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Comparative study on genital status, energy balance and mineral content of swamp buffalo (Luit) heifers and cows under organized system of rearing in Assam, India

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Abstract

A study was conducted on pubertal Swamp buffalo heifers and puerperal Swamp buffalo cows maintained at Network Project on Buffalo Improvement (Swamp), College of Veterinary Science, Assam Agricultural University, Guwahati, indicated that buffalo heifers of 1.5 to 3 years of age exhibited no genital changes indicative of cyclic activity. The ovary was active characterised by presence of palpable corpus luteum in 50.00 per cent buffalo cows within 45 days of parturition. Serum IGF-1 level in pubertal heifers increased significantly from 61.58 ± 0.66 ng/ml at 1.5 to 2 years to 69.31 ± 2.99 ng/ml at 2.5 to 3 years of age. Level of leptin, ghrelin and IGF-1 did not vary during the puerperal period in buffalo cows. Level of serum calcium and phosphorus was significantly higher in pubertal heifers of 2 to 3 years of age and the values were 9.50 ± 0.23 mg/dl for calcium and 3.70 ± 0.02 mg/dl for phosphorus as against 8.74 ± 0.21 mg/dl calcium and 3.44 ± 0.04 mg/dl phosphorus recorded in 1.5 to 2 year old heifers.

Keywords: Swamp buffalo, genital status, leptin, ghrelin, IGF-1, mineral content

1. Introduction

The Swamp Buffaloes found in the state of Assam, India are named as "Luit" by ICAR-National Bureau of Animal Genetic Resources with Accession number INDIA_BUFFALO_0212_LUIT_01014. These swamp buffaloes having 48 diploid no. of chromosome (2N) and distributed mostly in upper Brahmaputra valley of Assam. These are also found in some areas of Mizoram, Manipur and Nagaland bordering Assam. Buffalo bullocks are excellent draft animals for carting and ploughing especially in muddy field for paddy cultivation. However, Buffalo milk is superior to cow milk in terms of fat content (Zaman *et al.*, 2007) [27] ensuring higher sale price of milk.

Rate of reproduction is the basis of production in animals. There is a plenty of room for increasing productivity of buffaloes through improvement of 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. The incidence of anoestrus in buffalo cows and heifers varies in wider range of 11.25 to 66.28 per cent in different seasons (Kumar *et al.*, 2013) [8, 13-17]. The incidence is much higher in Swamp buffaloes due to poor reproductive hormone profile and negative energy balance after parturition. Leptin, ghrelin and IGF-1 are metabolic hormones which have been consider as indicators of energy balance in dairy animals. Higher level of leptin and lower level of ghrelin was found to be silent indicators of positive energy balance (Nowroozi-Asl *et al.*, 2016) [20].

Certain macro-elements like Ca, P and trace elements including Zn, Cu have been found to be crucial for normal animal reproduction (Akhtar *et al.*, 2009) [1]. Deficiency of macro and micro minerals lead to reproductive disorders in buffaloes.

Keeping the above points in mind the present study was designed to compare between the genital status, serum metabolic and minerals parameters in pubertal heifers and puerperal buffaloes (Luit) under the traditional farm management system.

2. Materials and Methods

2.1 Experimental animals

The experimental animals included in the present study comprised 24 pubertal Swamp buffalo heifers and 12 puerperal Swamp buffalo cows maintained at Network Project on Buffalo

improvement (Swamp), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam.

2.2 Grouping of animals

The experiment was conducted in heifers (n = 24) and cows (n = 12) separately. Heifers were divided in to three age groups such as 1.5 to 2 years, 2 to 2.5 years and 2.5 to 3 years comprising 8 heifers in each group. The 12 puerperal cows were within 15 days of calving.

2.3 Genital status

Rectal palpation of the genital organs was done in each pubertal heifer on day 0 and day 15 and in each puerperal cow at day 15, 30 and 45 after calving. On each day of examination the vulva, vagina, cervix, uterus and ovaries of the animals were examined to record the changes of the genital organs and the percentage frequencies were worked out. The animals were also observed for manifestation of behavioral signs of oestrus and development of interest by the bull. The vulva was recorded as congested when the vulvar mucous membrane appeared hyperemic. Vulva was considered as oedematous when the vulvar wrinkles were less prominent on visual examination. Vaginal mucus was recorded as free flowing, scanty and absent. Patency of the cervix as open or closed. Uterine tone as good or moderate. Ovaries for presence of graffian follicle or corpus luteum.

