

**STUDIES ON SUB-FERTILITY IN BUFFALOES IN  
RELATION TO SEASON AND  
NUTRITIONAL STATUS**

**Dissertation**

Submitted to the Punjab Agricultural University  
in partial fulfilment of the requirements  
for the degree of

**DOCTOR OF PHILOSOPHY**  
in  
**VETERINARY GYNAECOLOGY AND OBSTETRICS**  
(Minor Subject : Animal Production Physiology)

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**LUDHIANA - 141 004**

**2001**

To

My Dear Son  
Sanmukh

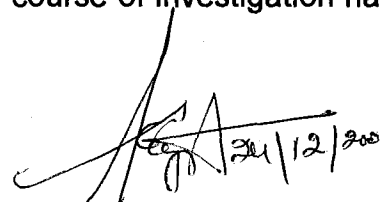
And

Wife  
Gurjinder

## CERTIFICATE I

This is to certify that the dissertation "**Studies on sub-fertility in buffaloes in relation to season and nutrition status**" submitted for the degree of Ph.D. in the subject of Veterinary Gynaecology and Obstetrics (Minor subject: Animal Production Physiology) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by Parkash Singh (L-97-66-D) under my supervision and that no part of this dissertation has been submitted for any other degree.

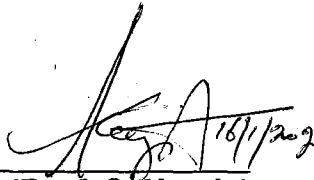
The assistance and help received during the course of investigation have  
• been fully acknowledged.



**Major Advisor**  
**(Dr. A.S. Nanda)**  
Professor-cum-Head  
Department of Animal Reproduction,  
Gynaecology and Obstetrics

## CERTIFICATE II

This is to certify that the dissertation entitled "**Studies on sub-fertility in buffaloes in relation to season and nutrition status**" submitted by Parkash Singh (L-97-V-66-D) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of Ph.D., in the subject of Veterinary Gynaecology and Obstetrics (Minor subject: Animal Production Physiology) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

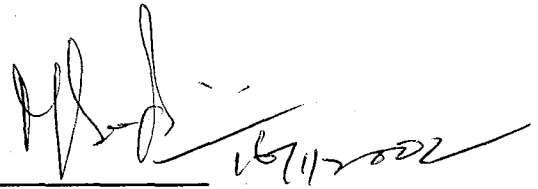


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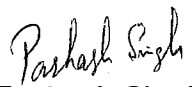
Mere words can hardly acknowledge the self-less sacrifice of my father during the completion of this work. I owe immensely to other members of my family for sincere wishes, loving emotions and for sparing me from my moral duties.

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All may not be mentioned but none is forgotten

Ludhiana

Dated 24-12-2021

  
(Parkash Singh)

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## ABBREVIATIONS USED

@	:	At the rate of
ADF	:	Acid detergent fiber
°C	:	Degree centigrade
cm	:	Centimeter
CP	:	Crude Protein
dl	:	Deciliter
EE	:	Ether Extract
Fig.	:	Figure
g	:	Gram (s)
hrs	:	Hours
kg	:	Kilogram
ml	:	Milliliter
mo	:	Month
ng	:	Nanogram
NDF	:	Neutral Detergent Fiber
%	:	Percent
P <sub>4</sub>	:	Progesterone
po.p	:	Postpartum
RIA	:	Radio-immunoassay
μl	:	Micro liter
UMMB	:	Urea Molasses Multi-nutrient Block
Vs	:	Versus

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## CHAPTER I

### INTRODUCTION

Water buffalo (*Bubalis bubalis*) is an important component of certain ecologically disadvantaged agricultural systems in the world providing meat, milk and drought power. The world population of buffaloes is estimated to be  $140-150 \times 10^6$  of which more than 50% is in India, accounting for 52% of the total milk production in this country (Acharya 1989, Aggarwal and Tomar 1998, Perera 1999). This otherwise disease resistant animal has traditionally been regarded as a poor breeder with delayed onset of puberty, long postpartum ovarian quiescence, poor heat symptoms and long inter-calving intervals. With the onset of summer (April) the breeding efficiency of buffaloes reduces, to attain a nadir in dry hot months of May and June when nearly 80% of the non-pregnant buffaloes are in anoestrus (Singh et al 1989). This phase coincides with the scarcity of green fodder, long day length and high ambient temperature. How far poor reproduction in buffaloes during summer is of genetic, climatic and/or nutritional origin is debatable.

Limited studies do indicate that buffaloes can have better fertility provided their genotypes match to the environment and they are managed and fed properly (Tullock 1979, Usmani et al 1985, Perera et al 1987, Vale 1997). They have the ability to utilize poor quality roughages and adapt to harsh environment more efficiently than cattle (Sebastian et al 1970). Still their reproductive performance remains poor especially in summer season. In Punjab, much is not known regarding the diversity in the management practices, availability of foodstuffs,

alternate measures to overcome summer stress and their impact on buffalo reproduction under different agro-climatic conditions and dairy systems.

