

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, Assam, India. 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 anoestrus 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

32 reproductive performance in buffaloes leading to delayed puberty in heifers and longer inter calving
33 period in cows (Das and Khan, 2010). The incidence of anoestrus in buffalo varies in wider range of
34 11.25 to 66.28 per cent in different seasons (Kumar *et al.*, 2013). The incidence is much higher in Swamp
35 buffaloes due to poor reproductive hormone profile.

36 Ovarian cyclicity is regulated by endocrine and neuroendocrine mechanisms involving namely
37 hypothalamic hormones, gonadotropins and ovarian steroids (Terzano *et al.*, 2012 and Oz S *et al.*, 2024).
38 In Swamp buffaloes, oestradiol-17 β profile during the oestrous cycle has been reported to be lower as
39 compared to that in riverine type (Mondal *et al.*, 2007). The progesterone levels raise and falls in
40 coincidence with the growth and regression of corpus luteum. Supplementation of fat, minerals and
41 vitamins in the diet improved the reproductive efficiency in cattle (Ramteke *et al.*, 2004). Under
42 nourishment brings about the loss of body weight and body condition prompting negative energy balance
43 (Bindari *et al.*, 2013 and Otto *et al.*, 2014). Bypass fats are normally alluded as ruminal dormant fat,
44 related with calcium particles rather than glycerol backbone. Bypass fat has low solvency in rumen and is
45 less defenseless to biohydrogenation. However, in abomasum at acidic pH it is dissociated and set free
46 fatty acids and calcium for absorption. Bypass fat not only act as a precursor of progesterone via
47 cholesterol and prostaglandins but also source of an energy supplement during the transition period
48 leading to improvement in reproductive performance (Staples *et al.*, 1998). The deleterious effect of acute
49 negative energy balance on lactation can be avoided by feeding bypass fat. Metabolic hormones such as
50 leptin, ghrelin and IGF-1 have been considered as the indicators of energy balance in animals. An
51 increase in the level of leptin and IGF-1 and decrease in the level of ghrelin indicate positive energy
52 balance (Tena-Sempere, 2005 and Perello and Zigman, 2012). The present study was performed to
53 understand the effect of nutritional supplementation on genital status in anoestrous buffalo cows and
54 heifers.

55 **2.MATERIALS AND METHODS**

56 **2.1Experimental animals**

57 The experiment was carried out in 24 aneestrous buffalo heifers not showing signs of estrous even
58 up to 3 years of age and 24 aneestrous buffalo cows not showing sign of estrous even after 8 month of
59 calving irrespective of parity maintained at Network Project on Buffalo improvement (Swamp), College
60 of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India.

61 **2.2 Allotment of treatment regimen**

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

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

69 *Bypass fat fortified with minerals and phosphorus:* Bypass Fat (Fatamax, Intas Pharmaceuticals
70 limited, Ahmedabad, India) @ 100 gm per day per animal with concentrate mixture for 30 days + Mineral
71 mixture (Minfa Gold, Intas Pharmaceuticals limited, Ahmedabad, India) @ 30 gm per animal daily with
72 concentrate mixture for a period of 30 days + Injectable Phosphorus (Tonophosphan Vet, containing
73 sodium salt of 4-dimethyl amino-2-methyl phenyl-phosphinic acid, MSD-Animal Health, Pune, India) @
74 2 gm intramuscularly for three occasions at alternate days commencing from the day of treatment (day 0).

75 **2.3 Schedule of examination and blood collection**

76 Rectal palpation of the genital organs was done in each anestrus buffalo heifer and cows at day
77 0, day 15 and day 30 of treatment. On each day of examination blood samples were collected via jugular
78 vein-puncture into a heparinized vial. It was then transported to the laboratory and serum was separated
79 from the coagulated blood by centrifugation at 3000 rpm for 10 minutes. The separated serum samples
80 were collected in sterilized screw-capped vials, labeled and stored at -20°C for analysis.

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

83 **2.4 Estimation of blood biochemical constituents**

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

93 **2.5 Statistical analysis**

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

96 **3.RESULT AND DISCUSSION**

97 **3.1 Genital status**

98 Genital changes observed in vulva, vagina, cervix, uterus and ovaries of anoestrous heifers and
99 anoestrous buffalo cows treated with bypass fat alone or bypass fat + minerals + injectable phosphorus

100 also of the control group on day 0, day 15 and day 30 of treatment have been presented in Table 1 and
 101 Table 2 respectively.

