



## Fertility response in post-partum buffaloes following bypass nutrient supplementation

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### ABSTRACT

The study was designed to deduce the effect of by-pass nutrient supplementation on follicular profile and fertility response in post-partum buffaloes. For this study, 40 post-partum buffaloes divided into 4 groups [control (10): C, no supplementation; T1 (10), bypass fat supplementation; T2 (10), bypass protein supplementation and T3 (10), bypass fat and protein supplementation] were supplemented with bypass nutrient for 90 days postpartum from day of calving. The study animals were examined at every 10 days interval for assessing the follicular profile and resumption of cyclicity. A number of large and small follicles among the groups remained comparable. However on day 90, number of large follicles was significantly reduced in T1 ( $0.90 \pm 0.18$ ) and T2 ( $0.70 \pm 0.15$ ) in comparison to control group ( $1.40 \pm 0.16$ ) with comparable dominant follicle size among groups. Cyclicity percentage was higher in control (90%) and T2 (80%) as compared to T1 (60%) and T3 (50%), though the difference was nonsignificant. Furthermore, days to first overt estrus, number of AIs/conception and pregnancy rate remained comparable between the groups. In summary, bypass fat and protein supplementation, either alone or in combination showed no effect on follicular profile and fertility in post-partum buffaloes.

**Key words:** Buffaloes, Bypass fat, Bypass protein, Fertility, Follicle, Postpartum

Post-partum anestrus is one of the major causes for economic loss to buffalo breeders. It leads to longer calving interval and lower reproductive efficiency in buffaloes (Perera 2011). Onset of post-partum cyclicity is controlled by several factors, viz. suckling, nutrition, body condition score (BCS), milk yield and season. Among these, nutrition plays an important role, as limitation in dietary energy and protein affects production and reproduction of post-partum buffaloes. In tropics, majority of the diets are dominated by crop residues, low in energy, protein, and minerals (Qureshi *et al.* 2002). Therefore, feeding low energy and protein diet to lactating animals leads to negative energy balance (NEB), thereby decreasing productive and reproductive performance (Ranjan *et al.* 2012). This eventually affects the ovarian function through several hormones, viz. luteinising hormone (LH), insulin-like growth factor-1 (IGF-1), insulin and glucose (Beam and Butler 1999). Occurrence of NEB is physiological and evokes non-esterified fatty acid mobilization and triglyceride accumulation in the liver (Bertics *et al.* 1992). Studies have shown that negative relationship exists

between high liver triglyceride concentration and subsequent fertility (Crowe *et al.* 2014); however, Gerloff *et al.* (1986) revealed no significant or unequivocal relationship. Therefore, it is crucial to prevent this steep NEB and shorten its duration post-partum acyclicity by increasing dry matter intake during early post-partum period. Reports suggest that cows losing  $\leq 1$  unit BCS have a longer post-partum interval to first ovulation, as compared to animals with high BCS ( $\leq 4$ ) (Baruselli *et al.* 2001). This can be reduced by feeding with bypass fat and/or protein in the diet (Aardema *et al.* 2014) as studies have shown positive effect on reproductive performance in dairy cows and buffaloes (Lopes *et al.* 2009, Tyagi *et al.* 2010).

Likewise, dietary protein utilization from rumen occurs during the poor efficiency of converting dietary protein into milk protein thereby resulting in higher ammonia absorption and nitrogen excretion in the urine (Naik *et al.* 2009). In addition, feeding higher rumen degradable protein (RDP) causes excessive ruminal ammonia production eventually result in gin increased blood urea. In this light, the present study was undertaken to study the effect of bypass fat and protein supplementation, either alone or in combination, on follicular profile and fertility response in post-partum buffaloes.

### MATERIALS AND METHODS

*Study location:* The study was performed at Animal Farm

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Section, ICAR-Central Institute for Research on Buffaloes, Hisar from November 2014 to May 2015 when humidity varied from 75–85% and the ambient temperature from 1.1–39°C. The farm is located 212 meter above sea level.