Milk production is a physiological process of highest priority in postpartum buffaloes utilizing a lot of available energy resources. If not met by the feed intake, the energy is obtained by utilizing body reserves. This leads to decrease in body weight and body condition postpartum. Higher the extent and longer the duration of this loss in body weight and condition, poorer is the post-partum reproductive performance (Takkar and Chaudhary 1994). Conventionally, the animals are fed high energy and protein rich diet at and after parturition. However, owing to limited voluntary dry matter intake and high nutrient requirements during early postpartum period, the animals undergo negative energy balance. More than 70% animals have been reported to be in negative energy balance during early postpartum period (Butler and Smith 1989, Domeq et al 1997). The animals having higher dry matter intake within few days of parturition had better reproduction (Staples et al 1990). Pre-partum feeding in addition to building up the body reserves, puts the habit of having more dry matter intake in the animals well before parturition. Increased prepartum feeding of concentrates and green fodder led to significant improvement in milk production and reproduction in cattle and buffaloes (Sharma 1989, Singh 1998)

In Northern India as also is elsewhere the green fodder production drastically goes down during two distinct lean periods of the year viz. May-June and Sept-Oct (Pandey 1995). In Punjab buffalo production is mainly in the hands of marginal farmers. The decrease in land holdings, stress on cereal cultivation and

poor economical status of the farmer limit round the year production of green fodder. Hence there is a strong need to develop economical, viable, and sustainable supplementary feeding strategies and assess their impact on productive and reproductive efficiency of the buffaloes. The Urea molasses multinutrient blocks (UMMB) is one such technology based on the locally available agro-industrial by-products involving easy procedures compared to its present technical, mechanical and costly procedure.

Use of UMMB has been indicated in some earlier studies (Kakkar and Makkar 1995, Kakkar et al 1997, Malik et al 1997) however it could not be widely adopted due to procedural complications and initial costs involved and its poor keeping quality. The technology may have wider application should these constraints be limited/reduced. UMMB fed prepartum, increased dry matter intake in ruminants (Tripathi 1997) Not much work has been done on prepartum feeding of UMMB to assess its effect on postpartum reproduction in buffaloes.

The present study, therefore, had been planned with the following objectives:

- 1 To work out the incidence of anoestrus in buffaloes during summer months under different agro-climatic conditions.
2. To assess the relationship between reproductive performance of buffaloes and managerial practices under field conditions.
- 3 To formulate urea molasses multinutrient blocks (UMMB) based on agro-industrial byproducts involving easy procedures.

- 4 To assess the impact of UMMB supplementation on anoestrus buffaloes.
- 5 To assess the impact of prepartum UMMB supplementation on postpartum reproduction during favorable and unfavorable periods for buffalo reproduction.

## **Chapter II**

### **REVIEW OF LITERATURE**

#### **2.1 Anoestrus in buffaloes –Incidence and management**

Anoestrus or absence of visible symptoms of oestrus can be due to complete ovarian inactivity (true anoestrus) or with corpus luteum and/ or follicle in the ovary (suboestrus). No comprehensive survey for the incidence of these conditions has been carried out in buffaloes in rural Punjab. Limited studies indicated the incidence of true anoestrus to be 78% in July and 14% in November (Singh et al 1985). Shah et al (1990) reported the incidence of post-partum anoestrus in buffaloes due to suboestrus (73%), true anoestrus (8.9%), persistent corpus luteum (7.8%), follicular cyst (5.5%) and luteal cyst (3.4%) in Pakistan. The incidence of anoestrus depends much on the method of heat detection. Behavioral signs of oestrus and/or use of teaser are mostly employed. However, buffaloes have weak symptoms of oestrus as compared to cows with less homosexual behavior, less mucus discharge and short duration of oestrus especially during summer months (Perera 1999). The oestrus remains undetected on occasions as frequent as 73% (Shah et al 1990). This may be the cause of inter calving intervals varying between 360-1230 days (Shah 1990).

The buffalo is a debatable seasonal breeder. Variations have been observed in the display of oestrus, conception rate and calving rate of buffaloes in different seasons. Reproduction in buffaloes is sub-optimum in summer months, which are characterized by high ambient temperature, long day length and scarcity of green fodder. A large proportion of breedable buffalo population pass these summer months in their pregnancy but majority of the rest, non-pregnant buffaloes, stop cycling in summer months (Singh et al 1989). Female buffaloes were sexually sub-active with lower conception rate (Tahir et al 1981) during hot summer months as compared to winter (Pandey and Raizada 1979). How far this seasonal breeding in buffaloes is of genetic, climatic and/or nutritional origin, is not clear (Gordon 1996). Buffaloes and Zebu cows kept under similar managemental conditions showed a different pattern of breeding. Reproduction was suppressed in buffaloes and not in cows in summer season (Shah 1988).