102 **Table 1.** Frequency of occurrence of different genital changes in anoestrous buffalo heifers at
 103 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) |

104 Figures in the parentheses indicate number of observations.

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

| Genital | Changes | Frequency of occurrence (%) |
|---------|---------|-----------------------------|
|---------|---------|-----------------------------|

| organ | observed | 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 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 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 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 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 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 0 (8) | 100.0 (8) | 75.00 (6) | 75.00 (6) | 100.0 (8) | 100.0 (8) | 100.0 (8) |

107 Figures in the parentheses indicate number of observations.

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

113 These characteristics were indicative of cyclic changes in treated anoestrous heifers and cows
 114 (Amonge, 1993; Singh *et al.*, 1984; Borah, 1994; Sinha, 1998, Deka, 2017 and **Gunun *et al.*, 2024**). These
 115 changes were not marked in the non treated heifers and cows under the control groups. However, 25 per
 116 cent buffalo heifers treated with bypass fat alone showed ovarian activity on day 15 of treatment which
 117 was not observed in anoestrous buffalo cow.

118 **3.2 Energy balance**

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

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

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

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

| Treatment Regimen | Leptin(ng/ml) | | | Ghrelin (pg/ml) | | | IGF-1(ng/ml) | | |
|-------------------|-----------------------------|-----------------------------|-----------------------------|---------------------|----------------------|----------------------|---------------------|----------------------|----------------------|
| | Day 0 Mean ± S.E | Day 15 Mean ± S.E | Day 30 Mean ± S.E | Day 0 Mean ± S.E | Day 15 Mean ± S.E | Day 30 Mean ± S.E | Day 0 Mean ± S.E | Day 15 Mean ± S.E | Day 30 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 (8) | 2.60 _A ± 0.03 | 2.48 _A ± 0.07 | 2.65 _A ± 0.09 | 319.50 ± 0.87 | 318.75 ± 0.14 | 319.68 ± 0.39 | 61.50 ± 1.49 | 61.50 ± 0.12 | 62.00 ± 0.14 |

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

139

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

| Treatment Regimen | Leptin (ng/ml) | | | Ghrelin (pg/ml) | | | IGF-1 (ng/ml) | | |
|---|---|--|--|--|--|--|---|---|---|
| | Day 0 Mean ± S.E | Day 15 Mean ± S.E | Day 30 Mean ± S.E | Day 0 Mean ± S.E | Day 15 Mean ± S.E | Day 30 Mean ± S.E | Day 0 Mean ± S.E | Day 15 Mean ± S.E | Day 30 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

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

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

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

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

167 **3.3 Reproductive hormone**

168 *Serum oestrogen*

169 The mean serum oestrogen level in anoestrous buffalo heifers treated with bypass fat alone was
170 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
171 heifers treated with bypass fat + minerals + injectable phosphorus the mean oestrogen values were
172 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
173 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
174 day 30 of treatment respectively. Serum oestrogen level differed significantly ($P < 0.01$) between treatment
175 regimens but not between days of treatment.

176 In the case of anoestrous buffalo cows treated with bypass alone the mean level of serum
177 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
178 respectively. On the corresponding days of treatment the mean values were 2.83 ± 0.17 , 2.89 ± 0.31 and
179 2.90 ± 0.17 pg/ml in anoestrous cows treated with bypass fat + minerals + injectable phosphorus against
180 the mean values of 2.59 ± 0.10 , 2.68 ± 0.10 and 2.72 ± 0.07 pg/ml in the control group.

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

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

190 *Serum progesterone*

191 The mean serum progesterone level in anoestrous buffalo heifers treated with bypass fat alone
192 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.
193 In the anoestrous heifers treated with bypass fat + minerals + injectable phosphorus the mean
194 progesterone values were 1.79 ± 0.26 , 1.24 ± 0.31 and 2.85 ± 0.482 ng/ml on the corresponding days of
195 treatment. In the control group the serum progesterone level was 1.72 ± 0.10 , 1.72 ± 0.04 and 1.55 ± 0.03
196 ng/ml on day 0, day 15 and day 30 of treatment respectively. Serum progesterone level differed
197 significantly ($P < 0.01$) between days of treatment but not between treatment regimens.

198 In case of anoestrous buffalo cows treated with bypass alone the mean level of serum
199 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
200 respectively. On the corresponding days of treatment the mean progesterone values were 1.75 ± 0.22 ,
201 1.43 ± 0.21 and 1.72 ± 0.07 ng/ml in anoestrous cows treated with bypass fat + minerals + injectable
202 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.

203 Feeding of bypass fat with or without supplementation led to increased serum progesterone level
204 on day 30 of treatment indicating a positive effect of bypass fat in addressing anoestrous in buffalo
205 heifers. Serum progesterone level did not change in the non treated control group of anoestrous heifers.
206 This variation in the level of serum progesterone during the treatment period might be due to the variation
207 in luteal activity in the treated heifers (Ahmed *et al.*, 1977, Mondal *et al.*, 2007 and Dixit *et al.*, 2023).

