

Therapeutic management of anoestrus in Swamp buffalo heifers and cows using bypass fat

ABSTRACT

The study was taken up to assess the effect of bypass fat supplementation on the performance of anoestrous Swamp buffalo heifers and cows maintained at Network Project on Buffalo Improvement (Swamp), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati. Efficacy of bypass fat in the treatment of anoestrus was studied on the basis of genital changes, level of some of the blood biochemical constituents and oestrus response. Twenty five per cent of the anoestrous heifers and 25 per cent of anoestrous cows treated with bypass fat + minerals + injectable phosphorus showed vulvar oedema and congestion, uterine tone and ovarian activity within 30 days of treatment. Level of serum leptin and IGF-1 was significantly higher in anoestrous cows on day 30 of treatment and the values were 4.11 ± 0.16 ng/ml for leptin and 74.11 ± 1.26 ng/ml for IGF-1 as against 3.40 ± 0.09 ng/ml leptin and 61.99 ± 4.87 ng/ml IGF-1 recorded at day 0 of treatment. Treatment of anoestrous heifer with bypass fat alone or bypass fat + minerals + injectable phosphorus for a period of 30 days resulted in increased serum oestrogen and progesterone level on day 30 of treatment indicating a positive effect of bypass fat in addressing anoestrus in buffalo heifers. In the anoestrous buffalo cows bypass fat treatment for a period of 30 days either alone or fortified with minerals and injectable phosphorus had no effect on serum oestrogen and progesterone concentration. Bypass fat treatment resulted in 37.50 per cent oestrus response in anoestrous buffalo heifers and 50.00 per cent oestrus response in anoestrous cows.

Key words: Anoestrous, IGF-1, leptin, oestrogen, Swamp Buffalo

1. INTRODUCTION

Buffalo is an integral part of traditional agriculture in India since centuries playing an important role in overall social development through contributions towards production of milk, meat, hides and strengthening draft power for agricultural operations. More than 50.00 per cent of India's milk production originates from buffaloes. Buffalo milk is superior to cow milk in terms of fat content (Zaman *et al.*, 2007) ensuring higher sale price of milk. Buffaloes in the state of Assam are however, of the swamp type having 48 diploid no. of chromosome (2N) and distributed mostly in upper Brahmaputra valley of Assam, India with low productive and reproductive performance. Anoestrus is the most important cause of poor

reproductive performance in buffaloes leading to delayed puberty in heifers and longer inter calving period in cows (Das and Khan, 2010). The incidence of anoestrus in buffalo varies in wider range of 11.25 to 66.28 per cent in different seasons (Kumar *et al.*, 2013). The incidence is much higher in Swamp buffaloes due to poor reproductive hormone profile.

Ovarian cyclicity is regulated by endocrine and neuroendocrine mechanisms involving namely hypothalamic hormones, gonadotropins and ovarian steroids (Terzano *et al.*, 2012). In Swamp buffaloes, oestradiol-17 β profile during the oestrous cycle has been reported to be lower as compared to that in riverine type (Mondal *et al.*, 2007). The progesterone levels raise and falls in coincidence with the growth and regression of corpus luteum. Supplementation of fat, minerals and vitamins in the diet improved the reproductive efficiency in cattle (Ramteke *et al.*, 2004). Under nourishment brings about the loss of body weight and body condition prompting negative energy balance (Bindari *et al.*, 2013). Bypass fats are normally alluded as ruminal dormant fat, related with calcium particles rather than glycerol backbone. Bypass fat has low solvency in rumen and is less defenseless to biohydrogenation. However, in abomasum at acidic pH it is dissociated and set free fatty acids and calcium for absorption. Bypass fat not only act as a precursor of progesterone via cholesterol and prostaglandins but also source of an energy supplement during the transition period leading to improvement in reproductive performance (Staples *et al.*, 1998). The deleterious effect of acute negative energy balance on lactation can be avoided by feeding bypass fat. Metabolic hormones such as leptin, ghrelin and IGF-1 have been considered as the indicators of energy balance in animals. An increase in the level of leptin and IGF-1 and decrease in the level of ghrelin indicate positive energy balance (Tena-Sempere, 2005 and Perello and Zigman, 2012). The present study was performed to understand the effect of nutritional supplementation on genital status in anoestrous buffalo cows and heifers.