*Experimental animals, management and study design:* Post-partum buffaloes (40) maintained at animal farm of the institute were divided into 4 groups (n=10 in each group) based on parity and milk production using completely randomized design (CRD). All buffaloes were maintained under iso-managerial condition with individual intensive feeding system. Animals were fed from day 0 to 120 post-partum as per nutrient requirement (ICAR 2013), while experimental feeding was continued up to first 90 days and thereafter for last 30 days feeding was common to all animals without supplementation. Recording of data was done till 120<sup>th</sup> day post-partum. Two different types of concentrate mixtures were prepared as per the ingredient composition given in Table 1, having different proportions of rumen undegradable proteins i.e. about 25% in control group concentrate mixture and about 40% in the concentrate mixture rich in rumen undegradable proteins. Bypass protein was provided with natural bypass protein rich source i. e. de oiled rice bran (DORB) and cotton seed cake by replacing the wheat bran and mustard seed cake in control group's concentrate mixture. By-pass fat was supplemented as calcium salt of long-chain fatty acids.

Feeding of different groups was as follows:

Control (C): Concentrate mixture + Available green fodder + Wheat straw

Treatment 1 (T1): Feeds and fodder as given in control + 15 g bypass fat per kg of milk production

Treatment 2 (T2): Concentrate mixture of bypass protein + Available green fodder + Wheat straw

Treatment 3 (T3): Feeds and fodder as given in T2 + 15 g bypass fat per kg of milk production

*Analysis of feed and fodder:* Feeds and fodder samples were analyzed for proximate principles, viz. dry matter (DM), total ash, organic matter, ether extract, crude protein, total carbohydrates, nitrogen free extract, crude fibre and DMI as per recommendations (AOAC 2005).

*Monitoring of follicular dynamics:* For monitoring of follicular dynamics, ultrasound scanning of genitalia was

done using a real time B-mode ultrasound scanner (Just Vision 200, Model 320A, Toshiba, Japan) equipped with an intraoperative 7.0 MHz micro convex transducer. Examination of follicular dynamics was done at 10 days interval starting from 20<sup>th</sup> day after parturition and continued up to 120<sup>th</sup> day of experiment. Each ovary was scanned in several planes by maneuvering the transducer along its surface to identify the ovarian structures (CL and follicles). Positions and sizes of follicles (>3mm) and CL were traced at each scanning and exact locations of the follicles and its size were recorded with the help of inbuilt callipers after freezing the image on the ultrasound screen. Follicles were defined non-echogenic rounded structures with a clear demarcation between the follicular wall and antrum. Ovulation was assessed by the disappearance of a largest follicle that was present at previous examination and subsequent development of a corresponding CL in the same location. Following ovulation, a CL was identified as a round to oval, grayish black structure with echogenic spots. The variables like cyclicity percentage, cyclicity starts percentage, days to first overt estrus after calving, number of artificial inseminations (AIs)/conception and conception rate were recorded for all buffaloes during post-partum study. Buffaloes were observed two times daily (04:00 AM and 03:00 PM) for signs of behavioural estrus, viz. excitement, bellowing, frequent urination, swollen vulva, chin resting on other animal, tail raising and mucus discharge. Per rectal examination was carried out for detection of overt estrus based on uterine tone and mucus discharge and animals showing estrus after 60 days post-partum were inseminated using frozen thawed semen. Pregnancy diagnosis was made by trans-rectal ultrasonography 30 days after AI and considered positive when an echogenic embryo surrounded by non-echogenic fetal fluids was detected in the uterus. Pregnancy rate in each group was calculated with formula: (Total no. buffaloes pregnant/total no. of buffaloes) × 100. Number of AIs per conception in each group was calculated as follows: (Total no. of inseminations/total no. of inseminated buffaloes).

*Statistical analysis:* Analyses were carried out by CRD as per the method described by Snedecor and Cochran (1989). Data related to cyclicity percentage and conception rate were analyzed with Chi-square test. Data represented as mean±SEM and considered significant at P<0.05.