2.4 Method of collection of blood

Blood was collected on each day of rectal palpation from the jugular vein and transferred to 6 ml vacutainers with serum clot activator (Becton, Dickinson and Company, New Jersey, USA). The separated serum samples were collected in sterilized screw-capped vials, labeled and stored at -20 °C for analysis.

2.5 Estimation of blood biochemical constituents

The blood mineral constituents of serum samples were estimated by using quality reagent kits of calcium, phosphorus, zinc (Greiner Diagnostic GmbH- Unter Gereuth 10-D-79353 Bahlingen- Germany) and Copper (Coral Clinical systems, Kolkata India).

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.

2.6 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

It was observed that in pubertal heifers the vulva appeared wrinkled and pale in all (100.00 per cent) animals under the age groups 1.5 to 2 years, 2 to 2.5 years and 2.5 to 3 years on

both day 0 and day 15 of examination. Percentage frequency of oedematous and congested vulva was 0.00 in all age groups of pubertal heifers on both days of examination. Vaginal discharge was absent in all heifers under different age groups and on both day 0 and day 15 of examination. Cervix was found closed in all heifers (100.00 per cent) under different age groups on both day 0 and day 15 of examination. Uterus was atonic in all heifers under different age groups and on both the days. The ovary was smooth in 100.00 per cent pubertal heifers of different ages.

In the present study none of the buffalo heifers under different age groups ranging from 1.5 to 3 years exhibited genital characteristics indicative of oestrous such as oedema and congestion of vulva, presence of vaginal discharge, relaxtation of cervix, uterine tone and active ovary. Earlier reports indicated that congestion of vulvar mucous membrane vaginal discharge and swelling of vulva were the common signs of oestrus in swamp buffaloes (Amonge, 1993; Sinha, 1998 and Deka, 2017) [2, 6] as well as in murrah buffaloes (Singh *et al.*, 1984 and Borah, 1994) [4, 8, 23, 26]. Result obtained in the present study indicated that swamp buffalo heifers under the present study did not reveal sign of attainment of puberty even at 3 years of age. On the contrary Perera (2011) [21] reported 21 to 24 month as the age at puberty in swamp buffaloes. The author further reported that riverine buffaloes attained puberty at early age of 15 to 18 month. According to Gupta *et al.*, (2016) [8, 12] average age at puberty in buffalo was 2.5 years. The author further reported that it was 33 months in Murrah and 32.50 month in Nili Ravi buffaloes. Reports in attainment of puberty at later ages were also on record. Ingawale and Dhoble (2004) [9] reported 36-42 months as the age at puberty in Indian buffaloes. Jainudeen and Hafez (1993) [10] gave a wider range of 18 to 46 months as the age at puberty in buffaloes. The wide variation in the age at puberty in buffaloes might be due to variation in level of nutrition, genotype, management system and climate as reported by Jainudeen and Hafez (1993) [10]. Ingawale and Dhoble (2004) [9] also reported that level of nutrition strongly influenced the age at puberty in buffaloes.

In buffalo cows on day 15, day 30 and day 45 after parturition frequency of oedematous vulva was recorded as 16.66, 0.00 and 0.00 per cent respectively. On the corresponding days of parturition frequency of wrinkled vulva was 83.34, 100.00 and 100.00 per cent. Congested vulva was not seen in any animals and the vulva remained pale in all (100.00 per cent) cows at all days of parturition. Vaginal discharge was absent in 100.00 per cent cows on all days of parturition. Cervix was found closed in 100.00 per cent cows on day 15, day 30 and day 45 after parturition. Uterus was atonic in all (100.00 per cent) puerperal cows on day 15, day 30 and day 45 of parturition. The ovary was found active in 100.00 per cent cows at all days of examination.

