ALTERATIONS IN HAEMATO-BIOCHEMICAL PROFILE FOLLOWING BY-PASS NUTRIENTS SUPPLEMENTATION IN EARLY LACTATING MURRAH BUFFALOES

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ABSTRACT

Present study was designed to decipher the haemato-biochemical and hormonal profile following bypass nutrient supplementation in Murrah buffaloes during early lactation. Forty Murrah buffaloes just after parturition divided randomly into four equal groups and fed basal diet constituting common green fodder and wheat straw, while two different types of concentrates mixture (CM) having 25 (CM1, for group fed control or bypass fat, BPF diet) and 40% (CM2 for group fed bypass protein, BPP and BPP with BPF, BPPF diet) of BPP using variable feed ingredients in CM. Animals of group BPF and BPPF additionally fed 15 g bypass fat (long chain fatty acid of calcium salt) for each kg of milk produced for initial 90 days of lactation. Blood sampling was carried out at 0 day (before starting supplementation) and thereafter at an interval of one month, till 120th day after parturition, to find out the effect of supplementation as well is after effects. Significant reduction ($P<0.05$) in serum urea on day 60 was observed in bypass protein fed groups (BPP and BPPF) remains indicative of efficient nitrogen utilization. Thus overall mean total protein in bypass protein fed groups (BPP and BPPF) was higher ($P<0.05$) than control group. Values of albumin also on day 90 ($P<0.01$), 120 ($P<0.05$) as well as overall mean remained higher ($P<0.001$) in group fed CM high in rumen protected protein (BPP and BPPF) than groups fed low bypass protein CM (control and BPF). Supplementation of BPPF improved ($P<0.05$) overall mean cholesterol level as compared to control. Reduction ($P<0.05$) in low density lipoprotein values was observed on day 60 in BPF group as compared to others, while overall mean high density lipoprotein (HDL) in bypass protein fed groups (BPP and BPPF) was found to be higher ($P<0.01$) as compared to others. IGF-1and BHBA showed non-significant difference in all treated groups at different intervals in addition of haematological parameters. It may be concluded that feeding of bypass protein (BPP and BPPF) improved serum overall total protein, albumin, cholesterol and HDL in post-parturient Murrah buffaloes during early lactation.

Keywords: buffaloes, bypass protein, bypass fat, haemato-biochemical, post-partum

INTRODUCTION

Feeding just after parturition and during early lactation is a greater challenge to exploit the

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productive potential of dairy animals. Dry matter intake remains compromised and causes additional hurdle to counteract the challenges. Fight against negative energy balance remains a major challenge during the period, otherwise leads to ketosis and related metabolic disorders. Supplementations with energy and protein rich diets have been followed to meet out the deficiency during early lactation. This is accomplished by incorporation of protected nutrients (rumen bypass fat and protein) in the diet of dairy animals. Bypass fat remains protected from rumen microbial digestion thus have positive effect during early lactation (Naik, 2013) but, excessive feeding have detrimental effect to animals health and reproduction (Kumar *et al*., 2006). Moreover, utilization of supplemental fat in cow rations has been described to have either positive or negative effects on hematological parameters (Rajora *et al*., 1997; Omer, 1999; Awad, 2001) and blood metabolites (Marreck, 1996; El-Shewy, 1997; Bremmer *et al*., 1998; Petit *et al*., 2004). Ranjan *et al*. (2012) found that supplementation of bypass fat to lactating buffaloes increased serum total protein and cholesterol without any alteration in triglyceride and glucose level. Wadhwa *et al*. (2012) reported increased plasma concentrations of triglyceride in crossbred cattle post bypass fat supplementation. However, in some studies, no change was found in blood cholesterol level in cows supplemented with 2.5% bypass fat (Tyagi *et al*., 2010; Wadhwa *et al*., 2012). Likewise, high protein diets to meet higher requirements during early lactation in post-partum cows resulted in increased blood urea (Roy *et al*., 2003), in addition to other physiological and external factors leads to compromised reproductive performance (Qureshi *et al*., 2002; Dhali *et al*., 2006).

Hormones *viz*. insulin like growth factor-1 (IGF-1) and beta-hydroxy butyric acid (BHBA) plays important role in regulation of neuroendocrine functions and dietary regulatory function as well. These hormones showed alteration in peripheral circulation following supplementation of bypass fat and/ or protein as reported earlier (Singh *et al*., 2014; Singh *et al*., 2015). A lot of work has been carried out to determine the effect of protected nutrients alone in the ration of dairy animals, while studies on combined effect of both these nutrients in dairy animals is lacking. Bhatt and Sahoo (2017) also reported beneficial effect of supplementing bypass protein in combination with bypass fat in ewes. Thus, present study was undertaken to decipher the haemato-biochemical and hormone profile following feeding both the by-pass nutrients alone or on combination in early lactating Murrah buffaloes.

