

Study of Factors Leading to Seasonality of Reproduction in Dairy Buffaloes. II. Non-Nutritional Factors

M. S. Qureshi*, G. Habib¹, H. A. Samad², L. A. Lodhi² and R. H. Usmani³
 Veterinary Research Institute, Peshawer, Pakistan

ABSTRACT : Fifty one Nili-Ravi dairy buffaloes in their last two months of gestation, were selected in NWFP Pakistan. Rectal palpation was carried out fortnightly, until the occurrence of first estrus. Ovulation was confirmed per rectum and milk progesterone levels (MPL). Body condition score (BCS) was recorded weekly. Milk samples were collected weekly for MPL and blood samples fortnightly for metabolites analysis. Milk yield was recorded and samples were collected for milk fat. The buffaloes calving during the normal breeding season (NBS, August to January) had short ($p < 0.01$) postpartum estrus interval of 55.95 ± 4.90 days versus 91.15 ± 11.61 days in the buffaloes calving during the low breeding season (LBS, February to July). MPL in the LBS calvers remained lower than NBS calves ($p < 0.01$). The incidence of silent ovulation was higher during LBS as compared to NBS (70.6% versus 29.4%). MPL showed a pattern opposite to atmospheric temperature. In NBS calvers serum glucose levels were higher ($p < 0.01$) and magnesium levels were lower ($p < 0.01$) than LBS calvers. Higher serum urea was found in summer and spring than that autumn and winter ($p < 0.05$). The reproductive performance in buffaloes calving in the LBS coincided with a low BCS ($p < 0.01$). Fat corrected milk production (FCM) was higher in NBS than LBS ($p < 0.01$) calvers. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 7 : 1025-1030*)

Key Words : Buffalo, Reproduction, Season, Milk, BCS, Metabolites

INTRODUCTION

Nili-Ravi is one of the best dairy buffaloes of the world with black color, well set head, hollow face, thick horns, long neck, short legs and a big prominent udder (Shah, 1991). Average milk yield is above 4082 kg in 1% buffaloes and 1815 kg in 72% buffaloes, with 8% fat (Waheed, 1976). Reproductive performance of Nili-Rabi buffalo is adversely affected by seasonality of breeding (Majeed et al., 1961; Shah and Shah, 1968). Seasonality of reproduction in dairy animals results in a reduction in milk supply during certain seasons of the year, hinderance in genetic improvement, decrease in the number of lactations and lactation peaks. In NWFP the year may be divided into four seasons i.e. autumn (August to October), winter (November to January), spring (February to April) and summer (May to July). The air temperature during the four seasons ranged from $8 \sim 39$, $-3 \sim 30$, $1 \sim 32$ and $14 \sim 48^\circ\text{C}$, average humidity was 76.5, 79.0, 72.9 and 70.8% and total rainfall was 107, 70, 228 and 106 mm, respectively (Amin and Khan, 1994-95). The green fodder available is non-leguminous during autumn and summer and leguminous during the winter and spring. During the extreme cold and hot weathers fodder scarcity occurs. The present project was designed to study seasonality of reproduction and its association with non-nutritional factors in buffaloes

kept under field conditions of the North-West Frontier Province (NWFP) of Pakistan, already described (Qureshi, 1995).

MATERIALS AND METHODS

Animals

Fifty one Nili-Ravi dairy buffaloes in their last two months of gestation were selected at seven peri-urban commercial farms located in the Central Valley of NWFP, $31\text{-}37^\circ\text{N}$ and $65\text{-}74^\circ\text{E}$. Selected animals were ear-tagged. The farms were visited twice a week. Reproductive history of the selected animals was recorded.

Clinical monitoring

After parturition, Rectal examination of reproductive organs was carried out on days 14 and 21, and then fortnightly, until the occurrence of the first estrus as described by Usmani et al. (1985). Position of the reproductive organs was recorded and the approximate size of the cervix, uterus and ovaries and the ovarian structures. Estrus detection was made twice daily, from 15 days postpartum until resumption of estrus. In addition to visual signs, an intact bull was used for heat detection at each farm. Postpartum ovulation was confirmed by palpation of an ovulation depression, a very soft corpus luteum or luteal tissue embedded in the ovary.

Milk yield, progesterone assay and body condition scoring

Milk yield was recorded in kg per day, once a week, until 150th day after parturition. Fresh milk

* Corresponding Author: M. S. Qureshi.

¹ NWFP, University of Agriculture, Peshawer.

