RELATIONSHIP OF PRE- AND POSTPARTUM NUTRITIONAL STATUS WITH REPRODUCTIVE PERFORMANCE IN NILI-RAVI BUFFALOES UNDER THE CONVENTIONAL FARMING SYSTEM IN N.W.F.P., PAKISTAN

BY

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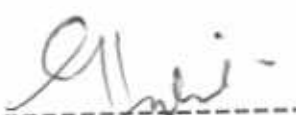(Dr. Nazir Ahmed)

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Chapter 1

INTRODUCTION

Buffaloes hold an important position in the economy of Pakistan. There are approximately 20.2 million heads of buffaloes in the country (Annonymous, 1996) which contribute to 76% milk and 50% beef produced. They also provide draught and traction power for agriculture. Buffalo population is scattered in herds of various sizes ranging from one head to several hundreds. The present farming community consists of decades-old farming families, financially sound businessmen and illiterate rich men. Economic return from the present farming system is not very attractive, as is the case with most of other agricultural sub-sectors. Many factors contribute to this state of affairs. Animals with poor genetic make up, nutritional disorders, infectious and parasitic diseases and poor reproductive performance are some of the major factors of economic importance. Usmani (1987) reported an annual loss of 10.66 million metric tons of milk, 4.4 million calves and 52 million metric tons of extra forage consumption by 6.5 million breedable buffaloes per anum, worth of Rs.61.35 billion on account of their late sexual maturity and long calving interval.

Reproductive performance in the buffalo is adversely affected by delayed age at first calving (Ashfaque and Mason, 1954; Wahid, 1975), poor expression of estrus symptoms, low number of primordial follicles in the ovaries, high frequency of follicular atresia, lower blood progesterone levels (Settergren, 1987), postpartum anestrus (Jainudeen and Wahab, 1987), repeat breeding (Ishaque, 1965) and prolonged calving interval (Wahid, 1975). Seasonal breeding is another factor contributing to the decreased

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reproductive efficiency of Pakistani (Majeed *et al.*, 1961, Shah and Shah, 1968) and Indian buffaloes (Goswami and Nair, 1965).

In the North-West Province (NWFP) of Pakistan reproductive efficiency of buffaloes is low and shows seasonal pattern (Shah, 1990). Prolonged postpartum estrus interval, more services required per conception, poor estrus symptoms and seasonality of estrus are the results of environmental stress including day-length, temperature and nutrition. Under the conventional farming system nutrition is manageable and reproductive process may be manipulated easily. Reproductive management of buffaloes kept under conventional farming system in NWFP Pakistan has been described (Qureshi, 1995). The province is exposed to severe alterations in atmospheric temperature, ranging from 0 to 50°C. Fodder availability is affected by the fluctuating temperatures resulting in scarcity during the severe hot and cold months. In the existing farming system, a highly fertile female buffalo gets a chance to survive and animals remaining non-pregnant beyond six months of parturition are culled off and slaughtered for meat purpose irrespective of their genetic make-up. Imbalanced nutritional status may be one of the causes of delayed breeding in such animals.

Under the conventional farming system in NWFP, there is no practice of ration formulation according to requirements of the individual animals, resulting in decreased production and poor health and reproduction (Qureshi, 1995). Excessive or deficient intake of some of the nutrients may decrease reproductive performance. The present project has been designed to study the reproduction-nutrition interaction in the buffalo with the following objectives:

1. To study the reproductive performance of buffaloes under the existing farming in NWFP in relation to thier pre- and postpartum nutritional status.

2. To investigate the association of nutritional factors with seasonality of reproduction with a view to minimize seasonal infertility through appropriate feed management.

3. To elucidate the association of concentration of blood metabolites and minerals with reproduction in buffaloes under the existing farming conditions.

CHAPTER 2

REVIEW OF LITERATURE

The available literature on various aspects of reproduction in buffaloes was reviewed under three sections. In the first section, reproductive performance of buffaloes under various managements is discussed. Relationship of pre- and postpartum nutritional status with various reproductive parameters is reviewed in the second section. Section three has described effect of body condition score, calf suckling, milk production, thermal stress and photoperiod on reproductive performance.

2.1 Reproductive performance under various managements

Major criteria of reproductive efficiency in dairy animals include postpartum estrus interval, postpartum ovulation interval, days open, first service conception rate, services per conception and calving interval. It is important to achieve optimum values for these criteria. For small scale farms reproductive goals in dairy cows have been defined as; a 12 month calving interval, 85 days open, 1.6 services per conception, 60% first service conception rate and 85% of cows observed in estrus by 60 days postpartum (Rowson, 1986).

Over 95% buffaloes belong to small holder farmers who do not consider it an economic necessity to maintain breeding records (Jainudeen, 1986). Calving interval has been reported as the most widely quoted fertility criterion for buffaloes. Calving interval is composed of a gestation period and days open, that is the service period. Calving interval depends upon days open which is a valuable index reflecting efficiency of estrus

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detection and the fertility of both females and males in a herd (Jainudeen and Hafez, 1987). Days open, in turn, depend upon postpartum estrous interval and number of services per conception.

Jainudeen and Wahab (1987) reported three types of postpartum ovarian activities in buffaloes: i) a corpus luteum with detectable estrus (cycling); ii) a corpus luteum with non-detected estrus (apparent anestrus and silent estrus); iii) no corpus luteum with non-detected estrus, "true" anestrus. Various parameters of reproductive performance are reviewed as follows:

2.1.1 Postpartum uterine involution

After parturition in buffaloes, the size of uterus decreases gradually during the first two weeks associated with a concomitant lochial discharge (Dobson and Kamonpatana, 1986). The process of uterine involution, depends upon myometrial contractions, elimination of bacterial infection and regeneration of endometrium (Jainudeen and Hafez, 1987). The rapid decrease in size is due to vasoconstriction and peristaltic contractions which occur at three to four minutes intervals and gradually diminish by day 4 postpartum (Leslie, 1983).

Uterine involution is completed sooner after normal than abnormal parturition, in suckled than non-suckled or milked buffaloes (Jainudeen, 1984) and in low than high milk producers (El-Fadaly, 1980b). It also occurs sooner with increasing parity (Peiris et al., 1980) and during winter and spring (Chauhan et al., 1977).

In 95 Egyptian buffalo heifers (El-Shafie et al., 1983), the postpartum intervals to uterine involution averaged 34.7 with a range of 19-57 days. Similarly Aboul-Ela et

al. (1988) reported a postpartum uterine involution interval of 40.3 ± 1.2 days in buffaloes. Perera *et al.* (1987) reported that in 55 Lanka buffaloes, involution of the uterus completed at an interval of 32.9 ± 8.2 days postpartum. The intervals to uterine involution and first service were correlated.

In a study on 20 Philippine carabao (PC) and 20 PC X Murrah buffaloes (Momongan *et al.*, 1991), uterine involution time was 73.0 ± 22.0 and 55.0 ± 27.0 days. In Thai Swamp buffaloes involution of uterus was reviewed by Virakul (1987); involution completed in 28 days postpartum (range 19-36 days) and the naval cord dropped off in 7.6 ± 3.7 days. In 22 Thai Swamp buffaloes under Surin Livestock Breeding Station management (Intaramongkol *et al.*, 1994), uterine involution completed in all cows between third and fourth week. Jainudeen (1984) reported a range for the uterine involution as 16-39 days in Swamp buffaloes.

Chaudhry *et al.* (1987) examined thirty five Nili-Ravi buffaloes, calving normally, for cervical and uterine involution. The mean time required for complete uterine involution of these organs was 27.5 ± 8.3 days, which was slightly affected by lactation number of the buffaloes. The rate of involution of the gravid horn was faster than the non-gravid horn. In Indian buffaloes uterine involution was completed within 23.5 and 45 days, respectively (Pargaonkar and Kaikini, 1974; Chauhan *et al.*, 1977).

A critical biological phase of the dairy cow, extending from 60 days before to 90 days after calving, has been reported to be performance related and assuming greater importance when annual yields are more than 4000 ± 400 kg. This phase was found to have a major impact on uterus involution in addition to other productive and reproductive traits (Slanina and Hlinka, 1991).

uterus through its myometrial contractile effect (Saiduddin et al., 1968). A period of 42 days has been reported for uterine involution in Nili-Ravi Buffaloes (Shah, 1988) and it was affected by parity, season of calving and retained placenta. Cervical involution completed by 28 days. In another study the cervical and uterine involution averaged 25.6 ± 1.0 days and it was not effected by parity and age in Nili-Ravi buffaloes (Usmani and Lewis, 1984).

It has been suggested that cows with longest duration of increased prostaglandin release undergo the most rapid uterine involution (Eley et al., 1981). Intramuscular injection of 10 mg dexamethasone in 6 buffaloes on days 1 and 8 postpartum (Weerasak and Wanapat, 1986) induced marked leucocytosis, neutrophilia, lymphopenia and eosinopenia. This corticosteroid treatment hastened the uterine involution, reduced the first postpartum estrus interval and increased the incidence of postpartum ovulatory estrus.

2.1.2 Relationship of uterine involution with fertility

Effect of uterine involution interval on postpartum reproductive performance was reported by some workers (Roy and Luktuke, 1962; Weerasak and Wanapat, 1986) but others (Dobson and Kamonpatana, 1986) found no significant effect.

Uterine involution and postpartum estrus was studied in 41 primiparous Nili-Ravi buffaloes calving normally during July-December in a government farm (Chaudhry et al., 1990). The mean time required for complete uterine involution was 28.37 ± 1.36 days and in majority of the buffaloes (92.68%) it was completed within 42 days postpartum. Age at first calving and gestation period significantly affected the interval to involution

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Postpartum uterine involution is effected by various factors, described in the following lines:

Factors affecting uterine involution

The relationship between nutrient balance and reproductive performance was studied in Finnish dairy cows (Miettinen, 1990). Uterine involution in cows maintained on the silage took significantly longer time than in those kept on the hay-urea diet. A low energy level in early puerperium, as indicated by increased ketone body levels, caused a delay in uterine involution.

In Lanka and Murrah buffaloes kept under free-range conditions, the duration from calving to complete involution of the uterus averaged 26.4 and 30.0 days, respectively (Mohamed and Jayaruban, 1991). Supplementary feeding of concentrate 1.2 kg per 100 kg body weight per day had no effect on the durations from calving to uterine involution.

Usmani (1987) found that limited-suckled buffaloes showed shorter periods of uterine involution (23.4 versus 38.7 days) and longer interval to first ovulation (54.4 versus 38.7 days) than the non-suckled buffaloes. In a subsequent study, postpartum interval to uterine involution were found longer for limited-suckled buffaloes than for those that were only milked (Usmani et al., 1990). The involuting uterus approaches its former non-pregnant size by 20 days postpartum in suckling Swamp buffaloes (Jainudeen et al., 1983b) and by 45 days in hand milked river buffaloes (Roy and Luktuke, 1962; El-Fouly et al., 1976b). It has been postulated that suckling stimulus cause frequent release of oxytocin from the posterior pituitary, which enhance the reduction in size of

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uterus through its myometrial contractile effect (Saiduddin *et al.*, 1968). A period of 42 days has been reported for uterine involution in Nili-Ravi Buffaloes (Shah, 1988) and it was affected by parity, season of calving and retained placenta. Cervical involution completed by 28 days. In another study the cervical and uterine involution averaged 25.6 ± 1.0 days and it was not effected by parity and age in Nili-Ravi buffaloes (Usmani and Lewis, 1984).

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of uterus. There was a positive correlation between the intervals from calving to involution and postpartum estrus.

Kirakofe (1980) concluded that uterine involution depress fertility during early postpartum period but it has no relationship with postpartum estrus interval. Grave *et al.*, (1968) found that fertilization and pregnancy rates were very low before day 20 after parturition but returned to normal later on. This poor fertility was attributed to physical barrier to sperm transport rather than any inherent defect in the ova or other physiological mechanism (Short *et al.*, 1974).

2.1.3 Postpartum interval to estrus and conception

The delay of first postpartum estrus and ovulation have been considered the major factor leading to a long service period in Egyptian buffaloes (El-Fouly, 1983). First detected estrus has been reported to occur sooner in dairy buffaloes (mean 75, range 35-185 days) as compared to swamp buffaloes (mean 90, range 40-275 days) (Jainudeen and Hafez, 1987). Postpartum estrous interval and service period of various breeds of buffaloes in various managements and locations are given in Table 2.1 and 2.2.

Postpartum estrus was studied in 41 primiparous Nili-Ravi buffaloes calving normally during July-December, 1987 (Chaudhry *et al.*, 1990). About 76% buffaloes came into estrus within two months after calving. There was a positive correlation between the intervals from calving to involution of uterus and postpartum estrus.

Thirty five Nili-Ravi buffaloes were studied for postpartum reproductive performance (Chaudhry *et al.*, 1989). The mean interval from parturition to formation of first palpable follicle (10 mm) after calving was 27.43 days. A positive correlation was

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Table 2.1. **Postpartum estrus interval of dairy buffaloes in various managements and locations**

Type of buffaloes	Postpartum estrus interval (Days)	Buffaloes population	Reference
Bangladesh (village)	179.32 < 120 121-240 > 240	Average 36.36% 47.74% 15.90%	Alam and Gosh (1993)
Egyptian	43.9	Average	El-Shafie *et al.* (1983)
Egyptian	66.5	Average	Aboul-Ela *et al.* (1985)
Egyptian	< 60 60-120 120-210 > 210	15.55% 22.39% 25.65% 36.41%	Yousuf *et al.* (1988)
Nili-Ravi	52.22	Average	Chaudhry *et al.* (1991)
Nili-Ravi	124 22-280	Average Range	Chaudhry (1989)
Nili-Ravi State farm	171.19	Average	Akhtar *et al.* (1994)
Nili-Ravi State farm	171.19	Average	Akhtar *et al.* (1994)
Murrah	191.7 162.2 135.9	Winter Summer Rainy season	Basu (1966)
Murrah	57	Average	Baruselli (1991)
Murrah	133	Average	Muhammad & Jayaruban (1991)
Lanka	97	Average	Muhammad & Jayaruban (1991)

Table 2.2. Service period of dairy buffaloes in various managements and locations

Type of buffaloes	Service period (days)	Buffaloes population	Reference
Bhadawari	192.6	Average	Sharma and Singh (1978)
Egyptian	99.8 to 165.31	Range	El-Shiekh & Muhammad (1967)
Egyptian	125.5	Average	El-Fouly et al. (1977)
Egyptian	304.07	Average	Kotby et al. (1987)
Kundi	274	Average	Shah (1991)
Murrah	123	Average	Roy (1960)
Nagpuri	96.49	Average	Kadu (1978)
Nili-Ravi	< 120	44%	Ishaque (1969)
	> 120	56%	
Nili-Ravi	< 101 days	31%	Waheed (1975)
	101-200	24%	
	201-300	18%	
	301-400	15%	
	> 400	12%	
Nili-Ravi	222	Average	Ahmad and Irfan (1979)
Nili-Ravi	204	Average	Shah and Khan (1987)
Nili-Ravi	217.03	Average	Rehman et al. (1988)
Nili-Ravi	243.81	Average	Chaudhry and Pasha (1988)
Nili-Ravi	249	Average	Jost (1979)
(village)	30-750	Range	
Nili-Ravi	212	Average	Zafar (1983)
(village)	21-915	Range	
Nili-Ravi	< 90	30%	Chaudhry et al. (1985)
(village)	> 90	70%	
Sri Lankan	71.5	Average	Perera et al. (1987)
(village)	23-502	Range	
Surti	175.73	Average	Rao et al. (1973)
	21 to 349	Range	

found between this interval and the interval to the formation of first corpus luteum. The average postpartum estrus interval highly correlated with resumption of follicular activity period ($r = 0.52$) and interval to formation of first corpus luteum ($r = 0.43$). The postpartum interval to formation of first follicle and estrus was not affected by month of calving, age and parity of buffaloes. The effect of year of calving on service period and services per conception was highly significant. The effect of parity on the service period was also significant ($P < 0.05$) and it ranged from 209.83 ± 12.55 to 263.28 ± 10.93 days in the fourth and the first lactations, respectively. The live weight at calving in buffaloes affected significantly, ($P < 0.05$) the service period which was longest in small buffaloes (278.06 ± 10.95 days) followed by medium (243.81 ± 7.29 days) and large (217.99 ± 9.37 days) buffaloes.

Pasha *et al.* (1986) reported 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 the month of May. The differences were significant.

According to Baruselli (1991), the first signs of ovarian activity in Murrah buffaloes occurred an average of 36.6 days postpartum while oestrus occurred an average of 57 days after calving, its duration averaged 14.76 hours and ovulation occurred an average of 16.92 hours after the end of oestrus. The conception rate to natural mating during the first postpartum oestrus was 65.5%. Visual estrus signs in swamp buffaloes were catagorized as; standing while being mounted by bull, vaginal mucus discharge, sniffing of genitali by the bull, bellowing and swollen labia (Chantaraprateep, 1987).

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In 791 Egyptian buffaloes (Yousuf *et al.*, 1988), a one-day increase in interval to service was associated with a 0.9-day increase in service period and calving interval. Postpartum service interval significantly affected the duration of lactation but not the milk yield.

In a study on days open of Thai Swamp buffaloes in Surin Livestock Breeding Station (Intaramongkol *et al.*, 1994), it was found that the longest days open (216 days) occurred in late dry season (April) with poorest pasture condition and highest atmospheric temperature. The days open and live-weight at calving were negatively correlated (r = -0.6, P<0.01). Similarly in Indonesian Swamp buffaloes (Lubis and Fletcher, 1987) body weight did not affect postpartum estrus interval. However postpartum intervals to first estrus and conception prolonged at low feeding levels. Earlier postpartum conception in cows on the high feeding level was associated with their lower body weight loss and/or their faster body weight recovery after calving.

2.1.4 Postpartum ovulation and services per conception

Analysis of the data on 1530 Nili-Ravi buffaloes for 1939-1985 (Khan *et al.*, 1989) showed that the average number of services per conception was the maximum (1.39) in heifers calving for first time during winter and it was minimum for those calving during hot dry season. The mean number of services per conception was the highest (1.95) for buffaloes bred within 30 days postpartum, while the lowest number (1.41) was recorded for those bred at an interval of 91-120 days.

El-Shafie *et al.* (1983) studied 95 heifers at a research station. The percentages of females that conceived after 1, 2, 3 and 4 matings were 55.8, 27.4, 10.5 and 6.3

respectively. The postpartum intervals to ovulation averaged 28.0 ± 1.4 days ranging from 16 to 47.

Number of services per conception and calving interval for 344 Egyptian buffalo cows averaged 1.87 ± 0.07 and 516.47 ± 9.23 days respectively (Kotby *et al.*, 1987). Number of services per conception was affected by season, year of calving, farm, parity and age of dam.

In 21 swamp and 14 Murrah buffaloes in one location and 26 swamp and 8 Murrah buffaloes in another location (Kamonpatana *et al.*, 1991), the percentage showing resumption of postpartum ovulation (as detected by progesterone assays) within 120 days after calving, was 38.1 and 35.7 in the two breeds, respectively in location 1 and 61.5 and 62.5 in location 2. In 16 Swamp, 16 river and 16 river X Swamp F_1 buffaloes, aged 2-5 years (Mahyuddin *et al.*, 1991), 33 and 57% of swamp and F_1 buffaloes respectively showed ovarian activity within 120 days after calving, but none of the river buffaloes did so.

It appears from the above review that water buffaloes show a diverse nature of postpartum interval to various reproductive events and the interval is greatly sensitive to variation in season, feed availability, breed and management.

2.1.5 Silent heat

Silent estrus, quiet estrus, sub-estrus, *estrus termidos* or silent ovulation is the ovulation without clinical signs of estrus. Jainudeen *et al.* (1983a) reported that silent estrus was not detected even by a vasectomized bull in swamp buffaloes.

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Buffaloes remain under stress during the summer as well as winter months as they are neither heat resistant, nor cold tolerant (Hafez, 1952). A large number of buffaloes remain unbred during summer months because of silent heat (Mudgal, 1992). Swamp buffaloes express heat symptoms with a varying intensity and pattern (Bodhipaksha et al., 1978). The heat symptoms are more difficult to detect and the mucus discharge and vulvav changes are less pronounced in buffaloes than cattle. Jainudeen et al., (1983a) reported the incidence of silent estrus as 11% in suckled Swamp buffaloes.

The percentage of silent estrus was 60% in Egyptian buffaloes (Khattab et al., 1988). In a later study by (Khattab et al., (1990), 82.4% silent ovulations were observed throughout the postpartum period, decreasing with advanced ovulation cycles. In those buffaloes that got pregnant, 100% ovulations were accompanied with detected signs of estrus, while in those that continued cycling and failed to conceive, incidence of silent ovulation was 82%.

Barkawi et al. (1986) reported that 79% first and 76% second postpartum ovulations were not accompanied by estrus sign in buffaloes. Borady et al. (1985), in a study on 14 Egyptian buffaloes, reported that silent ovulations ranged from 16.07% in the summer to 29.46% in the spring. Concentrations of tri-iodothyronine and thyroxine were significantly lower during cycles with silent oestrus than on the day of oestrus and during other oestrus cycles. Singh et al. (1979) reported 27.55% silent ovulations within 45 days postpartum in 98 Murrah-Nili Ravi buffaloes. A comparison between progesterone profiles and visible observation of estrus revealed that 50% of Indian dairy buffaloes showed at least one silent estrus during the postpartum period (Arora and Pandey, 1982).

In Nili-Ravi buffaloes 24% estrus periods were found as silent estrus while the remaining 76% were accompanied by true heat signs (Ishaque, 1956). Ali *et al.* (1983) studied 151 Nili-Ravi buffaloes and found that during silent estrus plasma calcium concentration and calcium - phosphorus ratio were lower and zinc concentration was higher as compared to the estrus group. Roberts (1971) suggested that silent estrus may be due to a lack of sufficient secretion of estradiol by the mature and secondary follicles or due to a need for a higher threshold of estrogen in the central nervous system of certain individuals at certain times to produce the nervous signs characteristic of estrus.

Comparatively lower progesterone concentrations observed during the luteal phase of the estrus cycle (Rosenberg *et al.*, 1977) and higher progesterone levels than basal levels during estrus (King *et al.*, 1976), lead to silent estrus. However, Khattab *et al.* (1990) observed no consistent trend for a relationship between progesterone levels and incidence of quiet ovulations in cattle and buffaloes.

Both prolonged under-nutrition and acute nutritional deficiency have been found to be associated with suppression of behavioral estrus in ewes (Knight, 1980). Lack of copper affects growth and causes silent oestrus (Wittkowski and Spann, 1993). Subnormal blood glucose levels supplying less energy to the gonads were associated with silent ovulations (Howland *et al.*, 1966).

Poor expression or even completely silent estrus has been attributed to the low profile of gonadotrophic hormone or disturbance in the ratio of certain minerals (Mudgal,

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1992). Maximum concentration of FSH and lowest concentration of LH are indicative of the peak breeding period i.e., when the FSH-LH ratio is highest. The FSH-LH ratio remain low in the low breeding period.

Optimum-fed buffaloes exhibited weak estrus of shorter duration during night hours in the summer months,whereas under-fed buffaloes did not exhibit any estrus and led to persistent infertility (Kaur and Arora, 1982). Thus,optimum feeding could partly overcome the effect of heat stress.

It has been reported in dairy cattle that thermal stress affects reproductive efficiency by reducing the length of estrus to about 10 hours, lower intensity of estrus behavior (Thatcher and Collier, 1982), silent ovulation to the tune of 80% and low conception rate (Thatcher et al., 1964).

The above review suggests that silent estrus is prevalent in various livestock species including dairy cows and Swamp and dairy buffaloes and that it is caused by seasonal and nutritional stress, in-efficient management and imbalanced hormonal levels. Adequate nutrition and improved heat detection methods are helpful in solving the problem.

2.1.6 Seasonality of reproduction

It is generally accepted that livestock productivity and reproductive efficiency in the temperate regions is higher than the tropical ones. The low productivity of livestock in the tropical regions has been attributed to: 1) poor forage availability (qualitatively and quantitatively) in the harsh, hot and often semi-arid or wild environment; 2) non-existence of genetic selection for high production traits unlike the temperate regions and

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3) thermal stress (Robertshaw, 1986). It was reported that most tropical species of animals have a metabolic rate lower than that predicted from Klieber's equation and this may be looked upon as an adaptation to the tropical environment in that the heat load of metabolism is relatively low and reduces the heat necessary for dissipation. On one hand it is beneficial as the maintenance requirements of animals are less, but on the other hand, the low metabolic rate is associated with slow growth rate.

The annual pattern of air temperature, solar radiation, humidity and rainfall constitute environmental stress factors depressing productive and reproductive efficiency of livestock during certain seasons (Thatcher and Roman-Ponce, 1980). Effects of air temperature, solar radiation and relative humidity on fertility of Jersey, Brown Swiss and Holstein cows was evaluated within a commercial dairy herd in Florida with sub-tropical environment (Thatcher et al., 1986) and it was concluded that heifers had a higher conception rate than cows. Conception rates of cows decreased during the summer months of June through August and did not improve until November, suggesting a carry-over effect during the months of September and October. Decrease in conception rate of lactating cows during summer was attributed to the fact that they were unable to maintain normal body temperature during heat stress conditions because of a high rate of internal heat production associated with milk yield.

Seasonality of reproduction in dairy animals is a factor of economic importance as it results in a reduced milk supply during certain seasons of the year, hinderance in genetic improvement, decrease in the number of lactations and lactation peaks in the life time of the animal.

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Reproductive performance in buffalo is adversely affected by seasonality of breeding (Majeed et al., 1961; Shah and Shah, 1968; Goswami and Nair, 1965). Buffaloes have a tendency to come in estrus mostly in late autumn or early winter in India (Nagaracenkar and Rao, 1987), Egypt (Shalash et al., 1969), Sri Lanka (Perera, 1982) and in Trinidad (Nagaracenkar, 1980). In Mediterranean climate as in Italy, calvings have been found mostly from July to October (Nagaracenkar and Rao, 1987). In Egyptian buffaloes most of calvings occur from October to January (Ragab et al., 1954; Youssef and Asker, 1959; El-Kiraby, 1995). It has been reported (Hossain and Ahmad, 1973) that the highest sexual activity was in the month of October to January when 67% of buffaloes were found to be in heat. Maximum calvings (75%) were reported during February to July in Swamp buffaloes in Malaysia (Fadzil, 1968) and September to January in the Philippines (Ocampo, 1939).

In Pakistan, autumn and winter have been identified as the normal breeding season for buffaloes. Ahmad et al. (1986) reported that on an average 48, 30, and 22% of Nili-Ravi buffaloes were bred in Punjab during the months of November to February, July to October and March to June, respectively. After an analysis of 1274 records of calving intervals of buffaloes maintained at Livestock Experiment Station, Qadirabad, Ahmad et al. (1981) reported that 71.9% buffaloes calved during the normal breeding season, i.e. autumn and winter. Similarly, Cady et al. (1983) reported that 60% calvings occurred from July to October and these calvings were followed by earlier return to estrus, fewer days open, and shorter calving intervals than for calvings in other months. Shah (1990) analyzed data for six years (1980-85) from eight Artificial Insemination Centers in

NWFP and found that significantly higher number of buffaloes (60.5%) were bred during their normal breeding season as compared to 39.5% during low breeding season.

Buffaloes calving in summer and autumn resumed ovarian activity earlier than those calving in winter or spring (Ahmad et al., 1981). In a study (Shah and Khan, 1987) 80% of the total fertile services took place during the period from June to November. Rehman et al. (1988) found that the service period was the minimum in buffaloes calving during spring. The calving interval also varied significantly due to years and season of calving.

Autumn and winter have been reported to be favorable seasons for occurrence of estrus in Murrah buffaloes in India (Abhi et al., 1973). In a study (Chauhan et al., 1977), autumn and winter calvings were favorable for early resumption of ovarian activity. Analysis of first lactation records of 699 Nili buffaloes showed that season influenced first-open period, service period, calving interval and dry period significantly, while service period had a significant influence on all the first lactation traits (Dutt and Yadav, 1988). In Surti buffaloes in India (Tailor and Jain, 1987), 31.77% estrus events occurred during winter and 12.34% during the rainy seasons. First-service conception rate was 21.96% in winter. The overall conception rate was highest (45.06%) and number of services per conception was lowest (2.21) during autumn.

In Sri Lanka (Perera et al., 1984), a marked seasonality of calving and conception was observed, with the highest percentage of conceptions occurring 2-5 months after the annual peak in rainfall.

It appears that autumn and winter constitute breeding season for buffaloes. Seasonality of breeding activities in buffaloes have been attributed to variation in the

thermal and humidity stress, photoperiod, rainfall, availability of fodder for the dam, affecting blood metabolites and hormonal levels (Stot *et al.*, 1972; Montgomery *et al.*, 1980; Chantalakhana *et al.*, 1979).

2.1.7 Hormonal levels

The elevated progesterone levels in blood of pregnant buffaloes decline rapidly following parturition to undetectable levels by day 3 or 4 of postpartum and remain thereafter low for a variable period until ovarian cyclicity is restored (Perera *et al.*, 1981; Jainudeen *et al.*, 1983a). Following the first postpartum ovulation, plasma progesterone level increases and remains above 0.7 ng for about 10 days, then decline to below 0.25 ng/ml at the next estrus.

In Murrah buffaloes plasma progesterone concentration declined gradually from 1.82 ± 0.13 ng/ml at 30 days before calving to 1.21 ± 0.11 at 2 days before calving; remained 0.77 ± 0.20 on the day before calving, then about 0.30 until day 3 after calving (Prakash and Madan, 1984).

Fifty five indigenous (Lanka) buffalo cows belonging to 5 farmers in a village in the dry zone, were monitored over 30 months (Perera *et al.*, 1987). The interval from calving to palpation of the first corpus luteum was 52.3 ± 16.8 days and to the first elevation of progesterone above 0.5 ng/ml of fat-free milk was 54.9 ± 17.9 days. Elevation of progesterone preceded first service in 19%; the resultant luteal phases were short in 4 and normal in 3 cases. Usmani *et al.* (1990) found that 86% Nili-Ravi water buffaloes showed at least 1 short luteal phase (8 to 13 days) before first estrus. Number

of rises of progesterone before first estrus was greater for limited-suckled buffaloes. Luteal activity after first estrus (15 to 19 days) did not differ with limited suckling.

The source of progesterone secretion is corpus luteum on the surface of ovary. A study could not confirm the diagnosis of a corpus luteum by rectal palpation in 24 of 38 buffaloes between days 30 and 60 postpartum, by progesterone assay due to embedded corpus luteum (Jainudeen *et al.*, 1983a). Corpus luteum size had fairly good relationship with the concurrent milk progesterone levels ($r=0.93$) (Qureshi and Khan, 1988). Qureshi *et al.* (1992) found that 50% of corpora lutea diagnosed per slaughter were not functional and failed to exhibit concurrent milk progesterone levels during premortem period in Nili-Ravi buffaloes.

In 12 non-lactating Murrah buffaloes (Kaur and Arora, 1984), progesterone level was low on the day of estrus and increased progressively, showing a peak value between days 14 and 18 depending upon the estrous cycle length. The progesterone concentrations remained basal throughout the anestrous periods in buffaloes on sub-maintenance ration.

It has been reported (Jain and Pandey, 1985) that buffalo heifers exhibited estrus only after 30 months of age and had higher concentrations of LH and oestradiol-17-beta and a lower concentrations of progesterone on the day of estrus. The progesterone concentrations were significantly affected by seasons and weaning and varied significantly between the periods of puberty and neonatal periods. The results suggested that increases in the concentrations of oestradiol-17-beta and progesterone after 30 months of age are probably indicative of the onset of puberty in buffalo heifers. However, a further increase in oestradiol-17-beta and LH, and a decrease in progesterone are essential for estrus and cyclicity to be exhibited in buffalo heifers.

In a study on cows (Kohno *et al.*, 1993), group 1 contained 6 cows that had a recurrence of distinct estrus 60 days after calving; group 2 contained 4 cows that had feeble signs of estrus 60 days after calving; and group 3 contained 3 cows that had no sign of estrus in that period. After calving, concentration of progesterone in plasma exceeded 1 ng/ml first in group 1 and last in group 3, indicating shorter and longer service intervals after calving in groups 1 and 3, respectively. The results showed that feeding level before calving is related to the time of recurrence of first estrus and fertility after calving. In 12 Murrah-type anestrous buffaloes (Jain, 1987), no palpable follicle or corpus luteum was detected on rectal examination. The plasma concentration of FSH, oestradiol-17-beta and progesterone ranged from 5.5 to 6.2 ng/ml, 7.6 to 15.0 pg/ml and 2.3 to 4.3 ng/ml. In 1 buffalo which showed estrus, the plasma concentrations of the 3 hormones on the day of estrus were 48.5 ng/ml, 50.1 pg/ml and 0.4 ng/ml, respectively.

According to Jain (1988), plasma progesterone concentration ranged from 0.27 $\pm$ 0.07 to 0.61 $\pm$ 0.15 ng/ml and was significantly higher in non-cycling heifers aged 811-1260 days than in younger animals. Pregnant and non-pregnant buffaloes did not differ significantly in plasma concentrations of progesterone up to 22 days after insemination. In Murrah buffaloes, Perera *et al.* (1984) found that progesterone profiles during the postpartum period showed ovarian inactivity to be a major problem. Most animals remained anestrous for 100-200 days, but conceived at the first or second postpartum ovulation.

In a study of infertility in cattle and buffaloes, the serum progesterone concentrations were higher in sub-fertile animals in the dry zone than in corresponding

animals in the wet zone, even though some of the later had palpable corpora lutea (Singal and Lohan, 1988).

Peripheral plasma concentrations of progesterone were measured in 13 mature Swamp buffaloes at 4 hours intervals from 36-40 hours before, until 36-40 hours after the onset of oestrus (Avenell et al., 1985). The progesterone concentrations remained around 0.1 ng/ml throughout the sampling period. There were no significant differences in hormone concentrations or changes between cows that conceived and those that did not conceive to artificial insemination 12-24 hours after the onset of estrus.

In 64 lactating buffaloes (El-Belely, 1993), plasma proges-terone concentrations were significantly higher in pregnant than in cyclic (between days 3 and 5) and repeat-breeder (between days 0 and 8) animals. Concentrations of total unconjugated estrogens were also higher in the pregnant group between days 6 and 16.

Plasma progesterone concentration on days 1, 13 and 16 of the cycle averaged 0.77, 5.18 and 5.49 ng/ml in normal cycling Murrah buffaloes versus 0.70, 2.95 and 3.23 ng/ml in repeat breeders (Agarwal et al., 1982).

Shelton et al. (1990) reported that the increase in post-ovulatory peripheral progesterone concentrations were delayed and occurred more slowly in sub-fertile cows than in heifers. It was suggested that luteal inadequacy, due to diminished responsiveness to luteotropic hormones, may contribute to embryo mortality in sub-fertile cows. In contrast,the presence of a viable embryo signalled an earlier increase in progesterone by day 3 that was not seen in cases of embryo failure (Maurer and Echternkamp, 1982) and may indicate a luteotropic effect of an early embryo.

Blood hormones and some metabolites were measured in 44 first-calf heifers once daily between 7 days before and 1 day after calving (Furstenberg *et al.*, 1990). The placenta was retained in 7 of them and these had lower concentrations of oestradiol-17-beta and progesterone throughout the period of observation.