208 In the case of anoestrous buffalo cows bypass fat feeding did not affect the level of serum
209 progesterone during the 30 days treatment period. This might be an indication of ineffective role of
210 bypass fat in treating anoestrous condition in buffalo cows. In this context, it could be mention that period
211 of feeding bypass fat might play an important role in determining the efficacy of bypass fat for treatment
212 of anoestrous buffaloes. Beneficial effect of feeding bypass fat for prolonged period on the reproductive
213 performance of cattle had been known. Wolf *et al.* (2002) recorded significant increase in pregnancy rate
214 of cows from 52.30 per cent to 86.40 per cent after supplementing Ca-LCFA, a bypass fat for a period of
215 120 days after parturition. Tyagi *et al.* (2010) using bypass fat for a period of 40 days pre partum to 90
216 days post-partum obtained higher calving rate, lesser time for expulsion of fetal membrane and involution
217 of uterus and lesser post-partum estrous interval in crossbred cows.

218 **3.4 Oestrus response to treatment with bypass fat**

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

221 **Table 5.** Percent responded and post treatment interval in anoestrous Swamp buffalo cows and
 222 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 |

223
 224 When bypass fat fortified with minerals and vitamins it enhanced the oestrus response rate and
 225 shortened the post treatment oestrus interval. Bypass fat feeding in periparturient period reduced post-
 226 partum oestrus interval in buffaloes (Ramteke *et al.* 2014). Several hypotheses have been postulated to
 227 explain the role of bypass fat in enhancing reproductive performance of dairy animals. These are (i)
 228 improved energy balance resulted in an earlier return to post-partum ovarian cyclicity; (ii) increased
 229 linoleic acid may provide increased PGF₂ α to stimulate return to ovarian cyclicity and improve follicular
 230 recruitment; and (iii) increase in progesterone secretion either from improved energy balance or from
 231 altered lipoprotein composition from dietary fat improves fertility (Naik, 2013). Lammoglia *et al.*, 1997
 232 reported that fatty acid supplementation had a positive effect on preovulatory follicles with formation of
 233 larger corpora lutea having optimum to high progesterone concentration.

234 **4. CONCLUSION**

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

237

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243 **7. DISCLAIMER (Artificial intelligence)**

244 Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT,
245 COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

246 8. REFERENCES

247 Ahima, R.S. and Filer, J.S. 2000. Leptin. *Annual Review Physiology*, 62: 413-437.

248 Ahmed, A., Agarwal, S.P., Agarwal, V.K., Rehman, S.A. and Laumas, K.R. 1977. Steroid hormones. Part
249 II. Serum progesterone concentrations in buffaloes. *Indian J. Exptl. Biol.*, 15: 591-593.

250 Amonge, T. K. 1993. *Aspects of productive and reproductive behaviours of Swamp buffaloes under*
251 *'Khuti' system of management in Assam*. Ph.D. Thesis, Assam Agricultural University,
252 Khanapara, Guwahati-22.

253 Bindari, Y. R., Shrestha, S., Shrestha, N. and Gairie, T. N. 2013. Effect of nutrition on reproduction - a
254 review. *Adv. Appl. Sci. Res.*, 4:421-429.

255 Borah, B. K. 1994. *Studies on certain aspects of reproduction in Murrah buffalo*. M. V. Sc. Thesis,
256 Assam Agricultural University, Khanapara, Guwahati-22.

257 Chilliard, Y., Delavaud, C. and Bonnet, M. 2005. Leptin expression in ruminants: nutritional and
258 physiological regulations in relation with energy metabolism. *Domestic Animal*
259 *Endocrinology*, 29(1):3-22.

260 Das G. K. and Khan F. A. 2010. Summer anestrus in buffalo—a review. *Reprod. Domest. Anim.* 45:
261 e483–e494. doi: 10.1111/j.1439-0531.2010.01598.x

262 Deka, R. 2017. *Reproduction in Swamp Buffalo*. M.V.Sc. Thesis, Assam Agricultural University,
263 Khanapara Guwahati-22.

264 Dixit CP, Bhuyan D, Bhuyan M, Ahmed K, Baruah A, Kalita D, Haloi S, Malik M. Ruling out efficacy of
265 hormonal protocols alone or fortified with mineral and bypass fat for treatment of postpartum
266 anoestrus in cattle. *Indian Journal of Animal Research*. 2023;57(12):1613-9.

267 Gunun N, Kaewpila C, Khota W, Kimprasit T, Cherdthong A, Gunun P. The effect of supplementation
268 with rubber seed kernel pellet on in vitro rumen fermentation characteristics and fatty acid
269 profiles in swamp buffalo. *BMC Veterinary Research*. 2024 May 6;20(1):177.

