



Mineral and hormonal profile in acyclic and cyclic Murrah buffaloes during summer in Chhattisgarh

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ABSTRACT

The experiment was conducted to study the incidence of cyclicity, biochemical profile (blood glucose, non esterified fatty acid (NEFA), macro minerals viz Ca, Pi, Mg, micro minerals viz Cu, Co, I, Zn, Mn, hormones, viz. progesterone and insulin in acyclic and cyclic Murrah buffaloes during summer (March to June 2009) in Chhattisgarh. Out of 108 Murrah buffaloes 49 (45.37%) were found without palpable follicles and corpus luteum at per rectal examinations performed twice at 12 days apart, indicating true anestrus. The biochemical and hormonal profile were estimated in 6 cyclic and 24 acyclic Murrah buffaloes during summer. There was no significant difference in blood glucose and NEFA level in cyclic and acyclic buffaloes. The level of inorganic phosphorus and Mg were significantly lower in acyclic buffaloes than cyclic buffaloes. There was no significant difference in the level of calcium, however there was significant difference in Ca: P in cyclic and acyclic buffaloes. The micro minerals Zn, Fe, Cu, Co were significantly lower in acyclic buffaloes, however, there was no significant difference in the level of Mn and iodine. The serum progesterone level was significantly higher in cyclic than acyclic buffaloes. There was no significant difference in the level of insulin.

Key words: Acyclic, Biochemical profile, Buffaloes, Cyclic, Cyclicity, Mineral profile

India has about 98 million buffaloes, one of the domestic livestock species with the greatest unexploited potential. Indian Murrah is the best riverine milk breeds in Asia, which is widely distributed all over India including Chhattisgarh. Buffaloes are polyestrous animals showing wide variation in reproductive efficiency throughout the year but they suffer from inherent reproductive problems such as poor estrus expression and long calving intervals, limiting their lifetime production (Paul and Prakash 2005). In buffaloes the important seasonal reproductive problems are weak or silent oestrus, irregular oestrous cycle lengths, low conception rates and early embryonic mortality (Bhattacharya *et al.* 1970, Luktuke *et al.* 1973). Breeding efficiency in buffaloes is reduced during the hot summer from March to August with longer day length, hence 80% of non-pregnant buffalo has quiescent ovaries (Razdan 1988, Manik *et al.* 2002). High environmental stress together with under-nutrition might

therefore be responsible for the long periods of seasonal anoestrus in buffaloes (Shah *et al.* 1989).

Postpartum reproductive function in dairy buffaloes seems directly dependent on the availability of nutrient energy relative to its utilization for lactation. Negative energy balance interfere with the primary LH pattern necessary for fostering ovarian and blood glucose concentrations during this period may limit the responsiveness of ovary of the gonadotrophin stimulation. Therefore, the present research work was designed to study the incidence of true anoestrus and hormonal and mineral profile in peripheral blood of cyclic and acyclic Murrah buffaloes during summer.

MATERIALS AND METHODS

Murrah buffaloes (108) belonging to private dairy farms nearby to Durg and Bhilai, that calved 90 days or more earlier to the March were observed for external signs of estrus while parading with teaser thrice a day and per rectal examination twice at 10 days interval to note the percentage of cyclic and acyclic animals during summer. True acyclic buffaloes (24) of 7 to 10 years of age at third to fifth parity having quiescent ovaries i.e., absence of palpable corpus luteum and ovarian follicle were selected as acyclic buffaloes while 6 buffaloes found to have corpus luteum and or palpable ovarian follicle were selected as cyclic buffaloes. The blood samples were collected in vacutainer tubes by jugular vein puncture with

