilled fat, sweetener, toxin binder on milk production, composition, lipid profile and hormones was studied in rural and urban Murrah buffaloes. Sweetener was added to enhance the palatability of feed. Further, to determine its economic impact the cost benefit ratio of feeding was also determined.

## Materials and Methods

### *Experimental design, diets and procedures*

Lactating Murrah buffaloes (24) on day 38 postpartum in 2<sup>nd</sup>- 3<sup>rd</sup> parity were selected from Gohargarh rural village (group I) and progressive urban dairy farm (group II). Buffaloes of both the group were supplemented with a mixture of prilled fat, sweetener and toxin binder, 100gm, 30gm and 20 gm/d respectively for

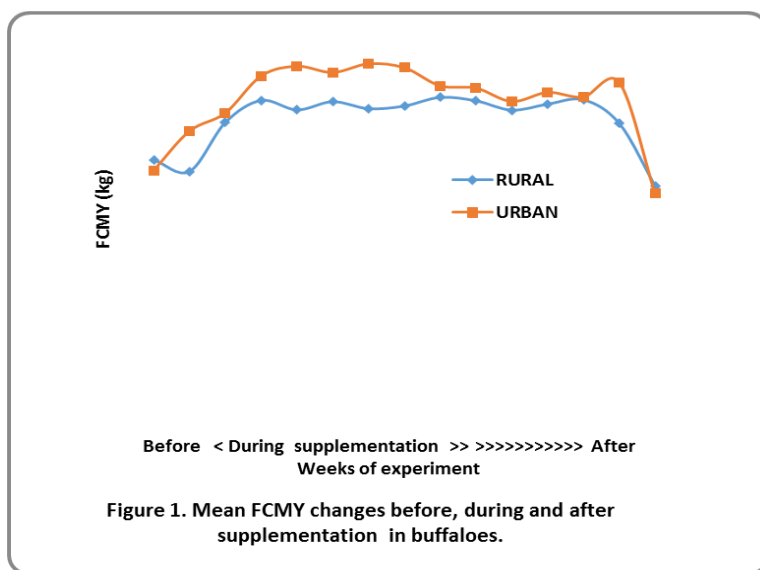
aa period of 90 days. The experiment included two weeks observations prior to supplementation (before) followed by feeding period of twelve weeks. The withdrawal effect of the supplementation on milk yield was recorded for one week. DMI was recorded daily based on the amount of green fodder, wheat straw and concentrate offered and the residue left (Table 1). Body condition score was recorded based on fat cover in the brisket, on the ribs, back, hooks, pins and around the tail head adopting a 5-point scale method. Body weight was recorded at fortnightly intervals. Milk yield of individual buffalo was recorded daily during morning (6am) and evening (6pm) milking. Milk composition viz., fat, protein, lactose and SNF were estimated by Lactoscan machine. Blood samples were collected from rural buffaloes before feeding and at monthly interval on day 30, 60 and 90 of experiment. Plasma growth hormone (GH), insulin like growth factors (IGF-1), leptin, ghrelin, progesterone and estrogen levels were determined by enzyme specific enzyme immunoassay kits. Plasma glucose was determined by analytical kit and plasma NEFA was estimated by *Shipe et al. (1980)* method. Plasma triglyceride, total cholesterol and HDL were estimated by the commercially available analytical kits. Statistical analysis of data i.e. two ways ANOVA was carried out by sigma stat-3 programme. Effects were considered to be significant at probabilities  $\leq 0.05$  while a trend was assumed for probabilities between 0.05 and 0.1. The mean and standard errors are being presented in table and figure.

**Table 1. Chemical Composition (% DM basis) of feeds**

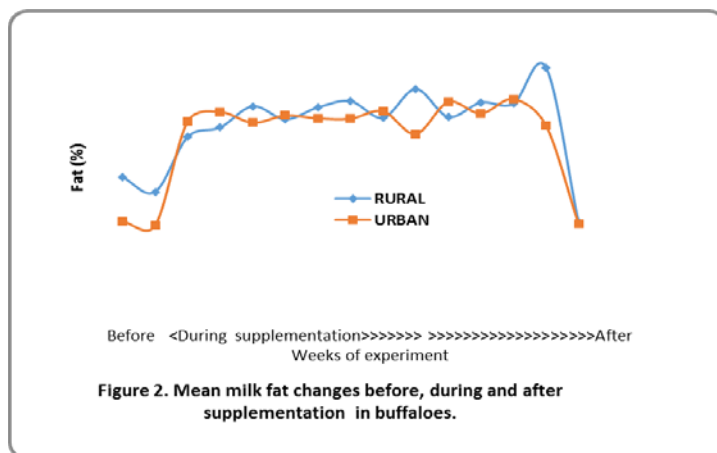
Parameter	Feed			
	Concentrate mixture	Maize	Berseem	Wheat Straw
DM	89.25	20.91	16.75	89.10
CP	20.95	8.75	16.92	3.60
EE	4.28	1.62	2.32	0.95
Total ash	3.75	11.02	9.88	10.11
NDF	30.60	63.91	50.71	75.27
ADF	18.14	44.52	35.95	52.30