² University of Agriculture, Faisalabad.

³ Buffalo Research Program, NARC, Islamabad.

Received July 24, 1998; Accepted November 27, 1998

Table 1. Mean values \pm SE of reproductive parameters in buffaloes during normal (NBS) and low breeding season (LBS)

Parameter	Overall	Calving period	
		NBS	LBS
Placenta expulsion (hours)	5.46 \pm 0.92 (38)*	4.40 \pm 1.03 ^a (23)	6.64 \pm 1.51 ^a (15)
Lochia discharge (days)	6.02 \pm 1.24 (50)	5.66 \pm 1.39 ^a (29)	6.52 \pm 2.23 ^a (21)
Postpartum uterine involution interval (days)	34.30 \pm 1.33 (50)	36.19 \pm 5.07 ^a (29)	31.52 \pm 3.09 ^a (21)
Postpartum estrus interval (days)	69.03 \pm 6.03 (35)	55.95 \pm 4.90 ^b (22)	91.15 \pm 11.61 ^a (13)
Postpartum corpus luteum interval (days)	59.37 \pm 4.76 (43)	55.24 \pm 5.77 ^a (25)	65.11 \pm 7.86 ^a (18)
Conception rate (%)	45.10 \pm 6.97 (51)	43.33 \pm 9.05 ^a (30)	47.62 \pm 10.90 ^a (21)

^{a,b} The value with different letters in the same row differ significantly ($p < 0.01$).

* Values in parenthesis represent the number of observations.

samples were collected weekly, preserved in 0.1% sodium azide and stored at -20°C until analysis for milk progesterone levels (MPL) using radioimmunoassay as described previously (FAO/IAEA, 1993). Milk samples were used once a month for milk fat determination (AOAC, 1980). The yield was converted to 4% fat corrected milk (FCM), as suggested by Morrison (1949). Body condition score (BCS) was recorded at weekly intervals on a scale of 0 to 5, using the procedure described by Peters and Ball (1987) for dairy and beef cows.

Analysis of blood samples

Jugular blood samples were collected fortnightly, beginning two months before parturition until the resumption of postpartum estrus or day 150 postpartum. Samples were centrifuged at 3,000 rpm for 10 minutes at 4°C , serum was collected and stored at -20°C until analyzed for glucose, urea, protein, calcium, phosphorus and magnesium, using Clonital kits (Italy).

Meteorological data and statistical analysis

Details on daily maximum and minimum ambient temperature were collected from two stations of the University of Agriculture Peshawar for the whole experimental period. The data obtained were statistically analyzed with general linear model procedures (Steel and Torrie, 1980). The following models were adopted:

Model 1. For comparing the effect of calving period on reproductive parameters (dependant variables)

$$Y_{ij} = u + \alpha_i + e_{ij}$$

Where

Y_{ij} = j th observation of the dependent variable during i th period of calving

u = population constant common to all records

α_i = The effect of i th period of calving; i = NBS

and LBS

e_{ij} = The random residual term associated with each Y_{ij}

Model 2. For comparing the effect of calving period and seasons on BCS, FCM and MPL (dependant variables)

$$Y_{ijk} = u + \alpha_i + \beta_j + e_{ijk}$$

Where

Y_{ijk} = k th observation of the dependent variable during i th period of calving and j th season

u = Population constant common to all records

α_i = The effect of i th period of calving; i = NBS and LBS

β_j = the effect of j th season; j = Autumn, winter, spring and summer

e_{ijk} = the random residual term associated with each Y_{ijk}

RESULTS AND DISCUSSION

Seasonality of reproductive performance

The buffaloes calving during the normal breeding season (NBS, August to January), had short postpartum estrus interval (PEI) of 55.95 ± 4.90 days while the interval was longer (91.15 ± 11.61 days), in the buffaloes calving during low breeding season (LBS, February to July) (table 1). Postpartum milk progesterone levels during the LBS remained lower than those in the NBS ($p < 0.01$). (table 2, figure 1). In spite of the higher incidence of estrus events (31.6%) and shorter postpartum uterine involution interval (33.4 days) during summer, the lower progesterone levels may be the cause of summer infertility. Shortest postpartum ovulation interval was noted during autumn, followed by winter, summer and spring (63.8, 77.5, 83.4 and 126.1 days, respectively). Placenta expulsion duration was also shorter in NBS than LBS calvers. The incidence of silent ovulations was higher during LBS

as compared to NBS (70.6% versus 29.4%).