In a study on five pregnant Murrah buffaloes (Prakash and Madan, 1986), it was suggested that for normal expulsion of foetal membranes a rise in oestradiol-17 beta before calving must be accompanied by a fall in progesterone and that this shift should occur in a gradual and sustained manner.

In prepubertal and postpubertal heifers, plasma progesterone levels were higher during winter and summer than during the other seasons and plasma LH levels were higher during winter than during the other seasons (Kumar and Rattan, 1992).

The reproductive performance of 74 Israeli Holstein-Frisian dairy cows was examined during summer (Wolfenson *et al.*, 1988). Progesterone concentrations were higher in inseminated, non-pregnant and in non-inseminated, cyclic, cooled cows than in non-cooled cows. It was similar in pregnant cows of cooled and non-cooled groups.

Nordin and Jainudeen (1991) reported that the interval from calving to the time when plasma progesterone was > 0.7 ng/ml was the longest in buffaloes with unrestricted suckling as compared to those with restricted suckling and calves weaned (85, 71 and 53 days).

Higher levels of progesterone was found to be one of the reasons for anestrous condition of buffaloes maintained on a low plane of nutrition, as often happen under rural conditions of buffalo management (Harjit and Arora, 1982, 1984). Similarly low serum

progesterone levels have been reported in cows fed low energy diets (Carruthers and Hafs, 1980).

Two groups of 8 Swamp buffalo cows were fed at low or high levels from 12 weeks before until 36 weeks after calving (Lubis and Fletcher, 1987). Plasma progesterone profiles indicated that normal postpartum luteal function at both feeding levels was commonly preceded by transient corpora lutea with subnormal progesterone secretion.

In Lanka and Murrah buffaloes kept under free-range conditions (Mohamed and Jayaruban, 1991), the duration from calving to first estrus was 97 ± 73 and 133 ± 91 days, and that from calving to conception 175 ± 106 and 250 ± 120 days. Supplementation with concentrate feed at the rate of 1.2 kg per 100 kg body weight per day significantly reduced the duration from calving to conception from 213.8 to 76.1 days in the Lankas and from 312.8 to 213.6 days in the Murrahs.

In a study, plasma progesterone level was not affected by the intake of crude protein (Elrod and Buttler, 1993). It was concluded that excess degradable protein acts through some undefined mechanism to decrease uterine pH during the luteal phase which may play a role in the reduction of fertility. But a decrease in serum progesterone has been suggested (Fergusson and Chalupa, 1989) at concentration of crude protein/dry matter (CPDM) that exceed rumen requirement of degradable intake protein (DIP). Endocrine function of the pituitary (luteinizing hormone secretion) and ovary (progesterone secretion), has been greatly elevated and depressed, respectively, in cows consuming feed high in protein (Jordan and Swanson, 1979a).

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Based on the above studies on buffaloes, it may be suggested that following parturition blood/milk progesterone concentrations remain at basal levels until resumption of ovarian activity, an interval influenced by season, nutritional status and suckling. A few elevations are observed before first estrus. Plasma progesterone concentrations in normal cycling buffaloes are higher than the repeat breeders.

2.2 Relationship of pre- and postpartum nutritional status with reproductive performance

Studies on buffalo indicate that all dietary nutrients are required in proper quantity and in proper proportion for reproductive performance. It may happen that any nutrient deficiency may affect more reproductive system without causing clinical signs of ill health (Hignett and Hignett, 1951). Low level of nutrition influences the estrus cycle rhythm not only in summer but also in winter (Arora, 1987).

Nutritional deficiency or excess affects reproductive efficiency of livestock in various ways. Dunn and Moss (1992) reported that insufficient dietary intake of energy by an animal lead to a negative energy balance accompanied by loss of body weight and body condition which in turn, results in various reproductive disorders. The authors suggested that inadequate nutrition inhibits reproduction through action on hypothalamic neurons responsible for release of GnRH. As a result, secretion of LH from pituitary is inhibited causing postpartum lactational anestrus, seasonal anestrus and delayed puberty in animals.

Endocrine status (Beal et al., 1978), interval from calving to first estrus (Dziuk and Bellows, 1983), occurrence of estrus (Imakawa et al., 1986, Johnson et al., 1987)

and pregnancy rate (Selk *et al.*, 1988) are altered when cows were subjected to reduced nutrient intake. Wilmut *et al.* (1986) found that under-nutrition or over-nutrition affects embryo survival and development by changing the uterine environment which is related to circulating levels of steroid hormones.

In an experiment, 60 buffaloes were fed rations according to the local standard or 10-15% above the standard and were exercised during the dry period or were not exercised. For buffaloes on standard rations and those on increased rations with and without exercise, service period averaged 95.3, 83.0 and 88.0 versus 85.4, 72.1 and 77.9 days and calving interval 408.4, 393.3 and 399.6 versus 397.8, 381.5 and 387.6 days (Ivanov and Gasanov, 1991).

In a study on lactating Murrah buffaloes (Sikka *et al.*, 1989), total number of expressed estruses was greatest in buffaloes given 4% ammonia gas ensiled wheat straw; however conception rate was highest in those given 4% urea-ensiled wheat straw.

Fourteen pre-pubertal Nili-Ravi buffalo heifers, aged 20-25 months, were assigned for 100 days, to 100% of the recommended ration (controls) or to 120% of the recommended ration and were checked thrice daily for estrus and monitored fortnightly by serum progesterone concentration and rectal palpation (Iqbal *et al.*, 1988). 28.6% of the supplemented heifers and 14.3% of the control, showed oestrus before the end of the 100-day period. At the end of the 100-day period, heifers not showing cyclic ovarian activity, were injected with 30 mg progesterone daily for 14 days. The response to treatment was higher in supplemented than control heifers (80% versus 50%).

Forty Angus X Hereford cows were given diets with 100 or 150% of NRC recommendations (NRC, 1988) for crude protein (Rusche et al., 1993). Treatment had no effect on profiles of luteinizing hormone or progesterone. Altering amount or source of crude protein did not significantly affect reproductive characteristics or hormonal profiles.

In 12 cows given supplementary or no supplementary feeding (control), with continuous or restricted (twice daily) suckling regimes, plasma progesterone levels were < 1 ng/ml in all cows until week 12 postpartum (Tegegne et al., 1993). Only 5 cows showed ovarian activity over the 33-week period. Cows that cycled expressed irregular and short-lived progesterone rises (> 1 ng/ml) lasting 8-12 days prior to establishment of normal patterns of progesterone secretion, with levels of 8-10 ng/ml for normal cycles.

In Murrah buffaloes with a normal breeding history (Agarwal et al., 1982), progesterone was negatively correlated with plasma potassium on day 1 and with cholesterol and calcium on day 16. In repeat breeder buffaloes, progesterone was significantly correlated with plasma protein, acid soluble phosphorus, sialic acid, potassium and calcium, on day 13 and 16. In sheep, Parr (1992) concluded that the inverse relationship between feed intake and progesterone concentrations was due to removal of progesterone by liver and gut due to an increased rate of blood flow.

Chohan et al. (1992) observed higher values of serum progesterone during estrous cycle in estrus synchronized buffaloes than normal cyclic buffaloes. The valves and pattern of serum progesterone during early pregnancy was non-significant and similar in both groups of buffaloes.

2.2.1 Relationship with endocrinological status

Luteinizing hormone (LH) is the primary luteotrophic factor in cattle (Hoffman et al., 1974) and is required for growth of ovarian follicles (Snook et al., 1969). Nutritional restrictions in heifers have been reported either to increase (Gombe and Hansel, 1973) or decrease (Apgar et al., 1975) plasma LH concentrations. Entwistle and Oga (1977) found no effect of undernutrition on GnRH-induced release of LH in postpartum cows.

In another study (Killer et al., 1989), it was concluded that nutritional restriction of beef heifers during gestation accompanied by body weight loss, was associated with an increase in LH release before parturition and a decrease in release of this hormone after parturition.

Changes in plasma progesterone concentration were studied in 12 non-lactating Murrah buffaloes for one year (Kaur and Arora, 1984). These buffaloes were fed a maintenance ration (group 1) or a sub-maintenance ration (group 2). Five out of 6 buffaloes in group 2 and 1 out of 6 buffaloes in group 1, showed long anestrous periods during the summer months. The progesterone concentration remained basal throughout the anestrous periods in group 2 as a result of inactive ovaries. It was concluded that under-nutrition coupled with high environmental temperature stress was responsible for long anestrous periods in buffaloes.

Buffaloes kept on low plan of nutrition (75% less digestible crude protein and 35% less total digestible nutrients than the maintenance requirements) had a higher concentration of progesterone in plasma and an increased length of estrous cycle (Harjit and Arora, 1982, 1984). Higher progesterone concentrations were probably due to low

metabolic rate. The higher levels of progesterone was considered to be one of the reasons for anestrus condition of buffaloes maintained on a low plan of nutrition as often happens under rural conditions of buffalo management.

2.2.2 Relationship with seasonality of reproduction

Goonewardene et al. (1983), in consensus with Chantalakhana et al. (1979), reported that the calving season in Thai and Sri Lankan buffaloes coincides with the availability of fodder. Roy and Pandey (1971) reported that plane of nutrition did not influence the incidence of estrus in buffaloes during summer months. However, fertility was significantly higher in buffaloes on a higher plane of nutrition (83.5% versus 66.6%). The buffaloes on high plane of nutrition were given 2.5 kg supplement of concentrate daily and those on a low plane of nutrition received 0.54 kg concentrate supplementation.

Putu et al. (1986) recorded ovarian activity, oestrus and conception in 75 Swamp buffaloes after estrus synchronization in two seasons (dry and wet) and at two levels of nutrition (fed grass ad libitum plus 1 or 5 kg concentrates per day). The number of acyclic animals was significantly higher on low plane of nutrition than those on higher plane of nutrition (35% versus 3%). It was concluded that ovarian inactivity associated with poor nutrition could be an important cause of low reproductive rates in Swamp buffalo cows.

Postpartum reproductive activity was studied in buffaloes kept under traditional and improved feeding conditions in Amazon basin (Vale et al., 1988). A comparison of monthly calving patterns showed that in the herd subjected to a deficiency of fodder

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between December and February, parturition occurred mainly between April and August. In contrast, in the herd kept on adequate pasture and dietary supplements throughout the year, calving occurred throughout the year. Milk progesterone profiles showed that cows under improved conditions first ovulated 30.2 ± 14 days after calving. Under traditional conditions, first ovulation was delayed until 102.4 ± 42.7 days postpartum. It has been postulated that, since serum protein influences the water retaining capacity of an animal, higher concentrations (7.14-8.51 g/100 ml) in buffaloes would favor heat regulation under hot conditions (Pandey and Roy, 1968b).

Decrease in the levels of vitamin C under the heat stress condition in buffaloes has been reported by Pandey and Roy (1968a) and Bahga et al. (1988), leading to lower conception rates during summer. Vitamin C (Ascorbic acid) has complex functions in the animal body. Due to it's oxidative-reduction properties vitamin C is involved in steroid biosynthesis (Searcy, 1969) and in the synthesis of antibodies (Panda and Ray, 1970) Bahga et al. (1988) studied seasonal variations in blood ascorbic acid levels in postpartum buffaloes in relation to reproductive efficiency. The overall blood concentrations of ascorbic acid were not different in summer and winter. However, significantly higher values were obtained in animals that conceived than those which did not conceive (0.65 ± 0.06 versus 0.53 ± 0.02 mg/100 ml of blood). Similarly, buffaloes with short postpartum estrus interval had higher blood ascorbic levels. It has been reported that adrenal and gonadal steroids depress plasma vitamin C levels as it is used for the synthesis of the former (Searcy, 1969).

Roy *et al.* (1964) concluded that hypothyroidism due to high ambient temperature was responsible for summer sterility in buffalo cows probably due to the reduced responsiveness of the ovary to pituitary gonadotrophin. Bahga and Gangwar (1988) studied seasonal variationw in plasma hormones and reproductive efficiency in 20 newly calved Murrah buffaloes. The results suggested that low reproductive performance during summer was associated with low progesterone and high prostaglandin $F_{2\alpha}$ concentrations.

From the above review it may be concluded that nutritional deficiency during some seasons may contribute to a decrease in the reproductive efficiency of buffaloes and proper feed management may improve the situation.

2.2.3 Energy feeding

An animal requires energy for the mechanical work of essential muscular activity, for chemical work such as the movement of dissolved substances against concentration gradients and for the synthesis of body constituents such as enzymes and hormones (McDonald *et al.*, 1984a). In lactating dairy cattle, an insufficient energy supply results in a decline in milk production and loss of live weight. Severe and prolonged energy deficiency depresses reproductive performance (NRC, 1988). Insufficient energy in the diet delayed puberty and caused either a complete anestrus or markedly increased the duration of postpartum anestrus (Guilbert, 1942; Topps, 1977; Ferrel, 1991).

Wiltbank *et al.* (1965) concluded that in beef cattle a deficiency of energy was more detrimental to reproduction than protein deficiency. They also reported that voluntary intake of feed was reduced when low protein diets were provided and

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consequently, cows consumed neither adequate energy nor protein. Ovarian activity is affected by low energy levels. Increased energy levels enhanced conception rates and the percentage of cows cycling early in the breeding season (Wiltbank *et al.*, 1964; Dunn *et al.*, 1969).

According to Miettinen (1990), a low energy level in early puerperium caused a delay in uterine involution and in the onset of ovarian activity and prolonged the interval from calving to the first insemination and service period in dairy cows.

In 3 consecutive years, spring-calving Hereford and Hereford X Angus cows (n = 348) were used to determine effects of level of supplemental energy or protein before and after calving on herd performance (Marston *et al.*, 1995). Cows fed on EN (20% crude protein, 2.44 kg/day) during gestation had greater body weight gain at calving than PR-fed cows (40% crude protein, 1.22 kg/day). Calf weaning weight was not affected by supplementation. Cows fed on EN before calving had an 11% higher pregnancy rate than cows fed on PR (P<0.002). Pregnancy rates were not influenced by treatments fed for a short period after calving. It shows that conception rates were improved by feeding higher levels of supplemental energy prepartum but not postpartum.

Hall *et al.* (1991) found that low energy intake lowered mean LH concentrations (0.97 versus 1.57 ng/ml) as compared to high energy intake in primiparous postpartum cows. It was suggested that hypothalamic-pituitary function increased in the early postpartum interval in the adequately fed beef cows. Concentration of plasma norepinephrine, a lipolytic factor in adipose tissue, was not affected by the treatment. Plasma levels of dihydroxyphenyl-alanine, another lipolytic agent, was higher in the high energy intake group. Cortisol concentration was not affected by the treatment and did not

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seem to suppress LH level. The authors suggested that adrenal cortical and medullary hormones do not directly mediate the effect of nutrition on reproduction.

In Swamp buffaloes, dietary metabolizable energy did not affect the utilization of digested nitrogen (Moran, 1983). However, it was noted that extreme difference in the intake of metabolizable energy generally tended to affect the utilization of dietary nitrogen. Bashi et al. (1981) reported that in Murrah buffaloes retention of nitrogen increased with increasing dietary energy (P < 0.001) and protein (P < 0.05).

2.2.4 Protein feeding

The absorption of essential amino acids from digested protein is vital to the maintenance, reproduction, growth and lactation of dairy animals. These essential amino acids must come either from dietary protein that escape rumen fermentation or from microbial protein produced in the rumen. In lactating cows, as the milk production increase, it becomes more and more important that dietary protein escape degradation in rumen fermentation (Kaufmann, 1982).

Food proteins are hydrolyzed to peptides and amino acids by rumen microorganisms but some amino acids are degraded further to organic acids, ammonia and carbon dioxide (McDonald et al., 1984b). The rumen degradable protein are either converted by ruminal microflora, to microbial protein and digested in small intestine, or they are hydrolyzed to ammonia and excreted as urea through kidney.

Supply of low-protein diets were found associated with a reduced intake of energy and protein in beef cattle (Wiltbank et al., 1965). Sasser et al. (1988) found that a deficiency of crude protein caused reduction of pre- and postpartum weight gains,

decreased the percentage of heifers showing estrus by 110 days after calving (89% versus 63%), decreased the first service conception rate (71% versus 25%), and increased the postpartum estrus interval (75 versus 86 days) in beef heifers.

Chalupa (1984) attributed the deleterious effect of high dietary protein on reproduction to a relative energy deficiency as increased energy is required for liver conversion of excess ammonia to urea.

Fergusson et al. (1991) suggested that when serum urea nitrogen concentrations, reflecting the quantity and degradibility of dietary protein, are > 20 mg/100 ml, fertility will be impaired. Kaim et al. (1983) reported that in cows fed a 20% crude protein diet with plasma urea nitrogen of 16.8 mg/100 ml pregnancy rate was lower than in cows fed a 15% crude protein diet with plasma urea nitrogen of 9.0 mg/100 ml. Services per conception were increased in cows fed diets with greater than 16% crude protein (Jordan and Swanson, 1979b) but in another study (Howard et al., 1987), the reproductive performance of cows was normal and essentially unaffected by increasing dietary protein from 14.5 to 19.4% of the dry matter.

In a study, 40 Angus X Hereford 2-year-old primiparous cows were given diets with 100 or 150% of NRC recommendations for crude protein in diets containing soybean meal (low escape) or maize gluten meal/blood meal (high escape) as the principal supplemental protein source (Rusche et al., 1993). Treatment had no effect on profiles of luteinizing hormone or progesterone.

Holstein heifers were fed on total mixed rations that met (control) or exceeded (high) ruminally degradable protein requirements, 73 and 82.5% rumen degradable protein as percent of crude protein, respectively (Elrod and Butler, 1993). First-service

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conception rates were 82 and 61% for control and high groups, respectively (P < 0.05). It was concluded that excess degradable protein acts through some undefined mechanism to decrease uterine pH during the luteal phase, which may play a role in the observed reduction of fertility. Kaufman (1982) concluded that cows fed high soluble protein show increasing difficulties in protein metabolism resulting in lack of protein in the intestine and high ammonia excess in the rumen leading to a load on the liver and fertility problems.

In an epidemiological case-control survey, data from 30, low-fertility Israeli Kibbutz dairy herds with a mean overall conception rate of 35% in multiparous cows were compared with data from 30, high-fertility Kibbutz dairy herds with a mean overall conception rate of 48% in multiparous cows (Francos et al., 1992). Among the factors which occurred more frequently in the low-fertility than in the high-fertility herds were a higher average protein density and lower energy/protein ratio fed during lactation.

The detrimental effect of excess protein has been attributed to the ruminally degradable fraction of dietary protein (Folman et al., 1981; Canfield et al., 1990). Jordan and Swanson (1979b) reported that in lactating dairy cows excess dietary protein decreased fertility and significantly altered the ionic composition of uterine fluid during the luteal phase, but not in the follicular phase.

A decrease in serum progesterone has been attributed (Fergusson and Chalupa, 1989) to high intake of degradable protein that exceed rumen requirement of fermentable nitrogen. Endocrine function of the pituitary (LH secretion) and ovary (progesterone secretion) has been greatly elevated and depressed, respectively, in cows consuming feed high in protein (Jordan and Swanson, 1979a).

Fergusson and Chalupa (1989) postulated several mechanisms through which high protein can affect fertility. These include: i) toxic by-products of nitrogen metabolism from the rumen which may impair sperm, ova or early embryo survival; ii) imbalances protein and energy supply; iii) nitrogen metabolites or deficiency of energy utilization may alter the functions of hypophysial-pituitary-ovarian axis. These effects may occur in additive or synergistic fashion.

Alterations in uterine secretions were reported in high producing cows fed diets high in crude protein and resulting in high plasma urea nitrogen (Jordan *et al.*, 1983). When heifers were fed diet with high rumen degradable protein, plasma urea nitrogen concentrations increased, uterine pH decreased and pregnancy rates decreased (Elrod and Butler, 1993).

Thirty two anoestrus buffaloes, 8 months postpartum, were allotted to 4 feeding groups (Singh *et al.*, 1985). The percentage of buffaloes coming into oestrus increased by 50% with addition of 20% digestible crude protein and by 100% with addition of green berseem to the diet. The intervals from the start of the experiment to estrus were 74.5, 54.6 and 69.0 days respectively.

2.2.5 Phosphorus feeding

Phosphorus is an important macro-element in animal's body. Animal's body contains 0.60-0.75 percent phosphorus on fresh tissue basis, 83% in skeleton in the form of hydroapatite and the remainder in soft tissues (Georgievskii, 1981). All-synthetic processes connected with growth and production involve phosphoric acid compounds.

Phosphorus requirement for reproduction in dairy cows is about 10 to 12 g daily except during lactation when an additional amount is required (Asdell, 1969). Feeding dairy cows according to requirements does not only include sufficient supply of energy and crude protein, but also major and trace elements (Wittkowski and Spann, 1993). Undersupply of calcium and phosphorus influence infection of reproductive organs.

Phosphorus deficiency produced small calf crops (Black *et al.*, 1943), resulted in lower birth weight (Read *et al.*, 1986) and an increased calving to first service interval in cattle (Little, 1975), delayed onset of puberty in heifers and failure of estrus in cows (Roberts, 1971).

Morrow (1969) reported that with proper supplementation the number of services required for conception declined from 3.7 to 1.3 in dairy heifers suffering from phosphorus deficiency. Cows supplemented with dicalcium phosphate provided as a free choice lick had 71% weaned calf crop compared with a 48% calf crop for un-supplemented cows (Read *et al.*, 1986). The supplemented cows weighed 121 kg more due to increased forage intake. However, Teleni *et al.* (1977) reported that phosphorus supplementation does not improve production and reproduction unless cattle received sufficient protein.

In rural Indian cows which did not become pregnant, phosphorus levels were significantly lower (Satish-Kumar and Sharma, 1991). Similarly, in a study on 73 lactating Egyptian buffalo cows (El-Belely *et al.*, 1994), plasma phosphorus concentrations were markedly increased in the buffaloes that did not return to service after mating, compared with those that returned. Concentrations were negatively correlated with the number of services per conception, decreasing substantially ($P < 0.01$) in animals conceiving after

$\geq$ 3 matings. Plasma concentrations were affected by lactation number and milk yield. There was a significant relationship between plasma levels of phosphorus with the numbers of days from parturition to first service and days open ($P < 0.01$).

Sixty anestrus buffaloes were divided into 2 groups (Veerapandian *et al.*, 1987). One group was fed green fodder *ad libitum* together with adequate amounts of concentrates. The other group was fed paddy straw and a variable amount of concentrates. The blood glucose and serum total protein concentrations were significantly low in anestrus buffaloes. Inorganic phosphorus levels were lower in the animals fed on paddy straw; but there was no difference between estrus and anestrus buffaloes.

El-Belely (1993) showed that concentrations of inorganic phosphorus were significantly higher in flushings from the uterus of cyclic Egyptian buffaloes than from repeat-breeders at all times. It was suggested that reduced luteal function, related to atypical profiles of plasma biochemical components, during the estrous cycle in repeat-breeder buffalo cows might provide the basis for altered synthesis and secretions of uterine calcium, phosphorus, glucose and proteins which are needed for embryo development.

Kumar and Sharma (1993) found that fertile non-discript buffaloes had higher serum concentrations of phosphorus than infertile ones (6.47 ± 0.38 versus 5.41 ± 0.29 mg/100 ml; $P < 0.05$). In a study on infertility in cattle and buffaloes in 2 villages in the dry zone and 2 in the wet zone of Haryana, the serum progesterone concentrations were higher in sub-fertile animals in the dry zone than in corresponding animals in the wet,

though some of the latter had palpable corpora lutea (Singal and Lohan, 1988). Mineral deficiency was a probable contributory factor to the infertility, the serum phosphorus concentration ranging from 1.02 ± 0.13 to 5.24 ± 0.70 mg/100 ml. When mineral supplements were fed for 5 weeks, 40% of the previously infertile animals showed estrus and conceived to mating.

Two groups of 4 multiparous Surti buffaloes were studied (Pathak *et al.*, 1991). The animals of group 1 (placanta expulsion < 4 hours) showed normal levels of serum calcium and corresponding phosphorus levels, maintaining their ratio at > 2 (2.37 : 1, calcium : phosphorus) throughout the period up to placental expulsion. Animals of group 2 (expulsion time > 5 hours) showed considerably lower levels of calcium with a significantly lower calcium - phosphorus ratio (1.50 : 1). Feeding lime water to these animals during the later part of their next pregnancy significantly increased the calcium level during the early postpartum period without any change in phosphorus concentration. The placental expulsion time was significantly reduced (4.07 ± 0.51 hours).

It may be concluded from the above discussion that sufficient phosphorus intake is essential for maintenance of optimal productive and reproductive processes. Phosphorus deficiency results in longer postpartum estrous interval, silent estrus and reduced conception rates.

2.2.6 Calcium feeding

Calcium is needed for skeletal growth and lactation and for the control of many intracellular processes mediated through calcium binding proteins (Hurely and Doane, 1989). Calcium-dependant mechanisms are involved in steroid biosynthesis in the testis,

adrenal glands and ovaries (Janszen *et al.*, 1976; Larson *et al.*, 1983; Veldhuis and Kalse, 1982) and gonadotropin-releasing hormone stimulation of luteinizing hormone release from pituitary cells (Naor and Catt, 1981).

Reduced blood calcium may delay uterine involution and increase incidence of retained placenta and prolapsed uterus (Morrow, 1980; Risco *et al.*, 1984). Excess calcium may impair reproductive function by causing a secondary deficiency of phosphorus, magnesium, copper and zinc, through inhibiting their absorption from the intestine.

Sixty four lactating buffalo cows (24 normal cycling, 8 pregnant and 32 repeat-breeder) were studied during the first 26 days after estrus (day 0) (El-Belely, 1993). Cyclic and pregnant cows had higher concentrations of plasma calcium between days 3 and 7 than repeat-breeders. Large differences were found in calcium levels, especially on day 12 when flushings from repeat-breeders contained more than 6 times the concentrations of this metabolite in uterine flushings from cyclic animals.

Agarwal *et al.* (1982) studied 5 Jersey-Sahiwal cows and 5 Murrah buffaloes. In the normal cows, progesterone was significantly correlated with plasma calcium on day 16 postpartum, while in repeat breeders it was significantly correlated with plasma acid soluble phosphorus and sodium. In normal buffaloes, progesterone was significantly correlated with plasma calcium on day 16 postpartum. In repeat breeder buffaloes, relationship between progesterone and plasma calcium was weak on day 16 postpartum.

According to Satish-Kumar and Sharma (1991) blood phosphorus levels were significantly lower in infertile Indian rural buffaloes than the fertile ones but calcium levels were not different. However, Sekerden *et al.* (1992) did not find any relationship

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between blood phosphorus and reproduction in Jersey cows. It has been reported (Mohanty *et al.*, 1994) that the cows with retention of placenta had a significantly lower level of calcium.

It may be concluded from the above review that calcium intake in proper quantity and with proper ratio with phosphorus is necessary for optimum reproductive performance in cattle and buffaloes.

2.2.7 Magnesium feeding

Delayed uterine involution and impairment in immune response have been attributed to magnesium deficiency in rats (Larvor, 1983). Ingraham *et al.* (1987) found that cows supplemented with magnesium combined with copper, showed improved fertility whereas those supplemented to copper or magnesium alone, did not.

Serum samples from 77 buffaloes aged 2-5 years, including 60 repeat breeders, were analyzed for their mineral content, to estimate the dietary intake of the animals while on a diet containing above the requirement of all the elements studied (Balakrishnan and Balagopal, 1994). The daily intake of 31.9 g magnesium was above the standard requirements. It was suggested that a mineral imbalance could be a cause of infertility in the repeat breeders. According to Quayam *et al.* (1987), during the peripartum period, magnesium concentration increased and it decreased at 60 days postpartum.

Khattab *et al.* (1995) reported that blood magnesium levels were not different in regular or irregular estrous cycles in Egyptian buffaloes. Similarly, Pathak and

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Janakiraman (1987) found no specific trends for the concentration of magnesium during pregnancy or estrus and averaged 3.37 mg/100 ml.

A study on 10 cows and 10 buffaloes in estrus, 10 of each species in the luteal phase of the estrous cycle and 10 each with inactive ovaries (Osman *et al.*, 1985) showed that cyclicity or stage of estrus cycle did not affect serum magnesium levels. Dvoracek (1988) reported that at cattle farms, where the conception rate was below 40% the potassium : calcium + magnesium ratio was in excess; 174.5% of standard. Wittkowki *et al.* (1993) reported that magnesium deficiency is involved in mastitis incidence and impairment of fertility.

2.2.8 Copper and zinc feeding

Several biological roles of zinc have been reported (Apgar, 1985; Chesters, 1978) and over 200 proteins and enzymes contain zinc. These authors reported zinc deficiency to be associated with impaired fertility. Zinc may act indirectly through the pituitary to influence gonadotrophic hormones or directly through complexing with specific ligand in the gonads (Hurley and Doane, 1989).

Availability of copper is reduced by an excess of other minerals such as iron, calcium and zinc (Blakemor and Venn, 1950). Suboptimal ovarian activity delayed or depressed estrus, reduced conception rates, increased incidence of early embryonic death and retained placenta, calving difficulties and congenital rickets have been attributed to copper deficiency (Hidiroglou, 1979). Lack of dietary copper affects growth and causes silent oestrus and zinc deficiency causes skin problems, slow growth, decrease in milk

yield and fertility disturbances (Wittkowki *et al.*, 1993). It was reported that reproductive problems were associated with over-supply of these minerals.

Deficiency of copper and zinc appear to be more pronounced on straw based diets in buffalo calves reflected in whole blood, plasma and rumen fluid (Randhawa *et al.*, 1993). In a study on 12 Lanka cows (Perera *et al.*, 1988) most animals were deficient in phosphorus and zinc.

Sixteen Murrah buffaloes in the second to fourth month of lactation received green fodder *ad libitum* and 3 kg concentrate mixture, containing 3% mineral mixture, daily (Sharma and Prasad, 1982). Blood zinc and copper were lower in lactating buffaloes than in growing buffalo calves indicating that lactation puts more drain on copper and zinc as compared to growth.

Attia *et al.* (1987b) found that high zinc supplement decreased serum copper. It was stated that the usual Egyptian rations were lacking in zinc. In a later study on Egyptian buffaloes (Khattab *et al.*, 1995), blood zinc and copper levels were significantly higher in buffaloes with regular estrous cycles.

In lactating Murrah buffaloes the average plasma zinc values were highest, 289 ± 19 mug/100 ml, in spring followed by those in winter, hot and humid and hot and dry summers (Mehta and Gangwar, 1984). In all the seasons, values were higher in pregnant than in non-pregnant buffaloes. The average values of copper were highest in winter (130 ± 7 mug/100 ml) and were higher in pregnant buffaloes. In another study zinc values were significantly lower in pregnant Nili-Ravi buffaloes (Hanif *et al.*, 1984).

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In a study on 30 Egyptian cows and 30 buffaloes, copper values were higher in oestrus than in the luteal phase, and significantly higher in cycling females than in females with inactive ovaries (Osman et al., 1985).

In 5 experiments, dry and lactating buffaloes in the Gyandza-Kazakh region of Azerbaidzhan were given or not given mineral supplement (injections of tetravitan, supplements of trivitan, potassium iodide, sodium selenite, calcium triphosphate, proserine, cobalt chloride or zinc sulphate) (Gasanov, 1991). Supplemented buffaloes had reduced service interval, improved conception to first service, increased calf birth weight, decreased calving problems, increased milk yield and colostral density, and blood immunoglobulin concentrations.

In Murrah buffaloes zinc concentration in whole blood and plasma declined progressively, whole blood and blood plasma copper concentrations revealed a non-specific but increasing trend during the last month of pregnancy and parturition (Setia et al., 1994). After parturition, zinc and copper concentrations reflected the increasing trends with the advancement of lactation.

In a study on buffaloes (Balakrishnan and Balagopal, 1994), the daily intake was 252 mg copper and 547 mg zinc and both of them were above the standard requirements. Serum levels of zinc were significantly lower in the repeat breeders, although it was within the normal range. Fayez et al., (1992) found that repeat breeders had significantly lower serum zinc concentrations than normal breeders. Supplementing repeat-breeding animals with 40 g sodium phosphate in the feed and 500 ppm zinc in the drinking water for 1 month improved conception rate by 80%.

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A study of blood samples from 57 anestrous, estrous and diestrous Murrah buffalo heifers (Chandolia and Verma, 1987), revealed that there were no significant differences between the 3 groups in plasma concentrations of copper, zinc, iron and selenium.

Up to 90 days postpartum, the difference between cyclic and anestrous Nili-Ravi buffaloes was not significant for plasma concentrations of zinc or copper (Ullah *et al.*, 1983). The calcium - phosphorus ratio was significantly wider in the last stage of pregnancy. Ali *et al.* (1983) found that Nili-Ravi buffaloes in estrus had a lower ($P < 0.01$) zinc concentration (1.48 ± 0.66 versus 2.04 ± 0.92 parts per million) as compared to those in anestrus.

2.2.9 Blood glucose levels

Blood glucose appear to be one of the key nutrients affecting estrous cyclicity in farm animals. Concentration of glucose in blood may determine the rate of steroidogenesis and gonadotropin synthesis and (or) secretion (Lynn *et al.*, 1965, Sen *et al.*, 1979). Hypoglycemia has been associated with reduced fertility in beef and dairy cattle (McClure, 1968; Oxenreider and Wagner, 1971; Selk, 1986). Secretion of LH in cows was increased by infusion of glucose (Garmendia, 1986).

Concentration of glucose and insulin reduced when body weight and body energy reserves were decreased in ruminants fed restricted diets (Trenkle, 1978; Richards *et al.*, 1989a). Concentration of non-esterified fatty acids (NEFA) in plasma of cows fed restricted diets were greater when they were losing body weight and body condition score. Increased concentration of NEFA in plasma of cow are an indication of a negative energy balance and fatty acid release from adipocytes (Bines and Hart, 1982). Reduced

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concentration of glucose in plasma and insulin and increased concentration of NEFA were associated with anestrus and continued throughout the anestrous period (Richards *et al.*, 1989a).

Brangus heifers with propionate-induced increases in blood glucose, released more LH in response to GnRH than control heifers (Rutter *et al.*, 1983). The number of LH pulses is reduced during initiation of anestrus in cows (Richards *et al.*, 1989b). It was concluded that reduced nutrient intake caused a reduction in glucose in the plasma of cows which resulted in inadequate concentrations of LH in serum to stimulate normal cyclic ovarian function. The mechanism by which nutrient deprivation regulates estrous cycle in cows was presumed to involve a metabolic signal that modulate LH secretion.

In 12 cows given supplementary or no supplementary feeding (control) with continuous or restricted (twice daily) suckling regimes (Tegegnae *et al.*, 1993), plasma glucose levels varied over time without consistent trends and were not influenced by feeding or suckling regimes, nor did they differ between cyclic and acyclic cows.

In 40 Angus X Hereford, 2-year-old primiparous cows (Rusche *et al.*, 1993), feeding high escape crude protein sources decreased plasma glucose and urea nitrogen. High crude protein increased plasma glucose and plasma urea nitrogen.

Sinclair *et al.* (1994) reported that plasma glucose concentrations were below 3 mmol/liter for twin-suckling cows until week 7 postpartum and were then similar to the values for single-suckling cows.

In 213 Italian Holstein-Frisian cows (Pedron *et al.*, 1993) glucose concentrations were relatively constant in cows calving at the highest body condition score.