2. MATERIALS AND METHODS

2.1 Experimental animals

The experiment was carried out in 24 anoestrous buffalo heifers not showing signs of estrous even upto 3 years of age and 24 anoestrous buffalo cows not showing sign of estrous even after 8 month of calving irrespective of parity maintained at Network Project on Buffalo improvement (Swamp), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22.

2.2 Allotment of treatment regimen

The anoestrous heifers and cows were divided separately into 3 different groups for allotting treatment regimens. Group I animals were treated with oral bypass fat alone, Group II animals were treated with oral bypass fat + oral mineral mixture + injectable phosphorus and Group III animals were treated as control animal. Group I and Group II animals were allotted the following treatment regimens

Bypass fat alone: Bypass fat (Fatamax, Intas Pharmaceuticals limited, Ahmedabad, India) @ 100 gm per day per animal with concentrate mixture orally for 30 days continuously commencing from the day of treatment (day 0).

Bypass fat fortified with minerals and phosphorus: Bypass Fat (Fatamax, Intas Pharmaceuticals limited, Ahmedabad, India) @ 100 gm per day per animal with concentrate mixture for 30 days + Mineral mixture (Minfa Gold, Intas Pharmaceuticals limited, Ahmedabad, India) @ 30 gm per animal daily with concentrate mixture for a period of 30 days + Injectable Phosphorus (Tonophosphan Vet, containing sodium salt of 4-dimethyl amino-2-methyl phenyl-phosphinic acid, MSD-Animal Health, Pune, India) @ 2 gm intramuscularly for three occasions at alternate days commencing from the day of treatment (day 0).

2.3 Schedule of examination and blood collection

Rectal palpation of the genital organs was done in each anestrous buffalo heifer and cows at day 0, day 15 and day 30 of treatment. On each day of examination blood samples were collected via jugular vein-puncture into a heparinized vial. It was then transported to the laboratory and serum was separated from the coagulated blood by centrifugation at 3000 rpm for 10 minutes. The separated serum samples were collected in sterilized screw-capped vials, labeled and stored at -20°C for analysis.

On each day during the treatment period the animals were let loose for grazing with a bull in morning hours for detection of signs of oestrus.

2.4 Estimation of blood biochemical constituents

The energy balance in the experimental animal was studied on the basis of levels of metabolic hormones *viz.* Leptin, Ghrelin and IGF-1 in blood collected from each experimental animals on each day of examination. ELISA techniques were used to estimate the serum leptin (Cloud-Clone Corp. marketed by 1304 Langham Creek Dr, Suite 226, Houston, TX 77084, USA), ghrelin (Diagnostics Biochem Canada Inc. marketed by 41 Byron Avenue, Dorchester, Ontario, Canada) and IGF-1 (Cloud-Clone Corp. marketed by 1304 Langham Creek Dr, Suite 226, Houston, TX 77084, USA) in the collected samples. The level of the reproductive hormone oestrogen and progesterone in the serum samples collected from the experimental animals was estimated by using a solid phase enzyme linked immunosorbant assay kit (Estradiol ELISA kit and Progesterone ELISA kit LDN, Germany).

2.5 Statistical analysis

The statistical analysis of the data was done using software IBM-SPSS-20 (<http://www.spss.co.in>) and Micro Soft Excel-2010 (<http://office.microsoft.com>)

3.RESULT AND DISCUSSION

3.1 Genital status

Genital changes observed in vulva, vagina, cervix, uterus and ovaries of anoestrous heifers and anoestrous buffalo cows treated with bypass fat alone or bypass fat + minerals + injectable phosphorus also of the control group on day 0, day 15 and day 30 of treatment have been presented in Table 1 and Table 2 respectively.