MATERIALS AND METHODS

Location of the study

The study was carried out on Murrah buffaloes of Animal Farm Section, ICAR-Central Institute for Research on Buffaloes, Hisar, Haryana, India, from November to May when humidity varied from 75 to 85% and the ambient temperature from 1.1 to 39^{o}C. The farm is located 212 meters above sea level.

Experimental animals, management and study design

Before starting the experiment proper approval for animal experimentation was obtained from the Institutional Animal Ethics Committee (IAEC). Forty post-partum Murrah buffaloes (Body weight 531.92±10.85 kg) from existing herd of animal farm were selected and divided randomly into four groups of 10 animals each on

the basis of their parity (2.68±0.28) and initial milk production (7.72±0.33 kg). Buffaloes were maintained with farm management practises, with individual feeding system in well-ventilated concrete floor shed. Animals were fed from day 0 to 120 postpartum as per their nutrient requirement suggested by ICAR (2013). Two different types of concentrate mixtures (CM, Table 1) were prepared having different proportions of rumen un-degradable proteins (using natural feed ingredients). De-oiled rice bran and cotton seed cake were included in CM2, in place of wheat bran and mustard cake of CM1. CM1 had about 25% rumen protected protein and fed to group control and bypass fat (BPF), while CM2 had 40% rumen protected protein fed to groups bypass protein (BPP) and BPP and BPF (BPPF). Buffaloes in group BPF and BPPF were additionally supplemented with BPF (commercially available calcium salt of unsaturated fatty acids) 15 g for each kg of milk produced. Experimental feeding was continued up to first 90 days only and thereafter a withdrawal effect was also investigated for a period of 30 days when feeding was shifted without supplementation and CM1 was used during the period till 120th day postpartum.

Analysis of feed and fodder

Feeds and fodder samples were analysed for proximate principles as per standard method (AOAC, 2000).

Blood sampling

Blood sampling was done on day 0, 30, 60, 90 and 120 postpartum, considering day 0 as the day of parturition. Blood samples were collected from jugular vein 3 to 5 h. post offering of the concentrate mixture at morning in serum clot activated vacutainer (Vacuette) for biochemical analysis. After sampling blood samples were chilled on ice, transported to the laboratory and centrifuged at 2500 rpm for 20 minutes. Serum was harvested and stored at -20^{o}C until analysis.

Estimation of haematological parameters

For hematological study blood samples were collected in EDTA coated vacutainer (Vacuette). Hematological parameters were determined immediately after blood collection by Vet Scan HM5 Hematology Analyzer (Abaxis, Inc.

Table 1. Ingredient composition of concentrate mixtures fed to Murrah buffaloes.

Attribute	(CM1)	(CM 2)
Barley	32	28.5
Wheat Bran	31.9	-
DORB	-	31
Mustard cake	32.2	
Cotton seed cake	-	33
Saturated fat	1.4	4
Urea	-	1
Mineral Mixture	1.5	1.5
Salt	1	1

CM= Concentrate mixture, CP1 for Control and BPF and CM2 for BPP and BPPF

CA, USA).

Blood biochemical and hormone analyses

Blood glucose estimation was done in freshly drawn blood by glucometer (Accu-Chek Active) immediately after collection of blood samples.

Serum total protein (TP), albumin, urea nitrogen, cholesterol, LDL, HDL and triglyceride concentration were determined using commercially available biochemical assay kits (Coral Clinical Systems, India) using automated biochemical analyser (Coralyzer 200, Tulip Diagnostics, India).

Insulin like Growth Factor-1 (IGF-1) and Beta-hydroxy butyric acid (BHBA) concentrations were estimated by enzyme linked immunosorbant assay (ELISA) using commercially available ELISA kits (Cloud-Clone Corp., Houston, USA; Sincere Biotech Co., Ltd. Beijing, China). The sensitivity of the IGF-1 and BHBA assay were <28.9pg/ml and <1.0 ng/ml, respectively.

Statistical analysis

Data generated in the experiment were analyzed statistically using SPSS computer package (version 16) with general linear model using LSD as Post Hoc test.

RESULTS AND DISCUSSION

Chemical composition of feeds and fodder

Ingredient composition of two different type of concentrate mixture prepared as per preference to get concentrate mixture iso-nutritional as well as variable levels of bypass protein presented in Table 1. Chemical composition of forage like green berseem (CP 12.6%) and wheat straw (CP 3.21%) were sufficient to meet the requirement of lactating buffaloes when fed with respective concentrate mixtures. The chemical composition of the concentrate mixture also remained comparable to each other (CP 18.1 and 17.6%, EE 7.18 and 7.21%, with rumen undegardable protein based on standard table value for Indian feeds, 24.72 and 39.96%, respectively, in CM1 and CM2).