Blood samples were collected from 25 nondescript buffaloes during estrus (Kumar and Sharma, 1993). There were no significant differences between fertile and non-fertile groups in concentrations of blood glucose and serum cholesterol. But on the the other hand Satish-Kumar and Sharma (1991) reported that in the cows which did not become pregnant, blood glucose levels were significantly lower on the day of insemination.

In 24 Frisian cows (Jones et al., 1988) serum glucose concentration was significantly correlated with service period (r=0.48). In Finish dairy cows during puerperium, low levels of glucose as well as high levels of ketone bodies were associated with poor fertility, although there was no linear correlation (Miettinen, 1990).

In 73 lactating buffalo cows (El-Belely et al., 1994), plasma concentrations of glucose (P<0.05) were markedly increased in females that did not return to service after mating compared with those that returned. The concentrations were negatively correlated with the number of services per conception, decreasing substantially (P<0.01) in animals conceiving after ≥ 3 matings. Glucose was affected by lactation number and milk yield.

In 33 buffaloes (Dutta and Dugwekar, 1983) blood glucose was high during late gestation and fell from 1 to 10 days postpartum. Values were higher in buffaloes not retaining the fetal membranes than animals with retained placenta.

In 64 lactating buffalo cows (El-Belely, 1993) cyclic and pregnant cows had higher concentrations of plasma glucose between days 2 and 8 of estrous cycle than repeat-breeders. It was suggested that reduced luteal function, related to atypical profiles of plasma biochemical components, during the oestrous cycle in repeat-breeder buffalo cows might provide the basis for altered synthesis and secretions of uterine glucose and proteins which are needed for embryo development.

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Quayam *et al.* (1988) reported that blood glucose level of 39.67 ± 0.61 mg/100 ml at 5 days postpartum was significantly correlated with the occurrence of estrus by 60 days.

In 17 anestrous prepubertal buffalo heifers, aged 42-96 months, serum concentration (per 100 ml) of glucose was 46.6 mg (Kumar *et al.*, 1992). The overall mean values for glucose levels were reported by Mullick (1964), Ahmad (1976) and Zaman (1984) as 79.4, 93.35 and 45-86 mg/100 ml, respectively.

In a study on 50 estrous, 50 sub-estrous and 50 anestrous Nili-Ravi buffaloes, blood glucose concentrations were not different between the groups, however the concentrations were significantly higher in animals yielding < 5 liters of milk daily (69.72 mg/100 ml) than in those yielding 6-10 or > 10 l (65.14 and 63.93 mg/100 ml, respectively) (Zaman, 1984).

2.2.10 Blood protein levels

In 12 cows given supplementary or no supplementary feeding, with continuous or restricted (twice daily) suckling regimes (Tegegnae *et al.*, 1993), plasma total protein, albumin, and globulin levels varied over time without consistent trends, and were not influenced by feeding or suckling regimes, nor did they differ between cyclic and acyclic cows. Similarly blood sampled from 16 Jersey cows that had calved over a 26-day period, were analyzed for total protein, albumin, and total bilirubin (Sekerden *et al.* 1992). None of the blood parameters studied was significantly correlated with milk fat concentration or conception rate to first insemination.

In studies on 178 high-yielding Holstein-Frisian cows, the serum albumin and milk protein values decreased in those with a reduced body condition score during the early- to mid-lactation periods (Yamada et al., 1994). El-Moneim et al. (1990) found that serum total protein and globulin tended to increase with lactation number.

Plasma protein level was depressed by heat stress (Bahga et al., 1985). It has been reported (Pandey and Roy, 1968b) that since serum protein influence the water retaining capacity of an animal, higher concentration (7.14 - 8.5 g/100 ml) in buffalo would favor heat regulation under hot condition. In a study on rural cows in Uttar Pradesh, India, (Kumar and Sharma, 1991), fascioliasis-infected cows had lower total serum protein levels. Over 36% normal cyclic, 50% anoestrus and 46.4% repeat breeder cows had chronic fascioliasis out of 110 rural cows.

Weekly blood samples were collected from 73 lactating buffalo cows, 3 times before and 3 times after mating (day 0), and were analyzed for plasma total protein (El-Belely et al., 1994). Plasma concentrations of total protein, did not markedly increase in the buffalo cows that did not return to service after mating compared with those that returned. The concentrations were negatively correlated with the number of services per conception, decreasing substantially (P < 0.01) in animals conceiving after $\geq$ 3 matings.

In buffaloes not retaining the fetal membranes, serum total protein, albumin and globulin values were higher during late gestation and declined as parturition approached, returning to prepartum levels by 10 days postpartum (Dutta and Dugwekar, 1983). A similar pattern occurred in buffaloes retaining the fetal membranes but at lower levels of the three values.

Sixty four lactating buffalo cows (24 normal cycling, 8 pregnant and 32 repeat-breeder) were selected from 2 buffalo farms near Cairo (El-Belely, 1993). Plasma protein concentrations were greater at all times, except between days 6 and 8 of estrous cycle, in the first 2 groups. Protein concentrations of uterine fluid were significantly higher in cyclic animals than repeat-breeders on day 2, 8 and 12. It was suggested that reduced luteal function, related to atypical profiles of plasma biochemical components, during the estrous cycle in repeat-breeder buffalo cows might provide the basis for altered synthesis and secretions of uterine proteins which are needed for embryo development.

A study on sixty anestrous buffaloes showed that serum total protein concentrations were significantly low in anestrous buffaloes (Veerapandian et al., 1987).

Blood samples were collected from 25 nondescript buffaloes during estrus (Kumar and Sharma, 1993). There were no significant differences between fertile and infertile groups in concentrations of serum protein and cholesterol.

In a study on 150 Nili-Ravi buffaloes (Zaman, 1984) the mean total plasma protein concentrations in cyclic, non-cyclic and sub-estrous buffaloes were 8.45 ± 0.49, 8.26 ± 0.64 and 8.47 ± 0.55 g/100 ml respectively. However the difference was non-significant. Similarly, Larson et al. (1980) also failed to find significant relationship between serum total protein and reproductive performance in a herd of 112 Holstein cows.

It may be concluded from the above studies that blood protein levels may be positively correlated with reproductive efficiency but the trend is not consistent in various studies.

2.2.11 Blood urea levels

Plasma urea nitrogen (PUN) and milk urea nitrogen (MUN) are useful indicators of protein metabolism and status in cows (Roseler *et al.*, 1993). McDonald *et al.* (1984b) reported that the rumen degradable protein are either converted by ruminal microflora, to microbial protein and digested in small intestine, or they are hydrolyzed to ammonia and excreted as urea through kidney.

Feeding high escape crude protein sources decreased PUN, while increased crude protein intake increased plasma urea nitrogen in Angus X Hereford primiparous cows (Rusche *et al.*, 1993). In a study on Holstein heifers (Elrod and Butler, 1993) PUN levels were elevated, (10.2 ± 0.17 versus 14.8 ± 0.19 mg/100 ml) in animals fed high ruminally degradable protein (82.5%) as compared to controls (73%).

According to Tegegnae *et al.* (1993), blood urea nitrogen levels varied over time without consistent trends and were not influenced by feeding or suckling regimes, nor did they differ between cyclic and acyclic cows.

In a study (Bertoni *et al.*, 1994) blood urea was higher with higher (14% versus 12%) protein level and after the meal. Kalchreuter (1991) recommended to use milk urea concentration to adapt protein and energy supply requirements during pregnancy and lactation.

Sekerden *et al.* (1992) reported that milk yield in the first 60 days of lactation was negatively correlated with blood urea concentration (r=-0.59) but the latter did not significantly correlate with milk fat concentration or conception rate to first insemination.

Concentration of PUN and MUN to pregnancy rate were compared in dairy cows (Butler *et al.*, 1996). Concentration of PUN or MUN greater than 19 mg/100 ml were

associated with decreased (P < 0.02) pregnancy rates. MUN was lower in pregnant cows and had a decreasing trend over time compared with non pregnant cows (P < 0.05). Plasma progesterone concentrations during mid-diestrus were reported to be approximately 30 percent points lower in cows with high PUN due to feeding high protein diets (Jordan and Swanson, 1979a).

Alterations in uterine secretions were reported in high producing cows fed diets high in crude protein and resulting in high PUN (Jordan *et al.*, 1983). When heifers were fed diet with high rumen degradable protein, PUN increased and uterine pH and pregnancy rates decreased (Elrod and Butler, 1993).

Concentration of serum urea nitrogen (SUN) has been found to be negatively associated with conception rate (Fergusson *et al.*, 1988) in dairy cows. Conception rate was not influenced by increasing SUN from 10 to 20 mg/100 ml but at concentrations > 20 mg/100 ml conception rate decreased dramatically. In a later study these results were confirmed (Fergusson *et al.*, (1991).

Kaim *et al.* (1983) reported that in cows fed a 20% crude protein diet with PUN of 16.8 mg/100 ml, pregnancy rate was lower than in cows fed a 15% crude protein diet with PUN of 9.0 mg/100 ml. Services per conception were increased in cows fed diets with greater than 16% crude protein (Jordan and Swanson, 1979b) but in another study (Howard *et al.*, 1987), the reproductive performance of cows was normal and unaffected by increasing dietary protein from 14.5 to 19.4% of the dry matter. This was probably due to inclusion of undegradable protein in the diet.

In Finnish dairy cows (Miettinen, 1990) uterine involution in cows in the silage group took significantly longer, the pregnancy rate at the first insemination was lower

and the interval from calving to conception was longer than in those of the hay-urea group. In late puerperium, low levels of urea were associated with poor fertility, although there was never any linear correlation. In puerperium, the threshold values of blood metabolites seem to be more important for subsequent fertility than the temporary absolute value. Differences in the quality of protein and the time of sampling influenced urea levels of serum and milk. Diurnal variations in urea levels were closely related to the feeding rhythm.

Zaman (1984) found that the mean urea concentration in lactating Nili-Ravi buffaloes during cyclic, non-cyclic and anestrous states were 34.29, 30.03 and 31.63 mg/100 ml respectively and the values were not different from each other significantly.

2.3 Effects of other parameters on reproductive performance

2.3.1 Body condition score

Jefferies (1961) initially developed a body condition score (BCS) system (scale 0 to 5) for ewes. The system involved palpating the backbone and lumber processes, feeling for the sharpness and covering of bones. This technique was adopted by Lowman et al. (1976) for beef cattle and modified by Mulvany (1981) for dairy cattle. Peters and Ball (1987) described a method for dairy and beef cows. In this method the thickness of fat cover over the lumber and tail head area is estimated and assigned a score from 0 (emaciated) to 5 (very fat).

Using a scoring scale of 0 (very thin) to 5 (very fat), scores of 3.5 and ≥ 2.5 at calving and peak milk yield, respectively, were recommended (Ruegg, 1991). A score

of 3.5-4.0 is optimal for cows at the end of lactation; this score should not increase during the dry period.

According to Rusche et al. (1993) increased crude protein (CP) intake increased average daily gain, but body condition score was unaffected in Angus X Hereford cows. Marston et al. (1995) found that conception rates were significantly improved by feeding greater levels of supplemental energy prepartum but not postpartum. Energy supplements can affect reproduction with minimal effects on body weight or condition.

Body condition scoring at calving is a direct measure of prepartum nutritional status and reproductive rates in beef herds might be increased by evaluating body condition score (using a scale of 1 to 9) as a criterion for determining nutritional status (Spitzer, 1986). The author suggested to sort the cows by condition 90 to 100 days prior to calving and feed the animals to maintain a body condition score of 5 to 7 to maximize reproductive performance and minimize supplemental feeding.

Postpartum estrus interval and reproductive performance are influenced by nutritional status (Wilmut et al., 1986) and body condition score (Jainudeen and Wahab, 1987). Similarly Osoro and Wright (1992) reported that body condition score at calving had a large influence on postpartum reproduction than changes in body weight or body condition score after calving. Another study (Lowman, 1985) suggested that direction of body condition change during the mating period was an important determinant of reproductive performance in cows in low body condition (≤ 2) but not in higher levels of body condition (> 2).

Jones et al. (1988) on the other hand, noted that diet and condition score at calving did not significantly affect the intervals from calving to first service and

conception, or number of inseminations per conception in Fiesian cows.

Sinclair *et al.* (1994) reported that in cows body condition at calving and postpartum energy intake did not influence the time taken for cows to overcome anovulation nor did they influence the proportion of cows which eventually resumed normal estrous cyclicity. Fertility variables were improved when twin-suckling cows were in moderate body condition at calving and / or offered metabolizable energy 130 MJ/day during the postpartum period. Roberson *et al.* (1992) reported that the pattern of LH in the circulation, responsiveness of the pituitary to LH-RH and releasable stores of luteinizing hormone in heifers fed diets of low energy content were influenced by body condition.

In a study on primiparous cows (DeRouen *et al.*, 1994) weight and body condition score (BCS; 1 = emaciated, 9 = obese) at calving were greater (P<0.05) for cows with a higher BCS 90 days prepartum (initial BCS) and those assigned to the high-energy diet. Calving and calf growth traits were not affected by initial BCS, energy level, prepartum changes in BCS and body weight or BCS at calving. Prepartum changes in BCS and weight, regulated by the 3 energy levels, had no effect on postpartum reproduction; however, BCS at calving influenced (P<0.03) subsequent pregnancy rate and interval to pregnancy.

Heuwieser *et al.* (1994) found that cows with a high BCS ($\geq$ 3.0) at the first breeding had 8.8 fewer days to first service but more services per conception (0.16) than cows with low BCS. Conception rate improved when GnRH was administered at the first breeding to cows with a BCS of < 3.0.

In Israeli Holstein-Frisian dairy cows (Wolfenson et al., 1988) body condition significantly affected only the postpartum interval to the start of ovarian activity (26 days for high and 32 days for low body condition groups). In the low body condition group, estrous behavior lasted significantly longer in cooled (16.0 ours) than in non-cooled (11.5 hours) cows.

Twenty four Holstein-Frisian cows were fed from 16 weeks before calving to reach fat or thin body condition scores of 3.25 and 2.0, respectively at calving (Jones and Garnsworthy, 1988). For thin and fat cows, service period averaged 92 and 84 days and number of services per conception 1.79 and 1.60 respectively with no significant difference among treatments.

In a study on Brahman crossbred cattle (O'Rourke et al., 1991), pregnant heifers averaged 13 kg more live weight than non-pregnant cows at the end of breeding season, after allowing for the weight of the fetus and gravid uterus. Cows with an adjusted weight of < 300 kg had a pregnancy rate 25% lower than those weighing > 350 kg. Cows with condition scores of ≥ 8 had a conception rate 33% higher than those with scores ≤ 5.

In Israeli Kibbutz dairy herds (Francos et al., 1992) 4.8% of the differences between low-fertility (conception rate 35%) and high-fertility herds (48%) could be related to body condition during the dry period. 67% of the difference was attributed to nutritional factors.

In high-yielding Holstein-Frisian cows (Yamada et al., 1994) the first insemination conception rate was significantly lower in cows with a markedly decreased body condition score during the early to mid-lactation period. These results indicated that

monitoring the BCS during a period from drying-off to early- and mid-lactation might be a useful tool in the prevention of peri-parturient diseases and infertility in dairy cows.

Kohno *et al.* (1993) found that BCS was highest in cows with recurrence of distinct estrus. BCS varied inversely with plasma non-esterified fatty acids and directly with blood sugar, 7 days after calving. The results showed that feeding level before calving was related to the time of recurrence of first estrus and fertility after calving.

Gary *et al.* (1989) observed that 45 days after calving, 62% of animals with good BCS were cycling, compared with 10% of animals with a poor score. However, dietary supplementation in the eighth month of pregnancy to increase energy intake did not improve subsequent ovarian activity. Similarly buffaloes in poor body condition and young females in their first lactation possess inactive ovaries and have extended periods of postpartum anestrus (Jainudeen and Wahab, 1987).

Thirty five buffaloes in poor body condition (average live weight 275 kg) and showing no ovarian activity were fed a level of nutrition for 30 weeks such that their average daily gain would be 0.18 or 0.41 kg (Gede-Putu *et al.*, 1983). The average body weight of the 8 anestrous cows at the end of the experiment remained low (was 291 kg). The results suggested that there was a critical body weight of about 320 kg associated with the beginning of ovarian activity.

Bhalaru *et al.* (1987) scored 180 buffaloes for body condition at calving by measuring fat cover on a scale 1 (very thin) to 5 (very fat). Subsequent conception rate was highest (91.7%) in females with a score of 3.5. The conception rate was significantly higher (88.3%) for females with moderate body condition, scoring 2.5-3.5, than for females scoring 1-2 and 4-5. The interval to first postpartum oestrus was significantly

shorter in moderate and fat females (66.2 and 66.9 days, respectively) than in thin females (77.7 days). The service period of moderate females was significantly shorter (128.3 days) than for fat and thin females (144.1 and 164.5 days respectively).

McClary (1991) reported that reduced fertility in the high-yielding cow is not directly related to increased milk yield but rather to loss of body condition. The magnitude of the negative impact of milk production on reproduction depends on the cumulative negative energy balance in early lactation. High-yielding cows can achieve optimal reproductive efficiency if they are fed adequately to minimize loss of body weight in early lactation, and replenish adequate body condition in late lactation and the dry period.

In a study on 213 Italian Holstein-Frisian cows (Pedron *et al.*, 1993), the cows calving at highest BCS lost more subcutaneous fat. Glucose concentration were relatively constant in cows calving at the highest BCS.

Richards *et al.* (1989b) concluded that anestrus occurs when non-lactating cows lose weight and have a BCS of about 3.5. Anestrus was associated with a decrease in frequency of LH pulses. Increased nutrient intake after a period of severe restriction resulted in resumption of estrous cycles and normal pregnancy rates.

It may be suggested on the basis of the above referred studies that BCS reflects the nutritional status of an animal and it's loss is associated with negative energy balance due to deficient energy intake or high milk production. Prepartum BCS and change in postpartum BCS are good indicators of postpartum fertility in cattle and buffaloes.

2.3.2 Calf suckling

Suckling is one exteroceptive stimulus that plays a major role in governing reproductive cycles in female mammals (Short, 1976). Biological effects in farm species range from a total blockade of ovulation in the domestic sow to little or no impact in the seasonally breeding ewes. Beef cow holds an intermediate position but has commanded an extraordinary attention due to its economic importance.

Early weaning of lactating beef cows at day 30 postpartum interval to first estrus, increased the number of conceiving and decreased the postpartum service period (Smith and Vincent, 1972). Williams (1990) reported that allowing calves to suckle their dams only once per day for 30 to 60 minutes increased conception rates and partial weaning extending beyond 10 days decreased the first postpartum estrus interval.

On the same pastures, cattle remained anestrous while suckling a calf, while a calf did not seem to affect the ability of Swamp buffaloes to conceive (Underwood *et al.*, 1982). Buffaloes held their body condition better than cattle on poor quality forage and weighed 100 kg more than cattle.

Twenty three buffaloes were assigned to the following treatments: unrestricted suckling; suckling restricted to once a day; and calves weaned (Nordin and Jainudeen, 1991). For the 3 groups, the interval from calving to first ovulation averaged 82 ± 30, 69 ± 24 and 50 ± 21 days respectively, that from calving to the time when plasma progesterone was > 0.7 ng/ml, 85 ± 30, 71 ± 24 and 53 ± 21 days, and that from calving to the first observed oestrus 89 ± 28, 78 ± 27 and 60 ± 24 days. Service period averaged 89 ± 17, 92 ± 26 and 58 ± 17 days and was significantly lower in the weaned group than in the suckled groups. Ovarian cyclicity was re-established earlier in non-

suckled than suckled river as well as Swamp buffaloes (El-Fouly *et al.* 1976a; El-Fadaly, 1980a; Jainudeen *et al.*, 1984).

Abeygunawardena *et al.* (1995) reported that free suckling, *ad libitum* buffaloes resulted in lowest pregnancy rate and weaning by day 60 and 90 did not improve postpartum fertility. Perera *et al.* (1984) reported that ovarian inactivity was the major problem in areas with poor fertility and was influenced by suckling. In a latter study (Perera *et al.*, 1992), 18 multiparous Lanka buffaloes were assigned to one of the three treatment groups immediately postpartum: restricted suckling (RS); *ad libitum* suckling (AS) and AS + supplementary feed (AS/S). The percentage of animals which had commenced ovarian activity in RS, AS and AS/S groups were 100%, 20% and 50% and endogenous episodic LH peaks were seen in 57, 0 and 33 respectively, up to day 90 postpartum. Restricted suckling resulted in early ovarian activity while the supplementary feed did not.

Plasma LH levels in lactating Murrah buffaloes with calves weaned at birth were significantly lower during the second and third week postpartum for animals which eventually experienced anestrus beyond 90 days postpartum than in those cycling within 50 days postpartum (Batra and Pandey, 1983).

Effects of limited suckling on postpartum reproductive performance of milked Nili-Ravi buffaloes were examined in two 2 X 2 factorial experiments (Usmani *et al.*, 1990). Half of the buffaloes were suckled by their calves twice daily (2 minutes at each milking) until second estrus. Postpartum intervals to uterine involution, resumption of follicular development, first rise in milk progesterone, first palpable corpus luteum and first estrus were longer for limited-suckled buffaloes than for those that were only

milked. Number of rises of progesterone before first estrus was greater for limited-suckled buffaloes in experiment 2 but not in experiment 1. Luteal activity after first estrus (15 to 19 days) and pregnancy rate to insemination at second estrus (average 50%) did not differ with limited suckling.

Williams (1990) reported that pituitary responsiveness to GnRH did not differ between suckled and non-suckled cows from day 3 to 20 postpartum. By day 20 after calving, non-suckled had begun to release both tonic and phasic bursts of gonadotropin, whereas most suckled cows had not.

Prolactin has been considered as a potential antigonadotropic agent mediating postpartum acyclicity in most species and suppression of prolactin release by ergcryptine blocked lactation and established early postpartum ovulatory cycles in human female (Rolland et al., 1975). Williams and Ray (1980) blocked prolactin release in early postpartum cows with CB-154 but were unable to effect gonadotropin concentrations or postpartum intervals by this treatment alone. Williams (1990) suggested that it is the suckling stimulus itself that suppresses gonadotropin secretion, rather than high prolactin concentrations associated with suckling.

It may be concluded from the above discussion that suckling exerts an inhibitory effect on resumption of postpartum ovarian activity in beef cattle and Swamp and dairy buffaloes and that temporary calf removal or weaning at birth shorten postpartum estrus interval.

2.3.3 Milk production

Harman (1994) found a quadratic relationship between milk yield and low occurrence of conception for adult cows. The highest and lowest yielding cows had a lower conception hazard than did average yielding cows. However there was no effect of milk yield after 120 days of lactation. Analysis of data on 389,063 German Simmental cows (Daubinger, 1994) showed that high milk and milk fat yields and a low milk protein yield had an adverse effect on subsequent fertility.

Nauk et al. (1991) found that the first postpartum estrus occurred significantly later and duration of estrus was shorter in cows with higher milk yields than in those with lower milk yields. The conception rate was significantly lower in cows with milk yields > 5000 kg than in cows with yields ≤ 4000 kg.

Marti and Funk (1994) reported that within herds days open were longer for cows with higher milk yields than for those with lower yields. Regressions of days open on production variables that were not adjusted for days open were significantly longer for low-producing than for high-producing cows.

In 15,320 Holstein cows in New York (Eicker et al., 1996), cows yielding at the highest level had 8% lower conception rate and 29% higher insemination rate as compared to cows yielding at the lowest level. Regardless of the strength of association between high yield and length of days open, the study suggested that milk yield per se has very little direct effect on conception. Daubinger et al., (1993) reported that an increase of > 0.5 in milk protein percentage at the end of lactation (compared with the mid-lactation value) leads to an increase in fertility problems in dairy cows.

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The relationships between milk production, subclinical ketosis and fertility were studied in 101 dairy cows (Miettinen and Setala, 1993). The yield of milk protein and of milk at 3-4 weeks postpartum affected the service period (r=0.31). The time from calving to conception also was associated with high protein yield, high milk yield and high fat yield at 7-8 weeks postpartum (r=0.41, r=0.40 and r=0.35, respectively). At the third milk recording, a low percentage of milk protein was associated with a long interval from calving to first insemination and to conception (r=0.22 and r=0.26, respectively).

In German Simmentals (Daubinger et al., 1993) it was found that milk yield had a greater effect than fat and protein percentages on fertility. Fertility tended to decrease as milk yield increased. A protein content > 3.2% and a fat percentage < 4% had a beneficial effect on fertility.

Comparison of milked and suckled Angus cows incriminated circulating cortisol levels as a possible component to the block of LH secretion, preventing postpartum ovulation and return to cyclicity (Short et al., 1972). Cortisol levels were higher in suckled cows as compared to machine milked ones and higher in high milk producers as compared to low milk producers.

Markusfeld (1987) reported that inactive ovaries were directly associated with high milk yield after calving in heifers. It was concluded that damage to the uterus and an energy shortage after calving were responsible for ovarian inactivity.

In a study on 281 lactations of 140 cows, high peak and 120-day milk yields were associated with long intervals from parturition to first ovulation, first ovulatory estrus and first conception, with low conception rate at first artificial insemination and with a high

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number of inseminations per service period (Berglund *et al.*, 1988). A high number of weeks with a positive keto-test reaction and a high energy deficiency were associated with long intervals to first ovulation and first ovulatory oestrus.

In 216 cows with 469 calvings (Fiss and Wilton, 1992), within breeding system, neither cow weight nor milk yield were significantly associated with reproductive traits of cows. No differences were detected among breeding systems in associations between food consumption and body weight or milk yield.

Beal *et al.* (1990) indicated that cows that produced more milk tended to loose more weight than low producing cows. Despite that relationship, neither the degree of weight change nor the amount of milk produced was related to the timing of the first ovulation post-calving.

Wanner (1991) suggested that high-yielding cows are pre-disposed to fertility problems, but often human error causes disturbances, especially in feeding. It was argued that high milk yield and good fertility are not mutually exclusive, provided that optimal rumen function can be achieved by feeding high-quality feeds correctly and by supplying essential nutrients strictly according to requirements.

It may be concluded from the above studies that higher milk production decrease fertility through mobilization of body fat, resulting in a loss of BCS. High milk yield and good fertility can be mutually maintained in case of adequate feeding for supplying energy, protein, minerals and vitamins strictly according to requirements.

2.3.4 Thermal stress and photoperiod

Thermal stress suppress exploitation of animal's full genetic potential through: (i) reduction in animal's appetite (Allen *et al.*, 1963) and; (ii) increased protein catabolism due to increased cortisol secretion (Christison and Johnson, 1972). Thermal stress affects reproductive efficiency of dairy cattle by reducing the length of estrus to about 10 hours and lowering intensity of estrus behavior (Thatcher and Collier, 1982). More-over heat stress increases the incidence of silent ovulation (to the tune of 80%) and lowers conception rate (Thatcher *et al.*, 1964).

Seasonal infertility associated with high ambient temperatures during summer in tropical and subtropical region has been reported in dairy cattle (Stot *et al.*, 1972). In a study on Friesian cows in Iraq, higher number of services per conception and a longer service period was recorded during summer then winter (Ali *et al.*, 1983). Infertility associated with thermal stress may be due to adverse effects of high temperature on the developing embryo and through maternal endocrine imbalance (Christenson, 1980). In dairy buffaloes, Bahga and Gangwar (1988) reported that although summer stress hastened uterine involution and first postpartum estrus interval, overall reproductive efficiency was impaired due to deficiency of progesterone which is essential for embryo survival. It has been suggested by Thatcher *et al.* (1986) that a thermal stress-induced reduction in uterine blood flow may elevate uterine temperature and likely affect availability of water, electrolytes, nutrients and hormones to uterus. Unfavorable uterine environment would have a high probability of increasing rates of early embryonic mortality. Acute thermal stress in cattle increases blood concentrations of prolactin

(Wettman and Tucker, 1974), catechol-amine and glucocorticoids (Alvarez and Johnson, 1973) and decreases aldosterone concentrations (Collier *et al.*, 1982).

Photoperiod has also been identified to be associated with cyclic activity in dairy cows (Montgomery *et al.*, 1980). Natural day light length was associated closely with monthly means of basal concentration of prolactin in lactating dairy cows throughout the year (Head *et al.*, 1976).

CHAPTER 3

MATERIALS AND METHODS

3.1. Selection of animals

The present study was conducted in the Central Valley of the North-West Frontier Province of Pakistan, situated at the latitude of $31-37^0$ North and longitude of $65-74^0$ East. Twenty one pregnant buffaloes expected to calve during the low breeding season (February to July) and 30 buffaloes expected to calve during the normal breeding season (August to January) were selected (Table 3.1). These animals belonged to seven private farms located in the rural areas within a radius of 70 Km around Peshawar city. Distribution of the animals according to location is given in Table 3.1. Period of calving and age-wise distribution of experimental buffaloes is given in Table 3.2. Selected animals were ear-tagged, dewormed, treated for ectoparasites and vaccinated. The selected animals were monitored from approximately two months prepartum till the occurrence of first estrus or 150 days postpartum.

The following criteria were adopted in selecting the farms for this study:

a.   The farms had at least 10 adult buffaloes.

b.   The farms followed conventional feeding system of green fodder and dry roughage with variable quantity and composition of concentrates.

c.   The animals were stall-fed (cut-and-carry system).

d.   The herd was free from brucellosis.

e.   The farm had a breeding bull for estrus detection.

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Limited suckling of the animals by calves at the time of milking was allowed according to the prevailing practice. Past history of the selected animals was recorded as follows:

- ► Date of birth, age or parity number (the age was assessed by counting the horn rings as suggested by FAO (1977))

- ► Age at puberty, service period and pregnancy stage

- ► Calving interval

- ► Heat detection and method of breeding (natural or artificial insemination)

- ► The use of calf suckling or oxytocin for milk let down

- ► Daily and cumulative milk yield and duration of the last lactation

- ► Feeding (types and quantities of roughages, concentrates and minerals) and watering practice

- ► Previous history of health status and reproductive performance.

3.2. Clinical monitoring of reproductive status

Deviation from the normal parturition was recorded using the following criteria:

- ► Calf delivery with or without assistance.

- ► Time taken for the expulsion of placenta after the calf delivery

- ► Incidence of any prepartum/postpartum metabolic or other diseases

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 cervix, uterus and ovaries and the ovarian structures including

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Table 3.1.   Distribution of experimental buffaloes by farms

| Farm code | Total number at the farm | Number of animals selected | | Total selected |
		Normal breeding* season calvers	Low breeding** season calvers	
A	51	5	7	12
B	27	8	0	8
C	16	5	4	9
D	10	2	2	4
E	47	8	0	8
F	16	2	3	5
G	35	0	5	5
Total	202	30	21	51

*   Normal breeding season (August to January)
*   Low breeding season (February to July)

Table 3.2.   Age and calving season-wise distribution of experimental buffaloes

Animal groups	Number of animals	Percent of selected animals
A. AGE GROUP		
1. 6-7 years	2	3.9
2. 8-12 years	24	47.1
3. 13-17 years	20	39.2
4. 18-22 years	5	9.8
Total	51	100.0
B. PERIOD OF CALVING GROUP		
1. Normal breeding season	30	58.8
2. Low breeding season	21	41.2
Total	51	100.0

Table 3.3.   Distribution of experimental buffaloes on the basis of body weight and body condition score 30 days prepartum

Animals group	Number of animals	Percent of total
A. BODY WEIGHT (kg)		
1. 416-449	6	11.8
2. 450-549	33	64.7
3. 550-599	12	23.5
Total	51	100.0
B. BODY CONDITION SCORE THIRTY DAYS PREPARTUM		
1. 1.0-2.0 (poor)	10	19.6
2. 2.5 (moderate)	28	54.9
3. 3.0-4.0 (good)	13	25.5
Total	51	100.0

Graafian follicles (GF) and corpora lutea (CL), or corpora albicancia (CA), was measured with fingers. Tonicity of the uterus was graded as good, fair, poor and meaty. The return of uterus to pelvic cavity was considered as an indication of uterine involution.

Normally, prepartum rectal palpation of the animals in advanced pregnancy was avoided to minimize stress to the animal. However, in case of delayed parturition, the animals were examined through rectal palpation.

3.3. Detection of postpartum estrus and ovulation

For the detection of first postpartum estrus, animals were examined twice daily, in the morning and evening, from 15th day postpartum until resumption of estrus. First postpartum ovulation was confirmed by palpation of an ovulation depression or a very soft corpus luteum developing on the ovary. In addition to visual signs of vulvular mucous, frequent micturition and bellowing at each farm, an intact bull was used for detection of estrus. Standing heat was used as a criterion for estrus confirmation. Natural breeding was allowed in buffaloes at least 60 days postpartum. On days 18 to 24 after mating, each buffalo was exposed to a bull to detect recurrence of estrus.

3.4. Milk yield, body condition scoring and body measurement

The animals were milked twice a day at the same 0100 and 1300 hours. Concentrate mixture was offered just before milking and calf was allowed to stimulate milk let down through suckling. In case of non-availability of calf, a calf from another dam was used for this purpose. If the dam was reluctant to accept suckling by a calf from

another dam, oxytocin injection was used for milk let down with a dose rate of 5 IU per time, intramuscularly, as per routine practice by the conventional farmers. In rare cases (11.8%) a buffalo was milked without the aid of suckling or oxytocin. Milk was collected through hand milking in steel buckets.

Milk yield was recorded (kg per day) once a week until 150th day postpartum. Fresh samples of milk were collected once a month and used for milk fat determination using Babcock Method (AOAC 1980). The yield was converted to 4% fat corrected milk (FCM) as suggested by Morrison (1949).

Body condition score was recorded at weekly intervals, using the method described for dairy and beef cows (Peters and Ball, 1987). In this method, the thickness of fat over the lumber and tail head area was estimated and assigned a score from 0 (emaciated) to 5 (very fat). Description of various scores is given below:

0 = Spine very prominent and transverse vertebral processes feel sharp with no fat cover

1 = Spine prominent and transverse process feel sharp with little fat cover

2 = Transverse processes can be felt but are rounded with a thin covering of fat

3 = Individual transverse vertebral processes can only be felt by firm pressure

4 = The transverse processes cannot be felt

5 = The transverse processes covered with a thick layer of fat

Distribution of experimental buffaloes on the basis of body weight and body condition score 30 days prepartum, is given in Table 3.3. Body measurements, including the height at withers, length from pin bone to scapular spine and girth (in inches) were recorded fortnightly. Body weight was calculated using the following formula recommended by Azhar (1976):

$$Y = -1604.790 + (1.268) X_1 + (30.902) X_2 + (3.960) X_3$$

(for 6-8 year old buffaloes) and

$$Y = -1263.663 + (8.060) X_1 + (18.924) X_2 + (8.565) X_3$$

(for buffaloes more than 8 years of age)

Where Y is weight of buffalo in pounds, X_1 is height at withers (in inches), X_2 is heart girth immediately behind the shoulders in (inches) and X_3 is length from the point of shoulder to the point of pin bone (in inches). Finally, the body weight was converted from pounds to kg.