Table 1. Frequency of occurrence of different genital changes in anoestrous buffalo heifers at different days of treatment with different treatment regimens

Genital organ	Changes observed	Frequency of occurrence (%)								
		Bypass fat (n=8)			Bypass fat + minerals + injectable phosphorus (n=8)			Control (n=8)		
		day 0	day 15	day 30	day 0	day 15	day 30	day 0	day 15	day 30
Vulva	Oedematous	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	25.00 (2)	25.00 (2)	0.00 (0)	0.00 (0)	0.00 (0)
	Wrinkled	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	75.00 (6)	75.00 (6)	100.0 (8)	100.0 (8)	100.0 (8)
	Congested	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	25.00 (2)	25.00 (2)	0.00 (0)	0.00 (0)	0.00 (0)
	Pale	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	75.00 (6)	75.00 (6)	100.0 (8)	100.0 (8)	100.0 (8)
Vagina	Discharge present	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
	Discharge absent	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)
Cervix	Relaxed and open	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
	Close	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)
Uterus	Tonic	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	25.00 (2)	0.00 (0)	0.00 (0)	0.00 (0)
	Atonic	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	75.00 (6)	100.0 (8)	100.0 (8)	100.0 (8)
Ovary	Active	0.00 (0)	0.00 (0)	25.00 (2)	0.00 (0)	0.00 (0)	50.00 (4)	0.00 (0)	0.00 (0)	0.00 (0)
	Smooth	100.0 (8)	100.0 (8)	75.00 (6)	100.0 (8)	100.0 (8)	50.00 (4)	100.0 (8)	100.0 (8)	100.0 (8)

Figures in the parentheses indicate number of observations.

Table 2. Frequency of occurrence of different genital changes in anoestrous buffalo cows at different days of treatment with different treatment regimens

Genital organ	Changes observed	Frequency of occurrence (%)								
		Bypass fat (n=8)			Bypass fat + minerals + injectable phosphorus (n=8)			Control (n=8)		
		day 0	day 15	day 30	day 0	day 15	day 30	day 0	day 15	day 30
Vulva	Oedematous	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	25.00 (2)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
	Wrinkled	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	75.00 (6)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)
	Congested	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	25.00 (2)	25.00 (2)	0.00 (0)	0.00 (0)	0.00 (0)
	Pale	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	75.00 (6)	75.00 (6)	100.0 (8)	100.0 (8)	100.0 (8)
Vagina	Discharge present	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
	Discharge absent	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)
Cervix	Relaxed and open	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
	Close	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)
Uterus	Tonic	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	25.00 (2)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
	Atonic	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	75.00 (6)	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)
Ovary	Active	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	25.00 (2)	25.00 (2)	0.00 (0)	0.00 (0)	0.00 (0)
	Smooth	100.0 (8)	100.0 (8)	100.0 (8)	100.0 (8)	75.00 (6)	75.00 (6)	100.0 (8)	100.0 (8)	100.0 (8)

Figures in the parentheses indicate number of observations.

Results obtained in the present study indicated that feeding of bypass fat fortified with minerals and injectable phosphorus for a period of 30 days had some beneficial effect in addressing anoestrous in both buffalo heifers and cows. It was observed that 25 per cent of the anoestrous buffalo heifers and 25 per cent of anoestrous buffalo cows treated with bypass fat + minerals + injectable phosphorus showed vulvar oedema and congestion, uterine tone and ovarian activity within 30 days of treatment.

These characteristics were indicative of cyclic changes in treated anoestrous heifers and cows (Amonge, 1993; Singh *et al.*, 1984; Borah, 1994; Sinha, 1998 and Deka, 2017). These changes were not marked in the non treated heifers and cows under the control groups. However, 25 per cent buffalo heifers treated with bypass fat alone showed ovarian activity on day 15 of treatment which was not observed in anoestrous buffalo cow.

3.2 Energy balance

Average levels of the metabolic hormones such as leptin, ghrelin and IGF-1 in anoestrous buffalo heifers at different days of treatment with different treatment regimens have been presented in Table 3 while that of anoestrous cows in Table 4.

Serum Leptin: As observed in the present study feeding of bypass fat either alone or fortified with minerals and injectable phosphorus for a period of 30 days led to an increase in the level of serum leptin in anoestrous Swamp buffalo heifers. In anoestrous buffalo cows feeding of bypass fat alone improved serum leptin level within 30 days of treatment.