Haematological profile

Haematological profile in different groups at day 0 (before starting supplementation) as well as at monthly interval is shown in Table 2.

Furthermore, haematological parameters *viz*. Hb, RBCs and WBCs including neutrophil, monocytes, lymphocytes and haematocrit, showed significant difference among overall mean values of groups. Critical elucidation of data indicate that in most of the cases initial difference among groups at day 0 of study might be the reason for the difference in overall mean among study groups and not the effect of feeding. Movaliya *et al*. (2013) also reported no change in haematological profile due to feeding of bypass methionine-lysine supplementation in Jaffarabadi heifers. Likewise, Liker *et al*. (1999) on feeding protected methionine to cattle did not report any effect on hematological parameters *viz*. WBC, RBC, Hb.

Blood biochemical parameters

Serum glucose, total protein, albumin, globulin, urea nitrogen, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides levels in buffaloes at different point of observation and their overall mean values are presented in Table 3.

Serum glucose showed non-significant (P>0.05) difference among different groups at different time period of study which was in

Table 2. Hematological parameters of buffaloes fed protected nutrients.

Parameter	Day	Control	BPF	BPP	BPPF	SEM	P value
Haematocrit (%)	0	40.52	40.02	44.19	41.30	0.701	0.149
	30	42.53	40.21	42.65	41.21	0.639	0.493
	60	40.14	39.40	44.37	40.24	0.797	0.107
	90	40.12	38.88	42.36	41.28	0.686	0.318
	120	40.99	38.01	40.85	39.81	0.993	0.712
	Overall mean	**40.86^{ab}**	**39.30^{a}**	**42.88^{b}**	**40.77^{ab}**	**0.345**	**0.003**
RBC (10^{12}/L)	0	7.38^{a}	7.08^{a}	8.21^{b}	7.39^{a}	0.153	0.048
	30	7.50	7.11	7.53	7.45	0.135	0.690
	60	7.63	7.16	8.17	7.46	0.174	0.223
	90	7.50	7.13	8.06	7.84	0.153	0.153
	120	7.83	7.13	8.40	7.69	0.237	0.312
	Overall mean	**7.57^{ab}**	**7.12^{a}**	**8.07^{b}**	**7.57^{ab}**	**0.077**	**<0.001**
Haemoglobin (g/dl)	0	11.89^{a}	11.88^{a}	13.27^{b}	12.07^{a}	0.193	0.024
	30	12.23	11.74	12.40	11.81	0.182	0.520
	60	11.03	11.52	12.66	11.65	0.260	0.157
	90	11.05	10.82	12.08	11.55	0.217	0.170
	120	11.59	10.81	11.35	11.09	0.235	0.695
	Overall mean	**11.56^{a}**	**11.36^{a}**	**12.35^{b}**	**11.63^{ab}**	**0.101**	**0.003**
Platelets (10^{9}/L)	0	1306.80	1608.70	1504.90	1132.30	240.10	0.908
	30	1343.70	1854.70	1024.50	1687.70	273.76	0.729
	60	1476.20	1136.80	1853.90	1042.40	255.83	0.686
	90	1671.40	1306.30	1284.40	2150.40	282.10	0.687
	120	1125.90	1136.00	563.10	574.90	178.11	0.492
	Overall mean	**1384.80**	**1408.46**	**1246.16**	**1317.46**	**111.62**	**0.957**
WBC (10^{9}/L)	0	9.27^{a}	12.31^{b}	10.19^{a}	10.37^{ab}	0.397	0.044
	30	10.62^{a}	13.92^{b}	11.14^{a}	10.84^{a}	0.441	0.021
	60	9.57	13.68	11.38	12.02	0.560	0.068
	90	9.50	14.44	10.52	12.96	0.737	0.064
	120	9.69	13.39	11.29	12.04	0.693	0.303
	Overall mean	**9.73^{a}**	**13.55^{c}**	**10.90^{ab}**	**11.65^{b}**	**0.259**	**<0.001**
Basophil (10^{9}/L)	0	0.07	0.08	0.11	0.09	0.008	0.448
	30	0.09	0.12	0.07	0.08	0.012	0.404
	60	0.07	0.03	0.06	0.03	0.009	0.254
	90	0.01	0.03	0.02	0.03	0.005	0.605
	120	0.01	0.01	0.03	0.01	0.004	0.289
	Overall mean	**0.05**	**0.05**	**0.06**	**0.05**	**0.004**	**0.940**