3.5 Feed sampling and analysis

Representative samples of all the ingredients fed to the experimental animals were collected individually at fortnight intervals. A portion of each sample were processed for dry matter contents (AOAC, 1980) and the remaining samples were air dried at 60°C for 48 hours, ground through 1 mm sieve in a Wiley mill and stored for further analysis. Due to large number of feed samples (444) collected, these were pooled period-wise for each of the farm and analyzed for ash, crude protein, crude fiber and ether extract according to AOAC (1980). The nitrogen free extract (NFE) was determined using the following formula:

$$NFE = 100 - (CF\% + EE\% + CP\% + TM\%)$$

Whereas   CF   =   Crude fiber (%)

               EE   =   Ether extract (%)

               CF   =   Crude protein (%)

               TM   =   Total mineral (%)

3.5.1 Energy value of feeds

Digestibility of various fodders was calculated using digestibility coefficients reported by Ishaque and Malik (1972) for Nili-Ravi buffaloes. Total digestible nutrients (TDN) values of feeds was estimated using the following equations suggested by Choo (1982):

%TDN (Protein supplements)   = $-133.726 - 0.254\,(CF) + 19.593\,(EE) + 2.784\,(NFE) + 2.315\,(Pr) + 0.028\,(CF)^2 - 0.341\,(EE)^2 - 0.008\,(CF)\,(NFE) - 0.215\,(EE)\,(NFE) - 0.193\,(EE)\,(Pr) + 0.004\,(EE)^2\,(Pr)$

%TDN (Energy feeds)   = $-202.686 - 1.357\,(CF) + 2.638\,(EE) + 3.003\,(NFE) + 2.347\,(Pr) + 0.046\,(CF)^2 + 0.647\,(EE)^2 + 0.41\,(CF)\,(NFE) - 0.081\,(EE)\,(NFE) + 0.553\,(EE)\,(Pr) - 0.046\,(EE)^2\,(Pr)$

%TDN (Dry forages)   = $92.464 - 3.338\,(CF) - 6.945\,(EE) - 0.762\,(NFE) + 1.115\,(Pr) + 0.031\,(CF)^2 - 0.133\,(EE)^2 + 0.036(CF)\,(NFE) + 0.207\,(EE)\,(NFE) + 0.100\,(EE)\,(Pr) - 0.022\,(EE)^2\,(Pr)$

The TDN, digestible energy (DE) and metabolizable energy (ME) of green fodders were calculated as recommended by NRC (1989), as follows:

$$TDN = DP\% + DCF\% + DNFE\% + (2.5 \times DEE\%)$$

Where   TDN   =   Total digestible nutrients

DP   =   Digestible protein (%)

DCF   =   Digestible crude fiber (%)

DNFE =   Digestible nitrogen free extract (%)

DEE   =   Digestible ether extract (%)

The TDN values were used for estimation of digestible energy (DE) and metabolizable energy (ME) as suggested previously (Crampton et al., 1957; Swift, 1957; Moe and Tyrell, 1976):

$$DE\ (MCal/kgDM) = 0.04409 \times TDN\%$$

and

$$ME\ (MCal/kgDM) = 0.45 + 1.01 \times DE$$

Nutrient composition of forages used for feeding to experimental buffaloes is given in Table 3.4 and of concentrate supplements in Table 3.5.

3.5.2 In sacco dry matter degradibility

Dry matter and protein degradibility in rumen was determined using the in sacco technique described by Orskov et al. (1980). Incubation of dacron bags was carried out

Table 3.4 Nutrient composition of forages used for feeding experimental buffaloes

Feed	Harvest-month	Cut number /growth stage	Dry matter (%)	Ash	Crude protein	Crude fiber	Ether extract	N-free extract	Metaboliza-ble energy (Mcal/kg DM)
Green Fodders									
Berseem	Nov-Dec	1st	13.62	14.91	20.96	17.38	2.20	44.55	2.67
	Jan-Feb	2nd	14.32	10.90	21.85	20.13	2.15	51.68	2.87
	Mar-May	3rd, 4th	15.36	13.02	20.27	24.54	2.10	40.08	2.47
Sorghum	Jul-Aug	Early	30.30	10.41	7.24	34.73	2.18	45.44	2.85
	Sep	Dough	35.25	8.20	5.69	34.67	3.82	49.63	2.97
	Oct-Nov	Full	37.50	7.86	5.33	39.84	3.80	43.18	3.35
Maize	Jun-Aug	Early	26.25	7.41	6.36	30.67	1.32	66.65	2.95
	Sep-Nov	Full	33.74	10.40	8.66	24.41	1.90	60.47	2.95
Wheat	Jan-Feb	Early	22.50	10.28	10.35	26.83	1.31	51.24	2.68
	Mar-Apr	Mid	27.53	7.83	7.67	29.01	1.36	54.13	2.78
	May	Full	34.12	5.93	7.20	31.83	1.25	68.31	2.49
Dry Roughages									
Maize stovers	-	-	95.09	6.04	3.72	1.08	1.02	48.36	1.73
Wheat straw	-	-	93.74	9.93	4.21	1.13	0.85	40.94	1.58

Table 3.5 Nutrient composition of concentrate supplements used for feeding experimental buffaloes

Feed	Dry Matter (%)	As percent in dry matter					Metabolizable Energy (Mcal/kg DM)
		Ash	Crude Protein	Ether Extract	Crude Fiber	Nitrogen-Free Extract	
Wheat bran	90.44	3.87	17.13	4.50	8.67	68.09	3.68
Cotton seed cake (undec.)	91.88	5.46	24.97	9.05	26.61	34.92	5.44
Mustard seed cake	92.01	6.79	31.61	13.81	10.32	36.99	2.93
Maize oil cake	95.38	1.94	20.50	11.68	14.17	51.72	2.72
Commercial Concentrate	92.60	6.68	15.66	3.21	13.02	61.43	2.98
Dried bread	82.66	2.49	20.39	2.00	2.52	72.59	4.57
Wheat grain	93.00	1.32	13.00	2.50	2.16	81.01	4.78
Beet pulp dried	95.06	4.49	11.96	2.08	27.07	54.40	2.31
Molasses	71.92	13.6	8.99	0.00	0.00	77.40	1.81

in two rumen fistulated buffalo steers (age 6 years and Body weight 420 kg). The animals had permanent rumen fistulas with an internal diameter of about 13 cm. They were fed 6 kg wheat straw (DM 91.0 %) and 20 kg fresh berseem fodder (DM 14.7%), once a day. The bags (140 x 90 mm) made of dacron cloth having a uniform pore size of 40 um were used. Feed samples, previously ground through a 2 mm mesh screen were sieved to remove fine particles and 3 g sample was weighed into each bag in triplicate. Ruminal incubation times were 4, 8, 12, 24, 48 and 72 hours. On termination of each incubation, the bags were taken out from the rumen and the loose material adhering to the outer surface of the bags was washed with cold running tap water. Finally, the bags were washed three times in a washing machine for 5 minutes each, using cold tap water (10-15°C). The bags were then dried in an oven at 70°C for 72 hours, cooled in a desiccator and reweighed.

Zero hour dry matter degradability of all the test samples was measured by suspending the bags containing sample for 10 minutes in water at 37°C, followed by washing and drying as described above. The percentage of disappearance of DM at each incubation time was calculated from the proportion remaining in the bag after incubation in the rumen. The degradation rate of dry matter (P) from the bag at time (t) in the rumen was described by the equation of the form:

$$P = a + b \left(1 - e^{ct}\right)$$

where a, b and c were fitted by an iterative least square procedure (Orskov and McDonald, 1979). The exponential constants a, b and c are measures of the rapidly soluble DM fraction, the degradable DM fraction and the rate constant for the

degradation of "b", respectively. The effective degradibility (ED) of dry matter in the rumen was calculated by the following equation:

$$ED = a + bc / (c + k)$$

(Orskov and McDonald, 1979)

The value of k which represents the estimated outflow rate of particulate from the rumen were chosen as 2.0, 4.0 and 6.0 % per hour.

Protein degradibility parameters of forages used for buffaloes feeding is presented in Table 3.6.

3.5.3 Mineral analysis of feed

3.5.3.1 Preparation and digestion of samples

Ten ml of the nitric acid and perchloric acid mixture (5:1) was added to 0.5 g of feed sample in a conical flask and heated slowly until 1 ml extract was left. The flask was cooled and 15-20 ml of de-ionized water was added to it. The volume was made up to 50 ml with de-ionized water and stored in plastic bottles until analysis.

3.5.3.2 Analysis for copper, zinc, calcium and magnesium

The digested samples were analyzed for copper and zinc using Atomic Absorption Flame Emission Spectrophotometer (Schimadzu, Japan). A series of standard solutions comprising blank, 0.2, 0.4, 0.6, 0.8 and 1.0 ppm were used for getting standard curve for zinc. For copper, the standard concentrations used were blank, 1.0, 2.0, 4.0, 8.0 and 16.0 ppm. Copper was determined at a wave-length of 324.8 nm and zinc at 213.9 nm.

Table 3.6   Protein degradability parameters of forages used for buffalo feeding

Feeds	*a (%)	b (%)	c (%/hr)	** Effective protein degradability			
				0.02	0.04	0.06	0.08
Green fodders							
Berseem (1st cut)	14.01	78.04	12.10	78.70	68.65	60.85	54.60
Berseem (2nd cut)	23.43	69.09	9.13	78.55	68.90	61.85	57.75
Berseem (3rd cut)	14.61	77.86	11.17	78.90	69.05	61.50	55.65
Maize (early stage)	19.64	49.51	5.48	55.85	48.20	43.25	39.75
Maize (late stage)	10.97	60.33	6.98	57.80	49.30	43.40	39.05
Sugar cane whole	25.45	36.29	2.57	45.80	39.60	36.30	34.25
Wheat (mid stage)	30.54	36.31	13.96	63.00	59.35	56.50	54.15
Sorghum (late stage)	21.08	40.11	5.56	50.20	44.00	40.05	37.20
Protein Supplements							
Cottonseed cake(undec.)	56.58	30.89	17.23	89.10	86.43	84.25	82.38
Mustard seed cake	54.50	38.91	19.75	89.87	86.95	84.47	82.37
Maize-oil cake	41.32	56.77	8.11	87.33	80.28	75.03	71.03
Wheat bran	56.41	38.53	29.43	92.20	89.90	87.95	86.25
Commercial Concentrate	63.24	31.53	5.88	85.80	80.70	77.45	75.15

*   a, b, c are measures of the rapidly soluble crude protein fraction at "zero" hour, during 48 hours other
      than "a" and rate constant disappearance of "b", respectively.
**   Effective Protein Degradability at different rumen outflow rates.

(5 ml of 5M HCl) and reagent-II (5 ml of ammonium molybdate vanadate reagent) and incubated at room temperature for 30 minutes. Absorbance of the mixture was read at 470 nm using Spectronic-20 (Bausch and Lomb, USA). Regression of absorbance of standards against concentrations was worked out. Constant factor and co-efficient of variation were determined for each batch of assay. Concentration of phosphorus in the sample was calculated as follows:

$$Y = (a + b \times X) / 100$$

Where

Y = concentration of Phosphorus in sample (% of dry matter)

a = constant

b = Coefficient of variation

X = Absorbance of sample

Mineral composition of feeds used for feeding to experimental buffaloes is given in Table 3.7.

3.5.4 Energy and protein requirements of lactating buffaloes

For calculating feed requirement of experimental buffaloes, the following values were used:

a) For maintenance, the values of 125 kcal/kg B.Wt$^{0.75}$, used by Sen et al., (1978) and followed by Kearl (1982) and Ranjhan and Pathak (1979), were used.

b) For milk production, the values of 1188 kcal per kg 4% fat corrected milk (FCM), as recommended by Ranjhan and Pathak (1979), were used.

The digestible protein requirements for maintenance were calculated as used by

The concentrations of copper and zinc were caculated in parts per million and converted into parts per million on dry matter basis as follows:

$$Min = PPM \times 100 + DM\%$$

Where Min is the mineral concentrations in the feedstuff as parts per million on dry matter basis, PPM is the concentration of mineral in the digested solution and DM% is the dry matter in the feedstuff.

Calcium and magnesium were also determined using atomic absorption spectrophotometry. The series of standard solutions used for getting standard curve comprised 0, 50, 100, 200 ppm for calcium and 0, 5, 10, 20 ppm for magnesium. Calcium was determined at a wavelength of 422.7 nm and magnesium at 285.2 nm. Calcium and magnesium concentrations were recorded in ppm and converted into percentage on dry matter basis as follow:

$$Min\% = PPM \times 100 \times 100 \times DM\% / 1000000$$

Where Min% = Mineral concentrations in the feedstuff (%) on dry matter basis

PPM = Concentration of mineral in the digested solution

DM% = Dry matter in the feedstuff (%).

3.5.3.3 Analysis for phosphorus

The prepared samples were used for phosphorus assay. A 5 ml of the sample or phosphorus standards (blank, 10, 20, 30, 40, 50 and 60 μg/ml) was mixed with reagent-1

(5 ml of 5M HCl) and reagent-II (5 ml of ammonium molybdate vanadate reagent) and incubated at room temperature for 30 minutes. Absorbance of the mixture was read at 470 nm using Spectronic-20 (Bausch and Lomb, USA). Regression of absorbance of standards against concentrations was worked out. Constant factor and co-efficient of variation were determined for each batch of assay. Concentration of phosphorus in the sample was calculated as follows:

$$Y = (a + b \times X) / 100$$

Where Y =   concentration of Phosphorus in sample (% of dry matter)

                a =   constant

                b =   Coefficient of variation

                X =   Absorbance of sample

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The digestible protein requirements for maintenance were calculated as used by

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Table 3.7   Mineral composition of feeds used for feeding experimental buffaloes (on dry matter basis)

Feed	Calcium (%)	Magneseum (%)	Phosphorus (%)	Zinc (ppm)	Copper (ppm)
Green forage					
Berseen	3.031	0.461	0.302	70.65	68.490
Sorghum	0.212	0.159	0.149	60.10	6.908
Maize	0.409	0.215	0.192	49.80	7.139
Wheat	0.272	0.141	0.181	43.66	6.630
Sugar cane	0.628	0.162	0.053	50.24	9.432
Dry roughage					
Maize stovers	0.492	0.298	0.078	57.13	8.698
Wheat straw	0.309	0.085	0.029	49.87	5.727
Concentrate					
Wheat bran	0.086	0.257	0.750	135.10	11.50
Cotton seed cake (undec.)	0.186	0.239	0.423	74.08	11.45
Mustard seed cake	0.961	0.320	0.592	107.10	17.83
Maize oil cake	0.101	0.061	0.235	91.77	11.05
Commercial concentrates	0.422	0.411	0.572	64.89	15.87
Dried bread	0.400	0.080	0.269	0.50	11.00
wheat grain	0.413	0.083	0.273	60.00	11.00
Beet pulp	0.600	0.200	0.056	102.80	10.00
Molasses	0.672	0.049	0.022	82.96	9.23

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Kearl (1982) (2.54 g/kg $BWt^{0.75}$) and requirements for milk production was calculated as recommended by Ranjhan (1980) (126 g/100 g of protein secreted in milk). Milk protein contents of Nili-Ravi buffaloes were assumed as 4.93% (Makhdum, 1983).

3.5.5 Feed intake

Feed components of the experimental animals comprised green fodders, concentrates and wheat straw. Green fodder during the winter and spring comprised Egyptian clover with higher crude protein and moisture. During autumn and early winter, sorghum and maize were available, having higher levels of dry matter and relatively low crude protein. During late winter and spring, wheat crop was fed while chopped sugar cane was fed during winter and spring. Wheat straw or dried maize stovers were fed through out the year with an increasing levels during winter and early summer. Concentrate feeds comprised wheat bran, cotton seed cake (undecorticated), mustard seed cake, maize oil cake, commercial concentrates, dried bread, wheat grain, beet pulp and molasses and were fed to lactating buffaloes at the same scale, irrespective of milk production, as per conventional practice.

Six out of seven owners of the experimental farms were landless and they either rented land for fodder cultivation or purchased standing fodder in the field. The common practice of feeding fodder to buffaloes included cut and carry system with no grazing throughout the year. With the existing land acquiring system for fodder by the farmers, the fodder availability was just enough to satisfy the timely requirements of the animals with no surplus for conservation into silage or hay. During the fodder scarcity periods (May, June and December), the shortage was compensated by including

more cereal straws or stovers in the buffalo rations with no increase in the concentrate allowance.

3.6 Blood sampling and analysis

Jugular blood samples were collected fortnightly, from two months before parturition until the resumption of postpartum estrus or day 150 postpartum, in case of anestrus. Immediately after collection, samples were taken to the laboratory in an ice box and centrifuged at 3000 rpm for 10 minutes at 4^0C. The serum was collected in screw capped bottles (5 ml in duplicate) and stored at -20^0C until used for chemical analysis.

A total of 499 serum samples were collected during the study from 51 animals. Due to large number of serum samples, animals were selected randomly representing each season, postpartum estrus interval and farm. From the selected animals, 261 serum samples were assayed in duplicate for glucose, urea, protein, calcium, phosphorus and magnesium, using Clonital kits. Assay particulars are summarized in Table 3.8. Each batch of assay contained standard in duplicate. Absorbance of the samples and standards was read at specific wavelengths recommended by the manufacturer, against the blank using cuvette with 1 cm light path in Lambda 1 UV/VIS Spectrophotometer (Perkin - Elmer). Principles and brief-character-istics of the assays are presented as follows:

3.6.1 Serum urea assay

The frozen serum was thawed, homogenized and assayed for urea levels with the Urea Color Berthelot modified method, using Urease Salicylate Kit (Cat. No. KC-022, Clonital). In the presence of urease, urea is hydrolyzed to ammonia and carbon

Table 3.8. Characteristics of serum metabolites assays (Clonital kits, Sede administrativa e productiva 24030-CARVICO (BG) ITALY)

Serum metabolite	Method	Standard/ sample volume(μl)	Reagents volume (ml)	Wavelength (nm)	Inter-assay coefficient of variation
Glucose	Trinder method, end point	20	2.0	546	2.60
Urea	Berthelot modified method, urease/ salicylate	20	2.5	600	6.40
Protein	Biuret method	25	2.5	546	5.35
Calcium	MTB colorimetric method	50	3.0	612	6.20
Magnesium	Xylidyl blue method	10	2.0	512	15.80
Inorganic Phosphorus	Colorimetric method	100	3.0	658	11.35

dioxide. The ammonia was then measured colorimetrically by a modified Berthelot's reaction which is linear up to 300 mg/100 ml. The color of the reaction is stable for 60 minutes, at least.

3.6.2 Serum glucose assay

Glucose levels were determined using the Glucose Liquid Formulation, Trinder Method End Point kit, Clonital (Cat. No.-KC-022). The principle of this method is that glucose is transformed by glucose oxidase to gluconic acid and hydrogen peroxide, which in the presence of peroxidase oxidizes the chromogen, 4-amino-phenazone/phenol to form red quinoneimine, color intensity of the later is proportionate to the concentration of glucose in the sample. Intensity of the color was measured colorometrically. The method is liner up to 500 mg/100 ml (27.8 mmol/l) of glucose. The color of the reaction is stable for at least 2 hours, at 15 to 25 ^{0}C, when protected from direct light.

3.6.3 Serum protein assay

Protein levels were determined using the Total Protein, Biuret Method, Clonital Kit (Cat. No. KC-025). In this method protein reacts with alkaline copper solution of biuret reagent producing a violet blue color, which is measured colorimetrically. The reaction is liner up to serum protein concentration of 120 g/l.

3.6.4 Serum calcium assay

Serum calcium concentrations were estimated using the Calcium MTB Colorimetric Method, Clonital Kit (Cat. No. KC-107). In this method calcium in an

alkaline medium forms, with MTB, a blue complex which is measured colorimetrically. The determination is not influenced by the presence of magnesium, phosphorus or bilirubin in the sample. In particular the interference caused by magnesium is eliminated by the 8-oxyquinoline complex. The reaction is linear up to serum calcium concentration of 14 mg/100 ml.

3.6.5 Serum inorganic phosphorus assay

Serum inorganic phosphorus levels were studied using the Inorganic Phosphorus, Colorimetric Method, Clonital Kit (Cat. No. KC-120). In this method inorganic phosphorus in the solution reacts with molybdate in an acid solution to form phosphomolybdic acid, which, in the presence of ammonium iron sulphate, is reduced to molybdenum blue, the intensity of which is proportional to the concentration of phosphorus in the sample. The method is linear up to 15 mg/100 ml (4.83 mmol/l). The color of the reaction is stable for, at least, one hour at 15 to 25°C.

3.6.6 Serum magnesium assay

Serum magnesium levels were determined using Magnesium Xylidyl Blue Method, Clonital Kit, (Cat. No. KC-037). In this method magnesium reacts with colorant xylidyl-blue to form a purple complex, intensity of which is proportional to the concentration of magnesium in the sample. Calcium in biological liquids does not influence the reaction. The method is linear up to serum magnesium concentration of 97.6 mg/l.

3.7 Milk sampling and progesterone assay

Milk samples were collected every week. Fat layer was removed and 100 μl of 0.1% sodium azide was added to 5 ml of milk sample, as a preservative. Samples were stored at -20°C until analysis for progesterone assay. A total of 1287 milk samples were collected and stored from 51 animals but due to large size of samples, 385 samples were selected randomly representing each season, postpartum estrus interval and farm and analyzed for milk progesterone concentrations. Radio-immunoassay (RIA) technique was used for determination of milk progesterone levels following the procedure proposed by FAO/IAEA (1993). The kits and protocols were prepared by the Animal Production Unit, Agriculture Laboratories, Seibersdorf, Austria, in collaboration with Diagnostic Products Corporation, Los Angeles, USA, in support of the programs of the Animal Production and Health Section of the Joint FAO/IAEA Division on Nuclear Technique in Food and Agriculture. The kits were developed for the purpose of measuring progesterone in milk to aid in monitoring the estrous cycle and pregnancy in domestic livestock species.

Milk samples and other assay components were brought to room temperature before starting the assay. The antibody coated polypropylene tubes were labelled for standard, quality control and samples, according to the protocols. Non-coated normal tubes were used for total count. 100 μl of standard, quality control or sample were pipetted into bottom of the corresponding tube. Then 1 ml of ^{125}I-progesterone was pipetted to each tube. Tubes were covered with parafilm and incubated at 4°C overnight. The next morning, the tubes were decanted vigorously except those for total count. The radioactivity was counted in a gamma counter (Vegacalc (c) NE Technology Limited). The data were analyzed by using Vegacalc program. Intra- and inter-assay coefficients

of variation were 3.54% and 9.21% respectively. Sensitivity (detection limit) of the assay was 0.09 ng/ml.

3.8 Meteorological data

Details on daily maximum and minimum ambient temperature, relative humidity and rain-fall were collected from two stations of the University of Agriculture Peshawar *viz* Agricultural Research Institute, Tarnab and Sugar Crop Research Institute, Mardan, for the whole experimental period of 18 months.

3.9 Statistical analysis

The data obtained were recorded in computer based files and statistically analyzed with correlation analysis and analysis of variance procedures (Steel and Torrie, 1980), using a computer package.

CHAPTER 4

RESULTS AND DISCUSSION

In this study various aspects of reproductive performance of buffaloes under conventional farming system in NWFP, were studied and results are discussed under three sections. Parameters of postpartum reproductive performance, comprising postpartum uterine involution (PUI), resumption of ovarian activity, postpartum estrus interval (PEI), seasonality of reproduction and milk progesterone levels are described in the first section. The second section describes relationship of pre- and postpartum nutritional status with reproductive performance. Effects of other factors, *viz.* body condition score (BCS), calf suckling duration and milk production are included in section three.

4.1 REPRODUCTIVE PERFORMANCE UNDER CONVENTIONAL FARMING IN NWFP

4.1.1 Uterine involution

The mean value for PUI interval was 34.30 ± 1.33 days (Table 4.1), ranging from 21 to 74 days. Uterine involution completed within 35 days in 55.0% buffaloes, within 50 days in 85.0%, and within 74 days in all buffaloes (Table 4.2). In all but two animals uterine involution was completed within two months after calving. Of these two buffaloes, one had dystokia and the other suffered from the retained placenta with subsequent metritis.

Almost similar PUI intertval was reported for Sri Lankan (32.9 days, Perera *et al.*, 1984) and Egyptian buffaloes (34.7 ± 1.5 days, El-Shafie *et al.*, 1983). Shorter mean intervals of 26.0 (Usmani *et al.*, 1985), 27.5 ± 8.3 and 28.37 ± 1.36 days (Chaudhry *et al.*, 1987 and 1990,

Table 4.1. **Mean values ± 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 ± 0.92 (38)*	4.40 ± 1.03a (23)	6.64 ± 1.51a (15)
Lochia discharge (days)	6.02 ± 1.24 (50)	5.66 ± 1.39a (29)	6.52 ± 2.23a (21)
Postpartum uterine involution interval (days)	34.30 ± 1.33 (50)	36.19 ± 5.07a (29)	31.52 ± 3.09a (21)
Postpartum estrus interval (days)	69.03 ± 6.03 (35)	55.95* ± 4.90b (22)	91.15 ± 11.61a (13)
Postpartum corpus luteum interval (days)	59.37 ± 4.76 (43)	55.24 ± 5.77a (25)	65.11 ± 7.86a (18)
Conception rate (%)	45.10 ± 6.97 (51)	43.33 ± 9.05a (30)	47.62 ± 10.90a (21)

a,b The value with different letters differ significnatly (P<0.01)
* Values in parenthesis represent the number of observations

Table 4.2. **Factors associated with postpartum uterine involution interval (PUI) in buffaloes**

PUI (days)	No. of animals (%)	BCS* loss 30 days postpartum	Placenta expulsion (hours)	Postpartum estrus interval (days)
< 30	9 (22.5%)	0.19	3.80	47.0
30 to 35	13 (32.5%)	0.21	5.06	67.8
36 to 50	12 (30.0%)	0.17	7.25	70.4
> 50	6 (15.0%)	0.36	11.40	87.1
r^2	-	0.66	0.99	0.82
b	-	0.01	0.17	0.74

BCS   Body condition score
r^2 Co-efficient of determination of regression
b   X co-efficient

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respectively) have been reported for complete uterine involution in Nili-Ravi buffaloes. However, these three studies were conducted at state farms where management practices seem better than those at private farms. Longer time required for completion of uterine involution in buffaloes was reported by some workers. Indian buffaloes completed uterine involution in 45 and 39 days (Roy and Luktuke, 1962; Chauhan et al., 1977, respectively). In Sri Lankan buffaloes it took 42 days (Peiris et al., 1980) and in Egyptian buffaloes, 40.3 ± 1.2 days (Aboul-Ela et al., 1988), have been reported. Difference in results among various studies might be due to variations in criteria of recording or methods of measuring involuting reproductive organs, seasons of study, breed or managemental practices.

Factors associated with PUI are summarized in Table 4.2. PUI interval was positively correlated with placental expulsion duration ($r^2 = 0.99$, $b = 0.17$). It appears that prolonged duration of placental expulsion causes stress to the animal and delays uterine involution. In agreement to this study, Jainudeen (1984) reported that uterine involution completed sooner after normal than after abnormal parturition.

Correlation of PUI with nutrients intake and serum mineral concentrations is presented in Table 4.3. Increasing crude protein (CP) intake ($r = -0.23$, $P < 0.01$) and CP:ME ratio ($r = -0.16$, $P < 0.01$) decreased PUI interval while metabolizable energy (ME) intake did not affect it. Intake of calcium ($r = -0.14$, $P < 0.05$), phosphorus-($r = -0.32$, $P < 0.01$), zinc ($r = -0.50$, $P < 0.01$) and copper ($r = -0.31$, $P < 0.01$) related negatively, while magnesium positively ($r = 0.37$, $P < 0.01$), with PUI interval. Serum calcium concentrations related positively ($r = 0.31$, $P < 0.01$) while magnesium negatively ($r = -0.20$, $P < 0.05$), with uterine involution interval and serum phosphorus concentrations had no effect on it.

PUI interval was positively correlated with prepartum body weight ($r^2 = 0.52$) and loss

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Table 4.3. Correlation of uterine involution interval with nutrients intake, serum mineral concentrations and other parameters in buffaloes

Parameter	Postpartum uterine involution	
	Number of observations	Pearson's corre-lation coefficient
Nutrients intake		
Crude protein (CP)	379	-0.23**
Metabolizable energy (ME)	379	0.07NS
CP/ME ratio	379	-0.16**
Calcium	301	-0.14*
Magnesium	326	0.37**
Phosphorus	326	-0.32**
Zinc	326	-0.50**
Copper	307	-0.31**
Serum mineral concentrations		
Calcium	124	0.31**
Magnesium	123	-0.20*
Phosphorus	103	0.06NS
Other		
Age	42	0.29**
Calf survival	271	0.05NS

NS   Non-Significant
*   Significant at P<0.05
**   Significant at P<0.01

Table 4.4   Effect of calving period and farm on postpartum uterine involution in buffaloes

Source	Degree of freedom	Sum of squares	Mean square	F value
Farm	6	2467.9	44.3	0.92 NS
Calving period	1	0.7	0.7	0.00 NS
Error	34	15260.3	448.8	-
Corrected total	41	17228.9	-	-

NS   Non-significant difference among groups

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in BCS 30 days postpartum ($r^2=0.66$, Fig. 4.1). This suggests that prevention of postpartum loss in BCS and maintenance of good health in postpartum buffaloes is important in preparing the reproductive organs for early resumption of their physiological functions. In some buffaloes (22.5%) the uterus was completely involuted by day 30 postpartum and these animals had lower body weight of 499.2 kg and BCS loss of 0.19.

PUI interval was negatively correlated with frequency of use of oxytocin ($r^2=0.59$, Fig. 4.2). Oxytocin injections are commonly used by local farmers for milk let down in buffaloes that have no calf in feet. Oxytocin stimulates contraction of myometrium (Reeves, 1987) and it's frequent use may lead to accelerated uterine involution. In the present study, calf suckling did not affect uterine involution or postpartum interval to first estrus (Fig. 4.3). The effect of suckling on these parameters was probably confounded by the use of exogenous oxytocin. This agrees with Wongsrikeao *et al.* (1991) who found that limited or *ad libitum* suckling had no effect on PUI in Swamp buffaloes.

However, in other studies uterine involution was completed quickly in suckled than in non-suckled or milked buffaloes (Jainudeen, 1984). Similarly, Usmani (1987) found that limited-suckled buffaloes showed shorter periods of uterine involution (23.4 versus 38.7 days) than non-suckled buffaloes. The suckling stimulus and milking have been reported to cause release of oxytocin from the posterior pituitary which enhanced uterine involution in dairy cows (Leslie, 1983), the effect of the former seems to be stronger than the latter due to it's high frequency.

Age of the animal, farm and calving period (normal or low breeding season) had non-significant effects on PUI in the present study (Table 4.3, 4.4). However, an increasing trend was observed in the PUI interval with the increasing age which support the findings of Roy and
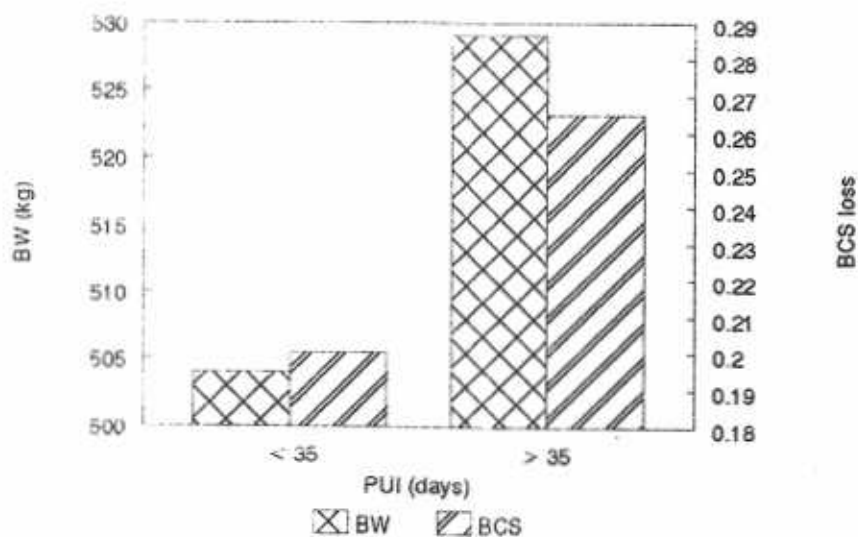
Figure 4.1. Effect of prepartum body weight (BW, $r^2=0.52$) and loss of body condition score (BCS, $r^2=0.66$) 30 days postpartum, on uterine involution (PUI) in buffaloes
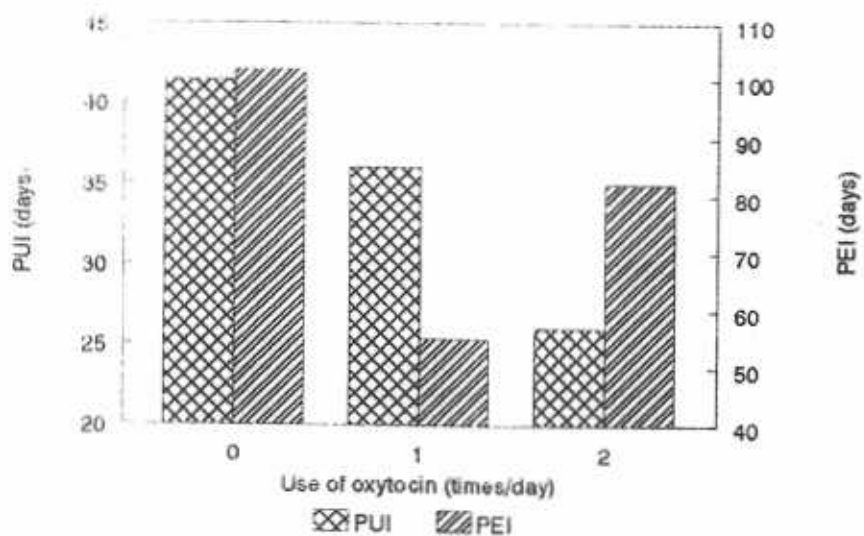
Figure 4.2. Effect of use of oxytocine injections on uterine involution (PUI, $r^2=0.59$) and estrus interval (PEI, $r^2=0.60$) in buffaloes
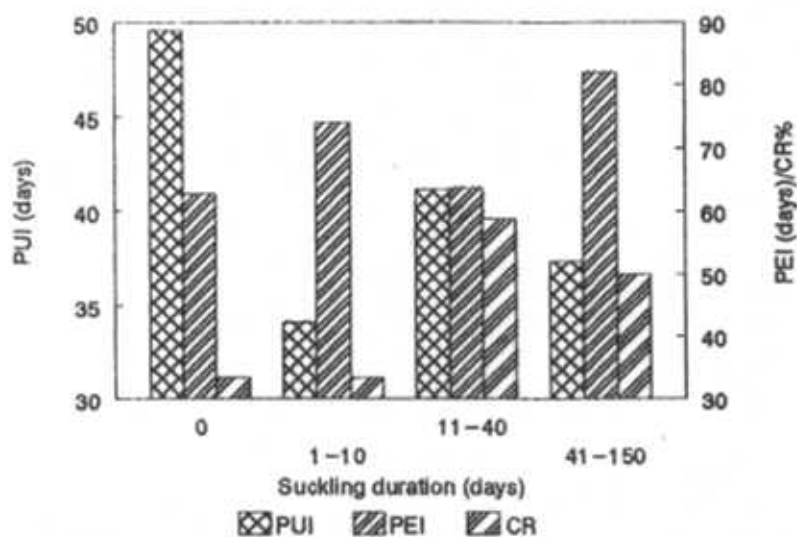
Figure 4.3. Effect of suckling duration on uterine involution (PUI), estrus (PEI)
and conception rate (CR) in buffaloes
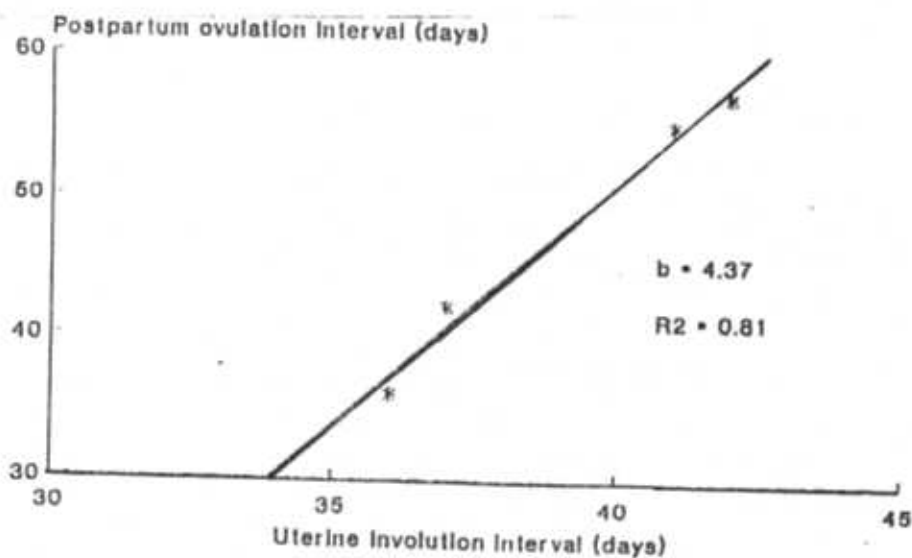
Figure 4.4. Relationship of uterine involution with ovulation in postpartum buffaloes

Luktuke (1962). Lack of the effect of calving period on PUI is not in line with Chauhan *et al.* (1977) who found faster rates during spring and winter than other seasons.