This indicated an improvement of energy balance in anoestrous Swamp buffalo heifers and cows due to feeding of bypass fat. The present findings were in good agreement with that of Deka (2017) who observed that feeding of bypass fat alone as well as bypass fat + minerals + vitamins for 45 days enhanced serum leptin level in both anoestrous Swamp buffalo heifers and cows. According to Jenkin and Palmquist (1984) bypass fat enhanced energy density of ration leading to increase energy intake in **Table 3**.

Table 3. Level of Leptin, Ghrelin and IGF-1 in the serum of anoestrous heifers at different days of treatment with different treatment regimens

Treatment Regimen	Leptin(ng/ml)			Ghrelin (pg/ml)			IGF-1(ng/ml)		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Bypass fat (8)	2.09 _A ± 0.23	2.64 _A ± 0.25	3.61 _B ± 0.18	320.08 ± 17.65	315.39 ± 9.50	316.64 ± 4.72	61.37 ± 2.30	62.88 ± 2.09	63.17 ± 3.13
Bypass fat + minerals + injectable phosphorus (8)	2.28 _A ± 0.14	2.65 _A ± 0.26	3.52 _B ± 0.22	312.95 ± 8.63	315.00 ± 9.10	316.75 ± 4.60	62.88 ± 1.09	62.66 ± 1.68	64.75 ± 0.92
Control	2.60 _A	2.48 _A	2.65 _A	319.50	318.75	319.68	61.50	61.50	62.00

(8)	± 0.03	± 0.07	± 0.09	± 0.87	± 0.14	± 0.39	± 1.49	± 0.12	± 0.14
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Figures in the parentheses indicate number of animals taken in the treatment regimen

^{A, B}Means bearing similar subscript in a row within the parameter do not differ significantly

Table 4. Level of Leptin, Ghrelin and IGF-1 in the serum of anoestrous buffalo cows at different days of treatment with different treatment regimens

Treatment Regimen	Leptin (ng/ml)			Ghrelin (pg/ml)			IGF-1 (ng/ml)		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Bypass fat (8)	3.40 ^a _A ± 0.09	4.12 ^a _B ± 0.16	4.11 ^a _B ± 0.16	326.30 ^a _A ± 8.24	262.36 ^a _B ± 6.26	262.05 ^a _B ± 7.25	61.99 ^a _A ± 4.87	74.64 ^a _B ± 4.00	74.11 ^a _B ± 1.26
Bypass fat + minerals + injectable phosphorus (8)	3.20 ^a _{AB} ± 0.20	2.94 ^b _B ± 0.21	3.61 ^b _A ± 0.18	325.71 ^a _A ± 6.97	262.05 ^a _B ± 7.25	223.67 ^b _C ± 3.24	57.74 ^a _A ± 1.70	70.94 ^a _B ± 2.84	70.19 ^b _B ± 0.95
Control (8)	3.22 ^a _A ± 0.19	3.05 ^b _A ± 0.09	3.10 ^c _A ± 0.06	323.75 ^a _A ± 0.14	322.50 ^b _A ± 0.29	323.25 ^c _A ± 0.72	57.94 ^a _A ± 0.74	56.75 ^b _A ± 2.17	56.25 ^c _A ± 0.14

Figures in the parentheses indicate number of animals taken in the treatment regimen.

^{a, b, c}Means bearing similar superscript in a column do not differ significantly

^{A, B, C}Means bearing similar subscript in a row within the parameter do not differ significantly

buffaloes. Another important observation in the present study was that bypass fat when fortified with minerals and injectable phosphorus did not improve serum leptin level within 30 days of treatment period in anoestrous buffalo cows. Rather the level decreased on day 15 of treatment after which it increased to the pretreatment level on day 30 of treatment. The reason for a decrease in the serum leptin level due to feeding bypass fat + minerals + injectable phosphorus on day 15 of treatment in anoestrous buffalo cows under the present study could not be explained. However, it was reported that leptin expression was modulated by physiological status of animals and body fatness, type of nutrition and lactation were important factors for leptin gene expression and leptinaemia in ruminants (Chilliard *et al.*, 2005 and Ahima and Flier, 2000). Chilliard *et al.* (2005) also stated that lactation down regulated leptinaemia even when energy balance was positive.