This study confirms earlier reports (Pasha et al., 1986; Shah, 1990) that in Nili-Ravi buffaloes the incidence of estrus during autumn was highest, while lowest incidence was observed during spring in Pakistan. The breeding and calving seasons have been reported to be February to April and December to March (Perera et al., 1987) for Sri Lankan buffaloes and autumn and winter (Tailor and Jain, 1987) for Indian buffaloes, respectively.

Table 2. Effect of calving period and season on body condition score, production of fat corrected milk and milk progesterone levels (MPL) (least square means \pm standard error)

Group	Body condition score (scale=0-5)	Fat-corrected milk (kg/day)	MPL (ng/ml)
Calving periods			
NBS**	2.82 \pm 0.02 ^a	15.31 \pm 0.33 ^a	1.97 \pm 0.30 ^a
LBS***	2.60 \pm 0.03 ^b	13.55 \pm 0.22 ^b	0.68 \pm 0.08 ^b
Seasons			
Autumn	2.91 \pm 0.03 ^a	14.16 \pm 0.29 ^a	0.84 \pm 0.72 ^a
Winter	2.86 \pm 0.02 ^a	14.24 \pm 0.34 ^a	1.77 \pm 0.32 ^b
Spring	2.61 \pm 0.03 ^b	15.41 \pm 0.51 ^a	3.00 \pm 0.12 ^c
Summer	2.22 \pm 0.05 ^c	13.96 \pm 0.53 ^a	0.25 \pm 0.04 ^c

* Means within a group in the same column with different letters, differs significantly from each other ($p < 0.01$).

** Normal breeding season.

*** Low breeding season.

Factors related with seasonality of breeding

Annual meteorological status and milk progesterone levels in the experimental area are presented in figure 2. Maximum ambient temperature started rising from 20°C in February to 46°C in June. From June onward the temperature decreased. Milk progesterone remained at basal levels during May to July, started rising up to February and then declined. This trend was almost opposite to that of atmospheric temperature. The differences among the four seasons were significant ($p < 0.05$) as shown in table 2. During start of the autumn the decreasing day-length and ambient temperature coincided with the commencement of breeding season in buffaloes. In NWFP summer period extends from May to July which is initially dry with ambient temperature reaching to a maximum followed by a decline up to December. In addition to the environmental heat stress, which resulted in low feed intake, feed during this period was also of low quality.

Similarly, in a study, maximum environmental temperature on the day after insemination was associated negatively with conception rate (Gwadzaukas

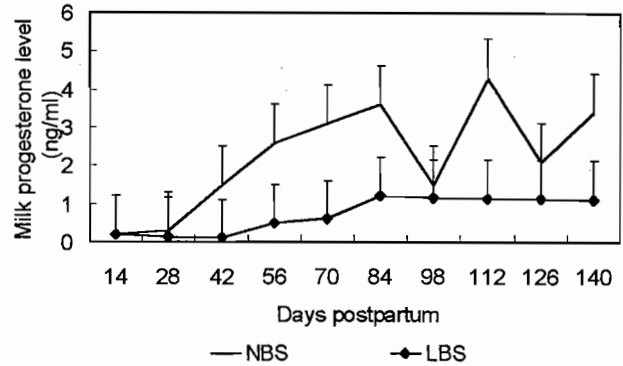


Figure 1. Milk progesterone levels at various postpartum intervals in normal (NBS) and low breeding season calvers (LBS) ($p < 0.01$)

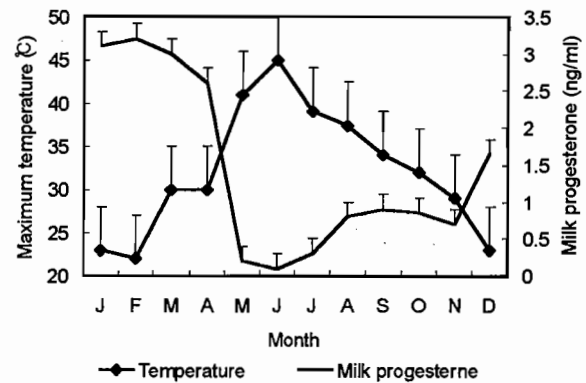


Figure 2. Annual maximum ambient temperature and milk progesterone concentration in buffaloes

et al., 1975). It has been suggested (Thatcher et al., 1986) that a thermal stress-induced reduction in uterine serum flow would preferentially elevate uterine temperature and likely affect availability of water, electrolytes, nutrients and hormone to uterus. Such induced inhibitory responses, as a consequence of thermal stress, would have a high probability of increasing rates of embryo death during early pregnancy.