Interestingly, the delayed uterine involution was associated with a delayed resumption of postpartum ovarian activity. Both PEI ($r^2=0.82$, $b=0.74$) and postpartum ovulation interval (POI) ($r^2=0.81$, $b=4.27$) were positively correlated with PUI which suggests that early completion of uterine involution may be a key factor in resumption of ovarian activity (Table 4.2, Fig. 4.4). Similarly, Usmani (1987) reported that longer interval to first ovulation was associated with longer PUI interval. Chaudhry *et al.* (1990a) reported that PUI interval and PEI were positively correlated. However, Kirakofe (1980) concluded that uterine involution has no relationship with the length of postpartum anestrus.

In the present study, majority (62.5%) of buffaloes completed uterine involution between 30 and 50 days postpartum which seems to be a desirable interval because according to Graves *et al.* (1968), PUI shorter than 20 days is associated with very low fertilization and pregnancy. This low conception rate was attributed to the physical barrier to sperm transport and implantation of zygote and not any inherent defect in the ova or other physiological mechanisms (Short *et al.*, 1990) .

The present findings suggest that buffaloes may be kept in good body condition through proper feeding to hasten uterine involution and thus reduce the postpartum interval to ovulation and resumption of ovarian activity.

4.1.2 Postpartum estrus and ovulation

Observations on postpartum reproductive events were recorded until 150 days postpartum. During this period, 68.63% buffaloes were found in estrus and the remaining 31.37% animals

remained anestrous (Table 4.5). The overall mean PEI was 69.03 ± 6.03 days, ranging from 21 to 147 days (Table 4.1). Mean postpartum ovulation interval (POI), based on detection of palpable corpus luteum per rectum, was 59.37 ± 4.76 days, ranging from 24 to 150 days.

Under the conditions of the present study, a shorter PEI in buffaloes was recorded than that reported by other workers. Ishaque (1969) reported that 44% of Nili-Ravi buffaloes showed estrus within 120 days postpartum, while 56% had a PEI longer than 120 days. Similarly, in studies conducted by Waheed (1975), 55% buffaloes came into estrus within 200 days after parturition. Under village conditions in Punjab, 30% buffaloes showed estrus within 90 days while 70% beyond 90 days postpartum (Chaudhry et al., 1985). A wide range of 30-750 and 21-915 days for PEI has been reported for Nili-Ravi buffaloes under village conditions in Pakistan (Jost, 1979; Zafar, 1983). However at state farms, relatively shorter PEI of 124 ± 14.58 days, ranging from 22 to 280 days, was reported for Nili-Ravi buffaloes (Chaudhry et al., 1989).

The shorter PEI recorded in this study might be due to better managemental and nutritional status of the experimental animals, as the animals were mostly kept on commercial basis, fed with high energy diets and maintained in a better BCS. In addition, the estrus detection regime during the study period was good.

A total of 66 estrus events were observed in the experimental animals during the study period (Table 4.6). Based on the observable estrus signs and milk progesterone levels, three types of estrus events were observed i.e. ovulatory estrus (43.9%), anovulatory estrus (4.6%) and silent estrus (51.5%). About 62.1% of the total 29 ovulatory estrus events occurred in NBS calvers while 37.9% in LBS calvers. Anovulatory estrus was not a notable problem in the experimental animals. However, silent ovulation was identified as the most prevalent

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Table 4.5   Postpartum estrus intervals in 51 buffaloes

Group	Postpartum estrus interval (days)	Number of Buffaloes	Percentage of total population	Cumulative Percentage
1	< 45	11	21.57	21.57
2	45 to 74	12	23.53	45.10
3	75 to 150	12	23.53	68.63
4	Anestrus	16	31.37	100.00
Total	-	51	100.00	-

Table 4.6.   Type of estrus events based on milk progesterone concentrations and clinical signs of estrus in 51 buffaloes during normal (NBS) and low breeding season (LBS)

Estrus groups	Estrus events		Calving period			
	Number	(Percent)	NBS		LBS	
			Number	Percent	Number	Percent
Ovulatory estrus	29	43.9	18	62.1	11	37.9
Silent estrus	34	51.5	10	29.4	24	70.6
Anovulatory estrus	3	4.6	2	66.7	1	33.3
Total	66	100.0	30	45.5	36	54.5

reproductive problem, with 29.4% of the cases occurring in NBS calvers and 70.6% in LBS calvers.

In a previous study, 27.7% of 47 Egyptian buffaloes showed normal sequences of estrus and ovulation, 10.6% of which had atretic follicles. Out of 50 ovulations detected, 64.0% ovulations were not accompanied by observable signs of estrus (Aboul-Ela et al., 1988). The high incidence of silent estrus was attributed to poor expression of estrus symptoms and inefficient detection of estrus in these buffaloes. In the present study, although intact bulls were used twice daily for detection of estrus, in addition to observation of visual estrus symptoms like frequent micturition, vulvar mucus and bellowing. A considerable number of estrous events may have been missed even by the bull, due to poor expression of symptoms (Jainudeen et al. 1983a).

The incidence of silent estrus in the present study (51.5%) was less than 82.4 and 56.0% reported for Egyptian buffaloes by Khattab et al. (1990 and 1988), respectively, but higher than 11.0% reported for suckled Swamp buffaloes (Jainudeen et al., 1983a). Poor expression or even complete absence of estrous signs has been attributed to lack of responsiveness of ovaries to gonadotrophins and poor folliculogenic activity (Mudgal, 1992), low concentration of tri-iodothyronine and thyroxine (Borady et al., 1985), low levels of calcium with a significantly lower calcium-phosphorus ratio (Pathak et al., 1991) and low profiles of gonadotrophic hormones or disturbances in the balance of micro-minerals (Janakiraman et al., 1975; Ullah et al., 1983).

4.1.3 Seasonality of reproduction

The buffaloes calving during the NBS had shorter PEI of 55.95 ± 4.90 days while this

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interval was longer (91.15 ± 11.61 days) in the buffaloes calving during LBS (P<0.01, Table 4.1). Only one among the 21 LBS calving buffaloes, exhibited estrus with ovulation at 38 days after calving and the remaining buffaloes in this group had either anovulatory estrus or silent estrus. Conversely, these reproductive problems were low in the NBS calving buffaloes. During NBS, postpartum estrus was accompanied by ovulation while during LBS, POI was shorter than PEI (65.11 versus 91.15 days), indicating occurrence of silent ovulation. When the estrus events of all the buffaloes in the seven farms were distributed among the four seasons of the year, highest number was recorded in autumn (39.86%) followed by summer (31.16%), spring (18.83%) and winter (10.15%) (Fig. 4.5). Placenta expulsion duration and POI were also shorter in NBS than LBS calvers, but the differences were not significant.

Similarly, Pasha et al. (1986) reported that in Nili-Ravi buffaloes the incidence of estrus during autumn was the highest, while the lowest incidence was observed during spring in Pakistan. They recorded highest monthly frequency in November and lowest in May. The differences in the incidence of estrus and conception rate were reported to be significant (P<0.01) among different seasons and months. 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 winter and autumn (Tailor and Jain, 1987) for Indian buffaloes, respectively.

Annual meteorological status in the experimental area is presented in Fig. 4.6. Maximum ambient temperature started rising from 20^0C in February to 46^0C in June and decreased thereafter. The relative humidity percentage during spring was higher.

A seasonal pattern was also noted in the annual milk progesterone concentrations. The differences among the four seasons were significant (P<0.01) as shown in Table 4.7. Highest values of milk progesterone concentrations were noted in spring followed by winter, autumn and

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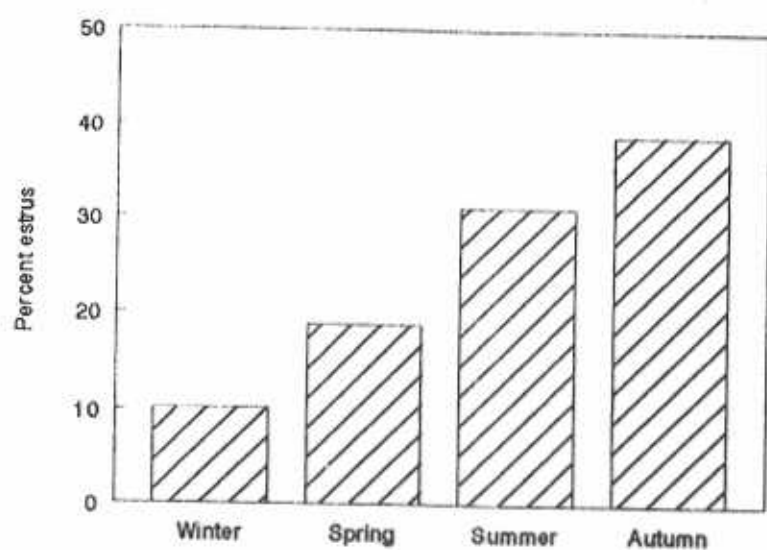
Figure 4.5. Distrubution of estrus events among various seasons of the year
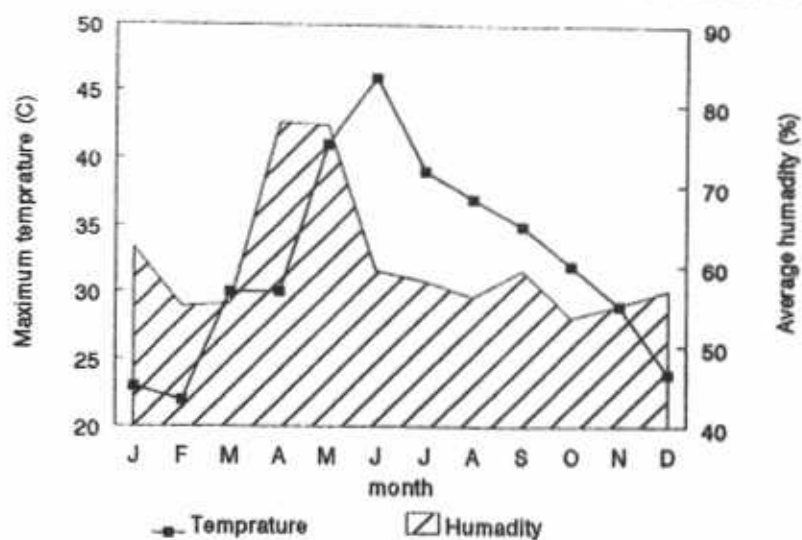
Figure 4.6. Annual maximum temprature and average humidity in the experimental area

summer (3.00 ± 0.12, 1.77 ± 0.32, 0.84 ± 0.72 and 0.25 ± 0.04 ng/ml, respectively). Fig. 4.7 indicates that milk progesterone remained at basal levels during May to July, increased up to February and then declined. This trend is almost opposite to that of atmospheric temperature in the region.

In spite of the higher incidence of estrus events (31.16%, Fig. 4.5) and shorter PUI intervals (33.4 days) during summer, the lower progesterone levels may be the cause of summer infertility. Postpartum milk progesterone levels in the LBS calvers remained lower than the NBS calvers (P<0.01, Table 4.7, Fig. 4.8).

In agreement with the present study, a study on seasonal variation in plasma hormones and reproductive efficiency in 20 newly calved Murrah buffaloes (Bahga and Gangwar, 1988), showed that although summer stress hastened uterine involution and first PEI, overall reproductive efficiency was impaired due to deficiency of progesterone which is essential for embryo survival. It was suggested that low progesterone levels during summer may be responsible for inadequate expression of estrus and lower conception rate.

McCool et al. (1987) reported a seasonal pattern of ovarian activity corresponded to a seasonal calving pattern. The depressed estrous activity during late dry and early wet seasons was attributed to the combined effect of poor pasture conditions and maximum ambient temperature. Perera et al. (1984) reported that seasonal rainfall, principally through its effect on quality and availability of fodder, favorably influenced fertility in buffaloes.

The incidence of silent ovulations was higher during LBS as compared to NBS (70.6% versus 29.4%, Table 4.6). These results are in agreement with those of Mudgal (1992) who attributed seasonality of breeding to difficulty in detection of estrus during summer.

In the present study, shortest POI was noted during autumn (63.8 days), followed by

Table 4.7. Mean values (±SE) and number of observations (n) of milk progesterone concentrations during various physiological and managemental conditions in buffaloes

Group	Milk progesterone concentrations (ng/ml)	n
Seasons		
Spring	3.00 ± 0.12 a	53
Winter	1.77 ± 0.32 b	150
Autumn	0.84 ± 0.72 c	101
Summer	0.25 ± 0.04 c	81
Calving periods		
Autumn-Winter	1.97 ± 0.30 a	207
Spring-Summer	0.68 ± 0.08 b	178
Postpartum intervals (days)		
Up to 15	0.26 ± 0.06 a	20
16 to 30	0.55 ± 0.08 a	127
31 to 60	1.78 ± 0.26 a	100
61 to 90	1.84 ± 0.53 a	69
91 to 120	1.87 ± 0.35 a	34
121 to 150	2.55 ± 0.59 a	35

a,b,c   Values with different letters in a group differ from each other significantly (P<0.01)
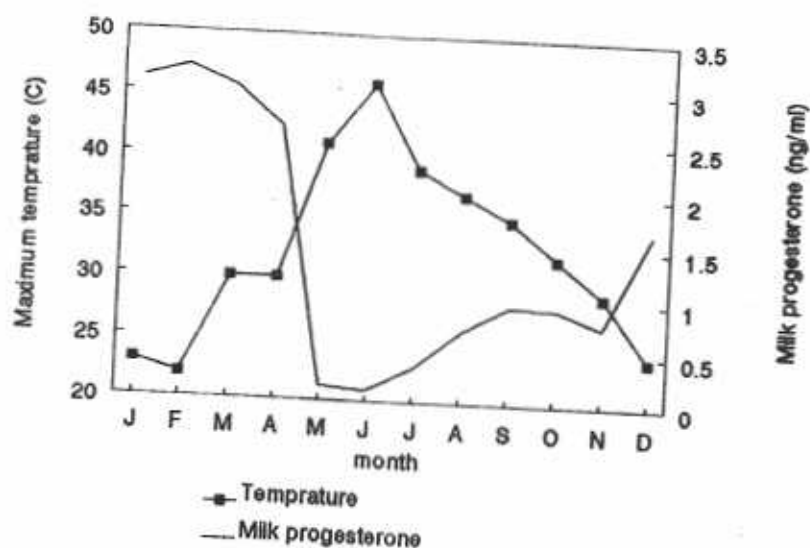
Figure 4.7. Annual maxiumum ambient temprature and milk progesterone concentrations in buffaloes
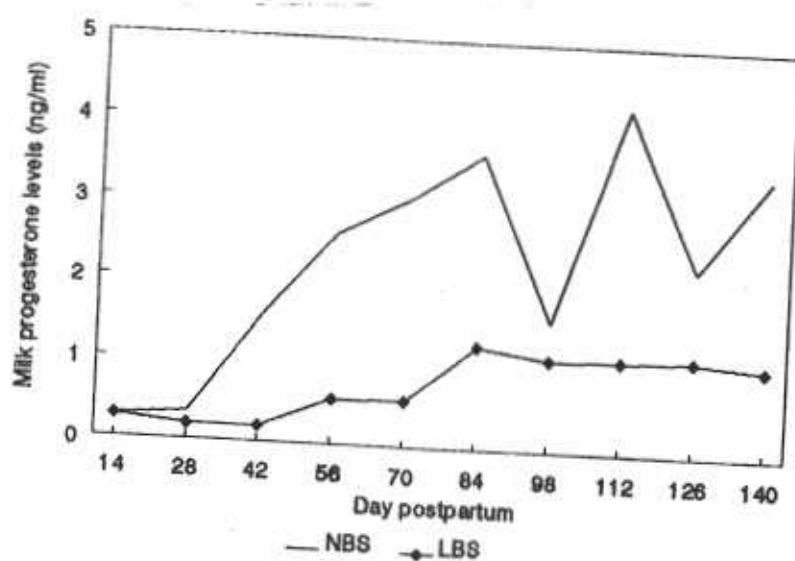
Figure 4.8. Milk progesterone levels at various postpartum intervals in normal (NBS) and low breeding season calvers (LBS) (P<0.01)

winter (77.5 days), summer (83.4 days) and spring (126.1 days), reflecting better reproductive performance during autumn and winter. These results agree with Shah (1990), who found that significantly higher number of buffaloes (60.5%) were bred during autumn and winter. Similarly, there was enhanced reproductivity in buffaloes and 66.0% to 69.6% of the animals were served during this period (Afiefy, 1967; Kodagali et al., 1973).

Seasonality of reproduction in the buffaloes may be attributed to large variation in the ambient temperature, relative humidity, changing day-length and feed availability. As shown in Fig. 4.6, the ambient temperature and relative humidity during the normal breeding season (NBS, autumn and winter) showed a decreasing trend. During start of the autumn the decreasing day-length and ambient temperature coincided with the commencement of breeding season in buffaloes. Commencement of ovarian activity in buffaloes after the summer was attributed to removal of under-nutrition coupled with high environmental stress (Kaur and Arora, 1984). In a previous study, it was observed that the frequency of estrus in buffaloes increases with decreasing day-length (Shah, 1990).

Maximum environmental temperature on the day after insemination was associated negatively with conception rate (Gwadzaukas et al., 1975). It has been suggested (Thatcher et al., 1986) that a thermal stress induced reduction in uterine blood flow would preferentially elevate uterine temperature and likely affect availability of water, electrolytes, nutrients and hormones 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.

Seasonal changes result in fluctuating feed supply. Better reproductive performance of the buffaloes, recorded during autumn (August to October) was associated with minimum intake

of crude protein ($P<0.01$) and maximum intake of metabolizable energy ($P<0.01$, Tables 4.8, 4.9). The ratio of crude protein to metabolizable energy intake was also low in autumn ($P<0.01$). The intake of metabolizable energy and crude protein was always above requirement in all the four seasons (Fig. 4.9, 4.10). 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. The crude protein content of the buffalo ration during this period is the highest. As the major components of the feed ingredient were ruminally degradable, which are not favorable for better reproductive performance (Jordan and Swanson, 1979b; Canfield et al., 1990), it may be one of the factors leading to seasonal breeding. Farmers generally substitute fodder scarcity with increasing amounts of cereal straws and beet pulp in the buffalo rations, resulting in inadequate nutrition of the animals. Dry matter and metabolizable energy increased while crude protein intake decreased from July onward, coinciding with the commencement of breeding season in buffaloes (Fig. 4.11 and 4.12).

Major portion of the crude protein from green fodders was degradable (Table 3.7). Berseem and wheat were found with highest degradability and were available during spring, the low breeding season. The late summer and winter fodders i.e. maize and sorghum, had higher dry matter contents, ranging from 50 to 56%, and contributed to the increasing metabolizable energy intake associated with commencement of breeding season. The concentrates comprising oil cakes, wheat bran and commercial concentrates, had 80% to 90% degradable protein contents.

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 tended to

Table 4.8. Effect of calving period, season and farm on body condition score, production of fat corrected milk and metabolizable energy (ME) intake (least square means* ± standard error)

Group	Body condition score (scale=0-5)	Fat-corrected milk (kg/day)	ME intake (MCal/day)
Calving periods			
NBS[**]	2.82 ± 0.02 a	15.31 ± 0.33 a	41.18 ± 0.32 b
LBS[***]	2.60 ± 0.03 b	13.55 ± 0.22 b	42.42 ± 0.40 a
Seasons			
Autumn	2.91 ± 0.03 a	14.16 ± 0.29 a	46.76 ± 0.40 a
Winter	2.86 ± 0.02 a	14.24 ± 0.34 a	39.14 ± 0.42 c
Spring	2.61 ± 0.03 b	15.41 ± 0.51 a	38.86 ± 0.46 c
Summer	2.22 ± 0.05 c	13.96 ± 0.53 a	42.29 ± 0.62 b
Farms			
A	2.68 ± 0.04 b	17.12 ± 0.32 a	40.12 ± 0.42 d
B	2.88 ± 0.03 a	17.04 ± 0.44 a	36.13 ± 0.44 e
C	2.74 ± 0.04 b	14.56 ± 0.67 b	45.38 ± 0.47 b
D	2.37 ± 0.08 d	7.58 ± 0.36 d	43.79 ± 1.13 c
E	2.55 ± 0.03 c	11.16 ± 0.39 c	36.92 ± 0.56 e
F	2.97 ± 0.04 a	14.46 ± 0.35 b	49.54 ± 0.31 a
G	2.71 ± 0.05 b	13.79 ± 0.33 b	42.79 ± 0.83 c

[*] Means within a group in the same column with different letters, differ significantly from each other (P<0.01)
[**] Normal breeding season
[***] Low breeding season

Table 4.9. Effect of calving period, season and farm on protein intake (least square means* ± standard error)

Group	Crude protein (kg/day)	Degradable protein (kg/day)	CP/ME Ratio (g/MJ)
Calving periods			
NBS**	1.81 ± 0.02 a	1.32 ± 0.02 a	12.15 ± 0.07 a
LBS***	1.83 ± 0.02 a	1.34 ± 0.02 a	11.78 ± 0.10 b
Seasons			
Autumn	1.79 ± 0.02 c	1.26 ± 0.02 c	11.47 ± 0.10 c
Winter	1.68 ± 0.02 d	1.22 ± 0.02 c	11.95 ± 0.09 b
Spring	1.89 ± 0.03 b	1.42 ± 0.02 b	12.19 ± 0.09 b
Summer	2.08 ± 0.05 a	1.55 ± 0.04 a	12.66 ± 0.19 a
Farms			
A	1.84 ± 0.02 c	1.36 ± 0.02 b	12.24 ± 0.13 bc
B	1.48 ± 0.03 f	1.05 ± 0.03 d	11.86 ± 0.06 c
C	1.98 ± 0.03 b	1.40 ± 0.03 b	12.73 ± 0.16 a
D	1.60 ± 0.02 e	1.13 ± 0.02 c	11.02 ± 0.21 d
E	1.55 ± 0.04 ef	1.13 ± 0.02 c	11.41 ± 0.13 d
F	2.59 ± 0.02 a	2.03 ± 0.03 a	11.34 ± 0.12 d
G	1.70 ± 0.02 d	1.18 ± 0.02 c	12.33 ± 0.12 ab

* Means within a group in the same column with different letters differ significantly from each other (P<0.01)
** Normal breeding season
*** Low breeding season

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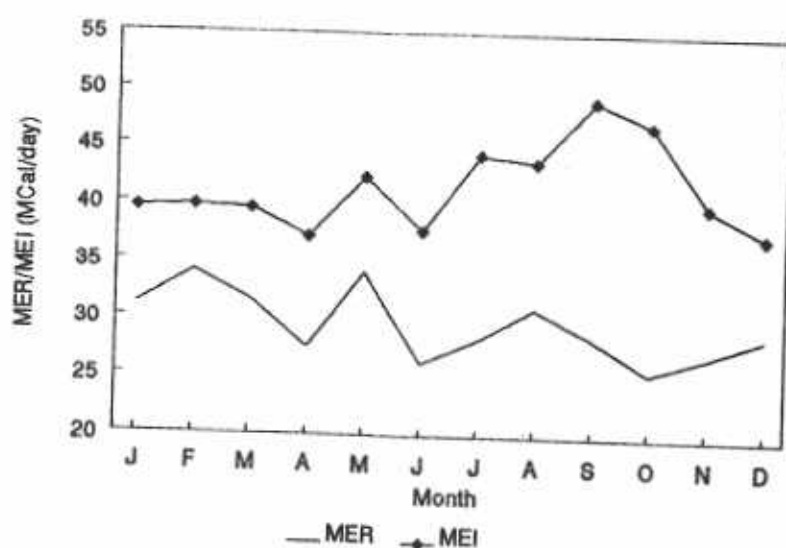
Figure 4.9. Requirements (MER) and intake (MEI) of metabolizable energy during various months
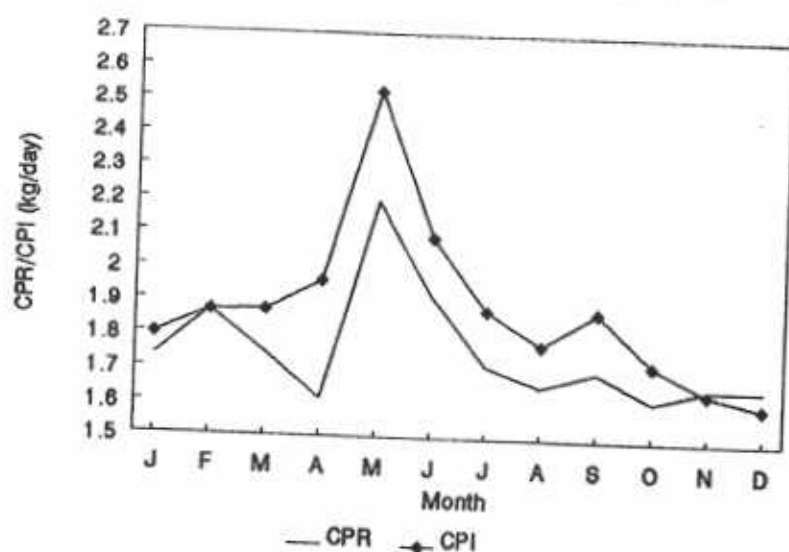
Figure 4.10. Requirements (CPR) and intake (CPI) of crude protein during various months
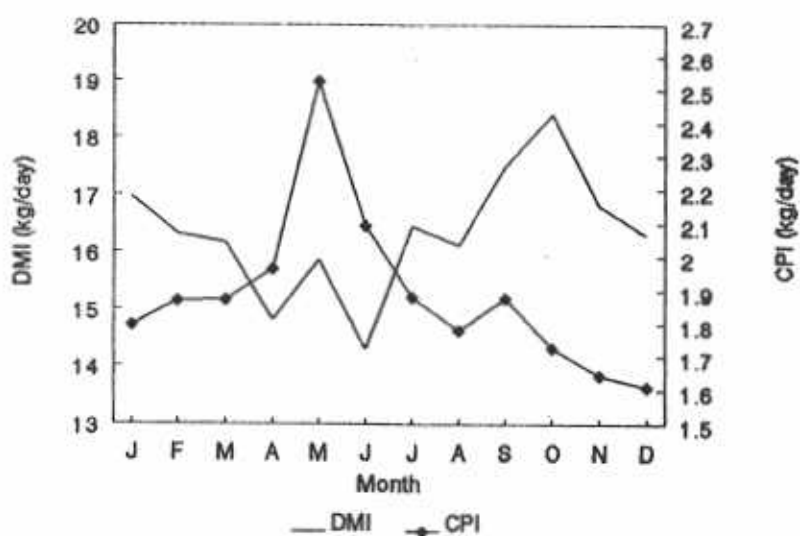
Figure 4.11. Average intake of dry matter (DMI) and crude protein (CPI) in buffaloes during various months
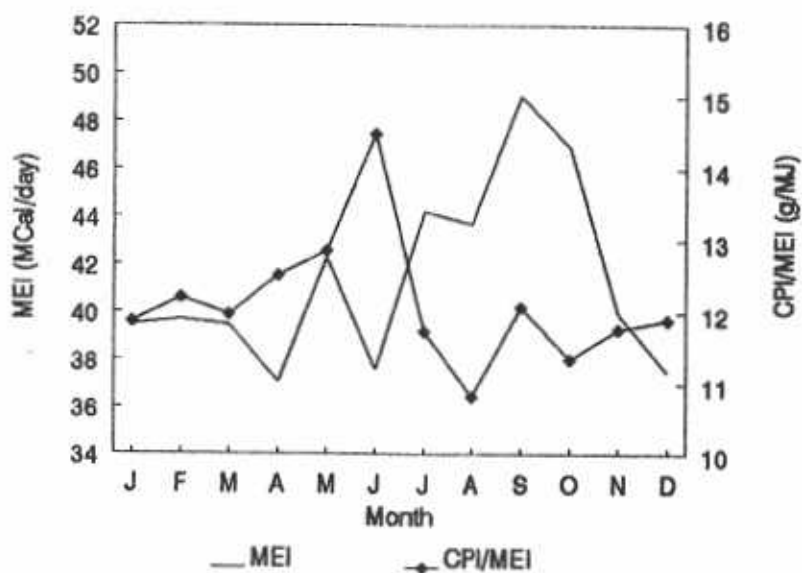
Figure 4.12. Average intake of metabolizable energy (MEI) and CPI/MEI ratio in buffaloes during various months

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remain lower (P<0.01) as compared to those calving in LBS (Table 4.10, 4.11). Higher serum glucose levels (P<0.01) were found in the NBS calvers (59.23 mg/100 ml) than the LBS calvers (55.15 mg/100 ml). Serum glucose levels were highest during spring and lowest during summer (60.9 versus 55.4 mg/100 ml, P<0.05, Fig. 4.13). 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 affected by seasons. Serum metabolic profiles reflect nutritional status of animals and their changing pattern is an indication of the fluctuating nutrient supply during various seasons of the year.

These findings are in line with a previous study in which hypoglycemia was found to be associated with reduced fertility in beef and dairy cattle (McClure, 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.* (1987) reported that serum glucose and serum total protein concentrations were significantly low in anestrous buffaloes. Neither of these variables had any effect on the serum calcium levels. Inorganic phosphorus levels were lower in the animals fed on paddy straw, but there was no difference between estrus and anestrus buffaloes.

Serum calcium, magnesium and phosphorus levels showed a seasonal changing pattern. From August onward the levels of these three minerals went on declining. Postpartum serum phosphorus and magnesium levels remained constantly higher during the LBS and never reached the levels of NBS calvers. Higher serum phosphorus and magnesium levels in this study seem to contribute to infertility.