Serum ghrelin: The level of serum ghrelin was not found to vary due to feeding bypass fat alone or bypass fat + minerals + injectable phosphorus for a period of 30 days in anoestrous heifers. But in case of anoestrous buffalo cows serum ghrelin concentration decreased to a significantly lower level within 30 days of treatment and the decrease was marked even on day 15 of treatment. This indicated that there was improvement in energy balance of anoestrous buffalo cows following treatment with bypass fat. Serum ghrelin values in the control anoestrous cows remained unchanged in 30 days treatment period.

Serum IGF-1: Bypass fat feeding did not affect level of energy in the anoestrous heifers. But in case of anoestrous buffalo cows bypass fat feeding proved to be beneficial for improvement in the energy balance as indicated by increased serum IGF-1 level even at day 15 of treatment.

From the result obtained in the present study on the metabolic hormones it could be concluded that feeding of bypass fat for a period of 30 days did not improve energy balance in anoestrous buffalo heifers but proved to be beneficial in anoestrous buffalo cows and its fortification with minerals and injectable phosphorus did not have any beneficial effect. Jenkin and Palmquist (1984) working on buffaloes reported that bypass fat could enhance energy density of ration and energy intake in early lactation and thus effectively avoided deleterious effect of negative energy balance on lactation.

3.3 Reproductive hormone

Serum oestrogen

The mean serum oestrogen level in anoestrous buffalo heifers treated with bypass fat alone was 3.00 ± 0.18 , 3.09 ± 0.18 and 3.90 ± 0.21 pg/ml on day 0, day 15 and day 30 of treatment respectively. In the heifers treated with bypass fat + minerals + injectable phosphorus the mean oestrogen values were 3.09 ± 0.18 , 5.81 ± 1.05 and 3.91 ± 0.14 pg/ml on the corresponding days of treatment. In control group the serum oestrogen level was recorded as 2.59 ± 0.10 , 2.57 ± 0.19 and 2.77 ± 0.04 pg/ml on day 0, day 15 and day 30 of treatment respectively. Serum oestrogen level differed significantly ($P < 0.01$) between treatment regimens but not between days of treatment.

In the case of anoestrous buffalo cows treated with bypass alone the mean level of serum oestrogen on day 0, day 15 and day 30 of treatment was 2.70 ± 0.21 , 2.84 ± 0.44 and 2.99 ± 0.25 pg/ml respectively. On the corresponding days of treatment the mean values were 2.83 ± 0.17 , 2.89 ± 0.31 and 2.90 ± 0.17 pg/ml in anoestrous cows treated with bypass fat + minerals + injectable phosphorus against the mean values of 2.59 ± 0.10 , 2.68 ± 0.10 and 2.72 ± 0.07 pg/ml in the control group.

It was clear that treatment of anoestrous heifers with bypass fat alone or bypass fat + minerals + injectable phosphorus for a period of 30 days resulted in increased serum oestrogen level on day 30 of treatment. Bypass fat fortification with minerals and injectable phosphorus led to elevated serum oestrogen level in the heifers even on day 15 of treatment. Increased level of serum oestrogen was indicative of an increased follicular activity following bypass fat treatment (Paltaet *al.*1998). Rectal

examination performed in the treated anoestrous heifers under the present study also revealed ovarian activity in 25 to 50 per cent heifers treated with the two regimens of bypass fat. In the anoestrous buffalo cows bypass fat treatment for a period of 30 days either alone or fortified with minerals and injectable phosphorus had no effect on serum oestrogen concentration.

Serum progesterone

The mean serum progesterone level in anoestrous buffalo heifers treated with bypass fat alone was 1.50 ± 0.18 , 1.72 ± 0.07 and 2.92 ± 0.07 ng/ml on day 0, day 15 and day 30 of treatment respectively. In the anoestrous heifers treated with bypass fat + minerals + injectable phosphorus the mean progesterone values were 1.79 ± 0.26 , 1.24 ± 0.31 and 2.85 ± 0.482 ng/ml on the corresponding days of treatment. In the control group the serum progesterone level was 1.72 ± 0.10 , 1.72 ± 0.04 and 1.55 ± 0.03 ng/ml on day 0, day 15 and day 30 of treatment respectively. Serum progesterone level differed significantly ($P < 0.01$) between days of treatment but not between treatment regimens.

