

## Study on Factors Leading to Seasonality of Reproduction in Dairy Buffaloes. I. Nutritional Factors

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**ABSTRACT** : Fifty one Nili-Ravi dairy buffaloes were studied in North-West Frontier Province of Pakistan. Rectal examination of reproductive organs was carried out on days 14 and 21 and then fortnightly. Milk samples were collected and analyzed for progesterone levels (MPL). Feed samples were collected fortnightly and analyzed. The buffaloes calving during the normal breeding season (NBS, August to January) had shorter ( $p < 0.01$ ) postpartum estrus interval of 55.95 days versus 91.15 days in those calving during the low breeding season (LBS, February to July). MPL in the LBS remained lower than the NBS ( $p < 0.01$ ). Shortest postpartum ovulation interval was noted during autumn (August to October), followed by winter (November to January), summer (May to July) and spring (February to April). The incidence of silent ovulations was higher during LBS than NBS (70.6% versus 29.4%). In autumn there was minimum intake of crude protein (CPI) and maximum intake of metabolizable energy (MEI,  $p < 0.01$ ). Calcium intake was higher in NBS than LBS calving buffaloes ( $p < 0.01$ ). Phosphorus, copper and magnesium intake was lower ( $p < 0.05$ ) and zinc intake was higher ( $p < 0.01$ ) in autumn, It was ( $p < 0.05$ ) and zinc intake was higher ( $p < 0.01$ ) in autumn. It was concluded that onset of breeding season was associated with increasing MEI and decreasing CPI and minerals intake. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 7 : 1019-1024*)

**Key Words** : Buffalo, Pakistan, Reproduction, Season, Nutrition

### INTRODUCTION

It is generally accepted that livestock productivity and reproductive efficiency in the temperate regions is higher than the tropical ones. A major factor associated with the low reproductive performance, is seasonal influence through poor forage availability and thermal stress in the harsh, hot and often semi-arid or wild environment (Robertshaw, 1986). Environmental stress factors depress reproductive efficiency of livestock during certain seasons (Thatcher and Roman-Ponce, 1980). 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.

Reproductive performance in the buffalo is affected by seasonality of breeding (Majeed et al., 1961; Shah and Shah, 1968; Goswami and Nair, 1965). Under field conditions of the North-West Frontier Province (NWFP) of Pakistan, nutritional stress coupled by seasonal fluctuations results in inactive ovaries (Qureshi, 1995). The present project was designed to study the role of nutritional factors in seasonality of reproduction in buffaloes.

### 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, 31-37° N and 65-74° 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 and the approximate size of the cervix, uterus and ovaries and the ovarian structures was recorded. 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 sampling and progesterone assay

Milk samples were collected weekly from each buffalo, fat layer was removed and 100  $\mu$ l of 0.1% sodium azide solution was added to 5 ml of milk sample as a preservative. Samples were stored at -20°C until analysis for milk progesterone levels

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(MPL) using radio-immunoassay technique (FAO/IAEA, 1993).

#### Analysis of feed samples

All the animals were stall-fed. Green fodder and concentrates were offered by the farmers irrespective of animal's requirements. Representative samples of all the feed ingredients, offered to the experimental animals, were collected at fortnight intervals. A portion of the samples were processed for dry matter contents in metallic crucibles and dried in a hot air oven to a constant weight at 65°C. The remaining portion of the samples was air dried at 60°C for 48 hours, ground through 1 mm sieve in a Wiley mill and stored for further analysis. The samples were analyzed for the contents of ash, crude protein, crude fiber, ether extract, calcium, magnesium, phosphorus, copper and zinc, according to AOAC (1980). Dry matter and intra-ruminal protein degradability of feed samples were determined using the in sacco technique similar to that described by Orskov et al. (1980). Intake of dry matter and various nutrients by the animals was calculated on the basis of intake of feed and feed composition (table 4).