## Results

Supplementation of the mixture significantly increased ( $p<0.01$ ) milk yield by 13.6% (1.25kg/d) and fat by 20.14% in urban buffaloes. In rural buffaloes the milk production increased ( $p<0.05$ ) by 17.00% (1.63 kg/d) and fat by 14.98% (Fig 1, 2).



However, milk protein and lactose content was not influenced ( $p>0.05$ ) by supplementation in both the groups. Solid not fat (SNF) was higher ( $p<0.01$ ) in urban buffaloes than the rural buffaloes. Supplementation did not influence body condition score (BCS) of both the groups. The dry matter intake (DMI) increased ( $P<0.05$ ) during the supplementation in rural and urban buffaloes. Body weight varied ( $P<0.05$ ) between the groups. The body weight was not influenced by supplementation in rural buffaloes however a significant increase ( $p>0.05$ ) in body weight of urban was observed (Table 2).



**Table 2. Mean body condition score, body weight changes and dry matter intake before, during and after supplementation**

Parameters	Before		Days during supplementation			After
			30	60	90	
BCS	Urban	3.58±0.12	3.58±0.12	3.54±0.11	3.50±0.08	3.72±0.09
	Rural	3.38±0.13	3.38±0.13	3.47±0.14	3.72±0.14	3.75±0.16
Body weight (kg)	Urban	480.0 <sup>a</sup> ±24.59	524.21 <sup>b</sup> ±17.23	519.3 <sup>b</sup> ±17.29	516.37 <sup>b</sup> ±16.38	523.42 <sup>b</sup> ±15.21
	Rural	493.9±7.94	515.11±16.68	510.92±15.73	509.03±15.08	508.66±13.77
DMI (kg)	Urban	14.78 <sup>ax</sup> ±0.52	16.62 <sup>bx</sup> ±0.87	17.59 <sup>bx</sup> ±1.04	17.35 <sup>bx</sup> ±0.80	17.02 <sup>bx</sup> ±0.99
	Rural	18.31 <sup>ay</sup> ±0.96 <sup>y</sup>	19.89 <sup>ay</sup> ±1.97	19.78 <sup>ay</sup> ±1.07	19.74 <sup>ay</sup> ±1.97	19.79 <sup>ay</sup> ±1.84

Values bearing different superscripts <sup>a,b,c,d</sup> differ ( $p < 0.05$ ) in a row.

Plasma GH level was numerically higher ( $p > 0.05$ ) before supplementation and was not influenced during the experimental period. Plasma leptin level was higher ( $p > 0.05$ ) before supplementation and a gradual decrease was observed during supplementation period (Table 3). However such decline in plasma ghrelin level was not observed. Plasma estrogen concentration was lower ( $p > 0.05$ ) before supplementation and increased upon supplementation during the experiment. Plasma progesterone level did not indicate any set pattern of change before and during supplementation periods. Plasma leptin, ghrelin and estradiol levels varied ( $p > 0.05$ ) between animal. Plasma IgG level was lower ( $p < 0.05$ ) before supplementation and increased during the supplementation period. Plasma IGF-1 level declined ( $p < 0.01$ ) during supplementation in comparison to before supplementation.