In the buffaloes calving during NBS, serum glucose levels were higher ($p < 0.01$) and serum protein ($p < 0.05$), urea ($p < 0.01$), calcium ($p < 0.01$) and magnesium levels were lower ($p < 0.01$), as compared to those calving in LBS (tables 3, 4). Highest serum urea was found in summer and spring than that in autumn and winter ($p < 0.05$). Serum calcium levels were highest during autumn ($p < 0.01$) while serum magnesium and phosphorus levels were not effected by season.

The present findings confirms previous study in which hypoglycemia was found to be associated with a reduced fertility in beef and dairy cattle (McClure,

Table 3. Effect of calving period and season on serum metabolites concentrations (least square means* ± standard error)

Group	Serum glucose (mg/100 ml)	Serum protein (g/1000 ml)	Serum urea (mg/100 ml)
Calving periods			
NBS**	59.23 ± 0.82 ^a	81.45 ± 0.79 ^b	31.69 ± 1.43 ^b
LBS***	55.15 ± 1.21 ^b	84.44 ± 0.96 ^a	39.42 ± 2.16 ^a
Probability	p<0.01	p<0.05	p<0.01
Seasons			
Autumn	59.23 ± 1.36 ^{ab}	85.32 ± 1.12	36.35 ± 2.92 ^{ab}
Winter	56.04 ± 1.01 ^b	80.80 ± 1.03	29.86 ± 1.96 ^b
Spring	60.91 ± 2.17 ^a	82.21 ± 1.52	38.62 ± 2.95 ^a
Summer	55.63 ± 1.44 ^b	83.32 ± 1.24	40.15 ± 2.53 ^a
Probability	p<0.05	Non-significant	p<0.05

* Means within a group in the same column with different letters, differs significantly from each other.

** Normal breeding season.

*** Low breeding season.

Table 4. Effect of calving period and season on serum mineral concentrations (least square means* ± standard error)

Group	Serum calcium (mg/100 ml)	Serum magnesium (g/1000 ml)	Serum phosphorus (mg/100 ml)
Calving periods			
NBS**	6.45 ± 0.21 ^b	21.70 ± 0.60 ^b	4.42 ± 0.15
LBS***	8.05 ± 0.17 ^a	28.64 ± 0.99 ^a	4.98 ± 0.21
Probability	p<0.01	p<0.01	Non-significant
Seasons			
Autumn	8.35 ± 0.23 ^a	23.61 ± 1.34	4.49 ± 0.22
Winter	5.94 ± 0.24 ^b	24.65 ± 0.82	4.82 ± 0.18
Spring	7.95 ± 0.35 ^a	21.45 ± 1.16	4.22 ± 0.33
Summer	7.28 ± 0.28 ^{ab}	26.59 ± 1.21	4.62 ± 0.32
Probability	p<0.05	Non-significant	Non-significant

* Means in the same column with different letters from each other.

** Normal breeding season.

*** Low breeding season.

1968). Similarly, high serum urea has been suggested to impair fertility (Fergusson et al., 1991). In a study on 12 cows (Tegegnae et al., 1993) plasma total protein, albumin and globulin were not influenced by feeding regimes, nor did they differ between cyclic and acyclic cows. However Veerapandian et al. (1992) reported that serum glucose and serum total protein concentrations were significantly low in anestrus buffaloes. Sekerden et al. (1992) reported that in Jersey cows, serum calcium levels were not correlated to conception rates at first insemination. However, favorable effects of magnesium on reproduction have been reported by Gabryszuk (1994). In rural Indian cows (Satish-Kumer and Sharma, 1991) and Egyptian buffaloes (El-Belely et al., 1994), plasma phosphorus concentrations were markedly higher in animals that conceived as compared with those that did not. The low reproductive performance in the LBS calvers coincided with a low BCS, (2.60 versus 2.82, p<0.01).

figure 3 shows that more animals in the higher BCS were available during NBS and more animals with lower BCS were available during LBS. BCS tended to increase from July onward reaching its peak in October and then declined up to June (figure 4). The increasing score coincided with the commencement of breeding season. The constant increase in BCS in autumn and early winter, apparently due to the increasing metabolizable energy, seems to support commencement of reproductive activities during this period. In addition to supporting enhanced reproductive performance, the increasing BCS supported increasing milk production from June onward. It confirms the findings of O'Rourke et al. (1991) and Derouen et al. (1994) on cows and Jainudeen and Wahab (1987) on buffaloes.