#### 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. Calving period of the buffaloes was categorized as the normal breeding season (NBS, August to January) and low breeding season (LBS, February to October), winter (November to January), spring (February to July). Seasons of the year were described on Autumn (August to April) and Summer (May to July). The data obtained were statistically analyzed using General Linear Model procedure (Steel and Torrie, 1980). The following models were adopted:

1. For comparing the effect of calving period on

reproductive parameters (dependant variables)

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

Where

$Y_{ij}$  = jth observation of the dependent variable during ith period of calving

$u$  = Population constant common to all records

$\alpha_i$  = The effect of ith period of calving;  $i$  = NBS and LBS

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

2. For comparing the effect of calving period and seasons on intake of various nutrients (dependant variables)

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

Where

$Y_{ijk}$  = kth observation of the dependent variable during ith period of calving and jth season

$u$  = Population constant common to all records

$\alpha_i$  = The effect of ith period of calving;  $i$  = NBS and LBS

$\beta_j$  = The effect of jth season;  $j$  = Autumn, winter, spring and summer

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

## RESULTS AND DISCUSSIONS

#### Seasonality of reproductive performance

NBS calving buffaloes had shorter postpartum estrus interval (PEI) of  $55.95 \pm 4.90$  days while the interval was longer ( $91.15 \pm 11.61$  days) in LBS calving buffaloes ( $p < 0.01$ , table 1). Postpartum milk progesterone levels during the LBS remained lower than those in the NBS ( $p < 0.01$ ) (table 2). In spite of the higher incidence of estrus events (31.16%) and

**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)

<sup>a,b</sup> The value with different letters differ significantly ( $p < 0.01$ ).

\* Values in parenthesis represent the number of observations.

**Table 2.** Effect of calving period and season on intake of crude protein (CP) metabolizable energy (ME) and milk progesterone levels (MPL) (least square means  $\pm$  standard error)

Group	ME intake (Mcal/day)	CP intake (kg/day)	CP/ME Ratio (g/MJ)	MPL (ng/ml)
<b>Calving periods</b>				
NBS**	41.18 $\pm$ 0.32 <sup>b</sup>	1.81 $\pm$ 0.02	12.15 $\pm$ 0.07a	1.97 $\pm$ 0.30a
LBS***	42.42 $\pm$ 0.40 <sup>a</sup>	1.83 $\pm$ 0.02	11.78 $\pm$ 0.10b	0.68 $\pm$ 0.08b
Probability	p<0.01	Non-significant	p<0.01	p<0.01
<b>Seasons</b>				
Autumn	46.76 $\pm$ 0.40 <sup>a</sup>	1.79 $\pm$ 0.02 <sup>c</sup>	11.47 $\pm$ 0.10 <sup>c</sup>	0.84 $\pm$ 0.72 <sup>c</sup>
Winter	39.14 $\pm$ 0.42 <sup>c</sup>	1.68 $\pm$ 0.02 <sup>d</sup>	11.95 $\pm$ 0.09 <sup>b</sup>	1.77 $\pm$ 0.2 <sup>b</sup>
Spring	38.36 $\pm$ 0.46 <sup>c</sup>	1.89 $\pm$ 0.03 <sup>b</sup>	12.19 $\pm$ 0.09 <sup>b</sup>	3.00 $\pm$ 0.12 <sup>a</sup>
Summer	42.29 $\pm$ 0.62 <sup>b</sup>	2.08 $\pm$ 0.05 <sup>a</sup>	12.66 $\pm$ 0.19 <sup>a</sup>	0.25 $\pm$ 0.04 <sup>c</sup>
Probability	p<0.01	p<0.01	p<0.01	p<0.01

\* Means in the same group within a column, with different letters differ from each other at the respective p value;

\*\* NBS = Normal Breeding Season; \*\*\* LBS = Low Breeding Season.

shorter PUI (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 comparatively shorter in NBS than LBS calving buffaloes. 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. They recorded highest monthly frequency in November and lowest in May. 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.

#### Factors related with seasonality of breeding

##### 1) Energy intake

The overall mean value of metabolizable energy (ME) intake was 41.70  $\pm$  0.25 Mcal/day, ranging from 20.2 to 60.4 Mcal/day. ME intake was lower in the NBS calving buffaloes than the LBS calving buffaloes (41.18  $\pm$  0.32 versus 42.42  $\pm$  0.40 Mcal/day, p<0.01) (table 2). Better reproductive performance of the buffaloes, recorded during autumn (August to October), was associated with maximum intake of metabolizable energy than winter and spring (46.76  $\pm$  0.40, 39.14  $\pm$  0.42 and 38.86  $\pm$  0.46 Mcal/day, respectively, p<0.01).

In agreement to our findings, a study on Thai Swamp buffaloes (Intaramongkol et al., 1994) revealed that the longest day open (216 days) occurred in late dry season (April) with poorest pasture condition and

highest atmospheric temperature. Vale et al. (1988) who found that deficiency of fodder supply interrupted calving frequency of buffaloes and the buffaloes having regular supply of fodder throughout the year, bred throughout the year. Low energy intake led to ovarian inactivity and anestrus in suckled beef cows. Similarly, Wongsrikeao and Taesakul (1984) reported that improved nutrition reduced the postpartum service period in Swamp buffalo cows and increased the growth rate of their calves.