Minerals intake by experimental buffaloes during the study period is given in Table 4.12

Table 4.10. Effect of calving period, season and farm 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 a	36.35 ± 2.92 ab
Winter	56.04 ± 1.01 b	80.80 ± 1.03 a	29.86 ± 1.96 b
Spring	60.91 ± 2.17 a	82.21 ± 1.52 a	38.62 ± 2.95 a
Summer	55.63 ± 1.44 b	83.32 ± 1.24 a	40.14 ± 2.53 a
Probability	P < 0.05	-	P < 0.05
Farms			
A	57.63 ± 1.68 a	80.69 ± 1.58 bc	32.11 ± 2.39 c
B	58.10 ± 1.33 a	85.01 ± 1.45 ab	31.21 ± 1.88 c
C	57.24 ± 1.24 a	83.02 ± 1.18 ab	28.21 ± 2.62 c
D	55.41 ± 2.09 a	80.34 ± 1.46 bc	29.97 ± 2.67 c
E	55.49 ± 2.28 a	78.10 ± 1.93 c	24.67 ± 2.90 c
F	60.75 ± 2.18 a	86.22 ± 1.69 a	45.68 ± 3.02 b
G	57.88 ± 2.41 a	84.94 ± 1.85 ab	59.92 ± 4.97 a
Probability	-	P < 0.01	P < 0.01

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

**   Normal breeding season

***   Low breeding season

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Table 4.11. Effect of calving period, season and farm on serum mineral concentrations (least square means[*] ± standard error)

Group	Serum calcium (mg/100 ml)	Serum magnesium (mg/1000 ml)	Serum phosphorus (mg/100 ml)
Calving periods			
NBS[**]	6.45 ± 0.21 b	21.70 ± 0.60 b	4.42 ± 0.15 a
LBS[***]	8.05 ± 0.17 a	28.64 ± 0.99 a	4.98 ± 0.21 a
Probability	P < 0.01	P < 0.01	-
Seasons			
Autumn	8.35 ± 0.23 a	23.61 ± 1.34 a	4.49 ± 0.22 a
Winter	5.94 ± 0.24 b	24.65 ± 0.82 a	4.82 ± 0.18 a
Spring	7.95 ± 0.35 a	21.45 ± 1.16 a	4.22 ± 0.33 a
Summer	7.28 ± 0.28 ab	26.59 ± 1.21 a	4.62 ± 0.32 a
Probability	P < 0.05	-	-
Farms			
A	7.60 ± 0.31 a	21.64 ± 1.09 cd	4.57 ± 0.20 abc
B	7.64 ± 0.35 a	18.30 ± 1.07 d	3.60 ± 0.20 c
C	6.51 ± 0.30 a	26.68 ± 0.79 bc	5.60 ± 0.31 a
D	6.42 ± 0.66 a	19.00 ± 0.71 d	3.84 ± 0.28 bc
E	7.41 ± 0.33 a	30.00 ± 1.12 ab	5.21 ± 0.30 ab
F	7.31 ± 0.31 a	26.95 ± 1.55 bc	5.75 ± 0.88 a
G	6.94 ± 0.36 a	34.96 ± 2.79 a	4.53 ± 0.47 abc
Probability	-	P < 0.01	P < 0.01

[*]   Means within a group in the same column with different letters differ significantly from each other
[**]   Normal breeding season
[***] Low breeding season

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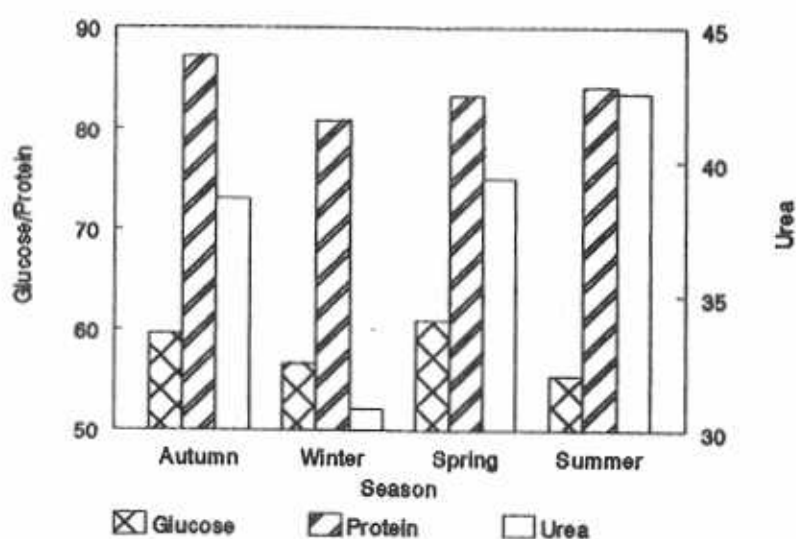
Figure 4.13. Serum glucose (mg/100 ml), protein (g/100 ml) and urea
concentrations (mg/100 ml) during various seasons
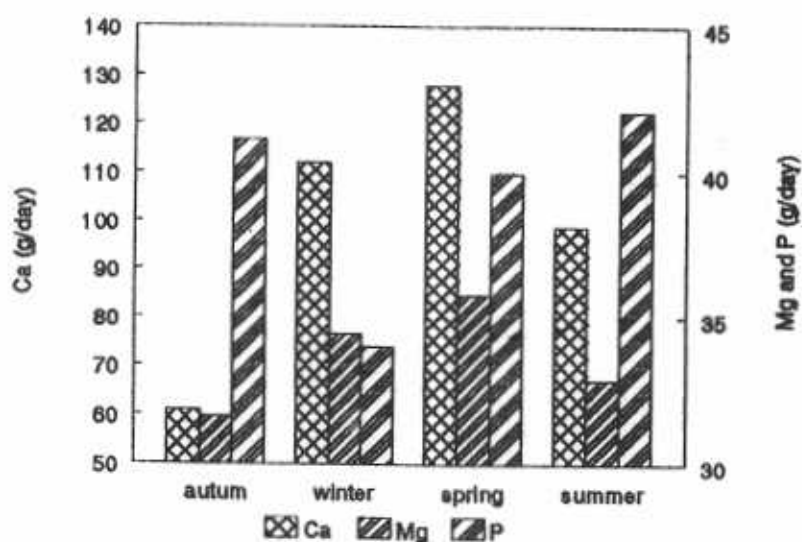
Figure 4.14. Macrominerals intake in various seasons

Table 4.12. Effect of calving period, season and farm on minerals intake (least square means* ± standard error)

Group	Calcium (g/day)	Magnesium (g/day)	Phosphorus (g/day)	Zinc (mg/day)	Copper (mg/day)
Calving periods					
NBS	115.15 a	33.00 a	40.17 a	1102 a	240 a
LBS	89.52 b	34.26 a	41.40 a	1087 a	202 a
Probability	P < 0.01	-	-	-	-
Seasons					
Autumn	62.25 d	31.60 c	39.81 bc	1127 a	150 d
Winter	117.71 b	34.40 b	35.08 c	1085 b	238 b
Spring	134.66 a	35.86 a	43.88 b	1096 b	295 a
Summer	100.33 c	32.65 c	51.19 a	1072 b	219 c
Probability	P < 0.01	P < 0.05	P < 0.01	P < 0.01	P < 0.01
Farms					
A	85.28 d	30.78 d	34.08 c	987 e	145 d
B	113.81 bc	34.69 b	32.52 c	1048 d	247 b
C	120.21 ab	40.28 a	46.36 b	1260 b	276 a
D	57.51 e	28.95 d	72.36 a	968 f	142 d
E	126.90 a	31.85 c	29.32 c	1015 de	272 a
F	108.38 c	36.00 b	51.91 b	1448 a	288 a
G	63.55 e	27.66 d	37.07 c	1208 d	175 c
Probability	P < 0.01	P < 0.01	P < 0.01	P < 0.01	P < 0.01

a..f   Means within a group in the same column with different letters differ significantly from each other

NBS   Normal breeding Season
LBS   Low breeding season

and Fig. 4.14 and 4.15. Calcium intake was higher in NBS calvers than LBS calvers ($P < 0.01$) and during spring as compared to the other three seasons ($P < 0.01$). Magnesium intake was highest during spring, followed by winter, summer and autumn ($P < 0.05$). Phosphorus intake in the buffaloes calving during normal breeding season was lower as compared to those calving during low breeding season from one month prepartum to two months postpartum (Fig. 4.16), although the difference was not significant. Highest phosphorus intake was recorded during summer followed by spring, autumn and winter ($P < 0.01$). Copper intake was higher during spring, followed by winter, summer and autumn ($P < 0.01$). Zinc intake was highest in autumn, followed by spring, winter and summer ($P < 0.01$). In the NBS calvers copper intake was constantly lower during various postpartum months as compared to NBS calvers (Fig. 4.17).

It is suggested that feed availability was one of the key factors in seasonal behavior of breeding in buffaloes. This is in line with the observations of 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.

The low reproductive performance in buffaloes in the LBS calvers coincided with a low BCS, (2.60 versus 2.82, Table 4.8). BCS tended to increase from July onward reaching to it's peak in October and then declined up to June (Fig. 4.18). The increasing BCS coincided with the commencement of breeding season. The constant increase in BCS in autumn and early winter, apparently due to the increasing metabolizable energy supply (Fig. 4.12), seems to support the commencement of reproductive activity during this period. Fig. 4.19 shows that more animals with the higher BCS were available during normal breeding season and more animals with the lower BCS were available during low breeding season. In addition to supporting enhanced reproductive performance, the increasing BCS also supported increasing milk
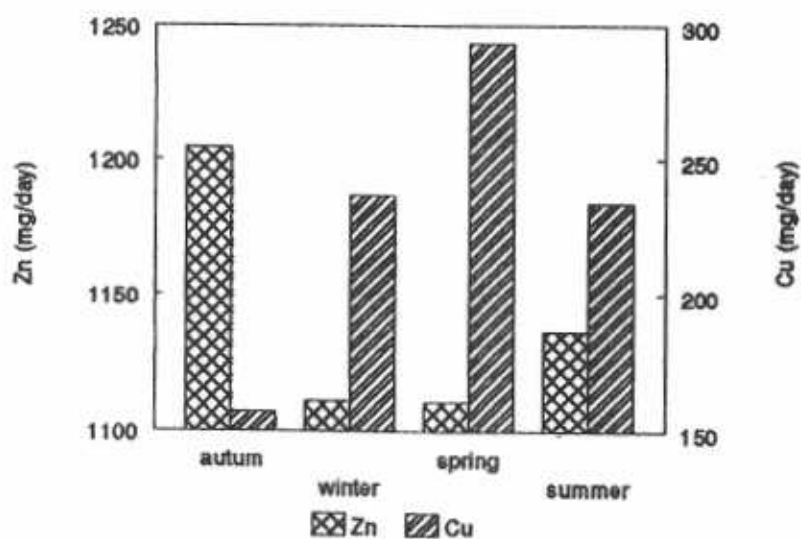
Figure 4.15. Microminerals intake in various seasons
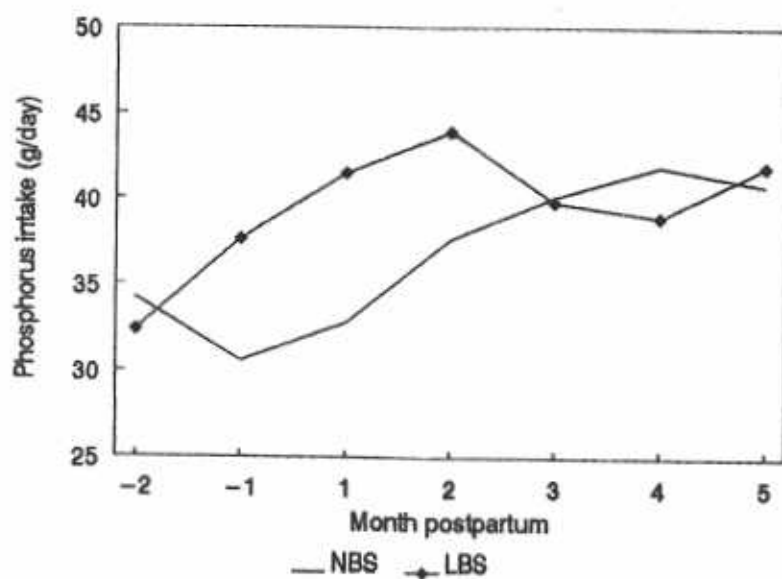
Figure 4.16. Phosphorus intake in normal (NBS) and low breeding season calving (LBS) buffaloes during various months

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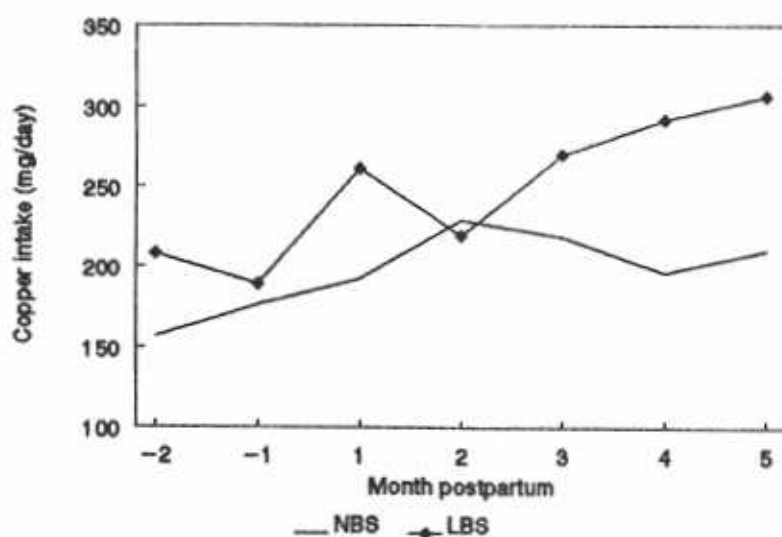
Figure 4.17. Copper intake in normal (NBS) and low breeding season (LBS) calvers
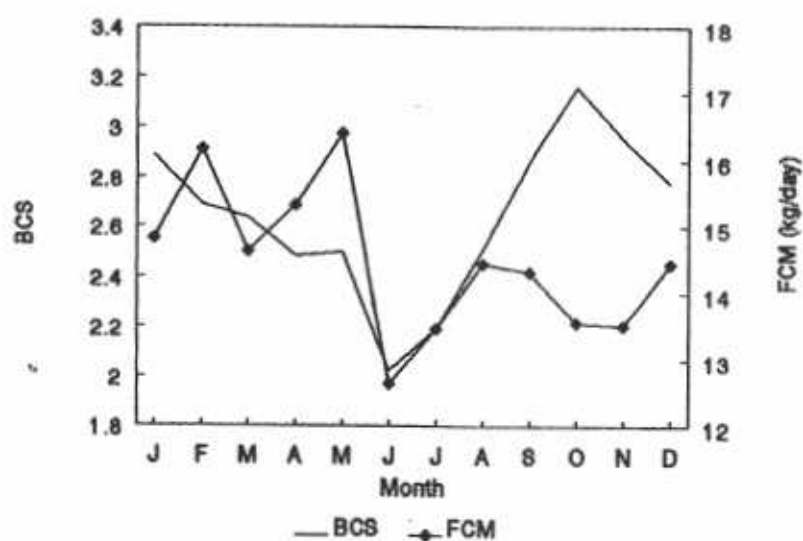
Figure 4.18. Annual pattern of body condition score (BCS) and 4% fat–corrected milk production (FCM) in buffaloes

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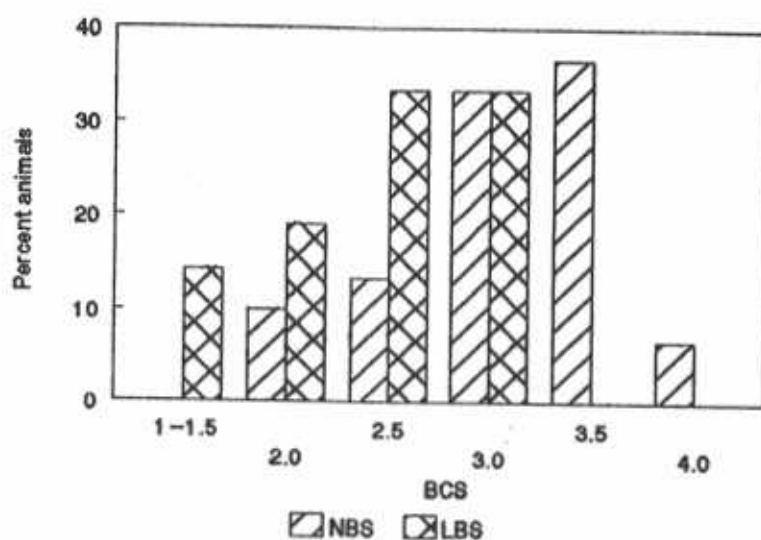
Figure 4.19. Distribution of buffaloes on the basis of body condition score (BCS) during normal (NBS) and low breeding season (LBS)
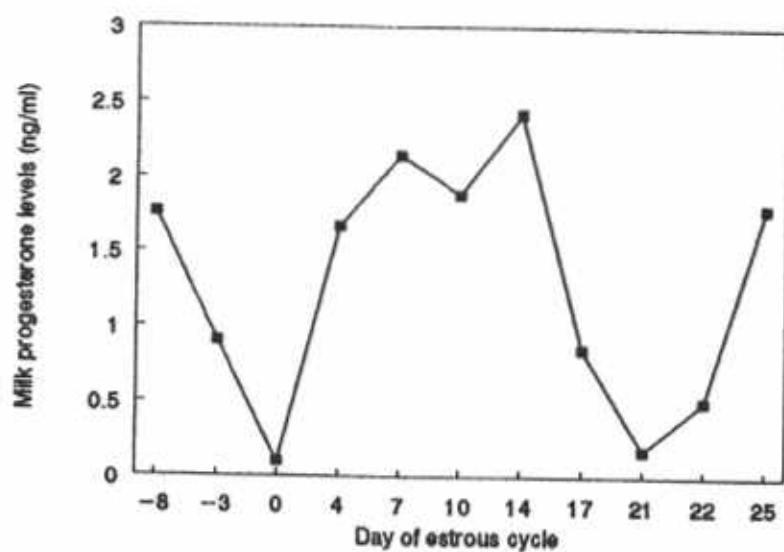
Figure 4.20. Milk progesterone levels at different days of estrus cycle in Nili-Ravi buffaloes

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production from June onward, as depicted in Fig. 4.18.

It may be concluded from the above results that Nili-Ravi buffaloes show a seasonal pattern of breeding, commencing from August to January. Onset of breeding season was associated with decreasing atmospheric temperature, increasing intake of dry matter and metabolizable energy, decreasing intake of crude protein and minerals, increasing BCS, high serum glucose levels and low serum urea, protein, magnesium and phosphorus levels.

4.1.4 Milk progesterone levels

The overall mean milk progesterone levels was 1.37 ± 0.17 ng/ml, ranging from 0.09 to 30.69 ng/ml. Values of MPL in different managemental and physiological conditions are presented in Table 4.7. Postpartum milk progesterone levels were higher ($P < 0.01$) in NBS calvers (1.97 ± 0.30 ng/ml) than LBS calvers (0.68 ± 0.08 ng/ml). In LBS calvers the milk progesterone levels remained < 0.30 ng/ml up to 6th week and started rising later reaching a peak of 1.27 ng/ml during 12 th week (Fig. 4.8). In NBS there was a sharp rise in milk progesterone level during the 4th week reaching 3.64 ng/ml during 12th week. These results suggest that during summer the corpus luteum does not function efficiently to maintain sufficient progesterone levels for maintaining reproductive cyclicity. These observations confirm the findings of Madan (1984) who attributed the low reproductive efficiency of buffaloes in summer to low luteal activities indicated by low blood progesterone levels.

Milk progesterone concentrations varied during various postpartum days. Lowest concentrations were recorded up to 15 days postpartum followed by 30, 60, 90, 120 and 150 days (0.26 ± 0.06, 0.55 ± 0.08, 1.78 ± 0.26, 1.84 ± 0.53, 1.87 ± 0.35, 2.55 ± 0.59 ng/ml, respectively). However, the difference was non-significant (Table 4.7). These findings are in

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agreement with previous studies (Perera *et al.*, 1981; Jainudeen *et al.*, 1983a) in which the elevated progesterone levels in serum of buffaloes declined rapidly following parturition to undetectable levels by day 3 or 4 of postpartum and remained low thereafter for a variable period until ovarian cyclicity was restored. Milk progesterone concentrations correlated negatively with suckling duration ($r = -0.13$, $P < 0.05$) and were significantly different at different experimental farms ($P < 0.01$).

Twenty four first postpartum estrous cycles were studied showing an average length of 20.0 days. Eleven second estrous cycles showed an average length of 23.6 days, ranging from 14 to 29 days. Milk progesterone levels during estrous cycle are shown in Fig. 4.20. These levels reached the lowest values on the day of estrus (0.10 ng/ml). Ovulation was indicated by a rise in milk progesterone levels which reached it's peak on day 14 and started declining on day 17 of the estrous cycle.

In previous studies (Perera *et al.*, 1981; Jainudeen *et al.*, 1983a), plasma progesterone level increased following the first postpartum ovulation and remained above 0.70 ng/ml for about 10 days, then declined to below 0.25 ng/ml at the next estrus. In agreement to this study, Kaur and Arora (1984) reported that plasma progesterone level was low on the day of oestrus, and increased progressively, showing a peak value between days 14 and 18 depending upon the estrous cycle length. Similarly, in Surti buffaloes serum progesterone levels were higher ($P < 0.05$) at diestrus phase followed by proestrus and estrus (2.78, 2.19 and 0.64 ng/ml, respectively) (Sarvaiya and Pathak, 1992).

In the present study milk progesterone levels showed two postpartum elevations, based on the data generated during the entire study period. The first rise was noted at a postpartum interval of 34.3 days (ranging from 15 to 66 days) and the second

rise was at 64.0 days (ranging from 28 to 143 days). According to Bahga and Ganwar (1988), plasma progesterone levels remained at basal levels from day 5 to day 30 postpartum and started rising thereafter. Increase in progesterone levels beyond day 30 was correlated with luteal function and ovarian cyclic activity (Madan, 1984). The interval from parturition to first ovulation, in studies based on rectal palpation, has been reported 38 days for milked river buffaloes in India (Singh et al., 1979) and 69 days in suckled buffaloes in Egypt (El-Fouly et al., 1976a).

Fig. 4.21 shows milk progesterone levels in buffaloes number A005, A255, B113, B300, which did not show estrus symptoms during the study period. Buffalo number A005 showed true anestrus, with neither estrous signs nor elevated milk progesterone concentrations. However, in the remaining three buffaloes, during the anestrous period, silent ovulations were noted, reflected by increasing milk progesterone levels.

In a study on 17 complete postpartum periods in Murrah buffaloes in Sri Lanka, plasma progesterone concentrations remained basal (< 0.25 ng/ml) for a period ranging from 92-210 days (Perera et al., 1984). In Swamp buffaloes, Perera (1982) found that post-partum anestrus was due to a failure in the resumption of ovarian cyclicity in the suckled buffaloes. Kaur and Arora (1984) stated that under-nutrition coupled with high environmental temperature stress was responsible for long anestrous periods in buffaloes.

In pregnant buffaloes in the peresent study, milk progesterone levels remained > 1.0 ng/ml. In a previous study (McCool et al., 1987), plasma progesterone levels were recorded as > 1.0 ng/ml for pregnant,-0.4-4.0 ng/ml for cycling and < 1 ng/ml for anestrous Swamp buffaloes.

The present results suggest that postpartum anestrus in Nili-Ravi buffaloes under field

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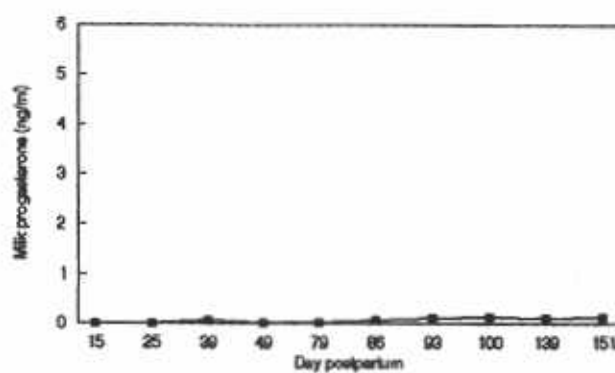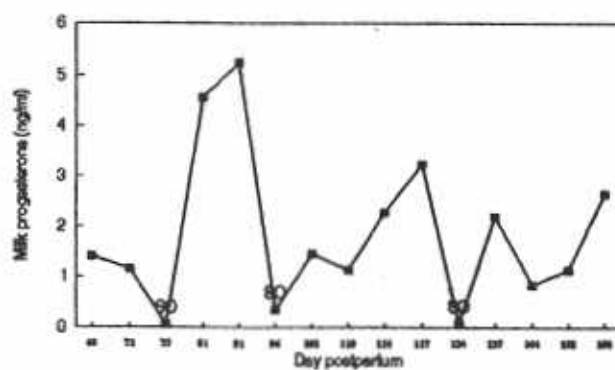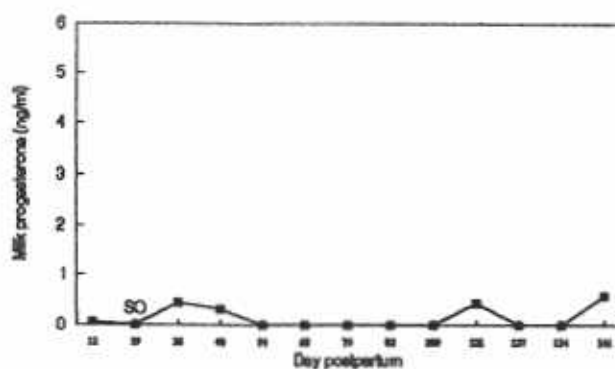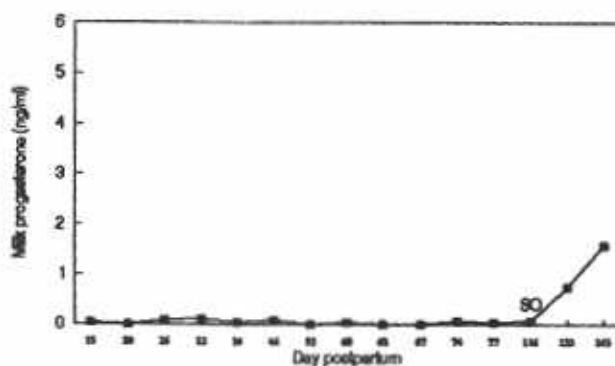
Figure 4.21. Milk progesterone levels in anestrus buffalo (A005) and those with silent ovulation (SO) (A255, B113, B300)

condition, is not true anestrous all the times and is frequently accompanied with ovulation. Careful observation of postpartum estrus symptoms and improved methods of estrus detection may reduce the incidence of postpartum anestrus.

4.2 RELATIONSHIP OF PRE- AND POSTPARTUM NUTRITIONAL STATUS WITH REPRODUCTIVE PERFORMANCE

Correlation of various parameters with reproductive performance in buffaloes is presented in Table 4.13. Placenta expulsion duration, lochia discharge, POI and PEI were significantly affected by intake of various nutrients, serum metabolites concentrations, age, BCS, fat corrected milk production and suckling duration. Relationship of nutritional status with reproductive performance is discussed as follows:

4.2.1 Energy intake

The overall mean value of metabolizable energy intake (MEI) was 41.70 ± 0.25 MCal/day, ranging from 20.2 to 60.4 MCal/day (Table 4.14). MEI was lower in the NBS calvers than the LBS calvers ($P<0.01$, Table 4.8). During autumn the MEI was the highest and during winter and spring it was the lowest ($P<0.01$). MEI was different at different farms ($P<0.01$). MEI also correlated with BCS ($r=0.16$, $P<0.01$, Table 4.15), expulsion of placenta ($r=0.19$, $P<0.01$) and discharge of lochia ($r=0.28$, $P<0.01$, Table 4.13). MEI was lowest during the first two months postpartum and highest during second to third month postpartum (Table 4.16). MEI correlated with serum magnesium ($r=0.18$, $P<0.01$) and phosphorus levels ($r=0.18$, $P<0.01$, Table 4.17). Increasing MEI above requirements reduced postpartum ovulation interval ($r=-0.27$, $P<0.01$)).

Table 4.13. Correlation of various parameters with reproductive performance in buffaloes (Pearson's correlation coefficients)

Parameter	Placenta expulsion duration	Lochia discharge duration	Postpartum	
			Ovulation interval	Estrus interval
Nutrients intake				
Crude protein (CP)	0.21**	-0.14	0.21**	0.08*
Degradable protein	0.10*	0.22**	0.01*	0.31**
Metabolizable energy (ME)	0.19*	0.28**	-0.03	0.03
CP/ME ratio	0.02	0.02	0.15**	0.04
CP above requirements	0.37**	0.06	-0.03	0.10**
ME above requirements	0.19*	0.24**	-0.27**	0.04
Calcium	0.08	-0.12	0.13*	-
Magnesium	0.17*	-0.27**	0.90**	-
Phosphorus	0.22**	-0.13	0.48**	-
Zinc	0.23**	0.22**	-0.52**	-
Copper	0.12	0.07	-0.32**	-
Serum metabolites levels				
Glucose	0.08	0.01	0.10	0.08
Protein	0.14	0.20	-0.20**	-0.06
Urea	0.16	-0.18	0.30**	0.28**
Calcium	0.15	0.19	0.05	-0.03
Magnesium	-0.12	-0.15	0.14*	0.03
Phosphorus	0.31*	0.14	-0.10	-0.07
Others				
Age	0.12	0.07	0.05	0.03
Body condition score	-0.17*	0.01	-0.06	-0.20**
Fat corrected milk	-0.09	0.07	0.31**	-0.02
Suckling duration	-0.19	-0.26*	0.19**	0.23**
Use of oxytocin	0.40**	0.02	0.17**	-0.18**

* Significant at P < 0.05
** Significant at P < 0.01

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Table 4.14. Number of observations (n), means and standard error (SE) for nutrients intake in buffaloes

Nutrients intake	n	Mean ± SE
Dry matter (kg/day)	1029	16.60 ± 0.08
Crude protein (CP) (kg/day)	1029	1.82 ± 0.47
CP above requirements (g/day)	1029	92.50 ± 17.08
Degradable protein (kg/day)	1027	1.32 ± 0.01
Metabolizable energy (ME) (MCal/day)	1027	41.70 ± 0.25
ME above requirements (MCal/day)	1027	12.10 ± 0.38
CP/ME ratio	1027	12.00 ± 0.06
Calcium (g/day)	899	100.40 ± 1.60
Magnesium (g/day)	899	33.70 ± 0.26
Phosphorus (g/day)	899	38.40 ± 0.40
Zinc (mg/day)	899	1138.70 ± 7.53
Copper (mg/day)	899	228.20 ± 3.60
Calcium above requirements (g/day)	899	43.80 ± 1.66
Phosphorus above requirements (g/day)	899	1.70 ± 0.50

Table 4.15. Effect of nutrients intake on body condition score, milk production and suckling duration (calf survival) in buffaloes (Pearson's correlation coefficients)

Nutrient Intake	Body condition score	Milk production	Suckling duration
Crude protein	0.03	0.12[**]	-0.16[**]
Un-degradable protein	0.08	0.12	-0.20[**]
Metabolizable energy	0.16[**]	0.00	-0.15[**]
CP/ME ratio	-0.10[*]	0.15[**]	-0.11[**]

[*] $P<0.05$
[**] $P<0.01$

Table 4.16. Body condition score, production of fat corrected milk and metabolizable energy (ME) intake during various peripartum periods and various reproductive states (least square means* ± standard error)

Group	Body condition score (Scale=0-5)	Fat-corrected milk production (Kg/day)	ME intake (MCal/day)
Peripartum month			
-2 to -1	2.97 ± 0.06 a	0.00 ± 0.00 a	40.95 ± 0.87 b
1 to 2	2.65 ± 0.03 b	15.01 ± 0.31 a	41.00 ± 0.36 b
2 to 3	2.73 ± 0.03 b	14.32 ± 0.32 a	42.72 ± 0.39 a
3 to 4	2.70 ± 0.05 b	14.03 ± 0.45 a	42.18 ± 0.82 ab
Probability	$P < 0.01$	-	$P < 0.05$
Postpartum ovulation interval (days)			
< 45	2.91 ± 0.03 a	14.59 ± 0.42 b	40.60 ± 0.54 bc
45 to 74	2.70 ± 0.05 b	12.89 ± 0.56 c	41.61 ± 0.47 ab
75 to 150	2.58 ± 0.02 c	16.69 ± 0.22 a	39.57 ± 0.34 c
Anestrus	2.74 ± 0.02 b	14.21 ± 0.22 bc	42.95 ± 0.34 a
Probability	$P < 0.05$	$P < 0.01$	$P < 0.01$
Postpartum estrus interval (days)			
< 45	2.91 ± 0.02 a	16.15 ± 0.27 a	42.33 ± 0.35 b
45 to 74	2.73 ± 0.04 b	14.79 ± 0.28 b	39.86 ± 0.60 c
75 to 150	2.58 ± 0.04 c	12.39 ± 0.48 c	44.04 ± 0.49 a
Anestrus	2.66 ± 0.03 bc	15.04 ± 0.31 ab	40.95 ± 0.39 c
Probability	$P < 0.01$	$P < 0.01$	$P < 0.01$
Pregnancy status			
Pregnant	2.83 ± 0.02 a	15.60 ± 0.22 a	39.97 ± 0.36 b
Non-pregnant	2.63 ± 0.03 b	13.75 ± 0.32 b	43.19 ± 0.35 a
Probability	$P < 0.01$	$P < 0.01$	$P < 0.01$

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

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Table 4.17. Correlation among nutrients intake and serum metabolite concentrations in buffaloes (Pearson's correlation coefficients)

Parameter	Serum metabolite concentrations					
	Glucose	Protein	Urea	Calcium	Magnesium	Phosphorus
Nutrients intake						
Crude protein	0.09	0.03	0.22"	0.04	0.11	0.05
Degradable protein	0.00	0.09	0.26**	0.03	0.17	-0.05
Metabolizable energy	0.09	0.11	0.04	-0.02	0.18"	0.18*
Calcium	-	-	-	-0.14*	-0.11	-0.16*
Magnesium	-	-	-	-0.27"	-0.22"	0.31"
Phosphorus	-	-	-	-0.14*	-0.11	-0.13
Zinc	-	-	-	0.17"	0.28"	0.68"
Copper	-	-	-	0.02	0.04	-0.17*
Serum metabolites concentrations						
Glucose	-	0.12	0.17"	0.17"	0.01	0.10
Protein	-	-	0.10	0.23"	0.22"	0.21"
Urea	-	-	-	0.05	0.22"	0.21"
Calcium	-	-	-	-	0.01	0.33"
Magnesium	-	-	-	-	-	0.12

* Significant at $P < 0.05$
** Significant at $P < 0.01$

Table 4.18. Number of observations (n), means and standard error (SE) for serum metabolites in buffaloes

Serum metabolites concentrations	n	Mean ± SE
Glucose (mg/100 ml)	260	57.80 ± 0.71
Protein (g/1000 ml)	261	83.50 ± 0.62
Urea (mg/100 ml)	252	38.90 ± 1.3
Calcium (mg/100 ml)	256	7.23 ± 0.14
Magnesium (mg/1000 ml)	243	26.40 ± 0.57
Phosphorus (mg/100 ml)	204	5.44 ± 0.12

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Prepartum MEI was higher in estrus animals as compared to the anestrus animals (42.34 versus 37.24 MCal/day, $P < 0.05$). Prepartum excess MEI was also higher in estrous group than the anestrus one ($P < 0.01$) (Fig. 4.22). The high prepartum excess MEI in the estrus group probably resulted in building of more body reserves which supported high postpartum milk production and early resumption of estrus activity. In the last month prepartum, the MEI was sufficient to support fetal growth (tissues and fluids). In parallel to excess MEI during prepartum period (Fig. 4.22), BCS remained higher in the estrus group (Fig. 4.23). This suggests that the animals receiving more MEI during prepartum period were able to maintain a relatively good BCS despite mobilization of body reserves. Maximum and minimum energy intake in this study were associated with anestrus and prolonged PEI (75 to 150 days) while moderate intake was associated with a PEI of less than 75 days (Table 4.16).

In agreement to our findings, increased energy levels enhanced conception rates and the percentage of cows cycling early in the breeding season (Wiltbank et al., 1964; Dunn et al., 1969). Low energy intake led to ovarian inactivity and anestrus in suckled beef cows. Shortening of the anovulatory period in primiparous beef heifers was suggested by increasing the dietary energy intake during gestation (Echternkamp et al., 1982). Miettinen (1990) reported that in dairy cows, a low energy level in early puerperium delayed the uterine involution, onset of ovarian activity and prolonged the PEI and service period. Marston et al. (1995) concluded that conception rates were significantly improved by feeding high levels of supplemental energy prepartum but not postpartum.

In agreement to these findings, 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. Wiltbank et al. (1965) reported that a deficiency of energy was more
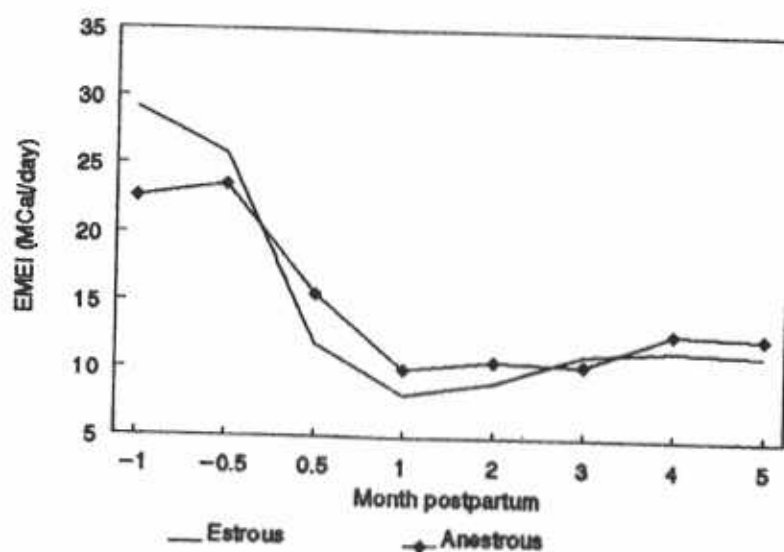
Figure 4.22. Excess metabolizable energy intake (EMEI) in estrous and anestrous
buffaloes
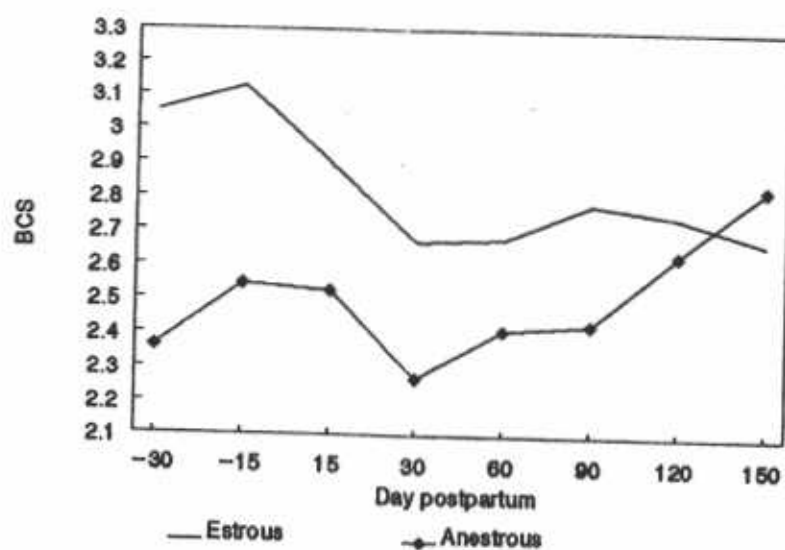
Figure 4.23. Postpartum body condition score (BCS) of estrous and anestrous
buffaloes

detrimental to reproduction than protein deficiency. However, cows reduced voluntary intake of feed when low protein diets were provided and, consequently, consumed neither adequate energy nor protein. Similarly, 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. Richards *et al.* (1989b) reported that increased nutrient intake after a period of severe restriction resulted in resumption of estrus.