Table 2. Hematological parameters of buffaloes fed protected nutrients. (Continue)

Parameter	Day	Control	BPF	BPP	BPPF	SEM	P value
Lymphocytes (10^9/L)	0	2.69^{a}	5.29^{b}	3.28^{a}	3.83^{ab}	0.323	0.023
	30	3.16^{a}	5.13^{b}	3.58^{a}	4.01^{ab}	0.243	0.021
	60	3.00	5.56	3.62	5.01	0.386	0.060
	90	2.98	6.60	3.48	5.96	0.587	0.066
	120	3.42	6.14	3.90	6.10	0.545	0.157
	Overall mean	**3.05^{a}**	**5.74^{b}**	**3.57^{a}**	**4.98^{b}**	**0.196**	**<0.001**
Monocytes (10^9/L)	0	0.45	0.53	0.47	0.55	0.029	0.561
	30	0.53	0.63	0.53	0.73	0.039	0.223
	60	0.45	0.59	0.52	0.57	0.036	0.537
	90	0.55	0.67	0.47	0.57	0.038	0.323
	120	0.48	0.66	0.61	0.52	0.039	0.333
	Overall mean	**0.49^{a}**	**0.62^{c}**	**0.52^{ab}**	**0.59^{bc}**	**0.016**	**0.021**
Neutrophil (10^9/L)	0	5.47	6.49	5.36	5.36	0.247	0.307
	30	6.20	7.24	6.51	5.51	0.275	0.160
	60	5.70	7.25	6.85	6.16	0.281	0.208
	90	5.82	7.06	6.48	6.26	0.257	0.408
	120	5.80	6.39	6.61	5.33	0.287	0.395
	Overall mean	**5.80^{a}**	**6.88^{b}**	**6.36^{ab}**	**5.72^{a}**	**0.121**	**0.002**
Eosinophil (10^9/L)	0	0.52	0.71	0.70	0.37	0.053	0.059
	30	0.51	0.67	0.46	0.53	0.071	0.756
	60	0.36	0.35	0.33	0.28	0.053	0.957
	90	0.13	0.17	0.09	0.14	0.016	0.438
	120	0.08	0.20	0.09	0.09	0.031	0.458
	Overall mean	**0.32**	**0.42**	**0.33**	**0.28**	**0.026**	**0.270**
Basophil (10^9/L)	0	0.07	0.08	0.11	0.09	0.008	0.448
	30	0.09	0.12	0.07	0.08	0.012	0.404
	60	0.07	0.03	0.06	0.03	0.009	0.254
	90	0.01	0.03	0.02	0.03	0.005	0.605
	120	0.01	0.01	0.03	0.01	0.004	0.289
	Overall mean	**0.05**	**0.05**	**0.06**	**0.05**	**0.004**	**0.940**

Table 3. Serum biochemical parameters of buffaloes fed protected nutrients.

Parameter	Day	Control	BPF	BPP	BPPF	SEM	P value
Glucose (mg/dl)	0	68.50	69.00	69.50	70.50	1.13	0.940
	30	68.30	63.30	59.40	65.70	1.51	0.195
	60	68.30	65.50	64.30	66.40	1.58	0.915
	90	63.50	60.30	57.40	60.40	1.04	0.846
	120	61.30	59.40	59.30	59.30	1.15	0.234
	Overall mean	**65.98**	**63.50**	**61.98**	**64.46**	**0.63**	**0.147**
Total protein (g/dl)	0	7.82	8.12	7.39	7.98	0.14	0.303
	30	7.40	7.61	7.79	8.32	0.14	0.113
	60	7.61	7.67	8.24	8.16	0.14	0.254
	90	7.41	7.75	8.10	8.03	0.15	0.337
	120	7.62	7.62	8.64	7.81	0.21	0.269
	Overall mean	**7.57^{a}**	**7.75^{ab}**	**8.03^{b}**	**8.06^{b}**	**0.07**	**0.039**
Albumin (g/dl)	0	2.93	2.91	3.02	3.02	0.037	0.608
	30	2.67	2.86	2.91	2.90	0.041	0.136
	60	2.79	2.83	2.89	3.05	0.048	0.235
	90	2.52^{a}	2.78^{ab}	2.98^{b}	2.94^{b}	0.049	0.002
	120	2.63^{a}	2.82^{ab}	2.97^{b}	2.98^{b}	0.046	0.017
	Overall mean	**2.71^{a}**	**2.84^{ab}**	**2.95^{c}**	**2.98^{b}**	**0.020**	**<0.001**
Globulin (g/dl)	0	4.89	5.21	4.37	4.96	0.143	0.208
	30	4.73	4.75	4.88	5.42	0.139	0.259
	60	4.82	4.84	5.35	5.11	0.128	0.426
	90	4.89	4.97	5.12	5.09	0.131	0.925
	120	4.99	4.80	5.67	4.83	0.207	0.420
	Overall mean	**4.86**	**4.91**	**5.08**	**5.08**	**0.068**	**0.563**
Urea nitrogen (mg/dl)	0	55.97	56.77	59.47	55.72	1.11	0.655
	30	57.91	59.64	60.59	53.63	1.36	0.281
	60	56.05^{b}	57.42^{b}	47.70^{a}	48.39^{a}	1.47	0.024
	90	46.12	44.92	42.23	41.57	1.66	0.749
	120	30.44	33.31	33.23	35.78	1.98	0.835
	Overall mean	**49.30**	**50.41**	**48.64**	**47.02**	**0.95**	**0.647**
Cholesterol (mg/dl)	0	55.27	60.05	57.15	62.91	2.49	0.731
	30	123.77	131.26	134.15	139.46	3.26	0.402
	60	127.13^{a}	120.67^{a}	136.49^{ab}	149.05^{b}	3.06	0.003
	90	113.87^{a}	117.81^{ab}	142.79^{c}	137.12^{b}	4.09	0.022
	120	111.32	102.03	128.83	122.28	4.85	0.217
	Overall mean	**106.27^{a}**	**106.36^{a}**	**119.88^{ab}**	**122.16^{b}**	**2.56**	**0.038**