## RESULTS AND DISCUSSION

Two specifically made concentrate mixture (CM) for two different groups i.e. control (C, having about 25% of bypass protein) and bypass protein rich concentrate mixture (T2, about 40% of bypass protein), wheat straw and green berseem fed to buffaloes are presented in Table 1. Average DMI of different experimental groups is presented in Table 2.

Mean number of small (<9mm) and large follicle (≥9 mm) at 10 days intervals in different groups is presented in Table 3. Nonsignificant (P>0.05) difference was found in overall number of small follicle in study groups. Likewise,

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

Attribute	Concentrate mixture (Control)	Concentrate mixture (Bypass protein, BPP)
Barley	32	28.5
Wheat bran	31.9	-
De-oiled rice bran	-	31
Mustard cake	32.2	-
Cotton seed cake	-	33
Saturated fat	1.4	4
Urea	-	1
Mineral mixture	1.5	1.5
Salt	1	-

Table 2. Chemical composition of feeds and fodder offered to buffaloes (% DM basis)

Attribute	CM (Control)	CM (BPP)	Wheat straw	Berseem
Organic matter	91.67	90.24	91.42	87.78
Crude protein	18.09	17.63	3.21	12.6
Ether extract	7.18	7.21	1.26	2.04
Crude fibre	8.26	10.59	33.21	29.19
Nitrogen free extract	58.16	54.81	53.74	43.95
Total carbohydrate	66.42	65.40	84.2	71.14
Total ash	8.31	9.76	8.58	12.22
Rumen undegradable protein	24.72	39.96		

CM, concentrate mixture.

nonsignificant ( $P>0.05$ ) difference in large follicle number in study groups was observed. However at 90<sup>th</sup> day, significant ( $P<0.05$ ) variation was observed among groups with highest in control ( $1.40\pm 0.16$ ) and lowest in T2 group ( $0.70\pm 0.15$ ). No significant ( $P>0.05$ ) difference was found in size of dominant follicle in different study groups (Table 3). In this study, nonsignificant difference in number of large and small follicles among different groups was in

accordance with Petit *et al.* (2002) in dairy cows. However, on 90<sup>th</sup> day of observation, number of large follicle was significantly ( $P<0.05$ ) reduced in T1 ( $0.90\pm 0.18$ ) and T2 ( $0.70\pm 0.15$ ) as compared to control group ( $1.40\pm 0.16$ ). In contrast, Bilby *et al.* (2006) reported an increase in number and size of developing follicles in cows fed calcium salts of trans-octadecenoic acids and linseed oil. Similarly, Shelke *et al.* (2012) and Savsani *et al.* (2013) reported higher ovarian activity in buffaloes following bypass protein supplementation. In this study, nonsignificant difference observed in size of dominant follicle in groups was in contrast to Robinson *et al.* (2002) and Ambrose *et al.* (2006) in cows. Likewise, other studies reported improvement in the size of dominant follicle by supplementation of bypass fat in cows (Moallem *et al.* 2007). The difference might be attributed to the feeding of selective fatty acids. The comparable follicular growth between groups can be attributed to the lack of energy deficiency in study animals (Mattos *et al.* 2000).

With respect to onset of cyclicity, nonsignificant ( $P>0.05$ ) difference among groups at different intervals was observed, being higher in control (90%, 9/10) and T2 (80%, 8/10) groups as compared to T1 (60%, 6/10) and T3 group (50%, 5/10). The results showed insignificant role of bypass supplementation in post-partum buffaloes. Similarly, nonsignificant difference in the onset of cyclicity observed