**Table 4. Mean plasma metabolite levels during different months of experiment in rural Murrah buffaloes**

	Days of experiment				
	Before	1	2	3	Av.
Glucose(mg/dl)	51.07 <sup>a</sup> ±2.40	57.83 <sup>b</sup> ±2.71	59.94 <sup>bc</sup> ±3.57	73.45 <sup>d</sup> ±2.15	63.74 <sup>b</sup>
NEFA (µm/l)	71.44 <sup>a</sup> ±5.82	55.07 <sup>b</sup> ±1.92	57.68 <sup>b</sup> ±2.36	54.00 <sup>b</sup> ±1.93	56.25 <sup>b</sup>
Triglycerides (mg/dl)	25.77 <sup>a</sup> ±1.76	26.77 <sup>a</sup> ±2.90	29.81 <sup>b</sup> ±2.94	31.84 <sup>bc</sup> ±2.36	29.47 <sup>b</sup>
Cholesterol (mg/dl)	103.88 <sup>a</sup> ±23.00	128.71 <sup>b</sup> ±17.22	113.17 <sup>ab</sup> ±6.09	113.84 <sup>ab</sup> ±4.73	118.57 <sup>b</sup>
HDL (mg/dl)	62.80 <sup>a</sup> ±11.17	86.32 <sup>b</sup> ±9.02	85.96 <sup>b</sup> ±7.20	86.01 <sup>b</sup> ±5.43	86.10 <sup>b</sup>
Urea mg/dl	23.10 <sup>a</sup> ±2.71	26.18 <sup>ab</sup> ±2.58	28.98 <sup>b</sup> ±2.85	32.76 <sup>c</sup> ±2.43	29.31 <sup>b</sup>
BUN (mg/dl)	10.79 <sup>a</sup> ±1.26	12.23 <sup>a</sup> ±1.21	13.54 <sup>b</sup> ±1.33	15.25 <sup>c</sup> ±1.12	13.67 <sup>b</sup>
IgG (mg/ml)	12.17 <sup>a</sup> ±5.87	34.34 <sup>b</sup> ±5.04	22.78 <sup>b</sup> ±7.48	22.88 <sup>b</sup> ±5.34	23.31 <sup>b</sup>

Values bearing different superscripts <sup>a,b,c,d</sup> differ ( $p < 0.05$ ) in a row.

The urban buffaloes exhibited first post-partum heat earlier (65 day) in comparison to rural Murrah buffaloes (87day) in spite of similar number of artificial inseminations. Conception rate was higher ( $p < 0.05$ ) in urban than the rural Murrah buffaloes. The supplementation resulted in lesser service period in urban buffaloes by 51 days in comparison to rural buffaloes. The overall conception rate was 46.87 % in buffaloes. Supplementation generated additional income of Rs 115/day, however withdrawal of feeding declined ( $p < 0.05$ ) milk yield leading to loss of Rs 274.5/ buffalo/day. The cost: benefit ratio during the experiment was 1:5.

## Discussion

The increases in milk production in both the groups of buffaloes suggest that the mixture of prilled fat was galactopoitic and sustained the milk production during the experiment. This was also evident from the significant decline in milk yield after the withdrawal of feeding. However inclusion of sweetener did not increased DMI though it was supposed to enhance the palatability of feed and increase in DMI. The greater response in milk yield and fat content in rural Murrah buffaloes indicated that energy was limiting the milk production. Such effects on milk yield have been reported earlier in mid-lactation cows fed with prilled fat 75g/d (*Singh et al., 2014*) and in buffaloes maintained in organized farm of the institute (*Singh et al., 2015*). The increase in milk fat content resulted probably due to elevated saturated fatty acids level in blood which is taken up by mammary gland for milk fat synthesis. The expected change in BCS was not observed in this study as increase in milk yield was persistent. Body condition score is used for