In contrast to the present findings, Usmani *et al.* (1990) reported that postpartum anestrus in Nili-Ravi buffaloes could be shortened by weaning calves at birth, regardless of prepartum supplemental feeding. Similarly, diet and condition score at calving did not affect the service period or number of inseminations per conception in lactating dairy cows (Jones *et al.*, 1988).

4.2.2 Protein intake

Crude protein intake (CPI) showed a mean value of 1.82 ± 0.47 kg/day, ranging from 0.95 to 2.64 kg/day (Table 4.14). CPI varied among different seasons ($P < 0.01$) and farms ($P < 0.01$, Table 4.9) and correlated positively with serum urea levels ($r = 0.22$, $P < 0.01$, Table 4.17). Degradable protein intake (DPI) was 1.32 ± 0.01 kg/day and was affected by seasons and farms ($P < 0.01$). CPI/MEI ratio related positively with milk production ($r = 0.15$, $P < 0.01$) and negatively with BCS ($r = -0.10$, $P < 0.01$) and suckling duration ($r = -0.11$, $P < 0.01$). Excess CPI negatively correlated with milk production ($r = -0.40$, $P < 0.01$).

CPI correlated positively with placenta expulsion duration ($r = 0.21$, $P < 0.01$), PEI ($r = 0.08$, $P < 0.05$) and POI ($r = 0.21$, $P < 0.01$) and negatively with PUI ($r = -0.23$, $P < 0.01$, Tables 4.19). Excess CPI was lower in estrus group as compared to the anestrus one ($P < 0.05$, Fig. 4.24). The difference was prominent from one month prepartum to four months postpartum.

Table 4.19. Protein intake during various peripartum periods and reproductive states (least square means[*] ± standard error)

Group	Crude protein (kg/day)	Degradable protein (kg/day)	CP/ME Ratio[**] (g/MJ)
Peripartum month			
-2 to -1	1.59 ± 0.04 c	1.10 ± 0.04 d	12.22 ± 0.17 a
1 to 2	1.79 ± 0.02 b	1.30 ± 0.02 c	12.07 ± 0.09 a
2 to 3	1.91 ± 0.02 a	1.40 ± 0.02 b	11.87 ± 0.09 a
3 to 4	1.97 ± 0.04 a	1.48 ± 0.04 a	11.97 ± 0.17 a
Probability	P < 0.01	P < 0.01	-
Postpartum ovulation interval (days)			
< 45	1.66 ± 0.03 b	1.18 ± 0.04 a	11.85 ± 0.11 b
45 to 74	1.72 ± 0.03 b	1.23 ± 0.03 a	11.93 ± 0.13 b
75 to 150	1.84 ± 0.02 a	1.35 ± 0.03 a	12.51 ± 0.07 a
Anestrus	1.90 ± 0.02 a	1.35 ± 0.02 a	11.85 ± 0.10 b
Probability	P < 0.05	-	P < 0.01
Postpartum estrus interval (days)			
< 45	1.90 ± 0.02 a	1.39 ± 0.03 a	12.24 ± 0.06 a
45 to 74	1.69 ± 0.03 b	1.22 ± 0.02 b	11.89 ± 0.12 b
75 to 150	1.87 ± 0.03 a	1.36 ± 0.03 a	11.60 ± 0.14 b
Anestrus	1.86 ± 0.02 a	1.37 ± 0.02 a	12.28 ± 0.10 a
Probability	P < 0.01	P < 0.01	P < 0.01
Pregnancy status			
Pregnant	1.70 ± 0.02 b	1.22 ± 0.02 b	11.94 ± 0.08 a
Non-pregnant	1.94 ± 0.02 a	1.43 ± 0.02 a	12.07 ± 0.08 a
Probability	P < 0.01	P < 0.01	-

[*]   Means within a group in the same column with different letters differ significantly from each other

[**]   Crude protein/metabolizable energy ratio

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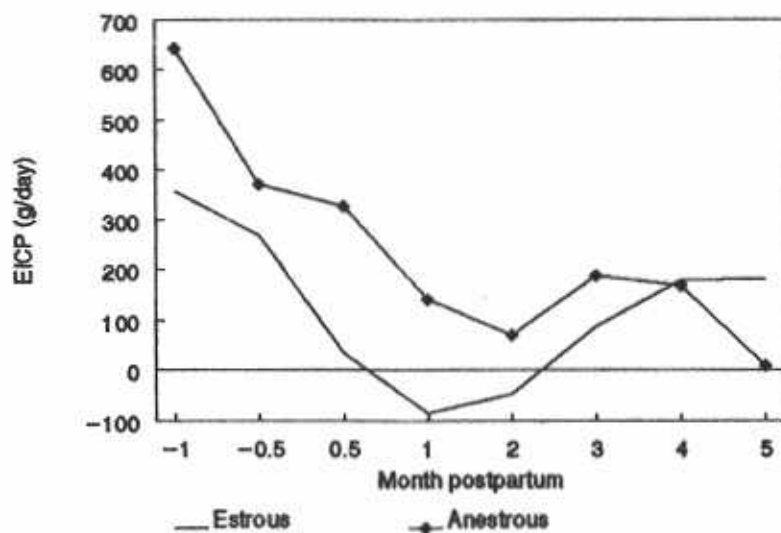
Figure 4.24. Excess intake of crude protein (EICP) in estrous and anestrous
buffaloes
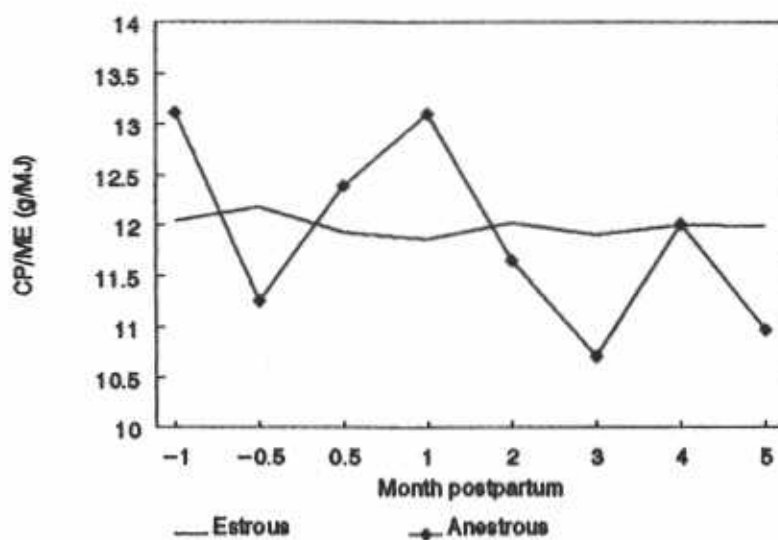
Figure 4.25. Ratio of crude protein/metabolizable energy intake (CP/ME) in
estrous and anestrous buffaloes

Excess CPI related positively with placental expulsion duration ($r=0.37$, $P<0.01$). The dietary ratio of crude protein - metabolizable energy (CP:ME) consumed by the buffaloes during prepartum and postpartum periods is given in Fig. 4.25. The comparison shows that the estrus animals had a narrow and almost constant CP:ME ratio (11.9-12.2 g/MJ), while the anestrus animals had a widely fluctuating ratio, ranging from 10.7 to 13.1 g/MJ. CP:ME was related negatively with PUI ($r=-0.16$, $P<0.01$) and positively with POI ($r=0.15$, $P<0.01$).

In agreement to the present study, Elrod and Butler (1993) reported detrimental effect of high CPI in cattle indicated by significantly lower first-service conception rates in Holstein heifers fed high ruminally degradable protein. In Israeli Kibbutz dairy cattle (Francos *et al.*, 1992), feeding of diets higher in protein with low energy/protein ratio during lactation and maintenance, was a factor contributing to low fertility. Similarly, Jordan and Swanson (1979b) found that services per conception were increased in cows fed diets containing more than 16% crude protein. In the present study, protein supplements fed to animals were highly degradable (Table 3.7) which was associated with a delayed resumption of ovarian activity in these animals, as mentioned in Section 4.1.3. Fig. 4.26 shows relationship of degradable protein intake/4% fat-corrected milk production ratio with conception rates. With the increasing ratio, conception rates linearly decreased. In the present study, farmers fed protein supplements to lactating buffaloes irrespective of their milk production as per their conventional practice. This over-burdened the low producers with intake of dietary protein. The relationship in Fig. 4.26 demonstrates that such a practice of indiscriminate use of CP of high degradibility adversely affects conception rates in low milk producing buffaloes. It seems that with the increasing milk production, the utilization of degradable protein increases and detrimental effects of the later decrease resulting in enhanced conception rates.
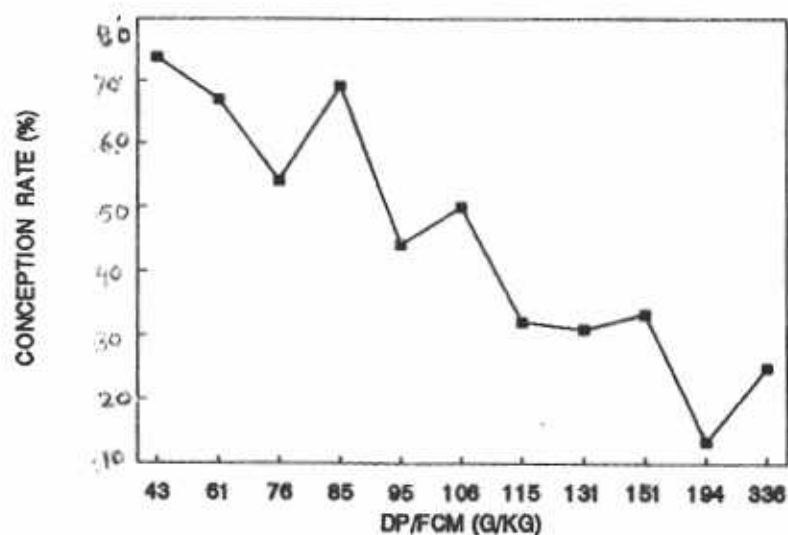
Figure 4.26. Relationsip of degradable protein intake/4% fat−corrected milk
production ratio (DP/FCM) with conception rate in buffaloes
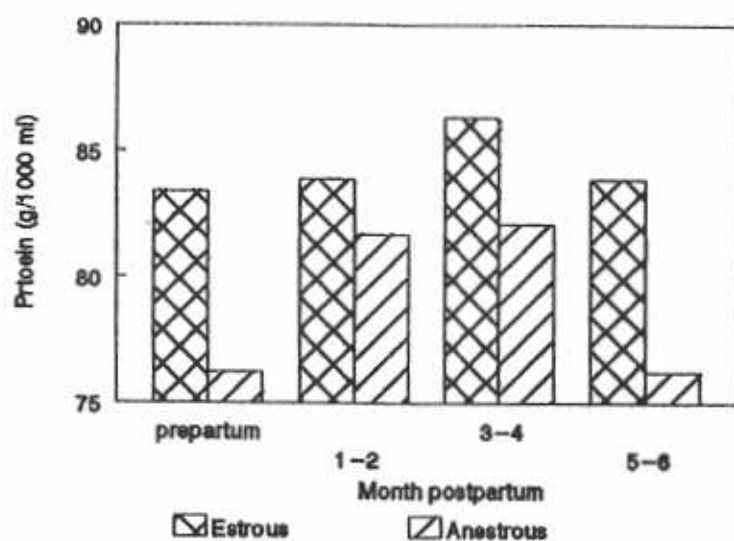
Figure 4.27. Serum protein concetrations in estrous and anestrous buffaloes
during postpartum months (P<0.05)

It may be concluded from results of the present study that under field conditions, higher protein intake is a cause of delayed postpartum estrus and the PEI may be shortened through feeding animals according to their requirements. Due to the high degradibility of crude protein, ammonia concentration in the rumen and consequently formation of urea in the liver would be high. This may increase the uterine pH and thus decrease fertility. The high serum urea levels may also result in endocrine imbalance, adversely affecting the ovarian cyclicity.

In agreement to the above results, detrimental effect of excess protein have been attributed to the ruminally degradable fraction of dietary protein (Folman et al., 1981; Canfield et al., 1990). Jordan and Swanson (1979b) reported that in lactating dairy cows, excess dietary protein decreased fertility and changed the ionic composition of uterine fluid during the luteal phase, but not in the follicular phase. A decrease in serum progesterone has been suggested (Fergusson and Chalupa, 1989) at concentration of crude protein/dry matter (CPDM) that exceed rumen requirement of degradable intake protein (DIP). 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, 1979a).

4.2.3 Serum glucose, protein and urea levels

4.2.3.1 Serum glucose levels

The overall mean value for serum glucose concentrations in buffaloes was 57.80 ± 0.71 mg/100 ml (Table 4.18). The concentrations were affected by calving period ($P < 0.01$), season ($P < 0.05$) and farm ($P < 0.01$, Table 4.10). Age had no effect on serum glucose levels. Serum glucose concentrations were lower during prepartum months as compared to postpartum months ($P < 0.01$, Table 4.17). In the animals resuming postpartum estrus at various intervals, the overall mean serum glucose levels were different ($P < 0.01$). Serum glucose concentrations

were higher in the NBS calvers than the LBS calvers (P<0.01). However, no consistent trend was noted in relationship of serum glucose levels with the reproductive performance of buffaloes.

The present study supports the results of Tegegnae et al. (1993), who found that plasma glucose levels varied over time without any consistent trends and were not influenced by feeding or suckling regimes, nor did they differ between cyclic and non-cyclic cows. These authors concluded that blood metabolite concentrations are not good indicators of nutritional status and are not related to postpartum ovarian activity. Zaman (1984) reported that the average plasma glucose concentrations in the lactating cyclic,-non-cyclic and sub-estrus buffaloes were 67.51 $\pm$ 8.96, 65.15 $\pm$ 7.38 and 66.09 $\pm$ 9.22 mg/100 ml, respectively, the difference being non-significant. Similar findings were reported by Blowey et al. (1973), Dhoble and Gupta (1987) and Kumar and Sharma (1993).

In contrast to the present study, hypoglycemia has been shown to be associated with reduced fertility in beef and dairy cattle (McClure, 1968; Selk, 1986; Satish-Kumar and Sharma, 1991). In anestrus buffaloes, the serum glucose concentrations were significantly low (Veerapandian et al., 1987). In Friesian cows, serum glucose concentration was significantly correlated with service period (Jones et al., 1988). Miettinen (1990) reported that in late puerperium, low levels of glucose, and high levels of ketone bodies, were associated with poor fertility. Reduced concentrations of glucose in plasma and increased concentrations of non-estrified fatty acid were associated with anestrus and continued throughout the anestrus period (Richards et al., 1989a), although there was never any linear correlation. El-Belely et al. (1994) reported that in Egyptian buffaloes plasma glucose levels were significantly (P<0.05) higher after fertile matings than infertile ones.

Quayam *et al.* (1988) reported that in multiparous buffaloes, serum glucose levels at 5 days postpartum were significantly correlated with the occurrence of oestrus by 60 days. Similarly, Ahmad (1976), while studying the serum glucose concentration in buffaloes during various phases of estrous cycle, found that the glucose concentrations were higher during estrus as compared to other phases i.e. proestrus, metestrus and diestrus. A distinct rise in glucose during estrus (Hewit, 1930; Hodgeson *et al.*, 1932; Deshpande, 1978) has been attributed to the excitement of the animal that increases its metabolic rate.

4.2.3.2 Serum protein levels

The overall mean value for serum protein levels was 83.50 ± 0.62 g/l (Table 4.18). Serum protein levels were significantly influenced by farm ($P < 0.01$) and calving period ($P < 0.05$, Table 4.10). Lower levels ($P < 0.05$) were found in NBS calvers (81.45 g/1000 ml) than LBS calvers (84.44 g/1000 ml).

Serum protein levels negatively correlated with POI ($r = -0.20$, $P < 0.01$). In anestrus buffaloes, serum protein levels decreased about two weeks before parturition and increased gradually postpartum. At various postpartum intervals serum protein levels in anestrus buffaloes never reached the values recorded in the estrus buffaloes, however the difference was statistically not significant (Fig. 4.27). These results show that higher serum protein levels tended to affect favorably postpartum resumption of ovarian activity in buffaloes.

In agreement to the present findings, significantly lower serum protein concentrations were reported in anestrus than the cyclic buffaloes (Veerapandian *et al.*, 1987). Since serum protein influences the water retaining capacity of an animal, high concentration-(7.14-8.5 g/100 ml) in buffalo would favor heat regulation under hot conditions (Pandey and Roy, 1968b). Dutta

and Dugwekar (1983) found that in buffaloes not retaining the fetal membranes serum total proteins, albumin and globulin values were high during late gestation, declined as parturition approached and increased again to prepartum levels by the 10th day postpartum.

In contrast to the present study, El-Belely *et al.* (1994) found that plasma concentrations of total proteins remained the same in conceived and non-concieved buffaloes, however concentrations were negatively correlated with the number of services required per conception. No differences could be seen between fertile and infertile groups in concentrations of serum protein in buffaloes (Zaman, 1984; Kumar and Sharma, 1993). Sekerden *et al.* (1992) found that serum total proteins were non- significantly correlated with milk fat concentration or conception rates to first insemination in Jersey cows. Tegegnae *et al.* (1993) reported that plasma total proteins, albumin and globulin levels varied over time without consistent trends and did not differ between cyclic and non-cyclic cows.

4.2.3.3 Serum urea levels

The overall mean serum urea concentration was 38.90 ± 1.30 mg/100 ml (Table 4.18). Serum urea levels correlated positively with intake of crude protein (r=0.22, P<0.01) and degradable protein (r=0.26, P<0.01) and serum glucose concentrations (r=0.17, P<0.01, Table 4.13).

Serum urea concentrations significantly varied due to calving period (P<0.01), season (P<0.05) and farm (P<0.01, Table 4.10). In NBS calvers serum urea levels were lower than those of the LBS calvers (31.69 versus 39.42 mg/100 ml). Serum urea concentrations were lowest one month prepartum and peaked at three months after parturition (P<0.01, Table 4.20).

Serum urea levels positively correlated with PEI (r=0.28, P<0.01) and POI (r=0.30,

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Table 4.20. Serum metabolites concentrations during various peripartum periods and reproductive states (least square means* ± standard error)

Group	Serum glucose (mg/100 ml)	Serum protein (g/1000 ml)	Serum urea (mg/100 ml)
Peripartum month			
-2 to -1	51.97 ± 2.00 b	80.78 ± 1.67 a	28.12 ± 2.50 c
1 to 2	57.61 ± 0.88 a	83.01 ± 0.80 a	34.32 ± 1.65 bc
2 to 3	61.59 ± 1.27 a	84.04 ± 1.24 a	43.84 ± 3.03 ab
3 to 4	58.21 ± 2.65 a	79.84 ± 2.59 a	43.10 ± 5.46 a
Probability	P < 0.01	-	P < 0.01
Postpartum ovulation interval (days)			
< 45	56.42 ± 1.10	83.53 ± 0.86 a	26.28 ± 1.68 a
45 to 74	57.50 ± 1.48	83.90 ± 1.34 a	36.50 ± 2.42 a
75 to 150	57.53 ± 1.14	79.64 ± 1.07 a	39.80 ± 2.79 a
Anestrus	58.05 ± 1.15	83.80 ± 1.08 a	38.72 ± 2.81 a
Probability	-	-	-
Postpartum estrus interval (days)			
< 45	58.72 ± 1.14 a	82.64 ± 0.99 a	30.97 ± 1.89 b
45 to 74	53.96 ± 1.14 b	85.36 ± 1.01 a	31.65 ± 1.93 b
75 to 150	56.30 ± 1.60 ab	82.74 ± 1.24 a	33.01 ± 2.80 b
Anestrus	60.27 ± 0.97 a	80.68 ± 0.85 a	46.00 ± 1.83 a
Probability	P < 0.01	-	P < 0.01
Pregnancy status			
Pregnant	57.43 ± 0.90 a	83.74 ± 0.79 a	33.15 ± 1.68 a
Pregnant	57.45 ± 1.15 a	81.98 ± 0.97 a	37.35 ± 2.02 a
Probability	-	-	-

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

P < 0.01, Table 4.13). Anestrus animals had highest serum urea concentrations (46.00 mg/100 ml) than those resuming estrus within 45 days (30.97 mg/100 ml), 45-74 days (31.65 mg/100 ml) and 75-150 days (33.01 mg/100 ml) postpartum (P < 0.01). From one month prepartum to six months postpartum, serum urea levels were repeatedly higher in anestrus than estrus buffaloes (Fig. 4.28). These results demonstrate negative effect of high serum urea levels on reproductive performance in buffaloes.

Results of the present study support the findings of Fergusson *et al.* (1991), who suggested that serum urea nitrogen concentrations > 20 mg/100 ml would impair fertility in dairy cattle. Similarly, Kaim *et al.* (1983) reported that in cows with plasma urea nitrogen of 16.8 mg/100 ml pregnancy rate was lower than in cows with plasma urea nitrogen of 9.0 mg/100 ml. However, according to Zaman (1984), the mean urea concentrations in cyclic, non-cyclic and anestrus Nili-Ravi buffaloes were 34.29 ± 10.04, 30.03 ± 9.85 and 31.63 ± 10.52 mg/100 ml, respectively and the differences were not significant.

It appears that increasing serum urea levels, associated with excess intake of crude protein may lead to delayed postpartum ovarian activity in Nili-Ravi buffaloes under field conditions and may be corrected by adopting proper feed supplementation strategy.

4.2.4 Intake and serum levels of Ca, Mg and P

4.2.4.1 Calcium

The overall mean value for calcium intake was 100.4 ± 1.60 g/day, ranging from 30.8 to 246.8 g/day (Table 4.14). Calcium intake correlated negatively with serum calcium (r=-0.14, P < 0.05) and phosphorus concentrations (r=-0.16, P < 0.05, Table 4.1). Season and farm significantly affected calcium intake (P < 0.01, Table 4.12).
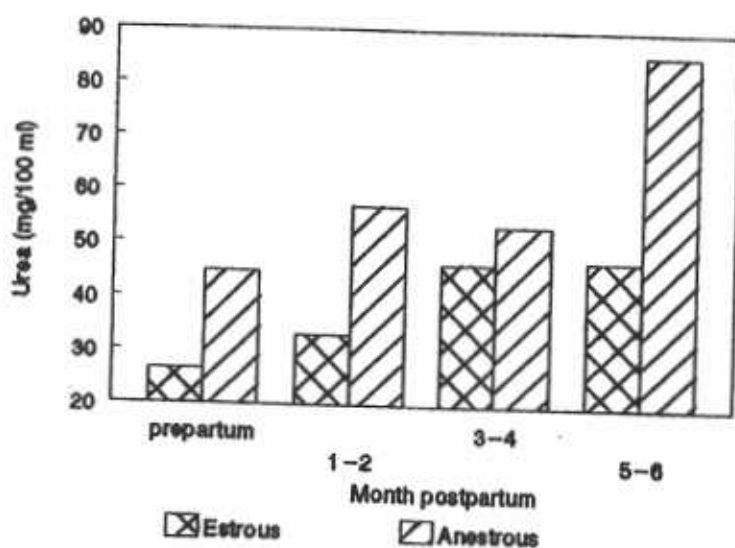
Figure 4.28. Serum urea concentrations in estrous and anestrous buffaloes
(P < 0.01)
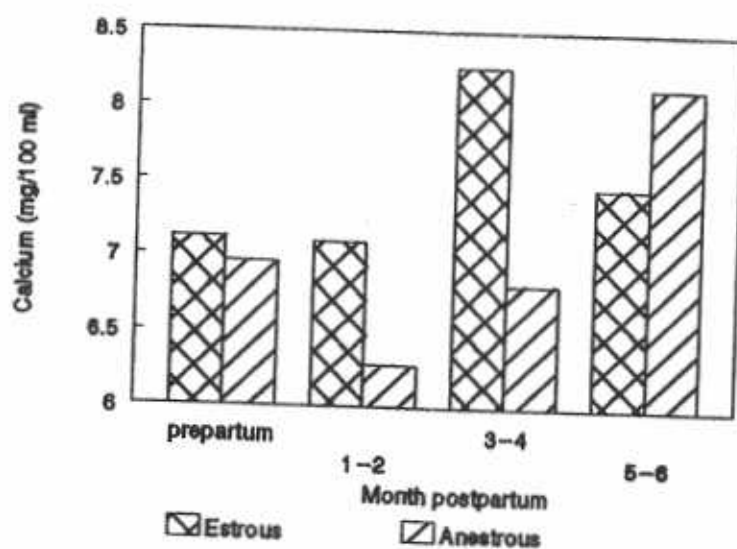
Figure 4.29. Serum calcium concentrations in estrous and anestrous buffaloes

Increasing calcium intake decreased postpartum uterine involution interval (r=-0.14, P<0.05, Table 4.3) and increased POI (r=0.13, P<0.05, Table 4.13). Calcium intake in anestrus animals was higher than those coming into estrus (112.7 versus 95.5 g/day, P<0.05). Daily calcium intake averaged 43.80 ± 1.66 g/day and was above the requirements of the buffaloes in the present study. It appears that excess calcium intake decreased reproductive performance in buffaloes.

In agreement to the present study, Morrow (1980) and Risco et al. (1984) attributed excess calcium intake to impairment in the reproductive function by causing a secondary deficiency of phosphorus, magnesium, copper and zinc, through inhibiting their absorption from the intestine. In line with the present study, these authors suggested that reduced serum calcium delayed uterine involution.

Mean serum calcium levels was 7.23 ± 0.14 mg/100 ml, ranging from 1.79 to 14.70 mg/100 ml (Table 4.18). Serum calcium levels correlated positively with serum glucose (r=0.17, P<0.01), protein (r=0.23, P<0.01) and phosphorus levels (r=0.33, P<0.01, Table 4.13). Serum calcium decreased with increasing intake of calcium (r=-0.14, P<0.05), magnesium (r=-0.27, P<0.01) and phosphorus-(r=-0.14, P<0.05) and decreasing intake of zinc (r=0.17, P<0.01). Calving period (P<0.01), season (P<0.05) and farm (P<0.05) had a significant effect on serum calcium levels (Table 4.11). The highest serum calcium values were noted in autumn (8.35 ± 0.23 mg/100 ml), followed by spring (7.95 ± 0.35 mg/100 ml), summer (7.28 ± 0.28 mg/100 ml) and winter (5.94 ± 0.24 mg/100 ml).

From two months prepartum to four months postpartum, serum calcium levels remained consistently higher in the buffaloes resuming estrus than the anestrus animals (Fig. 4.29), however the difference between the two groups was only significant (P<0.01) during third and

four months postpartum. Buffaloes becoming pregnant had apparently higher serum calcium than non-pregnant animals (7.65 versus 6.55 mg/100 ml). However, the difference was non-significant.

Results of the present study are partially in line with those of Kumar and Sharma (1993), who found that the fertile buffaloes had higher serum concentrations of calcium than the infertile ones (9.94 $\pm$ 0.26 versus 8.37 $\pm$ 0.45 mg/100 ml, P<0.05). Similarly,-El-Belely (1993) reported that cyclic and pregnant cows had higher concentrations of plasma calcium between days 3 and 7 postpartum than repeat breeders. However, Satish-Kumar and Sharma (1991) reported that in cows which did not become pregnant calcium levels were not different from those that became pregnant. Similarly, Sekerden *et al.* (1992) reported that in Jersey cows, serum calcium levels were not correlated to conception rates at first insemination.

4.2.4.2 Magnesium

The average magnesium intake was 33.70 $\pm$ 0.26 g/day, ranging from 16.7 to 60.2 g/day (Table 4.14) and was 15% higher than the NRC standard requirements of 0.20% (NRC, 1989). The major source of magnesium in this study was berseem, having 0.46% contents on dry matter basis, followed by oilseed cakes, maize stovers and maize fodder. Increasing magnesium intake decreased serum calcium and magnesium levels (r=-0.27 and -0.22, respectively, P<0.01) and increased serum phosphorus levels (r=0.31, P<0.01, Table 4.17). Farm (P<0.01) and season (P<0.05) had a significant effect on magnesium intake (Table 4.12). Highest magnesium intake was recorded during spring followed by winter, summer and autumn (35.86, 34.40, 32.65, 31.60 g/day, respectively, Fig. 4.14). However, Lu *et al.* (1995) reported that in Northern China magnesium and phosphorus were lowest in animal feeds during winter

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and early spring.

Magnesium intake tended to prolong placenta delivery duration (r=0.17, P<0.05), PUI (r=0.37, P<0.01) and POI (r=0.90, P<0.01) and shortened duration of lochia (r=-0.27, P<0.05, Table 4.13). Anovu-latory animals and those with POI of five months, had higher magnesium intake as compared to buffaloes with shorter POI (P<0.01). Animals becoming pregnant had lower magnesium intake as compared to non-pregnant animals (34.9 versus 32.2 d/day, P<0.01). Magnesium intake was higher in anestrus animals as compared to estrus animals (34.8 versus 33.4 g/day, P<0.05). These results indicate adverse effects of excess magnesium intake on reproductive performance in buffaloes under the conventional farming in NWFP.

In agreement with this study, in 77 female buffaloes wit 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 Gabryszuk (1994) who found that in ewes, after magnesium injections, the fertility was 92 and 94%, while in the control group the values were 72 and 70%, respectively.

The overall mean value for serum magnesium levels was 26.40 ± 0.57 mg/1000 ml, ranging from 3.10 to 60.30 mg/1000 ml (Table 4.18). The levels correlated positively with serum protein (r=0.22, P<0.01), urea (r=0.22, P<0.01) and metabolizable energy intake (r=0.18, P<0.01, Table 4.17). In previous studies, mean serum magnesium values were found to be 20 mg/1000 ml in cattle (Rojas *et al.*, 1993) and 21.2 mg/1000 ml (Dua *et al.*, 1986) in buffaloes.

Calving period and farm significantly affected serum magnesium levels (P<0.01, Table

4.11). Higher values were noted in LBS calvers than NBS calvers (28.64 versus 21.70 mg/1000 ml). The values during summer and winter were higher (26.59 and 24.65 mg/l) as compared to autumn and spring (23.61 and 21.45 mg/1000 ml).

Serum magnesium levels correlated negatively with PUI (r=-0.20, P<0.05) and positively with POI (r=0.14, P < 0.07, Table 4.13). Highest levels were found in animals coming into estrus during the first month postpartum and lowest in those coming into estrus in the third month (P<0.01, Table 4.21). The buffaloes becoming pregnant had lower serum magnesium levels than non-pregnant animals (18.74 versus 22.54, P<0.01). In the anestrus group, serum magnesium levels were constantly higher than the anestrus group, although non-significantly, from prepartum up to six months postpartum period (Fig. 4.30). These findings show an adverse effect of high serum magnesium on fertility in buffaloes.

Wittkowki et al. (1993) reported that magnesium deficiency is involved in impairment of fertility. According to Dvoracek (1988), at 8 farms where the conception rates were below 40%, the potassium : calcium + magnesium ratio was in excess, 174.5% of standard requirements. Studies on 426 pregnant goats in the semi-arid northeastern region of Brazil revealed subnormal blood magnesium, copper and zinc in animals with higher incidence of abortion (Unanian and Silva, 1989).

Analysis of blood samples from 136 dairy cows with low or normal fertility, revealed no differences between the groups in the plasma magnesium concentrations (Vijchulata, 1995). Pathak and Janakiraman (1987) did not find any specific trend in the concentration of magnesium during pregnancy or estrus and reported an average value of 33.7 mg/1000 ml. Similarly in Egyptian cows and buffaloes (Osman et al., 1985) serum magnesium levels were not related to cyclicity or stage of estrus cycle.