Table 3. Follicular profile in buffaloes from different study groups

Parameter	Day	Group				SEM
		C	T1	T2	T3	
Number of small follicles (<9 mm)	20	3.40±0.50	3.90±0.43	4.30±0.70	3.60±0.50	0.266
	30	4.20±0.59	4.20±0.44	4.20±0.44	4.50±0.58	0.251
	40	4.30±0.67	3.40±0.65	3.90±0.69	3.50±0.43	0.303
	50	2.80±0.47	3.70±0.45	3.00±0.45	3.40±0.50	0.231
	60	3.60±0.48	2.70±0.54	3.80±0.63	2.70±0.45	0.266
	70	3.80±0.49	3.50±0.62	3.50±0.62	4.20±0.42	0.265
	80	3.20±0.25	3.70±0.47	3.60±0.50	2.90±0.48	0.216
	90	3.70±0.56	4.00±0.52	3.70±0.47	4.00±0.54	0.252
	Overall mean		3.63±0.18	3.64±0.18	3.75±0.20	3.60±0.18
Number of large follicles (≥ 9 mm)	20	0.60±0.16	0.90±0.23	0.60±0.22	0.50±0.17	0.098
	30	0.80±0.13	0.90±0.28	0.70±0.26	0.80±0.25	0.114
	40	1.20±0.20	1.10±0.23	0.70±0.15	1.00±0.26	0.107
	50	1.40±0.16	1.00±0.21	0.80±0.20	1.10±0.10	0.090
	60	1.60±0.31	1.30±0.15	0.90±0.18	1.10±0.18	0.110
	70	1.10±0.18	1.00±0.00	1.10±0.18	1.20±0.13	0.070
	80	1.10±0.10	1.30±0.15	1.10±0.18	1.20±0.13	0.071
	90*	1.40 <sup>c</sup> ±0.16	0.90 <sup>ab</sup> ±0.18	0.70 <sup>a</sup> ±0.15	1.30 <sup>bc</sup> ±0.15	0.090
	Overall mean		1.15±0.07	1.05±0.07	0.83±0.07	1.03±0.07
Size of dominant follicle (mm)	20	9.80±0.73	10.60±0.73	9.00±0.92	9.50±0.97	0.416
	30	11.30±0.88	10.90±0.69	9.80±0.73	9.90±0.62	0.369
	40	11.50±1.00	11.50±0.79	10.40±0.95	9.70±0.50	0.418
	50	12.20±0.70	11.40±0.69	11.10±0.80	10.70±0.58	0.344
	60	10.90±0.31	10.70±0.42	9.50±0.70	11.10±0.48	0.261
	70	10.30±0.73	11.00±0.54	11.20±0.65	10.90±0.46	0.294
	80	10.50±0.54	10.10±0.50	10.70±0.58	11.30±0.30	0.247
	90	11.00±0.64	10.40±0.56	10.30±0.62	11.00±0.89	0.337
	Overall mean		10.94±0.26	10.83±0.22	10.25±0.27	10.51±0.23

Values expressed as mean±SE; \*( $P<0.05$ ), values with different superscript differ significantly.

among groups indicates the least effect of bypass protein rich feeding on resumption cyclicity in buffaloes. In accordance to these findings, Juchem (2010) reported nonsignificant difference in the resumption of cyclicity on feeding long chain fatty acid in post-partum cows. But in contrast, Shelke *et al.* (2012) and Savsani *et al.* (2013) reported enhanced ovarian activity (follicles growth and presence of CL) in buffaloes when fed with bypass protein. Similarly, Kridi *et al.* (2001) reported increased luteal activity, following feeding of 35% rumen undegradable protein (RUP) and 15.7% RDP in ewes. In addition, early onset of cyclicity following bypass nutrient supplementation was reported in cattle and buffaloes (Tyagi *et al.* 2010, Savsani *et al.* 2013). This discrepancy can be attributed to the species, season and composition of bypass nutrient supplements. Furthermore, the availability of fatty acids is less for ruminants as compared to non-ruminants which add up to the nonsignificance of the present findings.