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the help of sterile vacutainer needle from 24 acyclic and 6 cyclic buffaloes for estimation of glucose, non esterified fatty acid (NEFA), macro minerals (Ca, Pi, Mg), micro minerals (Cu, Co, Fe, Zn, Mn, I) and hormones (progesterone and insulin). Blood samples were allowed to clot at 4°C for 24h and then centrifuged at 3000 rpm for 20 min in refrigerated centrifuge machine. Serum was poured into sample tubes and stored at -20°C until analysis. Blood glucose was estimated by the glucometer, serum NEFA concentration was determined by soap extraction method (chloroform: heptane: methanol, 49:49:2) as per Ships *et al.* (1980). Ca was estimated by calcium kit. Pi was estimated by Fiske and Subbaraw (1925) method and serum magnesium concentration was determined by Titan Yellow method (Neill and Neely 1956). Micro minerals viz. copper, cobalt, iron, zinc, and manganese in cyclic and acyclic animals were estimated in the serum by atomic absorption spectrophotometer. The iodine in serum samples of cyclic and acyclic buffaloes was carried out by colorimetric method (Welshman *et al.* 1966) with modifications. Progesterone and insulin concentration in serum was estimated by RIA method using bovine RIA kit supplied by BRIT, BARC, Mumbai. Mean values of different parameters viz. macro and micro minerals, hormones (insulin, progesterone) and glucose between acyclic and cyclic animals were compared using t test following the standard statistical method described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Results revealed that out of 108 Murrah buffaloes 49 (45.37%) were without any palpable follicles and CL in either of the ovaries indicating true anoestrus, 23 (21.29%) had CL in the ovaries indicating silent oestrus or unobserved oestrus, 20 (18.51%) expressed overt oestrus, and 16 (14.81%) were in estrus as per per-rectal examination.

The biochemical and hormonal profiles in 6 cyclic and 24 acyclic Murrah buffaloes is presented in Table 1. There was no significant difference observed in blood glucose and NEFA in between cyclic and acyclic buffaloes. There was no significant difference observed in level of serum calcium in cyclic (10.31±0.28 mgdl⁻¹) and acyclic (9.62±0.02 mgdl⁻¹) Murrah buffaloes. However, Ca: Pi was found to be significantly (P<0.01) lower in cyclic buffaloes. The level of serum Pi, Mg was significantly higher in cyclic than acyclic buffaloes. The level of micro-minerals Fe, Cu, Co and Zn were significantly lower in acyclic than cyclic buffaloes while there was no significant difference in the level of manganese and iodine.

In present study the incidence of silent oestrus or unobserved oestrus in buffaloes during summer (21.29%) is similar to that reported by Luktuke and Sharma (1978) however, Chauhan and Singh (1979), Banerjee *et al.* (1987) reported lower incidence (5 to 7.94%) of silent oestrus and unobserved oestrus, while Rao and Kodagali (1983) reported

Table 1. Biochemical and hormonal profile in cyclic and acyclic Murrah buffaloes during summer

Parameters	Cyclic (n=6)	Acyclic (n=24)
<i>Macro minerals</i>		
Ca (mgdl ⁻¹)	10.31±0.28	9.62±0.02
Pi (mgdl ⁻¹)	5.37±0.14	3.25±0.02**
Mg (mgdl ⁻¹)	3.66±0.20	2.43±0.10**
Ca:P (mgdl ⁻¹)	1.93±0.02	3.02±0.10**
<i>Micro minerals</i>		
Zn (PPM)	1.71±0.21	0.59±0.13**
Mn (PPM)	0.23±0.10	0.36±0.02
Fe (PPM)	2.16±0.18	1.14±0.19*
Cu (PPM)	2.62±0.11	1.25±0.02**
Co (PPM)	0.21±0.002	0.17±0.01**
I ₂ (µgdl ⁻¹)	4.09±0.19	3.96±0.02
<i>Blood glucose and NEFA</i>		
Blood Glucose (mgdl ⁻¹)	47.33±4.64	51.25±3.27
NEFA (mM ml ⁻¹)	0.18±0.2	0.24±0.02
<i>Hormone</i>		
P ₄ (ngml ⁻¹)	4.70±1.27	0.67±0.02**
Insulin (µUml ⁻¹)	12.5±0.00	15.8±1.72

Superscripts column wise differ significantly; ** P<0.01, *P<0.05.

higher incidence (44.85%) of silent oestrus.

The buffaloes found with CL (silent or unobserved oestrus) in present study may be attributed to improper oestrus detection (i.e. missed oestrus), poor knowledge regarding the sign and symptoms of oestrus, method adopted for detection of heat and poor management regarding shading and watering of buffaloes during summer. The buffaloes were more sensitive to summer due to which they became more hyperthermic than cattle, but recovered rapidly if provided with shading, showering and wallowing (Chikamunu and Shimizu 1983) and could come into oestrus and conceive (as high as 80%) under rigorous summer condition if they are well sluiced, allowed to wallow and provided with better shelters (Gangwar 1980).