Table 3. Serum biochemical parameters of buffaloes fed protected nutrients. (Continue)

Parameter	Day	Control	BPF	BPP	BPPF	SEM	P value
Low density lipoprotein (mg/dl)	0	15.40	17.95	16.27	22.09	1.25	0.239
	30	36.67	36.98	40.48	45.37	1.94	0.361
	60	39.71^{b}	31.90^{a}	36.10^{ab}	42.59^{bc}	1.37	0.030
	90	30.69^{ab}	25.64^{a}	41.66^{b}	38.79^{b}	1.89	0.006
	120	64.97	55.83	65.96	62.01	2.49	0.487
	Overall mean	**37.49**	**33.66**	**40.09**	**42.17**	**1.29**	**0.110**
High density lipoprotein (mg/dl)	0	34.80	42.76	32.01	44.62	2.24	0.134
	30	65.44	70.93	74.45	79.68	2.24	0.146
	60	73.21	77.44	78.99	90.94	2.73	0.117
	90	65.07	72.59	75.27	76.22	1.69	0.076
	120	47.56^{a}	64.04^{b}	84.88^{c}	80.28^{c}	3.41	<0.001
	Overall mean	**57.22^{a}**	**65.55^{ab}**	**69.12^{b}**	**74.35^{b}**	**1.52**	**0.001**
Triglycerides (mg/dl)	0	42.82	43.28	42.12	42.39	1.38	0.992
	30	35.88	34.97	35.57	37.61	0.52	0.326
	60	37.18	37.42	35.99	42.11	0.94	0.097
	90	36.51	35.80	38.65	37.20	0.58	0.365
	120	37.96	37.54	39.87	38.20	0.84	0.789
	Overall mean	**38.07**	**37.80**	**38.44**	**39.50**	**0.43**	**0.527**

agreement with the study of Ranjan *et al.* (2012); Shelke *et al.* (2012a) in buffaloes. This could be due to high metabolic rate of utilization of glucose during early lactation and homeostatic mechanism of animal's body does not allow appreciable changes in glucose level.

In this study, feeding of bypass protein rich diets increased (P<0.05) serum total protein in BPP and BPPF groups as compared to control. However, supplementation of BPF did not influence TP level. Similar to present study, Ranjan *et al.* (2012); Wadhwa *et al.* (2012) also did not report any improvement in TP levels by supplementation of BPF in Murrah buffaloes and crossbred cows, respectively. In contrast to our findings, Movaliya *et al.* (2013) did not reported any significant differences in total protein concentration using BPP rich diets in Jaffarabadi heifers but the reason might be the use of rumen protected amino acids in spite of protein itself.In addition, serum albumin was high in bypass protein supplemented groups (BPP and BPPF) on day 90 (P<0.01) and 120 (P<0.05) thus overall mean values (P<0.001) too. No effect of supplementing BPF on albumin level was in concordant with the findings of Wadhwa *et al.* (2012), who also reported no change in albumin levels by supplementation of bypass fat in crossbred cattle, but in contrast to present study, Movaliya *et al.* (2013) observed non-significant differences in albumin concentration when fed BPP rich feeds in buffalo heifers. Here again the reason might be the use of bypass methionine and lysine in the

experiment in spite of complete protein.