##### 2) Protein intake

Crude protein (CP) intake showed a mean value of 1.82  $\pm$  0.47 kg/day ranging from 0.95 to 2.64 kg/day. Better reproductive performance of the buffaloes, recorded during autumn and winter was associated with minimum intake of crude protein (1.79  $\pm$  0.02, 1.68  $\pm$  0.02 kg/day), followed by spring and summer (1.89  $\pm$  0.03, 2.08  $\pm$  0.05 kg/day, respectively, p<0.01, table 2). The ratio of crude protein to metabolizable energy intake was also low during autumn (p<0.01). Degradable protein (DP) intake was lower in autumn and winter as compared with spring and summer (p<0.01).

The crude protein content of the buffalo ration during LBS was higher and the major components of the feed ingredient were ruminally degradable, which is not favorable for better reproductive performance (Jordan and Swanson, 1979a; Canfield et al., 1990). It may be one of the factors leading to seasonal breeding. Crude protein intake decreased from July onward, coinciding with the commencement of breeding season in buffaloes. In agreement to the present study, Elrod and Butler (1993) reported detrimental effect of high CP intake in cattle indicated by significantly lower first-service conception rates in Holstein heifers, fed high ruminally degradable protein.

Endocrine functions of the pituitary (LH secretion) and ovary (progesterone secretion) have been greatly elevated and depressed, respectively, in cows consuming feed high in protein (Jordan and Swanson, 1997b).

### 3) Minerals intake

Mineral intake by experimental buffaloes during the study period is given in table 3. Calcium intake was higher in NBS calving buffaloes than LBS calving buffaloes (115.15 versus 89.32 g/day,  $p < 0.01$ ) and

during spring as compared to winter, summer and autumn (134.66, 117.71, 100.33 and 62.25 g/day respectively,  $p < 0.01$ ). Magnesium intake was not effected by calving period. However it was highest during spring, followed by winter, summer and autumn ( $p < 0.05$ ). Phosphorus intake was effected by season ( $p < 0.01$ ) and in the buffaloes calving during NBS, it was lower as compared to those calving during LBS from one month prepartum to two months postpartum (figure 1). Zinc intake was highest in autumn, followed by spring, winter and summer ( $p < 0.01$ ).

**Table 3.** Effect of calving period and season on dry matter and mineral intake (least square means  $\pm$  standard error)

Group	Dry matter (kg/day)	Calcium (g/day)	Magnesium (g/day)	Phosphorus (g/day)	Zinc (mg/day)	Copper (mg/day)
Calving periods						
NBS**	16.5	115.15 <sup>a</sup>	33.00	40.17	1,102	240
LBS***	16.7	89.52 <sup>b</sup>	34.26	41.40	1,087	202
Probability	NS****	$p < 0.01$	NS	NS	NS	NS
Seasons						
Autumn	17.6 <sup>a</sup>	62.25 <sup>d</sup>	31.60 <sup>e</sup>	39.81 <sup>bc</sup>	1,127 <sup>a</sup>	150 <sup>d</sup>
Winter	16.7 <sup>b</sup>	117.71 <sup>b</sup>	34.40 <sup>b</sup>	35.08 <sup>c</sup>	1,085 <sup>b</sup>	238 <sup>b</sup>
Spring	15.9 <sup>c</sup>	134.66 <sup>a</sup>	35.86 <sup>a</sup>	43.88 <sup>b</sup>	1,096 <sup>b</sup>	295 <sup>a</sup>
Summer	15.8 <sup>c</sup>	100.33 <sup>c</sup>	32.65 <sup>c</sup>	51.19 <sup>a</sup>	1,072 <sup>b</sup>	219 <sup>c</sup>
Probability	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.01$	$p < 0.01$	$p < 0.01$

\* Means in the same group within a column, with different letters differ from each other at the respective p value;

\*\* NBS = Normal Breeding Season; \*\*\* LBS = Low Breeding Season; \*\*\*\* NS = Non-significant.