The weak signs of oestrus in buffaloes in present study during summer may be attributed to their inherent reproductive problems such as poor expression of oestrus, long calving interval, seasonality in breeding behavior, seasonal trend of calving, prolonged post partum service period, unequal ovarian biometry and lower concentration of plasma progesterone and estradiol which is the prime need for the behavioral manifestation of oestrus (Buglio *et al.* 2000, Campo *et al.* 2002, Paul and Prakash 2005).

Contrary to several reports, viz. Derashri *et al.* (2004), Singh *et al.* (2006), Patel *et al.* (2007) and Balusami *et al.* (2008), present study indicated nonsignificant difference in the level of blood glucose and NEFA between cyclic and acyclic buffaloes which might be attributed to similar energy balance in both groups (Richards *et al.* 1989).

Similar to this study Shah *et al.* (2003) reported

nonsignificant difference in serum concentration of Ca in cyclic and acyclic buffaloes. Our results pertaining to level of serum calcium in cyclic buffaloes are in close agreement with Ali *et al.* (1991), Dutta *et al.* (2001), Shah *et al.* (2003), Kumar *et al.* (2004) and Patel *et al.* (2007). However, Dutta *et al.* (1988) and Singh *et al.* (2006) reported comparatively higher level of calcium in cyclic buffaloes (range- 7.86 ± 0.12 to $9.30 \pm 0.3 \text{ mgdl}^{-1}$).

Significant higher level of serum Pi and magnesium in cyclic buffaloes are in close agreement with Shah *et al.* (2003) and Khan *et al.* (2007). Our results pertaining to serum Ca: P in acyclic buffaloes are in close agreement with Ali *et al.* (1991) who reported the level of Ca: P in cyclic buffaloes in range of 2.48 ± 0.11 to $4.82 \pm 0.08 \text{ mgdl}^{-1}$.

Micro minerals (copper, cobalt, iron, iodine, zinc and manganese): Serum copper, cobalt and iron and zinc levels in cyclic buffaloes are nearly similar to the level reported by Singh *et al.* (2006), Ahmed *et al.* (2009), and Talvelkar *et al.* (2008). The higher level of these trace elements may be correlated with higher level of progesterone concentration as it was reported that the lower concentration of these alter the bioactivity of LH molecule in cows (Randel 1990).

There was no significant difference observed in level of serum manganese and iodine in cyclic and acyclic Murrah buffaloes. However, the level of serum copper, cobalt and zinc is significantly ($P < 0.01$) higher in cyclic buffaloes as compared to the acyclic buffaloes. The serum Fe concentration was significantly ($P < 0.05$) higher in cyclic as compared to acyclic Murrah buffaloes.

Hormones (progesterone and insulin): The higher level of serum progesterone in cyclic buffaloes in present study might be attributed to their cyclicity (i.e. dioestrus phase of oestrous cycle), presence of corpus luteum of various functional stages and positive mineral balance. The level of serum progesterone in cyclic buffaloes pertaining to present study are in close agreement with Dutta *et al.* (1989) who reported the serum level of progesterone in cyclic buffaloes to range from 4.30 ± 0.24 to $4.43 \pm 0.92 \text{ ngml}^{-1}$. However, Deka *et al.* (2007) reported comparatively higher level of serum progesterone in cyclic ($6.50 \pm 0.69 \text{ ngml}^{-1}$) cows, while Kumar *et al.* (1991) reported comparatively lower (range- 0.18 ± 0.03 to $2.68 \pm 0.75 \text{ ngml}^{-1}$) level of serum progesterone in cyclic buffaloes. The level of serum progesterone pertaining to present study in acyclic buffaloes are in close to agreement with Qureshi *et al.* (1999) and Kumar *et al.* (1991) who reported the level of serum progesterone in buffaloes ranging from 0.67 ± 0.05 to $0.61 \pm 0.1 \text{ mgdl}^{-1}$. The lower level of progesterone in acyclic buffaloes in present study might be attributed to their smooth ovaries (true anoestrus). In present study there is no significant difference observed in serum insulin level in cyclic and acyclic Murrah buffaloes. The level of serum insulin in cyclic buffaloes are in close agreement with Jindal and Ludri (1990) who reported the serum level of insulin in cyclic buffaloes as $14.72 \pm 1.42 \text{ } \mu\text{Uml}^{-1}$.