Serum globulin showed non-significant (P>0.05) difference among four study groups being in accordance with Wadhwa *et al*. (2012) who also did not report difference in globulin levels due to BPF supplementation in crossbred cattle. Likewise, Movaliya *et al*. (2013) after supplementing bypass lysine and methionine did not report any difference in globulin concentration in buffalo heifers.

In present study a decreasing trend (P>0.05) in the values of serum urea nitrogen was observed in bypass protein fed groups (BPP and BPPF) and hence the overall mean values remained lower (P>0.05) in groups fed bypass protein (BPP and BPPF). Higher serum urea nitrogen remains indicative of less efficient utilization of dietary nitrogen for microbial protein synthesis due to higher ammonia level. Feeding of rumen protected protein not only results in more supply of amino acids, but also saves energy loss in urea synthesis. Similar results were reported by Tiwari and Yadav (1994) on feeding formaldehyde treated mustard cake (bypass protein source) to buffalo calves and Sahoo and Walli (2005) to lactating goats. Similar to Liker *et al*. (1999) in cattle and Movaliya *et al*. (2013) in Jaffarabadi buffalo heifers, present study also revealed decreasing serum urea nitrogen at 60th day of supplementation in groups fed with bypass protein rich concentrate mixture (BPP and BPPF). Likewise, Shelke *et al*. (2012b) showed reduction in blood urea nitrogen levels by feeding of bypass protein in combination with bypass fat in diet of buffaloes. Considering the effect of BPF supplementation on serum urea, the results in this study were analogous to report in crossbred cattle (Garg *et al*., 2012).

Supplementation of bypass fat with protein (BPPF) improved (P<0.05) overall mean cholesterol as compared to control and BPF groups. Similar to the present findings, Tyagi *et al*. (2010); Wadhwa *et al*. (2012) also did not observe any effect of BPF supplementation on serum cholesterol levels in crossbred cattle. But, significantly high cholesterol level was reported by Ranjan *et al*. (2012) in Murrah buffaloes. In contrast to present findings, Shelke *et al*. (2012a) reported no effect on cholesterol levels due to combined effect of BPF and BPP. The reason might be variation in source of BPP used, as Shelke *et al*. (2012a) used formaldehyde treated cakes, while in present study BPP values of CM was improved through replacement of feed ingredients.With respect to LDL, non-significant (P>0.05) difference in overall mean values was observed among different groups. Similar to the present findings, Ranjan *et al*. (2012) Shelke *et al*. (2012b) also did not report any effect on serum LDL content by supplementation of BPF either alone or with BPP, respectively in lactating buffaloes.

There was no significant (P>0.05) difference was reported in mean values of HDL at monthly intervals during supplementation, but one month after ceasing supplementation all the treatment groups were having higher (P<0.001) HDL values over control. Thus, overall mean values of HDL cholesterol in groups fed bypass protein (BPP and BPPF) found to be higher (P=0.001) as compared to control. In agreement with our study, Shelke *et al*. (2012b) also reported increased level of HDL cholesterol using bypass fat and protein in combination. Likewise, Ranjan *et al*. (2012) found that supplementation of BPF to lactating buffaloes increased HDL cholesterol in fat supplemented group. Increased HDL cholesterol in BPF group might be contributed by long-chain fatty acids (Shelke *et al*., 2012b) incorporation.

In the present study, triglycerides showed no significant (P>0.05) variation during different intervals after starting supplementation. Overall

mean values also found comparable among groups, which was in agreement with the studies of Delbecchi *et al*. (2001) in crossbred heifers, and Shelke *et al*. (2012a) in buffaloes. In contrast to present study, Wadhwa *et al*. (2012) reported increased plasma triglyceride concentrations in bypass fat supplemented crossbred cattle.

Hormone profile

Hormone profile (IGF1 and BHBA) of buffaloes under different groups is presented in Table 4.

Non-significant (P>0.05) difference was observed in IGF-1 values during the whole study. This was in contrast to Childs *et al*. (2008), where higher blood IGF-1 concentrations was deduced by supplementation of BPF in diet of beef heifer. This could be due to species difference. Likewise, BHBA showed non-significant (P>0.05) difference among different groups. This was similar to study of Badiei *et al*. (2014) who also reported comparable (P>0.05) value of BHBA in cattle supplemented with BPF. Numerical changes in BHBA indicates that animals were in low body weight loss in the supplemented groups as compare to control. Present study may be an indicative of appropriate feeding of buffaloes which was as per the requirement of animals and hence no animals were in negative energy balance to affect the indicators of negative energy balance (IGF-1 and BHBA).