Fat-corrected milk production of the NBS calvers was higher than that of the LBS calvers (15.31 ± 0.33 versus 13.55 ± 0.22 kg/day, p<0.01). As the protein

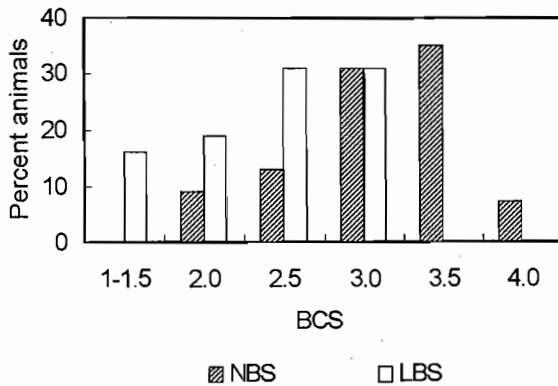


Figure 3. Distribution of buffaloes on the basis of body condition score (BCS) during normal (NBS) and low breeding season (LBS)

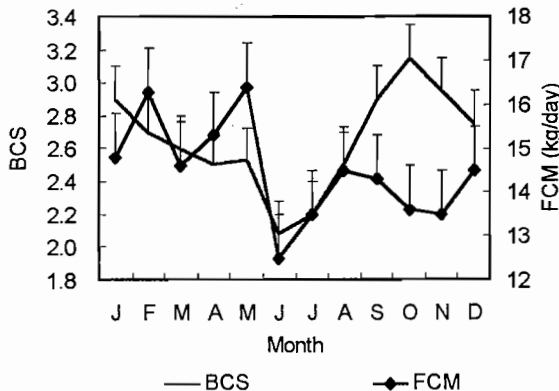


Figure 4. Annual pattern of body condition score (BCS) and 4% fat-corrected milk production (FCM) in buffaloes

and minerals intake in this study was above requirements and was associated with adverse effect on reproductive performance (Qureshi et al., 1998), higher milk production in the NBS calvers was associated with better reproductive performance. Highest production was recorded during spring, followed by winter, autumn and summer. However the difference among the four seasons was not significant.

It was concluded that Nili-Ravi buffaloes show a seasonal pattern in breeding, commencing from August up to January. Onset of breeding season was associated with decreasing atmospheric temperature, increasing BCS and milk production, high serum glucose levels and low serum urea, protein, calcium and magnesium levels.

ACKNOWLEDGEMENT

Financial sponsorship of National Coordinated

Buffalo Research Program/ARP-II/PARC Islamabad and donation of RIA kits by Animal Production and Health Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA Vienna, are acknowledged.

REFERENCES

- AOAC. 1980. Official methods of analysis (13th Ed.). (Ed. W. Horwitz). Association of Official Analytical Chemists, Washington.
- Amin, M. and M. J. Khan. 1994-95. Meteorological observations. NWFP Agricultural University Peshawar.
- Derouen, S. M., D. E. Franke, D. G. Morrison, W. E. Yatt, D. R. Coombs, T. W. White, P. E. Humes and B. B. Greene. 1994. Parturition body condition and weight influences on reproductive performance of first-calf beef cows. *J. Anim. Sci.* 72:1119-1125.
- El-Belely, M. S., M. M. Abou-Ahmed and H. M. Eissa. 1994. The relationship between concentrations of certain plasma metabolites and fertility levels in buffalo cows (*Bubalus bubalis*). *Buffalo J.* 10:53-59.
- FAO/IAEA. 1993. Progesterone RIA kit protocol, version 2.0, Joint FAO/IAEA Program in Animal Production and Health, Seibersdorf, Austria.
- Fergusson, J. D., D. T. Galligan and T. Blanchard. 1991. Blood urea nitrogen and conception rate. The usefulness of test information. *J. Dairy Sci.* 74(Suppl.):242(Abstract).
- Gabryszuk, M. 1994. The effect of selected minerals and vitamin E on the reproduction of the Polish Merino sheep. II. Reproduction and rearing lambs. *Jastrzebiec* 12:53-61.
- Gwadzaukas, F. C., C. J. Wilcox and W. W. Thatcher. 1975. Environmental and managerial factors effecting conception rate in subtropical climate. *J. Dairy Sci.* 58:88.
- Jainudeen, M. R. and S. Wahab. 1987. Postpartum anestrus in dairy buffalo. Proc. International symposium on milk buffalo reproduction. Pakistan Agric. Res. Council Islamabad. pp. 69-77.
- Majeed, M. A., G. K. Garlick and I. R. Khan. 1961. Seasonal trend in frequency of estrus of buffaloes and cows. *Agric. Pakistan.* 12:181-193.
- McClure, T. J. 1968. Malnutrition and infertility of cattle in Australia and New Zealand. *Australian Vet. J.* 44:134.
- Morrison, F. B. 1949. Feeds and feeding. The Morrison Publishing Company, Ithaca, New York. pp. 653.
- O'Rourke, P. K., V. J. Doogan, T. H. McCosker and A. R. Egginton. 1991. Prediction of conception rate in extensive beef herds in north-western Australia. 1. Seasonal mating and improved management. *Australian J. Exp. Agric.* 31:1-7.
- Pasha, T. N., M. A. Chaudhry and M. A. Jabbar. 1986. Effect of month and season on breeding behavior of Nili-Ravi buffaloes in rural condition. *Buffalo J.* 2:135-139.
- Perera, B. M. A. O., L. N. A. de Silva, V. Y. Kuruwita and A. M. Karunaratne. 1987. Postpartum ovarian activity, uterine involution and fertility in indigenous buffaloes at a selected village location in Sri Lanka.