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REFERENCES

- Ali M D, Kanjlal B C, Bondopadhyay S K, Roychoudhary R and Ghosh, B B. 1991. Serum calcium, inorganic phosphorus and serum calcium-inorganic phosphorus ratio in anestrus rural crossbred heifers. *Indian Journal of Animal Reproduction* **12**: 32–35.
- Balusami C, Sivakumar T and Alike N. 2008. A study on blood biochemical profiles of lactating buffaloes. *Indian Veterinary Journal* **85**:1011–12.
- Banerjee A K. 1987. Studies on the incidence of anoestrus in the Murrah grade buffalo cows (*Bubalus bubalis*) in the city khatahs of Calcutta, West Bengal. *Indian Journal of Animal Health* **44**: 139–44.
- Bhattacharya A R, Luktuke S N and Roy D J. 1970. Incidence of normal and pathological conditions of she-buffalo genitalia in different months. *Indian Journal of Animal Sciences* **40**: 425–29.
- Bughio S, Shahni A X, Mirani A A, Oad F C and Bughio B A. 2000. Seasonaltrend of calving and subsequent service period in buffaloes. *Pakistan Journal of Biological Science* **3**: 2169–70.
- Campo E, Alons O J C, Hincapie J J, Garcia L, Faure O and Fernandez. O. 2002. Seasonal influence on uterine involution and postpartum ovarian activity in river buffaloes. *Bubalus Bubalis, Journal of Animal Science* **83**:59–63.
- Chauhan F S. and Singh M. 1979. Anoestrus in buffaloes. *Indian Veterinary Journal* **56**: 583–89.
- Chikamunu T and Shimizu H. 1983. Comparison of physiological response to climatic condition in swamp buffaloes and cattle. *Indian Journal of Animal Sciences* **53**: 595–604.
- Deka B N, Borgohain B N, Nath K C, Goswami J, Biswas R. K and Das P K. 2007. A comparative appraisal of serum progesterone level during estrous cycle in normal and repeat breeder crossbred cows with ovulatory problems. *Indian Journal of Animal Reproduction* **28**:83–85.
- Derashri H J, Kavani F S, Panchal M T, Khasatiya C T, Ahlawat A R and Sarvaiya N P. 2004. Metabolic profile in fertile and infertile postpartum anoestrus Surti buffaloes. *Annual convention and National Symposium on advanced reproductive technologies for management of fertility in livestock*. FIM-22: 52.
- Dutta J C, Baruah R N, Dutta Leena and Talukdar S C. 1988. Blood biochemical studies in anoestrus and normal cyclic cattle. *Indian Veterinary Journal* **65**: 239–41.
- Dutta J C, Baruah R N, Dutta Leena and Talukdar S C. 1989. Serum progesterone levels in repeat breeder jersey cows. *Indian Journal of Animal Reproduction* **10**: 9–11.
- Dutta A, Baruah B, Sarmah B C, Baruah K. K. and Goswami R N. 2001. Macro mineral levels in cyclic, postpartum anoestrus and repeat breeding local cows in lower Brahmaputra valley of Assam. *Indian Journal of Animal Reproduction* **22**: 41–44.
- Fiske G H and Subbaraw Y. 1925. The colorimetric determination of phosphorus. *Journal of Biology and Chemistry* **66**:375–400.
- Gangwar P C. 1980. Climatic effects and reproduction in buffaloes- a review. *Indian Journal of Dairy Science* **36**: 28–33.