The genital changes recorded in the puerperal buffalo cows during the period of 15 to 45 days after parturition as observed in the present study were pale vulvar mucous membrane, absence of vaginal discharge, closed cervix and atonic uterus occurring in 100.00 per cent cows. The ovary however, was found to be active characterised by presence of palpable corpus luteum in all cows on both day 15 and day 30 of parturition and in 50.00 per cent cows on day 45 of parturition. This might be an indication that ovary in swamp buffaloes resumed activity within 45 days of parturition. It was however difficult to comment on the status of palpable corpus luteum detected on day 15 after parturition as there appeared varying reports on the time of regression of pregnancy corpus luteum in buffaloes. Usmania *et al.*, (1985)

reported 22 days as the period required for regression of pregnancy corpus luteum and 21 days as the time of resumption of follicular activity in Nili Ravi buffaloes. Bhuyan (1997) [3, 19], on the other hand reported shorter time of regression of pregnancy corpus luteum (9.17 ± 0.04 days post partum) in swamp buffalo.

Table 1: Level of leptin, ghrelin and IGF-1 in the serum of pubertal heifers of different age groups at different days of examination

Age groups	Leptin (ng/ml)		Ghrelin (pg/ml)		IGF-1 (ng/ml)	
	Day 0 Mean \pm S.E	Day 15 Mean \pm S.E	Day 0 Mean \pm S.E	Day 15 Mean \pm S.E	Day 0 Mean \pm S.E	Day 15 Mean \pm S.E
1.5 to 2 years (8)	2.37 \pm 0.09	2.39 \pm 0.03	367.25 \pm 10.37	367.13 \pm 10.99	61.58 ^a \pm 0.66	61.50 ^a \pm 0.57
2 to 2.5 years (8)	2.35 \pm 0.12	2.30 \pm 0.06	354.25 \pm 1.78	354.63 \pm 1.37	62.32 ^a \pm 0.20	61.47 ^a \pm 0.63
2.5 to 3 years (8)	2.14 \pm 0.19	2.08 \pm 0.21	339.75 \pm 13.49	345.75 \pm 16.83	69.31 ^b \pm 2.99	68.51 ^b \pm 2.58

Figures in the parentheses indicate number of animals taken in the age group; ^{a, b} Means bearing similar superscript in a column do not differ significantly

Table 2: Level of leptin, ghrelin and IGF-1 in the serum of puerperal buffalo cows at different days of parturition (n = 12)

Parameter	Days of parturition		
	Day 15 Mean \pm S.E	Day 30 Mean \pm S.E	Day 45 Mean \pm S.E
Leptin (ng/ml)	2.90 \pm 0.16	2.96 \pm 0.15	2.97 \pm 0.19
Ghrelin (pg/ml)	278.00 \pm 4.02	277.75 \pm 4.23	278.05 \pm 3.97
IGF-1 (ng/ml)	58.92 \pm 2.68	59.11 \pm 2.62	59.40 \pm 2.63

Figures in the parentheses indicate number of observations.

Results of analysis of variance indicated that the level of leptin, ghrelin and IGF-1 in pubertal heifers did not vary significantly between age groups and between days of examination. Similarly, in puerperal buffalo cows also did not vary significantly between days of parturition.

In the present study, it was observed that the serum leptin

3.2 Energy balance

Average levels of the metabolic hormones such as leptin, ghrelin and IGF-1 in pubertal buffalo heifers under different age groups have been presented in Table 1 while that of puerperal cows in Table 2.

level did not vary significantly in different age groups; age ranging from 1.5 to 3 years. Similarly, serum ghrelin level also did not vary with age of the heifers. Serum IGF-1 level in the heifers also remained unchanged till 2.5 years of age but slightly higher level was recorded in heifers at 2.5 to 3 years of age. The elevated serum IGF-1 level in the older heifers might be due to the growth promoting effect of IGF-1 in adult animals as reported by Kumar and Laxmi (2015) [8, 13-17]. Results obtained in the study of metabolic hormones indicated that energy level in pubertal heifers remained unchanged from 1.5 to 2.5 years of age after which it increased slightly.

3.3 Mineral content of blood

Average levels of calcium, phosphorus, zinc and copper in pubertal buffalo heifers under different age groups have been presented in Table 3 while those of puerperal cows in Table 4.