Table 4.21. Serum mineral concentrations during various peripartum periods and reproductive states (least square means* ± standard error)

Group	Serum calcium (mg/100 ml)	Serum magnesium (mg/1000 ml)	Serum phosphorus (mg/100 ml)
Peripartum month			
-2 to -1	6.81 ± 0.35 a	21.02 ± 1.09 a	3.41 ± 0.27 a
1 to 2	6.78 ± 0.19 a	22.42 ± 0.80 a	4.19 ± 0.17 a
2 to 3	7.78 ± 0.24 a	19.11 ± 1.25 a	4.19 ± 0.25 a
3 to 4	6.42 ± 0.62 a	17.44 ± 2.02 a	3.96 ± 0.37 a
Probability	-	-	-
Postpartum ovulation interval (days)			
< 45	6.60 ± 0.24 a	22.61 ± 0.74 a	4.13 ± 0.19 a
45 to 74	7.71 ± 0.21 a	17.83 ± 1.15 a	2.74 ± 0.22 b
75 to 150	6.83 ± 0.22 a	22.94 ± 1.15 a	4.15 ± 0.20 a
Anestrus	6.96 ± 0.26 a	19.24 ± 1.16 a	4.07 ± 0.23 a
Probability	-	$P < 0.05$	$P < 0.05$
Postpartum estrus interval (days)			
< 45	7.01 ± 0.22 a	25.31 ± 0.92 a	4.11 ± 0.18 ab
45 to 74	8.34 ± 0.28 a	18.04 ± 0.71 a	3.62 ± 0.20 ab
75 to 150	6.32 ± 0.23 b	17.24 ± 1.23 a	3.30 ± 0.26 b
Anestrus	6.71 ± 0.18 a	23.25 ± 0.75 a	4.35 ± 0.18 a
Probability	$P < 0.01$	-	$P < 0.05$
Pregnancy status			
Pregnant	7.65 ± 0.18 a	18.74 ± 0.69 b	3.61 ± 0.16 b
Non-pregnant	6.55 ± 0.22 b	22.54 ± 0.97 a	4.14 ± 0.19 a
Probability	$P < 0.05$	$P < 0.01$	$P < 0.01$

* Means within a group in the same column with different letters differ significantly from each other
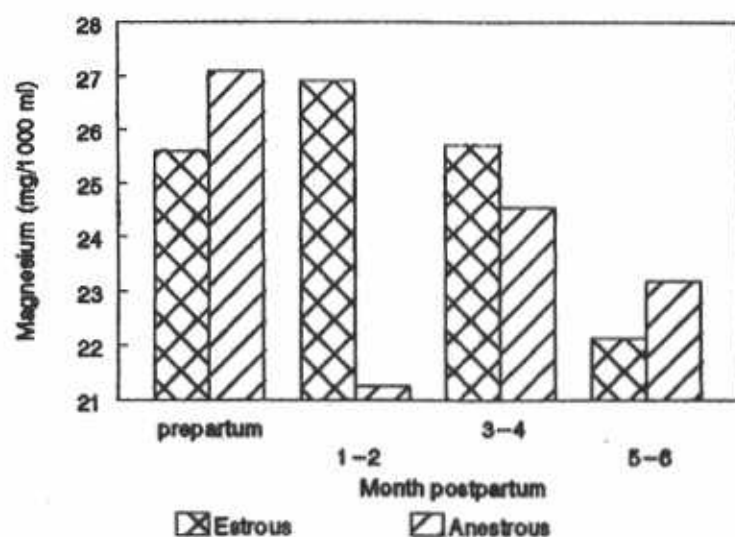
*Figure 4.30. Serum magnesium conentrations in estrous and anestrous
buffaloes (P <0.08)*
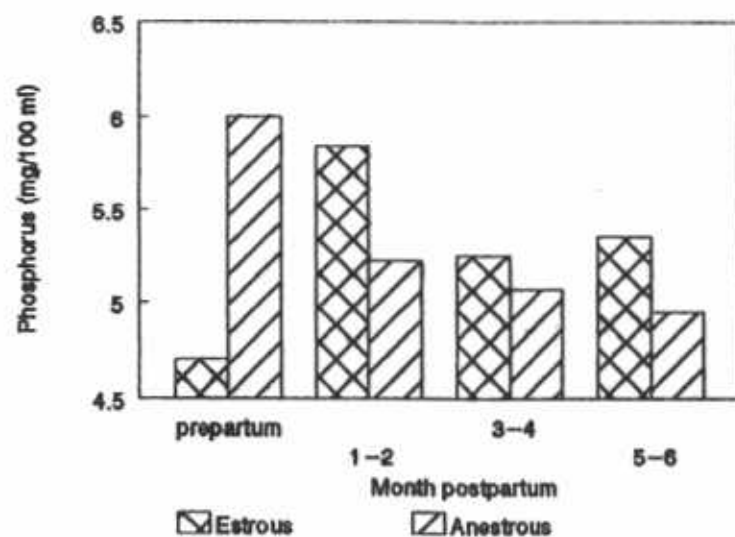
*Figure 4.31. Serum phosphorus concentrations in estrous and anestrous
buffaloes (P <0.05)*

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4.2.4.3 Phosphorus

Phosphorus intake averaged 38.40 ± 0.40 g/day (Table 4.14), ranging from 16.0 to 64.4 g/day. Phosphorus intake correlated negatively with serum phosphorus (r=-0.13, P < 0.08) and serum calcium levels (r=-0.14, P<0.05, Table 4.17). Phosphorus intake delayed placenta delivery duration (r=0.22, P<0.01) and POI (r=0.48, P<0.01, Table 4.13) and shortened uterine involution interval (r=-0.32, P<0.01, Table 4.3). Phosphorus intake was different in different seasons (P<0.01) and farms (P<0.01, Table 4.12). During summer, highest phosphorus intake was noted, followed by autumn, winter and spring (51.19, 43.88, 39.81, 35.08 g/day, respectively). The difference in phosphorus intake during various seasons and farms may be due to differences in availability of feed ingredients. In the present study the intake of phosphorus was in excess with an average of 1.70 ± 0.50 g/day and the increasing intake levels were associated with a decline in fertility.

The overall serum phosphorus levels were 5.44 ± 0.12 mg/100 ml, ranging from 1.23 to 11.18 mg/100 ml (Table 4.18). Serum phosphorus levels correlated positively with serum protein (r=0.21, P<0.01), serum calcium (r=0.33, P<0.01) and serum urea levels (r=0.21, P<0.01, Table 4.17). Serum phosphorus levels also correlated positively with metabolizable energy intake (r=0.18, P<0.05). Serum phosphorus levels were different in different farms (P<0.01) but not in different seasons.

Serum phosphorus levels correlated with placenta expulsion duration (r=0.31, P<0.05, Table 4.13). The buffaloes coming into estrus within 150 days postpartum had lower serum phosphorus levels than anestrus ones. However, postpartum serum phosphorus concentrations were higher in estrus group as compared to anestrus group (Fig. 4.31). It reflects the favorable effect of increasing serum phosphorus concentrations on reproductive performance.

In agreement with the present findings, in rural Indian cows (Satish-Kumar 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 number of services required for conception declined from 3.7 to 1.3 with proper supplementation of ration with phosphorus in dairy heifers (Morrow, 1969) and the weaned calf crop increased from 48 to 71 % in cows supplemented with dicalcium phosphate provided as a free choice lick (Read et al., 1986). Quayam et al. (1988) reported that in pluriparous buffaloes, serum concentrations of inorganic phosphorus, 4.35 ± 0.28 mg/100 ml at 5 days postpartum, were significantly correlated with the occurrence of estrus within 60 days postpartum.

In contrast to the present study, Sekerden et al. (1992) found that serum phosphorus levels were not correlated with conception rates to first insemination in Jersey cows. Similarly, Veerapandian et al. (1987) reported that inorganic phosphorus levels did not differ between estrus and anestrus buffaloes.

4.2.4.4 Calcium-Phosphorus ratio

The ratio of intake of Ca:P ranged from 0.7 to 6.4. The increasing ratio was associated with delayed ovulation as depicted in Fig. 4.32. Serum Ca:P ratio varied from 0.4 to 3.5. Reproductive performance was optimum for serum Ca:P ratio from 0.4 to 2.0 but

higher ratio from 2.1 to 3.5 was associated with prolonged postpartum estrus and ovulation intervals and lower conception rates

It suggests that higher intake and blood concen-trations of Ca:P may adversely affect reproductive performance of buffaloes.

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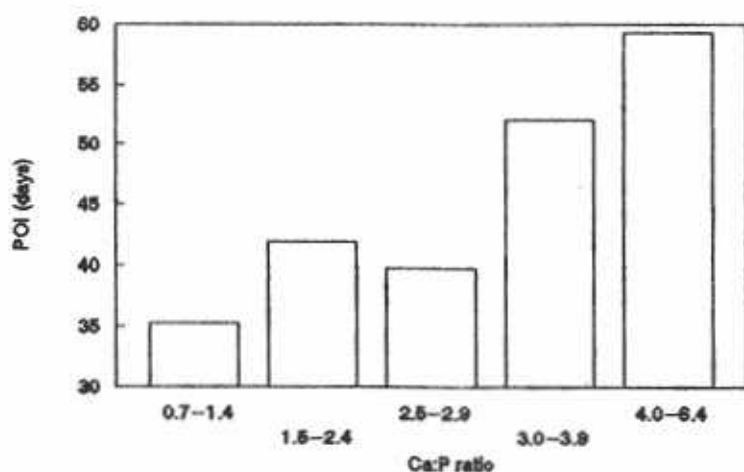
Figure 4.32. Effect of intake of Ca:P ratio on postpartum ovulation interval (POI) in buffaloes
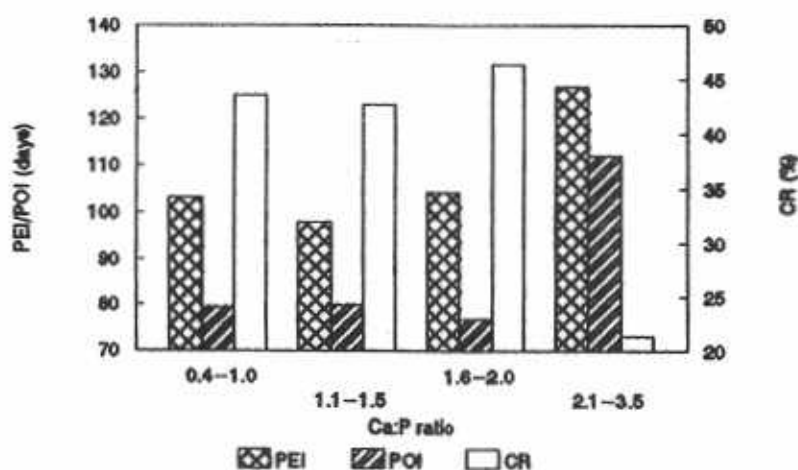
Figure 4.33. Effect of serum Ca:P ratio on postpartum estrus (PEI) and ovulation interval (POI) and conception rate (CR) in buffaloes
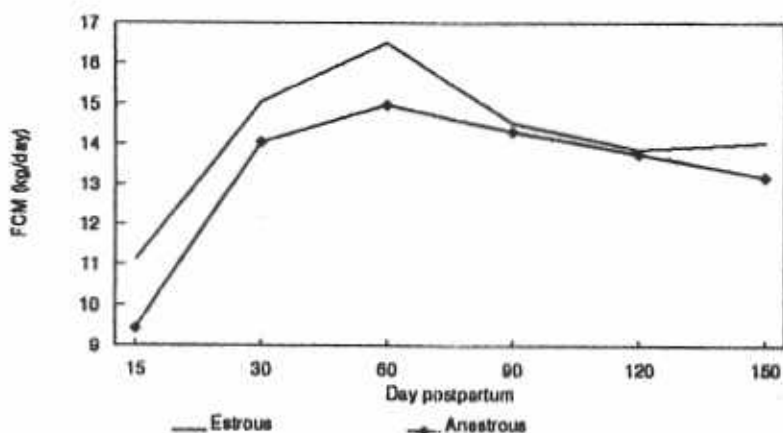
Figure 4.34. Postpartum 4% fat corrected milk production (FCM) in estrous and anestrous buffaloes

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In a previous study on Nili-Ravi buffaloes, the serum Ca:P ratio was narrower in cyclic animals than non-cyclic ones (2.4 versus 2.9, Ali *et al.*, 1983). Optimum fertility in cattle has been reported when the ratio of calcium to phosphorus in the diet was 1.0 to 3.0 (Annenkov, 1982). The fertility was adversely affected by both excess and deficiency of calcium and phosphorus.

4.2.5 Zinc and copper intake

The average intake of zinc was 1138.70 ± 7.53 mg/day, ranging from 644.1 to 1611.5 mg/day (Table 4.14). On dry matter basis, this intake was equivalent to 69 ppm which is higher by 72.5% than NRC (1989) requirements of 40 ppm. Zinc intake was correlated positively with serum calcium, magnesium ($r=0.17$ and 0.28, $P<0.01$) and negatively with phosphorus levels ($r=-0.68$, $P<0.01$, Table 4.17). Increasing zinc intake increased placenta expulsion duration and lochia ($r=0.23$ and 0.22, $P<0.01$) and decreased postpartum uterine involution and ovulation intervals ($r=-0.50$, -0.52, $P<0.01$, Tables 4.3, 4.13). Zinc intake was different at various seasons ($P<0.01$) and farms ($P<0.01$).

In agreement to this study, Khattab *et al.* (1995) reported that serum zinc levels were significantly 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.

The overall average value for intake of copper was 228.20 ± 3.60 mg/day, ranging from 82.30 to 584.50 mg/day (Table 4.14). On dry matter basis, this intake was equivalent to 14 ppm which was higher by 40% than NRC requirements of 10 ppm (NRC, 1989). Intake of copper correlated with serum phosphorus levels ($r=-0.17$, $P<0.05$, Table 4.17). Increasing copper

intake decreased postpartum uterine involution and ovulation intervals ($r = -0.31$, -0.32, $P < 0.01$). Intake of copper was different at different calving periods ($P < 0.01$), seasons ($P < 0.01$) and farms ($P < 0.01$, Table 4.12). The favorable effect of copper intake in the present study may be due to the lower copper levels during low breeding season (Fig. 4.17)

In agreement to the present study, serum copper levels were significantly higher in buffaloes with regular estrus cycles (Khattab *et al.*, 1995). Copper intake of 118 to 245 mg/day has been reported in a previous study on Egyptian buffaloes (Attia *et al.*, 1987a). Lack of copper affected body growth and caused silent estrus (Wittkowski and Spann 1993). Suboptimal ovarian activity, delayed or depressed estrus, reduced conception rates, early embryonic death, increased incidence of retained placenta, calving difficulties and congenital rickets have been associated with copper deficiency (Hidiroglou, 1979).

4.3 EFFECT OF OTHER PARAMETERS ON REPRODUCTIVE PERFORMANCE

4.3.1 Body condition score

On a scale ranging from 0.0 to 5.0, body condition score (BCS) of 51 buffaloes during the late prepartum and early postpartum periods, varied from 1.0 to 4.0. The overall average BCS was 2.72 ± 0.02 (Table 4.22).

Based on BCS, the buffaloes were grouped into three categories i.e. poor (BCS 1.0-2.0), moderate (BCS 2.5) and good (3.0-4.0, Table 3.3). Fig. 4.19 shows that majority of the buffaloes having poor BCS (1.5 to 2.5) belonged to the LBS calving group. Conversely, most of the NBS calving buffaloes had good BCS, which was apparently due to a higher intake of metabolizable energy ($P < 0.01$) during summer and autumn i.e. NBS (Table 4.8). BCS positively correlated with production of fat-corrected milk ($r = 0.16$, $P < 0.01$) and negatively with suckling duration ($r = -0.16$, $P < 0.01$). The prepartum BCS correlated positively with postpartum loss of BCS ($r = 0.56$, $P < 0.01$) and production of fat corrected milk ($r = 0.26$, $P < 0.05$).

BCS was significantly affected by the period of calving ($P < 0.01$), season of the year ($P < 0.01$) and farm ($P < 0.01$, Table 4.8). Animals calving during the NBS had significantly high BCS as compared to those calving during LBS (2.82 versus 2.60), which is the probable cause of low reproductive efficiency. Prepartum BCS was the highest (2.97) and during first two months postpartum it was the lowest (2.65, $P < 0.01$).

BCS negatively correlated with placenta expulsion duration ($r = -0.17$, $P < 0.05$) and PEI ($r = -0.20$, $P < 0.01$, Table 4.13). Fig. 4.23 shows that BCS in the buffaloes resuming estrus was constantly higher than those failing to resume estrus activities. This clearly suggests that under field conditions poor heath condition of buffaloes may be a

Table 4.22. Number of observations (n), means and standard errors (SE) of various traits in Nili-Ravi buffaloes

Traits	n	Mean	±	SE
Overall body condition score	1023	2.72	±	0.02
Body condition score (prepartum)	51	2.77	±	0.10
Body condition score loss	51	0.25	±	0.08
Body weight prepartum (kg)	50	510.00	±	5.90
Body weight loss 30 days postpartum	50	3.70	±	2.12
Body height (cm)	50	135.80	±	0.67
Heart girth (cm)	50	196.00	±	1.39
Body length (cm)	50	147.40	±	0.99
4% Fat-corrected milk (kg/day)	898	14.50	±	0.20
4% Fat-corrected-milk (kg/150 days)	50	1989.00	±	118.37
Calf weight (kg)	33	33.60	±	0.75
Calf survival (days)	43	26.40	±	5.57

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key factor for their low reproductive efficiency.

Results of this study confirm the findings of Osoro and Wright (1992) and De Rouen *et al.* (1994), who concluded that body condition at calving significantly affected postpartum reproductive performance in cows. O'Rourke *et al.* (1991) reported that cows with BCS of $\geq$ 8 had a conception rate 33% higher than those with score $\leq$ 5 (scale 3-9). Buffaloes in poor body condition had inactive ovaries and long periods of postpartum anestrus (Jainudeen and Wahab, 1987). Bhalaru *et al.* (1987) reported that the conception rates were significantly higher (88.3%) for buffaloes with moderate body condition, scoring 2.5-3.5, than for females scoring 1-2 (65.8%) or 4-5 (70.8%). The interval to first postpartum estrus was significantly shorter in moderate and fat females (66.2 and 66.9 days, respectively) than in thin females (77.7 days). The service period of moderate BCS females was significantly shorter (128.3 days) than fat or thin females (144.1 and 164.5 days, respectively). Dunn and Kaltenbach (1990) found that in cows with moderate BCS, a prepartum gain in weight resulted in 25% better cyclicity than those loosing weight. In cows with poor BCS the cows gaining weight showed 45% higher cyclicity.

Analysis of data on 172 primiparous and 821 multiparous cows from 55 herds showed that 45 days after calving, 62% of animals with good BCS were cycling, compared with 10% of animals with a poor BCS (Gary *et al.* 1989). In spite of low BCS at calving, 67% of the thin cows that gained weight before calving showed estrus by 60 days postpartum (Dunn and Moss, 1992). Richards *et al.* (1989b) reported that anestrus occurred when non-lactating Hereford cows lose weight and had a BCS of approximately 3.5 (scale 1-9). Anestrus was associated with a decrease in LH pulses. Contradicting to

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our findings, Jones *et al.* (1988) found that BCS at calving did not effect the intervals from calving to first service and conception, or number of insemination per conception. This lack of relationship may be due to maintenance of constant average BCS values through controlled feeding while during the present study the animals were exposed to variations in BCS due to seasonal and nutritional variations during various time periods.

The findings of the present study clearly suggest that under field condition, poor health condition of buffaloes (BCS < 2.75) may be a key factor for low reproductive efficiency in the animals. Approaches to improve pre- and postpartum nutrition of such animals are likely to be of great help in overcoming the problem.

4.3.2 Calf suckling

Calf suckling duration (calf survival) averaged 26.40 ± 5.57 days, ranging from 0 to 150. Increasing suckling duration delayed POI ($r=0.19$, $P<0.01$) and PEI ($r=0.23$, $P<0.01$) and shortened duration of discharge of lochia ($r=-0.26$). As the calves were allowed to suckle two times daily, it probably resulted in adverse effect on resumption of postpartum ovarian activity be increasing postpartum ovulation and estrus intervals. Suckling duration stimulated uterine involution and improved conception rates (Fig. 4.3).

Suckling was used by the farmers for milk let down, twice a day for approximately five minutes each time. Under the conventional system the calves are not used for herd replacement and their management is given a little attention. Out of the 51 calves born in the experimental herd, only 8 (15.69%) could survive beyond 150 days while the rest died or were slaughtered, although some of them were born of excellent milk producers. In case of death of the calves,   the dams were given oxytocin

injections for milk let down at a dose rate of 5 IU intra-muscularly, exceeding sometimes to 25 IU. 33 buffaloes (64.7%) were milked with the use of oxytocin and use of oxytocin enhanced uterine involution. One injection per day shortened while no injection or two injections per day increased PEI (Fig. 4.2).

In agreement with this study, ovarian cyclicity was re-established earlier in non-suckled than suckled river, as well as swamp buffaloes, (El-Fouly et al. 1976, El-Fadaly 1980, Jainudeen et al. 1984). In Nili-Ravi buffaloes, Usmani et al. 1990) reported that postpartum intervals to uterine involution, resumption of follicular development, first rise in milk progesterone, first palpable corpus luteum and first oestrus were longer for limited-suckled buffaloes than for non-suckled buffaloes.

Similarly, Williams et al. (1990) reported that only two or three suckles per day were required to delay the occurrence of postpartum estrus in cows. The delay due to suckling was attributed to either an interference with the release of GnRH from the hypo-thalamus or the inability of pituitary gland to respond appropriately to GnRH stimulation. Weaning at birth resulted in resumption of ovarian activity by day 10 to 14 postpartum which occurred coincidently with a transient increase in pulsatile release of LH between day 7 and 14 postpartum (Carruthers and Hafs, 1980). Early weaning of calves from lactating beef cows at day 30 postpartum, alone or in combination with hormone therapy, reduced the PEI and increased conception rates (Smith and Vincet, 1972).

Prolactin has been considered as a potential antigonadotropic agent mediating postpartum acyclicity in most species and suppression of prolactin release by CB-54 (ergcryptine, a dopamine agonist) blocked lactation and established early postpartum ovulatory cycles in human female (Rolland et al. 1975). Williams and Ray (1980)

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blocked prolactin release in early postpartum cows with CB-154 but they were unable to change blood gonadotropin concentrations or postpartum intervals by this treatment alone. Williams (1990) suggested that it is the suckling stimulus itself that suppresses gonadotropin secretion, rather than high prolactin concentrations associated with suckling.

4.3.3 Effect of milk production

Average daily production of 4% fat-corrected milk (FCM) was 14.50 ± 0.20 kg per animal, ranging from 2 to 35 (Table 4.22). FCM was significantly effected by calving period and farm ($P < 0.01$) but not by season of the year (Table 4.8). FCM was positively correlated with BCS ($r = 0.16$, $P < 0.01$), intake of crude protein ($r = 0.12$, $P = 0.05$) and negatively with excess intake of crude protein ($r = -0.40$, $P < 0.01$) and metabolizable energy (-0.65, $P < 0.01$). It was also correlated positively with use of oxytocin injection for milk let down ($r = 0.15$, $P < 0.01$). These results suggest that protein intake favors milk production but excess intake of protein and energy adversely effect milk production. More-over milk production is increased by use of exogenous oxytocin, probably by completely evacuation of the udder.

The animals calving in the NBS showed significantly higher milk production ($P < 0.01$) as compared to those calving during the LBS (15.26 versus 13.61 kg/day). It was probably the higher BCS in the NBS supporting the higher milk production and better reproductive performance. Maintenance of better body condition as well as higher milk yield in this study was probably due to the fact that buffaloes can hold their body condition better than cattle under stressed conditions (Underwood et al. 1982).

FCM was significantly higher in estrus group as compared to anestrus one, during

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the first two months postpartum (15.09 versus 13.56 kg/day, P<0.01, Fig. 4.34).
Under field conditions in dairy buffaloes used in this study, lactating animals are fed on
same scale irrespective of their milk yield and feed requirements. Increasing degradable
protein/milk production ratio decreased conception rates (Table 4.26). Therefore it is
suggested that due to less requirements of nutrients for milk production in the low
yielding buffaloes as compared to high yielders, the detrimental effect of excess intake
of crude protein on the reproductive performance of low yielders was pronounced.
Detrimental effects of excess protein intake on reproductive performance has already
been discussed in Section 4.2.2 of this thesis.

In the present study, FCM correlated positively with POI (r=0.31, P<0.01).
These results support the findings of Slanina and Hlinka (1991), who reported that
performance of dairy cows was effected when annual yields were more than 4000 ± 400
kg. Nauk et al. (1991) found that the conception rates were significantly lower in cows
with milk yields > 5000 kg (47.1%) than in cows with yields ≤ 4000 kg (63.7%).
McClary (1991) reported that reduced fertility in the higher-yielding dairy cows was not
directly related to increased milk yield but rather to loss of body weight and body
condition. Investigations in buffaloes had indicated that lactation number and milk yield
might have an adverse effects on it's fertility (El-Belely 1984). Zia-ul-Hasnain (1981)
reported that milk yield in the cyclic buffaloes was not different from non-cyclic Nili-
Ravi buffaloes.

It appears that high milk yield in buffaloes prolonged postpartum ovulation.
Moreover proper pre- and postpartum feeding will help in attaining higher BCS and
maintaining postpartum milk production and resumption of ovarian activity.

4.4   CONCLUSIONS AND RECOMMENDATIONS

4.4.1 CONCLUSIONS

Relationship of pre- and postpartum nutritional status with reproductive performance was studied in Nili-Ravi buffaloes under the conventional farming system in the North-West Frontier province (NWFP) of Pakistan. Based on results of the study, the following conclusions can be made:

1.   Under the field conditions of NWFP the normal breeding season (NBS) for Nili-Ravi buffaloes is autumn and winter while low breeding season (LBS) is spring and summer.

2.   NBS was associated with higher body condition score (BCS), higher intake of metabolizable energy (ME) and zinc, lower intake of crude protein (CP), CP:ME ratio, phosphorus and copper, ᵇⁱᵍʰᵉʳ low serum urea and/milk progesterone levels.

3.   Postpartum uterine involution interval (PUI) is delayed by increased loss in BCS 30 days postpartum, higher magnesium intake and blood calcium levels, decreased intake of CP, CP/ME ratio, calcium, phosphorus, zinc and copper and blood magnesium levels. Administration of oxytocin injections for milk let-down hastened PUI. The delayed uterine involution resulted in a delayed resumption of postpartum ovarian activity.

4.   During the postpartum period of 150 days, estrus resumption occurred in 68.63% animals, while 31.37% buffaloes remained anestrous. The overall mean for postpartum estrus interval (PEI) was 69.03 days and that of postpartum ovulation interval (POI) was recorded as 59.37 days. Estrus events comprised 43.9% ovulatory estrus, 4.6% anovulatory estrus, 51.1% silent estrus; 29.4% of silent

ovulations occurred in the NBS and 70.6% in the LBS calvers.

5. Optimum BCS, greater than 2.75 on scale of 1 to 5, supported higher milk yield and resulted in earlier resumption of postpartum estrus activity.

6. Excess intake of CP is one of the major factors contributing to delay in the onset of ovarian activity. With the increasing ratio of intake of degradable protein / milk production, conception rates linearly decreased. Buffaloes resuming estrus had a narrow /ratio of 11.9-12.2 g/MJ versus 10.7-13.1 g/MJ for those not coming into estrus.

CP/ME

7. Higher CP and calcium intake resulted in higher serum urea and low serum calcium levels respectively, which in turn were associated with anestrus.

8. Suckling duration enhanced uterine involution but delayed postpartum ovulation.

4.4.2 RECOMMENDATIONS

Based on findings of this study, the following recommendations can be made for improvement of reproductive performance of dairy buffaloes under field conditions of NWFP:

1. Seasonality of reproduction may be minimized by adopting proper pre- and postpartum feeding strategies, to achieve BCS ($>$ 2.75) during the LBS and minimize heat stress during the hot summer months. This would hasten uterine involution and resumption of ovarian activity.

2. Feeding buffaloes according to their maintenance and production requirements will ensure efficient exploitation of production potential of the animals. Excessive feeding of degradable protein should be avoided to curtail its deterimental effects on reproduction. Protein supplements should be analyzed regularly, for ruminal degradability.

3. Serum metabolic and mineral profiles need intensive investigations so that their role as indicators of nutritional and fertility status becomes more clear.

4. Adoption of proper calf weaning schedule would accelerate uterine involution during the first month postpartum, which in turn would ensure earlier resumption of ovarian activity and save a lot of milk lost through calf suckling.

CHAPTER 5

SUMMARY

Reproductive performance of dairy and Swamp buffaloes under village conditions, is low due to substandard management and imbalanced nutritional status, making the farming a non-profitable business. Studies were conducted to investigate the relationship of per- and postpartum nutritional status with reproductive performance in Nili-Ravi buffaloes under the conventional farming system in the North-West Frontier Province (NWFP) of Pakistan.

Fifty one pregnant buffaloes were selected at seven private farms in the Central Valley of NWFP. These animals were monitored from two months prepartum to five months postpartum, for expulsion of placenta, discharge of lochia, uterine involution (PUI), postpartum ovulation (POI) and estrus interval (PEI), production of 4% fat corrected milk (FCM), milk progesterone levels (MPL) and body condition score (BCS). Feed samples were collected fortnightly and analyzed for dry matter, ash, crude protein (CP), crude fiber, ether extract, copper, zinc, phosphorus, calcium and magnesium levels and dry matter degradibility. Metabolizable energy (ME) value of feeds and nutrient requirements for the buffaloes were calculated. Blood samples were collected at fortnightly intervals and assayed for glucose, urea, protein, calcium, magnesium and phosphorus.

The mean value for PUI interval was 34.30 ± 1.33 days. Uterine involution completed within 35 days in 55.0% buffaloes, within 50 days in 85.0% and within 74 days in all buffaloes. PUI interval was positively correlated with placental delivery duration, prepartum body weight and loss in BCS 30 days postpartum. Intake of CP,

CP/ME ratio, calcium, phosphorus, zinc and copper related negatively, while magnesium positively, with PUI interval. Serum calcium concentrations related positively, while magnesium negatively, with PUI interval. PUI interval negatively correlated with frequency of use of oxytocin. Both PEI and POI were positively correlated with PUI.

During 150 days postpartum, 68.63% buffaloes were found in estrus and 31.37% animals remained anestrus. The overall mean for PEI was 69.03 ± 6.03 days and POI was 59.37 ± 4.76 days. Three types of estrus events were observed i.e. ovulatory estrus (43.9%), anovulatory estrus (4.6%) and silent estrus (51.1%). About 62.1% of the total 29 ovulatory estrus events occurred in normal breeding season (NBS, August to January) calvers, while 37.9% in low breeding season (LBS, February to July) calvers. Silent ovulation was identified as the most prevalent reproductive problem, 29.4% of the cases occurring in the NBS calvers and 70.6% in the LBS calvers.

The overall mean for MPL was 1.37 ± 0.17 ng/ml. MPL was higher in NBS calvers (1.97 ± 0.30 ng/ml) than LBS calvers (0.68 ± 0.08 ng/ml). For 24 first and 11 second estrus cycles the average length was 20.0 days and 23.6 days, respectively. MPL reached to the lowest levels on the day of estrus (0.10 ng/ml). Ovulation was indicated by a rise in progesterone levels which reached to its peak on day 7. In anestrus buffaloes MPL remained constantly low and in pregnant buffaloes these levels remained > 1.0 ng/ml.

Buffaloes calving during the NBS had shorter PEI than LBS calvers. Highest number of estrus events were recorded in autumn (39.86%), followed by summer (31.16%), spring (18.83%) and winter (10.15%). MPL 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 in the region. Better performance of

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buffaloes during the NBS was associated with minimum intake of CP, maximum intake of ME and low CP/ME ratio. Calcium intake was higher in NBS clavers than LBS calvers. Phosphorus and copper intake in the buffaloes calving during NBS was lower than those calving during LBS. Zinc intake was highest in autumn, followed by spring, winter and summer. In the buffaloes calving during the 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 tended to remain lower ($P < 0.01$) than those calving during the LBS. BCS tended to increase from July onward reaching to its peak in October and then declined up to June.

The overall mean for ME intake was 41.70 ± 0.25 MCal/day. ME intake correlated with BCS, expulsion of placenta and discharge of lochia. Increasing ME intake above requirements reduced POI. Prepartum MEI intake was higher in estrus group than the anestrus one (42.34 versus 37.24 MCal/day, $P < 0.05$). CP intake showed a mean value of 1.82 ± 0.47 kg/day and correlated positively with serum urea levels, placenta expulsion duration, PEI, POI and negatively with PUI. CP/ME ratio related positively with milk production and negatively with BCS. The estrus animals had a narrow and almost constant ratio (11.9-12.2 g/MJ) while the anestrus animals had a widely fluctuating one (10.7-13.1 g/MJ). Degradable protein intake (DPI) was 1.32 ± 0.01 kg/day. With the increasing DPI/FCM ratio, conception rates decreased.

The overall mean value for serum glucose concentrations in buffaloes was 57.80 ± 0.71 mg/100 ml. The overall mean value for serum protein levels was 83.50 ± 0.62 g/1000 ml. Serum protein levels negatively correlated with POI. Serum protein concentrations increased gradually postpartum and the levels in anestrus buffaloes never reached those of estrus ones. The overall mean serum urea concentration was 38.90 $\pm$

1.30 mg/100 ml and correlated positively with intake of CP, DP, PEI and POI. Anestrus animals had higher serum urea concentrations (46.00 mg/100 ml) as compared to those resuming estrus within 45, 45-74 and 75-150 days postpartum, showing and adverse effect of higher serum urea levels on reproductive performance in buffaloes.

The overall mean value for calcium intake was 100.4 ± 1.60 g/day. Calcium intake decreased serum calcium and phosphorus concentrations and PUI but increased POI. Calcium intake in anestrus animals was higher than those coming into estrus. Calcium intake was above the requirements with a mean value of 43.80 ± 1.66 g/day. Therefore the increasing calcium intake was associated with decreasing reproductive performance in buffaloes. Mean serum calcium levels was 7.23 ± 0.14 mg/100 ml and was correlated positively with serum glucose, protein and phosphorus levels and intake of zinc and negatively with intake of calcium, magnesium and phosphorus. From two months prepartum to four months postpartum, serum calcium levels remained constantly higher in the buffaloes resuming estrus than the anestrus ones.

The mean magnesium intake was 33.70 ± 0.26 g/day and was 15% higher than the National Research Council, USA (NRC) standard requirements. Increasing magnesium intake decreased serum calcium and magnesium levels and increased serum phosphorus levels. Magnesium intake prolonged placenta delivery duration, PUI and POI, while shortened duration of lochia. Animals becoming pregnant had less magnesium intake as compared to non-pregnant animals (34.9 versus 32.2 g/day, P<0.01). Magnesium intake was higher in anestrus animals than estrus animals (34.8 versus 33.4 g/day, P<0.05). Thus magnesium intake had an adverse effect on reproductive performance, probably due to its intake above requirements. Mean for serum magnesium levels was 26.40 ± 0.57 mg/1000 ml and correlated positively with serum protein, urea,

ME intake and POI and negatively with PUI. The buffaloes becoming pregnant had lower serum magnesium levels than non-pregnant ones (P < 0.01).

Phosphorus intake averaged 38.40 ± 0.40 g/day and correlated negatively with serum phosphorus and calcium levels. Phosphorus intake prolonged placenta delivery duration and POI and shortened PUI. The mean serum phosphorus level was 5.44 ± 0.12 mg/100 ml and correlated positively with serum protein, calcium and urea levels ME intake and placenta expulsion duration. Postpartum serum phosphorus concentrations were higher in estrus group as compared to anestrus group. The ratio of intake of Ca/P ranged from 0.7 to 6.4. The increasing ratio was associated with delayed ovulation. Serum Ca/P ratio varied from 0.4 to 3.5 and with the ratio from 0.4 to 2.0, reproductive performance was optimum but higher ratio from 2.1 to 3.5 was associated with prolonged PEI, POI and low conception rate.

The average intake of zinc was 1138.70 ± 7.53 mg/day which was 72.5% higher than NRC requirements. Zinc intake was correlated positively with serum calcium and magnesium and negatively with phosphorus levels. Its increase resulted in increased placenta expulsion duration and lochia discharge and decreased PUI and POI. The overall average value for intake of copper was 228.20 ± 3.60 mg/day which was 40% higher than NRC requirements. Increasing copper intake decreased serum phosphorus levels, PUI and POI.

The overall average for BCS was found to be 2.72 ± 0.02. BCS positively correlated with FCM and negatively with suckling duration, placenta expulsion duration and PEI. The prepartum BCS was positively correlated with postpartum loss of BCS and FCM. BCS in the buffaloes resuming estrus was constantly higher than those failing to resume postpartum estrus activity. Suckling duration averaged 26.40 ± 5.57 days.

Increasing suckling duration delayed POI and PEI and shortened duration of discharge of lochia. As the survival of calf was accompanied by two times suckling, it probably resulted in adverse effect on resumption of postpartum ovarian activity. Average daily production of FCM was 14.50 ± 0.20 kg/day. CPI favored FCM but excess intake of CP and ME adversely affected FCM. FCM was significantly higher in estrus group than anestrus one during the first two months postpartum ($P < 0.01$). FCM positively correlated with POI.

It may be concluded that autumn and winter constitute breeding season for buffaloes in NWFP. Better reproductive performance was associated with higher BCS and intake of ME, relatively lower intake of CP and minerals, lower blood urea and higher milk progesterone levels. Reproductive performance was adversely effected by higher intake of CP in low yielding buffaloes. Excess intake of ME and CP decrease milk production.

CHAPTER VI

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