Days to first overt estrus after calving, number of AIs/conception and conception rate (%) showed nonsignificant ( $P>0.05$ ) difference observed in days to first overt estrus among the study groups (control:  $37.88\pm 6.57$ , T1:  $42.33\pm 12.94$ , T2:  $29.37\pm 5.35$ , T3:  $30.6\pm 6.73$ ). Similarly, number of AIs/conception did not show any significant difference among 4 groups; however, it was highest in T1 group ( $2.16\pm 0.65$ ) and lowest in control ( $1.22\pm 0.20$ ), and comparable between T2 ( $1.5\pm 0.05$ ) and T3 ( $1.6\pm 0.10$ ). Overall pregnancy rate (%) till day 90 remained comparable ( $P>0.05$ ) among groups (control: 40, T1: 10, T2: 10, T3: 30). On the contrary, reduced number of AIs/conception in cross breed cattle and buffaloes fed on bypass fat supplementation have been reported earlier (Shelke *et al.* 2012, Savsani *et al.* 2013). This discrepancy might be due to season, composition of supplemented bypass fat. But, in accordance to our findings, Campanile *et al.* (2003) and Javaid *et al.* (2007) reported non-significant difference in nutrient supplemented post-partum buffaloes. In addition, supplementation of fat resulted in significantly ( $P<0.05$ ) improved service period and conception rate in buffaloes (Shelke *et al.* 2013, Savsani *et al.* 2013). But the present findings was contrary to the above results, but was in agreement with Petit and Twaqiramungu (2006) in cows. Discrepancy observed might be due to several factors controlling conception rate i.e. environment, genetics and health, and the role of bypass nutrient (fat and protein). In addition, fertility response differs with the nature of fatty acid supplementation.

In summary, bypass fat and protein supplementation, either alone or in combination, had no effect on follicular profile, resumption of cyclicity and pregnancy rate in post-partum buffaloes, and effect of specific bypass supplementation on the reproductive outcome in post-partum buffaloes needs further investigation. Furthermore, better fertility response in non-supplemented group hints other factors playing role in reproductive processes in postpartum buffaloes.

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## REFERENCES

- Aardema H, Gadella B M, van de Lest C H A, Brouwers J F H M, Stout T A E, Roelen B A J and Vos P L A M. 2014. Free fatty acid levels in fluid of dominant follicles at the preferred insemination time in dairy cows are not affected by early postpartum fatty acid stress. *Journal of Dairy Science* **98**: 2322–36.
- Ambrose D J, Kastelic J P, Corbett R, Pitney P A, Petit H V, Small J A and Zalkovic P. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in alpha-linolenic acid. *Journal of Dairy Science* **89**: 3066–74.
- AOAC International. 2005. *Official Methods of Analysis*. AOAC International, 18th edn. Gaithersburg MD USA Association of Analytical Communities.
- Baruselli P S, Barnabe V H, Barnabe R C, Visintin J A and Porto R. 2001. Effect of body condition score at calving on postpartum reproductive performance in buffalo. *Buffalo Journal* **17**: 53–65.
- Beam S W and Butler W R. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *Journal of Reproduction and Fertility* **54**: 411–24.
- Bertics S J, Grummer R R, Cadorniga-Valino C and Stoddard E E. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. *Journal of Dairy Science* **75**: 1914–22.
- Bilby T R, Guzeloglu A, MacLaren L A, Staples C R and Thatcher W W. 2006. Pregnancy bovine somatotropin and dietary n-3 fatty acids in lactating dairy cows: II Endometrial gene expression related to maintenance of pregnancy. *Journal of Dairy Science* **89**: 3375–85.
- Campanile G, Palo R D, Infaseelli F, Gasparrini B, Neglia G, Zicarelli F and D'Oeohio M J. 2003. Influence of rumen protein degradability on productive and reproductive performance in buffalo cows. *Reproduction, Nutrition and Development* **43**: 557–66.
- Crowe M A, Diskin M G and Williams E J. 2014. Parturition to resumption of ovarian cyclicity: Comparative aspects of beef and dairy cows. *Animal* **8** (1): 40–53.
- Gerloff B J, Herdt T H and Emery R S. 1986. Relationship of hepatic lipidosis to health and performance in dairy cattle. *Journal of American Veterinary Medical Association* **188**: 845–50.
- Juchem S, Cerri R and Villasenor M. 2010. Supplementation with calcium salts of linoleic and trans-octadecenoic acids improves fertility of lactating dairy cows. *Reproduction in Domestic Animals* **45**: 55–62.
- Khan S, Singh M, Mehla R K, Thakur S and Meena B S. 2016. Plasma hormones and milk production performances in early lactation buffaloes supplemented with a mixture of prilled fat sweetener and toxin binder. *Biotechnology in Animal Husbandry* **32** (1): 15–26.
- Kridi R T, Haddad S G and Muwalla M M. 2001. The effect of feeding ruminally undegradable protein on postpartum reproduction of Awassi Ewes. *Asian Australasian Journal of Animal Science* **14**: 1125–28.
- Lopes C N, Scarpa A B, Cappellozza B I, Cooke R F and