accurate determination of energy reserves as energy balance during entire lactation period (Coffey et al., 2003). Due to this reason BCS have strongest genetic association with cow fertility (Banos and Coffey, 2010). The decline in plasma NEFA concentration which is an important energy marker and transporters of fatty acids in blood remain unaffected by prilled fat supplementation (Quiroz-Rocha et al., 2009). However in this study decline in plasma NEFA reflected less adipose tissue triacylglycerol mobilization (Pullen et al., 1989). Stressors and poor nutritional management during peripartum results in large increases in NEFA immediately after calving due to decrease in voluntary dry matter intake (Drackley, 1999). The enhanced milk production therefore could be attributed to prilled fat. The significant increase in DMI could be attributed to bypassing of the prilled fat and not affecting the rumen microflora. Cows in NEB have lower leptin concentrations, higher NEFA and produce more milk with lighter live weight compared to cows in positive EB (Liefers et al., 2003). Moreover plasma leptin concentration is positively correlated with glucose and insulin concentrations and negatively with plasma NEFA concentrations (Block et al., 2001). However such type of correlation and pattern was not evident as buffaloes were producing less milk yield than the cows. But in medium producer cows similar effect of prilled fat supplementation on plasma leptin concentration has been reported (Singh et al., 2014). The non-significant decline in plasma ghrelin was probably due to increasing stage of lactation and maintenance of milk yield in buffaloes. Ghrelin, a 28-amino acid octanoylated peptide is secreted primarily by cells in the abomasum in ruminants (Huang et al., 2006) and is the “ultimate anabolic hormone” because it causes the body to consume and store energy (Litwack et al., 2008). Singh et al. (2014) report no effect of prilled fat feeding on plasma ghrelin concentration in cows as observed in this study. Plasma GH did not vary significantly though its role as galactopoietic hormone in buffaloes have been established (Prasad and Singh 2010; Singh et al., 2014). GH partitions nutrient towards the mammary gland at the expense of other tissues (Aschenbach et al., 2011). An increase in plasma cholesterol, triglycerides was observed as expected but lower HDL level during supplementation suggest that animal health remains unaffected by prilled fat supplementation. The improved reproductive performance could be attributed to higher plasma cholesterol and higher IGF-I levels as both play important role in reproduction (Wadhwa et al., 2012). Plasma insulin, leptin and insulin-like growth factor- I control ovarian follicular development and serve as mediators of energy balance on cow's fertility (Diskin et al., 2003; Webb et al., 2004). The feeding supplement was highly economical for dairy farmers in urban and rural conditions and corroborates the similar findings on additional income generation in prilled fat fed buffaloes (Singh et al., 2015) and with calcium salts of fatty acids in cows (Naik et al., 2009; Parnerkar et al., 2011).



## Conclusion

Supplementation of a mixture of prilled fat along with sweetener and toxin binder significantly improves milk yield and fat percent without affecting milk protein, lactose and body condition score. The supplementation increased plasma glucose, decreased NEFA and improves the reproductive performance. Further being cost effective it could be used successfully to augment the productive performance of rural and urban reared buffaloes.

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## **Hormoni u plazmi i osobine mlečnosti u ranoj laktaciji bivola hranjenih dodatkom mešavine zaštićenih masti, zaslađivača i sredstva za vezivanje toksina**

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## Rezime

Uticao kombinovanih dodataka hrani (zaštićena mast, zaslađivač i sredstvo za vezivanje toksina) je ispitan na 24 grla murah bivola, zdravih i na početku laktacije, u ruralnim i urbanim uslovima držanja. Kombinovani dodatak obroku je korišćen u periodu od 90 dana. DMI (konzumiranje suve materije), BCS (ocena telesne kondicije), telesna masa su evidentirani u dvonedeljnim intervalima, a sastav mleka je analiziran u nedeljnim intervalima. Uzorci krvi su analizirani na hormone, metabolite u plazmi i lipidni profil. Dopuna hrani je uticala na povećanje ( $p < 0,01$ ) prinosa mleka za 13,6 i 17,0% u urbanim odnosno ruralnim uslovima držanja murrah bivola sa odgovarajućim povećanjem od 20,14 i 14,98% sadržaja mlečne masti ( $p < 0,01$ ). BCS i DMI nisu značajno varirali ( $P > 0,05$ ) između grupa. Telesna masa povećana je kod ruralnih bivola u odnosu na one u urbanim sredinama. Plazma GH je bila veća ( $p < 0,05$ ) pre suplementacije i varirala je nesigifikantno ( $P > 0,05$ ) tokom perioda suplementacije. Srednji nivo leptina se

smanjio ( $p < 0,05$ ), dok su estradiol plazme i IgG nivo povećani u periodu suplementacije. Progesteron plazme i nivo grelina pokazali su nesigifikantne razlike pre i tokom suplementacije. Plazma IGF-1 i nivo glukoze bili su viši a nivo NEFA niži ( $p < 0,05$ ) tokom eksperimenta. Srednja vrednost HDL, koncentracija triglicerida i holesterola je povećana ( $P < 0,05$ ) tokom suplementacije u odnosu na period pre suplementacije. Nivo uree u krvi i plazmi je bio niži pre ishrane sa dodatkom i povećan je tokom eksperimenta. Koncepcija je bila viša i servis period kraći ( $p < 0,05$ ) kod bivola u urbanim u odnosu na bivole u seoskim uslovima držanja. Suplementacija kompletnog obroka je bila veoma ekonomična i generiše dodatni prihod od Rs. 114.45/dan/ bivo sa odnosom isplativosti 1: 5. Zaključeno je da je potpuna hrana koja se sastoji od zaštićene masti, zaslađivača i sredstva za vezivanje toksina povećava ukupni produktivni učinak bivola u seoskim i gradskim uslovima držanja.

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