In case of anoestrous buffalo cows treated with bypass alone the mean level of serum progesterone on day 0, day 15 and day 30 of treatment was 1.62 ± 0.14 , 1.55 ± 0.25 and 1.79 ± 1.14 ng/ml respectively. On the corresponding days of treatment the mean progesterone values were 1.75 ± 0.22 , 1.43 ± 0.21 and 1.72 ± 0.07 ng/ml in anoestrous cows treated with bypass fat + minerals + injectable phosphorus against the mean values of 1.71 ± 0.10 , 1.74 ± 0.40 and 1.81 ± 0.32 ng/ml in the control group.

Feeding of bypass fat with or without supplementation led to increased serum progesterone level on day 30 of treatment indicating a positive effect of bypass fat in addressing anoestrous in buffalo heifers. Serum progesterone level did not change in the non treated control group of anoestrous heifers. This variation in the level of serum progesterone during the treatment period might be due to the variation in luteal activity in the treated heifers (Ahmed *et al.*, 1977 and Mondal *et al.*, 2007).

In the case of anoestrous buffalo cows bypass fat feeding did not affect the level of serum progesterone during the 30 days treatment period. This might be an indication of ineffective role of bypass fat in treating anoestrous condition in buffalo cows. In this context, it could be mentioned that period of feeding bypass fat might play an important role in determining the efficacy of bypass fat for treatment of anoestrous buffaloes. Beneficial effect of feeding bypass fat for prolonged period on the reproductive performance of cattle had been known. Wolf *et al.* (2002) recorded significant increase in pregnancy rate of cows from 52.30 per cent to 86.40 per cent after supplementing Ca-LCFA, a bypass fat for a period of 120 days after parturition. Tyagi *et al.* (2010) using bypass fat for a period of 40 days pre partum to 90 days post-partum obtained higher calving rate, lesser time for expulsion of fetal membrane and involution of uterus and lesser post-partum estrous interval in crossbred cows.

3.4 Oestrus response to treatment with bypass fat

Percent responded and post treatment oestrous interval in anoestrous Swamp buffalo cows and heifers treated with different treatment regimens have been presented in Table 5.

Table 5. Percent responded and post treatment interval in anoestrous Swamp buffalo cows and heifers treated with different treatment regimens

Treatment Regimens	No. of animals Treated	No of animal responding to Treatment	Per cent conceived	Average Post treatment oestrus interval (days)
Buffalo Heifers				
Bypass fat alone	8	4	50.00	55
Bypass fat + Minerals + Vitamins	8	6	75.00	40
Buffalo Cows				
Bypass fat alone	8	5	62.50	45
Bypass fat + Minerals + Vitamins	8	7	87.50	38

When bypass fat fortified with minerals and vitamins it enhanced the oestrus response rate and shortened the post treatment oestrus interval. Bypass fat feeding in periparturient period reduced post-partum oestrus interval in buffaloes (Ramteke *et al.* 2014). Several hypotheses have been postulated to explain the role of bypass fat in enhancing reproductive performance of dairy animals. These are (i) improved energy balance resulted in an earlier return to post-partum ovarian cyclicity; (ii) increased linoleic acid may provide increased PGF₂ α to stimulate return to ovarian cyclicity and improve follicular recruitment; and (iii) increase in progesterone secretion either from improved energy balance or from altered lipoprotein composition from dietary fat improves fertility (Naik, 2013). Lammoglia *et al.*, 1997 reported that fatty acid supplementation had a positive effect on preovulatory follicles with formation of larger corpora lutea having optimum to high progesterone concentration.

4. CONCLUSION

Bypass fat alone or fortified with minerals and injectable phosphorus could be effectively used for the treatment of anoestrus in Swamp buffalo heifers and cows.

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