In conclusion, this study reports the alteration of certain biochemical parameters following by-pass nutrient supplementation in Murrah buffaloes during early lactation.

Table 4. IGF1 and BHBA hormones levels in buffaloes fed protected nutrients.

Attribute	Day	Control	BPF	BPP	BPPF	SEM	P value
(IGF1) (ng/ml)	0	110.78	101.46	100.18	105.27	4.09	0.810
	30	134.71	131.70	124.10	122.23	4.76	0.772
	60	121.35	124.62	136.03	142.80	6.55	0.645
	90	127.22	128.90	115.48	112.43	4.39	0.461
	120	87.13	108.84	105.76	112.15	7.27	0.636
	Overall mean	**116.24**	**119.10**	**116.31**	**118.97**	**2.60**	**0.964**
(BHBA) (mmol/l)	0	0.683	0.639	0.688	0.655	0.072	0.995
	30	1.118	0.630	0.777	0.723	0.098	0.184
	60	1.080	0.743	0.820	0.766	0.124	0.664
	90	0.934	0.647	0.779	0.646	0.079	0.513
	120	0.810	0.624	0.695	0.670	0.076	0.877
	Overall mean	**0.925**	**0.656**	**0.752**	**0.692**	**0.041**	**0.057**

IGF1= Insulin-like Growth Factor 1, BHBA= beta-hydroxy butyric acid

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REFERENCES

AOAC, International. 2000. *Official Methods of Analysis of AOAC International,* 17th ed. Gaithersburg, MD, USA.

Awad, A.S.S. 2001. *Performance of fattening bulls fed diets supplemented with protected fat.* Ph.D. Thesis, Faculty of Agriculture, Cairo University, Giza, Egypt.

Badiei, A., A. Aliverdilou, H. Amanlou, M. Beheshti, E. Dirandeh, R. Masoumi, F. Mosakhani and H.V. Petit. 2014. Postpartum responses of dairy cows fed different durations of omega 3 fatty acid supplementation during the peripartal period. *J. Dairy Sci*., **97**: 6391-6399.

Bhatt, R.S. and A. Sahoo. 2017. Effect of feeding complete feed block containing rumen protected protein, non-protein nitrogen and rumen protected fat on improving body condition and carcass traits of cull ewes. *J. Anim. Physiol. Anim. Nutr.,* Available on doi.org/10.1111/jpn.12628

Bremmer, D.R., L.D. Ruppert, J.H. Clark and J.K. Drack Ley. 1998. Effects of chain length and unsaturation of fattty acids mixtures infused into the abomasums of lactating dairy cows. *J. Dairy Sci*., **81**: 176-188.

Childs, S., F. Carter, C.O. Lynch, J.M. Sreenan, P. Lonergan and A.K.D. Hennessy. 2008. Embryo yield and quality following dietary supplementation of beef heifers with n-3 PUFA. *Theriogenology*, **70**: 992-1003.

Delbecchi, L., C.E. Ahnadi, J.J. Kennelly and P. Lacasse. 2001. Milk fatty acid composition and mammary lipid metabolism in Holstein cows fed protected or unprotected canola seeds. *J. Dairy Sci*., **84**: 1375-1381.

Dhali, A., D.P. Mishra, R.K. Mehla and S.K. Sirohi. 2006. Usefulness of milk urea concentration to monitor the herd reproductive performance in crossbred Karan fries cows. *Asian Austral. J. Anim*., **19**: 26-30.

El-Shewy, A.A. 1997. *Feeding lactation animals on some agricultural wastes and its effect on milk yield and composition*. Ph.D. Thesis, Faculty of Agriculture, Ain Shams University, Egypt.

Garg, M.R., B.M. Bhanderi and P.L. Sherasia. 2012. Effect of supplementing bypass fat with rumen protected choline chloride on milk yield, milk composition and metabolic profile in crossbred cows. *Indian J. Dairy Sci*., **65**: 319-323.

Gargouri, A., G. Caja, R. Casals and I. Mezghani. 2006. Lactational evaluation of effects of calcium soap of fatty acids on dairy ewes. *Small Ruminant Res*., **66**: 1-10.

Indian Council of Agricultural Research, ICAR. 2013. *Nutrient Requirements of Animals - Cattle and Buffalo*, Indian Council of Agricultural Research, National Institute of Animal Nutrition and Physiology, India.

Kumar, M.R., D.P. Tiwari, A. Kumar and N. Gupta. 2006. Effect of undegradable dietary protein level and plane of nutrition on milk yield and serum biochemical constituents in crossbred cattle. *Indian J. Anim. Sci*., **76**: 733-736.