Table 3: Level of calcium, phosphorus, zinc and copper in the serum of pubertal heifers of different age groups at different days of examination

Age groups	Calcium (mg/dl)		Phosphorus (mg/dl)		Zinc (μ g/dl)		Copper (μ g/dl)	
	Day 0 Mean \pm S.E	Day 15 Mean \pm S.E	Day 0 Mean \pm S.E	Day 15 Mean \pm S.E	Day 0 Mean \pm S.E	Day 15 Mean \pm S.E	Day 0 Mean \pm S.E	Day 15 Mean \pm S.E
1.5 to 2 years (8)	8.74 ^{ab} \pm 0.21	8.79 ^a \pm 0.29	3.44 ^a \pm 0.04	3.58 ^a \pm 0.17	290.93 \pm 26.71	290.63 \pm 27.01	65.82 \pm 0.31	66.12 \pm 0.53
2 to 2.5 years (8)	7.82 ^a \pm 0.41	7.78 ^b \pm 0.29	3.70 ^b \pm 0.02	3.50 ^a \pm 0.17	290.10 \pm 3.73	290.38 \pm 3.40	66.42 \pm 3.25	66.50 \pm 3.74
2.5 to 3 years (8)	9.50 ^b \pm 0.23	9.57 ^a \pm 0.27	3.33 ^a \pm 0.05	3.46 ^a \pm 0.19	277.14 \pm 6.77	271.75 \pm 6.63	61.38 \pm 0.70	60.98 \pm 0.93

Figures in the parentheses indicate number of animals taken in the age group; ^{a, b} Means bearing similar superscript in a column do not differ significantly.

Table 4: Level of calcium, phosphorus, zinc and copper in the serum of puerperal buffalo cows at different days of parturition (n = 12)

Parameter	Days of parturition		
	Day 15 Mean \pm S.E	Day 30 Mean \pm S.E	Day 45 Mean \pm S.E
Calcium (mg/dl)	8.87 \pm 0.27	8.86 \pm 0.20	8.86 \pm 0.30
Phosphorus (mg/dl)	4.05 \pm 0.10	4.10 \pm 0.21	3.95 \pm 0.15
Zinc (μ g/dl)	245.83 \pm 13.09	245.83 \pm 13.00	246.29 \pm 12.80
Copper (μ g/dl)	56.26 \pm 1.49	56.74 \pm 1.15	56.54 \pm 1.20

Figures in the parentheses indicate number of observations.

3.3.1 Serum calcium

In pubertal buffalo heifers the level of serum calcium varied significantly ($P < 0.01$) between age groups but did not vary significantly between days of examination. From the results of critical difference test presented in Table 3 it was evident that level of calcium was significantly higher in pubertal heifers of 2.5 to 3 years of age as compared with that of 2 to 2.5 years of age, but similar to that recorded in 1.5 to 2 years age group heifers on both day 0 and day 15 of examination. In case of puerperal buffalo cows the serum, calcium level did not differ significantly between days of parturition.

In the present study mean level of serum calcium in pubertal buffalo heifers in different age groups ranging from 1.5 to 3 years varied from 7.78 ± 0.29 mg/dl to 9.57 ± 0.27 mg/dl. In the puerperal buffalo cows recorded on day 15, day 30 and day 45 of parturition the mean level was in the range from 8.86 ± 0.20 to 8.87 ± 0.27 mg/dl. These values irrespective of heifers or cows were similar to that reported by Jayachandran *et al.*, (2013) [11], Khan *et al.*, (2015) [12] and Virmani *et al.*, (2018) [26] in different breeds of buffaloes; mean serum calcium levels being within the range of 8.12 ± 0.22 to 9.62 ± 0.24 mg/dl. Mean serum calcium values recorded in the present study were slightly higher than that reported by Akhtar *et al.*, (2014) [1] in Nili Ravi buffaloes (7.46 ± 0.29 mg/dl) and Deka (2017) [6] in Swamp buffaloes (7.34 ± 0.16 to 7.68 ± 0.05 mg/dl) and slightly lower than that reported by Kumar *et al.*, (2015) [8, 13-17] in water buffaloes (11.75 ± 0.86 mg/dl).