270
271 Jenkins, T. C. and Palmquist, D. L. 1984. Effect of fatty acids of calcium soaps on rumen and total
272 nutrient digestibility of dairy rations. *Journal of Dairy Science*. 67: 978-986.

273 Kumar, P. R., Shukla, S. N., Shrivastava, O. P. and Purkayastha, R. D. 2013. Incidence of postpartum
274 anestrus among buffaloes in and around Jabalpur. *Veterinary World*, 6: EISSN 2231-0916.

275 Lammoglia, M. A., Willard, S. T., Hallford, D. M., and Randel, R. D. 1997. Effects of dietary fat on
276 follicular development and circulating concentrations of lipids and insulin on follicular
277 development and circulating concentrations of lipids, insulin, progesterone, estradiol 17b,13,14-

278 dihydro-15-ketoprostaglandin F₂ α and growth hormone in estrous cyclic Brahman cows. *Journal*
279 *of Animal Science*, 75: 1591-1600.

280 Mondal, S., Prakash, B.S. and Palta, P. 2007. Endocrine aspects of oestrous cycle in buffaloes (*Bubalus*
281 *bubalis*): An overview. *Asian-Aust. J. Anim. Sci.*, 20(1): 124-131.

282 Naik, P.K. 2013. Bypass Fat in Dairy Ration - A Review. *Animal Nutrition and Feed Technology*, 13:
283 147-163.

284 Otto, J. R., M. J. Freeman, B. S. Malau-Aduli, P. D. Nichols, P. A. Lane, and A. E. O. Malau-Aduli.
285 2014. "Reproduction and Fertility Parameters of Dairy Cows Supplemented With Omega-3 Fatty
286 Acid-Rich Canola Oil". *Annual Research & Review in Biology* 4 (10):1611-36.

287 Öz S, KÜÇÜKERSAN S. Determining the effects of different bypass fat supplementation on
288 performance, milk yield, and milk composition of Anatolian buffaloes. *Turkish Journal of*
289 *Veterinary & Animal Sciences*. 2024;48(2):117-25.

290 Palta, P., Bansal, N., Prakash, B. S., Manik, R. S. and Madan, M. L. 1998. Interrelationship between
291 follicular size and follicular fluid estradiol-17 β , progesterone and testosterone concentrations in
292 individual buffalo ovarian follicles. *Asian-Aust. J. Anim. Sci.*, 11:293-299.

293 Perello, M. and Zigman, J. M. 2012. The role of ghrelin in reward-based eating. *Biol. Psychiatry*, 72
294 (5):347-53.

295 Ramteke, P.V., Patel, D.C., Parnerkar, S., Shankhpal, S.S., Patel, G.R. and Pandey, A. 2014. Effect of
296 bypass fat supplementation during prepartum and postpartum on reproductive performance in
297 buffaloes. *Livestock Research International*, 2(3): 54-58.

298 Singh, G., Singh, B.C., Sharma, S.S. and Sharma, R.D. 1984. Studies on oestrous symptoms of buffalo
299 heifers. *Theriogenology*, 21(6):849-858.

300 Sinha, S. 1998. *Studies on certain aspects of oestrus and oestrus cycle in Swamp buffalo of Assam*. Ph.D.
301 Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

302 Staples, C.R., Burke, J.M. and Thatcher, W.W. 1998. Influence of supplemental fats on reproductive
303 tissues and performance of lactating cows. *Journal of Dairy Science*, 81, 856–871.

304 Tena-Sempere, M. 2005. Ghrelin: novel regulator of gonadal function. *J. Endocrinol. Invest.*, 28:26-29.

305 Terzano, G. M., Barile, V. L. and Borghese, A. 2012. Overview on Reproductive Endocrine Aspects in
306 Buffalo. *Journal of Buffalo Science*, 1:126-138.

307 Tyagi, N., Thakur, S.S. and Shelke, S.K. 2010. Effect of bypass fat supplementation on productive and
308 reproductive performance in crossbred cows. *Trop. Anim. Health Prod.*, 42(8):1749-1755.

309 Wolf, E., Arnold, G.H., Bauersachs, S., Beier, H.M., Blum, H., Einspanier, R., Frohlich, T., Herrier, A.,
310 Hiendleder, S., Kolle, Prella, S., Reichenbach, H-D., Stojkovic, M., Wenigerkind, H. and

- 311 Sinowatz, F. 2002. Embryo-maternal communication in bovine-strategies for deciphering a
312 complex cross-talk. *Reprod. Dom. Anim.*, 38: 276-289.
- 313 Zaman, G., Goswami, R.N. and Aziz, A. 2007. Milk constituents of swamp buffalo of Assam. *Buffalo*
314 *Bulletin*, 26(1): 25-28.