Liker, B., N. Vranesic, D. Grbesa, B.H. Lina, M. Ivana, M. Knezevic, S. Marcela, J. Leto

and D. Macesic. 2006. Blood metabolites and hematological indices of beef cattle fed rumen-protected Methionine. *Acta Veterinaria*, **56**: 3-15.

Marreck, Y.A. 1996. *Effect of feeding some by products on the performance of dairy buffaloes.* Ph.D. Thesis, Faculty of Agriculture, Ain Shams University, Egypt.

Movaliya, J.K., K.S. Dutta, R.J. Padodara, A.R. Bhadaniya and H.H. Savsani. 2013. Effect of bypass methionine-lysine supplementation on haematological and blood biochemical parameters of Jaffarabadi heifers. *Veterinary World*, **6**: 147-150.

Naik, P.K. 2013. Bypass fat in dairy ration - A Review. *Anim. Nutr. Feed Techn*., **13**: 147-163.

Omer, F.M.A. 1999. *Using protected fat prepared from soap industry by - Products in finishing rations of Friesian bulls.* Ph.D. Thesis, Faculty of Agriculture, Cairo University, Giza, Egypt.

Petit, H.V., C. Germiquet and D. Lebel. 2004. Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition and prostaglandin secretion in dairy cows. *J. Dairy Sci*., **87**: 3889.

Qureshi, M.S., G. Habib, H.A. Samad, M.M. Siddiqui, N. Ahmad and M. Syed. 2002. Reproduction-nutrition relationship in dairy buffaloes. I. Effect of intake of protein, energy and blood metabolites levels. *Asian Austral. J. Anim.,* **15**: 330-339.

Rajora, V.S., S.P. Pachauri and G.C. Gupta. 1997. Blood profiles in dairy animals of different locations and productivity. *Indian J. Dairy Sci*., **50**: 388.

Ranjan, A., B. Sahoo, V.K. Singh, S. Srivastava, S.P. Singh and A.K. Pattanaik. 2012. Effect of bypass fat supplementation on productive performance and blood biochemical profile in lactating Murrah (*Bubalus bubalis*) buffaloes. *Trop. Anim. Health. Prod*., **44**: 1615-1621.

Roy, B., R.K. Mehla and S.K. Sirohi. 2003. Influence of milk yield, parity, stage of lactation and body weight on urea and protein concentration in milk of Murrah buffaloes. *Asian Austral. J. Anim*., **16**: 1285-1290.

Sahoo, B. and T.K. Walli. 2005. Effect of feeding bypass protein as formaldehyde treated mustard cake along with energy supplement on blood metabolites and milk production in lactating goats. *Indian J. Dairy Sci*., **58**: 184-190.

Shelke, S.K., S.S. Thakur and S.A. Amrutkar. 2012a. Effect of feeding protected fat and proteins on milk production, composition and nutrient utilization in Murrah buffaloes (*Bubalus bubalis*). *Anim. Feed Sci. Tech*., **171**: 98-107.

Shelke, S.K., S.S. Thakur and S.M. Shete. 2012b. Productive and reproductive performance of Murrah buffaloes (*Bubalus bubalis*) supplemented with rumen protected fat and protein. *Indian J. Anim. Nutr*., **29**: 317-323.

Singh, M., A.K. Roy and S. Sharma. 2015. Augmentation of milk production by supplementing bypass fat in dairy animals. *Asian J. Anim. Vet. Adv*., **10**: 476-488.

Singh, M., J.P. Sehgal, A.K. Roy, S. Pandita and G. Rajesh. 2014. Effect of prill fat supplementation on hormones, milk production and energy metabolites during mid lactation in crossbred cows. *Veterinary World*, **7**: 384-388.

Tiwari, D.P. and I.S. Yadav. 1994. Effect on growth,

nutrient utilization and blood metabolites in buffalo calves fed rations containing formaldehyde treated mustard cake. *Indian J. Anim. Sci*., **64**: 625-630.

Tyagi, N., S.S. Thakur and S.S. Shelke. 2010. Effect of bypass fat supplementation on productive and reproductive performance in crossbred cows. *Trop. Anim. Health Prod*., **41**: 1749-1755.

Wadhwa, M., R.S. Grewal, M.P.S. Bakshi and P.S. Brar. 2012. Effect of supplementing bypass fat on the performance of high yielding crossbred cows. *Indian J. Anim. Sci*., **82**: 200-203.

Westwood, C.T., I.J. Lean and J.K. Garvin. 2002. Factors influencing fertility of Holstein dairy cows: A multivariate description. *J. Dairy Sci*., **85**: 3225-3237.