- Vasconcelos J L M. 2009. Effects of rumen-protected polyunsaturated fatty acid supplementation on reproductive performance of *Bos indicus* beef cows. *Journal of Animal Science* **87**: 3935–43.
- Mattos R, Staples C R and Thatcher W W. 2000. Effects of dietary fatty acid on reproduction in ruminant. *Reviews of Reproduction* **5**: 38–45.
- Moallem U, Katz M, Arieli A and Lehrer H. 2007. Effect of peripartum propylene glycol or fats differing in fatty acid profiles on feed intake production and plasma metabolites in dairy cows. *Journal of Dairy Science* **90**(8): 3846–56.
- Naik P K, Saijpaal S and Rani N. 2009. Effect of ruminally protected fat on *in vitro* fermentation and apparent nutrient digestibility in buffaloes (*Bubalus bubalis*). *Animal Feed Science and Technology* **53**: 68–76.
- Perera B M. 2011. Reproductive cycles of buffalo. *Animal Reproduction Science* **124**(3–4): 194–99.
- Petit H V and Twagiramungu H. 2006. Conception rate and reproductive function of dairy cows fed different fat sources. *Theriogenology* **66**: 1316–24.
- Petit H V, Dewhurst R J, Scollan N D, Proulx J G, Khalid M and Haresign W. 2002. Milk production and composition ovarian function and prostaglandin secretion of dairy cows fed omega-3 fats. *Journal of Dairy Science* **85**: 889–99.
- Qureshi M S, Habib G, Samad H A, Siddiqui M M, Ahmad N and Syed M. 2002. Reproduction-nutrition relationship in dairy buffaloes I Effect of intake of protein energy and blood metabolites levels. *Asian Australasian Journal of Animal Science* **15**: 330–39.
- Ranjan A, Sahoo B, Singh V K, Srivastava S, Singh S P and Pattanaik A K. 2012. Effect of bypass fat supplementation on productive performance and blood biochemical profile in lactating Murrah (*Bubalus bubalis*) buffaloes. *Tropical Animal Health and Production* **44**: 1615–21.
- Robinson R S, Pushpakumara P G A, Cheng Z, Peters A R, Abayasekara D R E and Wathes D C. 2002. Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. *Reproduction* **124**: 119–31.
- Savsani H H, Murthy K S, Padodara R J, Bhadaniya A R and Kalaria V A. 2013. Effect of bypass fat supplementation on haematology growth and reproductive performance in Jaffarabadi buffaloes. *Asian Journal of Animal Science* **8**(1): 12–15.
- Shelke S K, Thakur S S and Shete S M. 2012. Productive and reproductive performance of Murrah buffaloes (*Bubalus bubalis*) supplemented with rumen protected fat and protein. *Indian Journal of Animal Nutrition* **29**: 317–23.
- Snedecor G W and Cochran W G. 1989. *Statistical Methods*. 8<sup>th</sup> edn. Iowa State University Press.
- Tyagi N, Thakur S S and Shelke S K. 2010. Effect of bypass fat supplementation on productive and reproductive performance in crossbred cows. *Tropical Animal Health and Production* **42**: 1749–55.