- Jindal S K and Ludri R S. 1990. Insulin concentration in lactating crossbred cows and buffaloes. *Indian Journal of Animal Sciences* **5**: 249–52.
- Khan J R, Munde V K, Tiwari R P and Jogi S. 2007. Changes in biochemical parameters during various reproductive disorders in cows and buffaloes of Chhattisgarh region. *XXIII Annual convention of ISSAR and National Symposium*. OP-45:344.
- Kumar P, Jindal R and Rattan P J S. 1991. Plasma hormonal profile during estrous cycle of Murrah buffalo heifers. *Indian Journal of Animal Sciences* **61**:382–85.
- Kumar M G, Das P K, Sanyal S, Roy S and Rajendran D. 2004. Hematology and biochemical profile of Hariyana heifers during estrus and anestrus condition. *Indian Veterinary Journal of Animal Health* **43**: 32–36.
- Luktuke S N, Bhattacharya A R, Singh S.K. and Khan B U. 1973. Studies on aberrations in functional activity of the ovaries in buffaloes. *Indian Veterinary Journal* **50**: 876–81.
- Luktuke S N and Sharma C. 1978. Studies on incidence of true anestrus in rural cattle and buffaloes. *Indian Veterinary Journal* **55**: 940–42.
- Manik R. S, Palta P, Singla S K and Sharma V. 2002. Folliculogenesis in buffalo (*Bubalus bubalis*): a review. *Journal of Reproduction and Fertility Development* **14**:315–325.
- Neill and Neely 1956. Iodine, iron and cobalt, sulphur, magnisium. *Practical Clinical Biochemistry*. (Ed.) Harold Varley. 4th edn. Pp 468–84.
- Patel B P, Shukla S P, Nema S P and Baghel R P S. 2007. Serum biochemical changes in relation to anestrus in buffaloes. *XXIII Annual convention of ISSAR and National Symposium*. PP-07:57.
- Paul, V and Prakash B S. 2005. Efficacy of Ovsynch protocol for synchronization of ovulation and fixed time artificial insemination in Murrah buffaloes (*Bubalus bubalis*) *Theriogenology* **64**:1049–60.
- Qureshi Z I, Lodhi L A, Bajwa M A and Naz N A. 1999. Bubaline progesterone concentration in serum, fore milk and saliva during different phases of estrus cycle. *Pakistan Journal of Biological Science* **2**: 583–85.
- Randal R D.1990. Role of minerals in reproduction. *Animal Breeding Abstract* **58**:4273.
- Rao M N and Kodagali S B.1983. Onset of estrus sign and optimum time of artificial insemination in Surti buffaloes. *Indian Journal of Animal Sciences* **53**: 553–55.
- Razdan M N. 1988. Buffalo performance in relation to climatic environment. *Proceedings of the 2nd World Buffalo Congress*. 12–17 December, New Delhi, India. (Ed.) Bhat P N. pp. 173–86. ICAR, New Delhi.
- Richards M W, Wettelman R P and Schenemann, M. H. 1989. Nutritional anestrus in beef cows: concentration of glucose and nonesterified fatty acids in plasma and insulin in serum. *Journal of Animal Science* **67**: 2354–62.
- Shah R G, Dhami A J, Patel K P and Kavani F.S. 2003. Biochemical and trace minerals profile in fertile and infertile Surti buffaloes. *Indian Journal of Animal Reproduction* **24**: 16–21.
- Shah S N H, Willemse A H and Van de Wiel D F M.1989. Influence of season and parity on several reproductive parameters of Nili-Ravi buffaloes in Pakistan. *Animal Reproduction Science*. **21**:177–190
- Shipe W F, Senyk G F and Fountain K B. 1980. Modified copper soap solvent extraction method for measuring free fatty acid in milk. *Journal of Dairy Science* **63**: 193 –98.
- Singh B, Rawal C V S and Singh J P. 2006. Serum zinc, copper and cobalt in normal cyclic, anoestrus and repeat breeder buffaloes. *Indian Journal of Animal Reproduction* **27**: 34–36.
- Snedecor G W and Cochran W G.1967. *Statistical Methods*.6th edn. Oxford and IBH Publishing company. Kolkata.
- Talvelkar B A, Jain V K, Kumar R and Sridhar N B. 2008. Trace element profile during gestation, lactation and peripartum in buffaloes. *Indian Journal of Animal Sciences* **78**: 811–13.
- Wellshman S S, Walter J W, Rogger G A and Baltimore D V.1966. Serum estimation of protein free iodine in serum. *Journal of Biological Chemistry* **63**: 538 – 41.