**Table 4.** Nutrient composition of feeds used for experimental buffaloes

Feed	Month	Dry matter	As % in dry matter		Metabolizable energy (Mcal/kg DM)
			Minerals	Crude protein	
Green fodders					
Berseem	Nov-May	14.43	12.94	21.03	2.67
Sorghum	Jul-Nov	34.35	8.82	6.09	3.06
Maize	Jan-Nov	30.00	8.91	7.51	2.95
Wheat	Jan-May	28.05	8.01	8.41	2.65
Dry roughage					
Maize stovers	Jan-Dec	95.09	6.04	3.72	1.73
Wheat straw	Jan-Dec	93.74	9.93	4.21	1.58
Concentrate supplements					
Wheat bran	Jan-Dec	90.44	3.87	17.13	3.68
Cotton seed cake	Jan-Dec	91.88	5.46	24.97	5.44
Mustard seed cake	Jan-Dec	92.01	6.79	31.61	2.93
Maize oil cake	Jan-Dec	95.38	1.94	20.50	2.72
Commercial conc.	Jan-Dec	92.60	6.68	15.66	2.98
Dried bread	Jan-Dec	82.66	2.49	20.39	4.57
Wheat grain	Jan-Dec	93.00	1.32	13.00	4.78
Beet pulp dried	Apr-Jun	95.06	4.49	11.96	2.31
Molasses	Jan-Dec	71.92	13.6	8.99	1.81

Copper intake was highest during spring, followed by winter, summer and autumn ( $p < 0.01$ ) and in the NBS calving buffaloes, it was constantly lower as compared to NBS calving buffaloes (figure 2). These results suggest association of lower intake of magnesium, phosphorus and copper and higher intake of calcium and zinc with NBS.

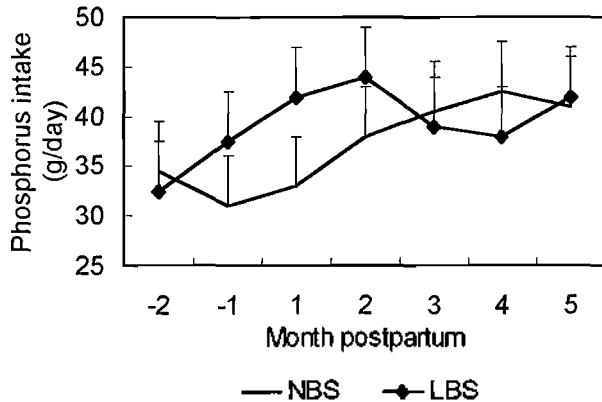


Figure 1. Phosphorus intake in normal (NBS) and low breeding season calving (LBS) buffaloes various months post-partum

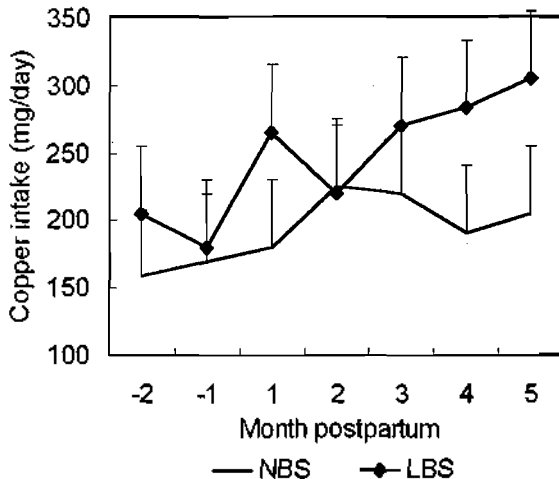


Figure 2. Copper intake in normal (NBS) and low breeding season (LBS) calvers

In agreement to the present findings, Wittkowski and Spann (1993) reported that undersupply of calcium decreased reproductive performance. In buffaloes, an intake of 31.9 g magnesium above the standard requirements, resulted in mineral imbalance and was considered a cause of infertility in the repeat breeders (Balakrishnan and Balagopal, 1994). However, favorable effects of magnesium on reproduction have been reported by Gabryszak (1994). In contrast to the present findings, a favorable effect of supplementation

of ration with phosphorus in dairy heifers was reported (Morrow, 1969).

In agreement to this study, Khattab et al. (1995) reported that serum zinc levels were higher in buffaloes with regular estrus cycles. However, Kirchgessner et al. (1976) found that diets very deficient (6 ppm) or very high (436 ppm) in zinc had no effect on blood gonadotrophic hormones levels in cattle. Copper intake of 118 to 245 mg/day has been reported in a previous study on Egyptian buffaloes (Attia et al., 1987). Lack of copper affected body growth and caused silent estrus (Wittkowski and Spann, 1993).

It may be concluded from the above results that Nili-Ravi buffaloes show a seasonal pattern in breeding, commencing from August up to January. Onset of breeding season was associated with higher intake of metabolizable energy, zinc and calcium and lower intake of crude protein, magnesium, phosphorus and copper.

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