3.3.2 Serum phosphorus

Serum phosphorus level in the pubertal heifers varied significantly ($P < 0.05$) between age groups but not between days of examination. It was evident from the results of critical

difference test (Table 3) that level of serum phosphorus was significantly higher in pubertal heifers of 2 to 2.5 years of age as compared to that in heifers of 1.5 to 2 years and 2.5 to 3 years of age on day 0 of examination. But on day 15 of examination the values did not differ significantly among the three age groups.

In puerperal buffalo cows mean serum phosphorus level did not differ significantly between days of parturition.

Mean serum phosphorus level in pubertal heifers and puerperal cows as observed in the present study ranged from 3.33 ± 0.05 to 4.10 ± 0.21 mg/dl which were nearer to the values reported by Jayachandran *et al.*, (2013) [11] in anoestrous buffaloes (4.22 ± 0.13 mg/dl) and Deka (2017) [6] in Swamp buffaloes (4.25 ± 0.14 to 4.35 ± 0.13 mg/dl). However, these values were lower than that reported by Newer *et al.*, (1999) [19] in Swamp buffaloes (5.94 ± 0.04 mg/dl) and Shahzad *et al.*, (2016) in Nili Ravi buffaloes (5.55 ± 0.32 mg/dl).

3.3.3 Serum zinc and serum copper

Serum zinc and copper level did not vary significantly between different age groups as well as between days of examination in the pubertal heifers and in puerperal buffalo cows no significant variation was recorded between days of parturition.

Mean serum zinc level in the Swamp buffalo heifers and cows under the present study ranged from 271.75 ± 6.63 to 290.93 ± 26.71 µg/dl, which was higher than that reported by Akhtar *et al.*, (2009) [1] in Nili Ravi buffalo cows and Kumar *et al.*, (2016) [8, 13-17] in Murrah buffaloes; the range being 181.40 to 286.00 µg/dl.

Mean serum copper level in pubertal heifers and puerperal cows under the present study ranged from 60.98 ± 0.93 to 66.50 ± 3.74 µg/dl and 56.26 ± 1.49 to 56.74 ± 1.15 µg/dl respectively. These values were much lower than that reported by Kumar *et al.*, (2005) [8, 13-17] in buffalo heifers and non-pregnant buffalo cows (0.72 ± 0.03 ppm). Mean serum copper level of 70.59 µg/dl in cyclic Nili Ravi buffalo as reported by Akhtar *et al.*, (2009) [1] was comparatively higher than that observed in Swamp buffalo under the present study.

The variation in the level of calcium, phosphorus, zinc and copper in the serum of buffalo cows and heifers might be due to variations in the type of feeds and fodder and feeding and management system practiced in different regions, as because animals obtained minerals through consumption of natural feeds, fodders and supplementation of inorganic salt in the ration (Kumar 2015) [8, 13-17]. Status of reproduction was also recorded to be an important factor for variation in the level of minerals in blood. Anoestrous females were found to maintain lower serum levels of calcium, phosphorus, zinc and copper as compared to cyclic cows (Dutta *et al.*, 2001; Das *et al.*, 2012 and Sahoo *et al.*, 2016) as well as in buffalo cows (Newer *et al.*, 1999; Akhtar *et al.*, 2009; Jayachandran *et al.*, 2013 and Kumar *et al.*, 2016) [1, 5, 7, 19, 22].

Result obtained in the present study also indicated that there was significant variation in the level of serum calcium and serum phosphorus between different age groups of buffalo heifers which might be due to individual variation.

4. Conclusion

The study revealed that Swamp buffalo heifers at 1.5 to 3 years of age exhibited no genital changes indicative of cyclic activity. Fifty per cent buffalo cows resumed ovarian cyclicity within 45 days of parturition. Of the metabolic hormones,

serum IGF-1 concentration increased at 2.5 to 3 years of age. Ghrelin and leptin levels did not vary up to 3 years of age. Level of metabolic hormones did not vary during the puerperal period. Serum level of zinc and copper did not vary with age of the heifers and during the 45 days puerperal period.

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