- Anim. Reprod. Sci. 14:115-127.
- Perera, A. R. and P. J. H. Ball. 1987. Reproduction in cattle. Butter-worth, London. 99. 167-168.
- Qureshi, M. S. 1995. Conventional buffalo farming system in the North-West Frontier Province of Pakistan. buffalo bulletin. 14:38-41.
- Qureshi, M. S., G. Habib, H. A. Samad, R. H. Usmani and M. M. Siddiquee. 1998. Study on factors leading to seasonality of reproduction in dairy buffaloes - I. Nutritional factors. Asian-Aus. J. Anim. Sci. (Accepted).
- Satish-Kumar and M. C. Sharma. 1991. Level of haemoglobin and certain serum biochemical constituents in rural cows during fertile and non-fertile oestrus. Indian Vet. J. 68:361-364.
- Sekerden, O., E. Cekgul and H. Erdem. 1992. Effects of blood traits on conception rate to first insemination and milk yield. Hayvancilik Arastirma Dergisi. 2;18-20.
- Shah, S. K. 1991. Buffaloes of Pakistan. Pakistan Agricultural Research Council, Islamabad.
- Shah, S. N. H. 1990. Prolonged calving interval in the Nili-Ravi buffaloes. Ph. D thesis. Utrecht Univ., the Netherlands.
- Shah, N. H. and S. K. Shah. 1968. Seasonal incidence on estrus incidence and conception rates in buffaloes. Agric. Sci. Pakistan. 5:222-227.
- Steel, R. G. D. and J. H. T. Torrie. 1980. Principals and procedures of statistics - A biometrical approach. 2nd Ed. McGrawhill Book Company, New York.
- Taylor, S. P. and L. S. Jain. 1987. Reproductive performance of medium sized buffaloes. Indian J. Dairy Sci. 40:179-182.
- Tegegne, A., K. W. Entwistle and E. Mukasa-Mugerwa. 1993. Plasma progesterone and blood metabolite profiles in postpartum Small East African Zebu cows. Trop. Anim. Hlth. Prod. 25:101-110.
- Thatcher, W. W., R. I. Collier, D. K. Beede and C. J. Wilcox. 1986. Interaction of environment and reproductive process in cattle. nuclear and related techniques for improving productivity of indigenous animals in harsh environments. Proceedings of an advisory group meeting, Ankara 3-8 June 1985, IAEA-SR-115/104 Vienna. pp. 61-73.
- Usmani, R. H., A. Ahmad, E. K. Inskeep, R. A. Dailey, P. E. Levis and G. S. Lewis. 1985. Uterine involution and postpartum ovarian activity in Nili-Ravi buffaloes. Theriogenology. 24:435-448.
- Veerapandian, C., L. S. Narasimhan, S. A. Quayam, S. R. V. Pattabiraman. 1987. Variation in certain biochemical constituents in the blood of anoestrous buffaloes maintained under farm and urban conditions. Cheiron. 16:19-22.
- Wahid, A. 1976. Livestock Resources of Pakistan. Monogram No. 7, Pakistani Buffaloes. University of Karachi. p. 13.