### **Seasonal Breeding - Role of Nutrition**

Buffaloes kept on high plane of nutrition had better conception rates during summer than in their counterparts on normal plan of nutrition (Raizada et al 1969). Improved management and feeding conditions with adequate pasteur and dietary supplementation induced round-the-year breeding in Brazilian buffaloes (Vale 1990). Aggarwal and Purbey (1981) and Singh et al (1989) also attributed poor performance of buffaloes during summer to meager availability of green fodder. During summer months, onset of ovarian activity was delayed and progesterone levels were lower (Kaur and Arora 1984). The influence of heat

stress on plasma progesterone was hypothesized to be mediated through poor nutrition.

### **Seasonal Breeding – Role of Heat Stress**

Buffaloes are unable to adapt to extreme hot weather, probably due to sparsely distributed sweat glands and black body color. High ambient temperature is stressful to buffaloes reflected from 2-6 fold rise in circulatory prolactin during summer as compared to in winter (Sheth et al 1978, Razdan and Kaker 1980). Higher circulatory prolactin concentration retards the process of reproduction through suppression of gonadotropins (Heranjal et al 1979). Further, improvement of reproductive performance following cold treatment i.e. wallowing and sprinkling suggested that heat stress might contribute to high incidence of infertility in summer. Allowing the buffaloes to once or more frequent wallowing and/or water sprinkling has been found effective in alleviating the heat stress to some extent (Srivastava et al 1978, Perera 2000). Higher proportion of buffaloes kept in relatively cooler sheds 33°C provided with extra shade experienced oestrus (100% Vs 60%) and had better conception rate (80 % Vs 13 %) as compared to the buffaloes kept under conventional sheds having 41°C average environmental temperature (Roy et al 1968). Repeated water sprinkling or wallowing (Srivastava et al 1978) helped in reducing the incidence of summer anoestrus.

### **Seasonal Breeding – Effect of Day Length Pattern**

The proportion of buffaloes exhibiting oestrus during the period of short day length was significantly greater than during the period of long day length

(74% Vs 26%, respectively; Tailor et al 1990). Singh and Nanda (1993) recorded a negative correlation ( $r = - 0.658$ ;  $p < 0.01$ ) between the day length and the interval from parturition to first oestrus in buffaloes. Only 23% of all the detected oestrus during two successive years were recorded in the months with increasing temperature suggesting some detrimental effect of high temperature. However, 15 % oestrus occurred in September against only 5 % in March despite of same temperature ( $19^{\circ} \text{C}$ ) in both the months (Singh et al 2000). Borghese and Terzano (2000) proved that epifisis melatonin production is stimulated by decreasing photoperiod, which had a pivotal role in hypothalamus- hypophysis axis activity in buffaloes. Hassan et al (2000) could induce oestrus in 50% anoestrus buffaloes by keeping them in artificial short photoperiod and in 100% animals by single or double injection of 18mg melatonin during months of mid July to September compared to none in controls.

## **2.2 Urea Molasses Multi-nutrient Blocks (UMMB): Formulation and preparation**

The ability of the ruminants to utilize urea through ruminal microorganisms had been reported more than half a century ago (Morrow 1981). Urea has been fed in the form of uromol (Chopra et al 1974), urea molasses liquid supplement (Kaur 1993), urea treated straw (Bakshi et al 1986), or urea molasses multinutrient blocks (Malik et al 1993). Easy preservation, transport and convenient feeding made the use of blocks more common than rest of the methods and it also has the merit of providing nitrogen over a longer period of

time as compared to any other urea formulation. "Hot Process" of UMMB preparation has been the initial practice wherein the urea molasses mixture was heated to have Maillard type reaction (Van Soest 1964). Of late "Cold Method Preparation" of UMMB has been introduced that requires no heating (Tripathi et al 1997).

Kakkar and Makkar (1995) reviewed that 21 ingredients have been used for making UMMB by various workers in India and abroad. The proportion of molasses varied from 27-76% and of urea from 10-18%. Other commonly used ingredients have been the deoiled mustard cake (0-10%), cotton seed meal (0-10%), deoiled ground nut cake (0-9%), deoiled rice polish (9-20%), ground nut extraction (0-10%), wheat bran (0-28%). Cement (10-15%) or bentonite (3%) were used as binders. Some additives like vitablend, lipids or anthelmithics have also been in used. The choice of ingredients depended upon their availability and costs. The proportion of various ingredients used and method of preparation determined the characteristics of UMMB. Blocks with higher molasses were softer but had lesser shelf life due to early liquefaction and rancidity especially during rainy season. Blocks with high level of cement tended to be harder and hence less acceptability by the animals (Kakkar and Makkar 1995).

### **UMMB intake**

Adult cows and buffaloes consumed, on an average, 400 –500 g of UMMB (Makkar and Saijpaul 1996, Malik et al 1997). The intake of UMMB depended upon the basic diet being fed to the animals and the quality and texture of the blocks. Calves kept on lower amount of concentrate consumed more UMMB (695

Vs 559g; Kakkar et al 1997). Higher content of cement reduced the intake of UMMB. Higher concentration of salt also decreases the UMMB intake.

### **Effect of UMMB on production and reproduction**

UMMB supplementation in adult buffaloes increased intake of the wheat straw based diet by 53% without affecting normal health and reproduction of the animals (Tripathi 1997). This saved up to 40% concentrate in buffalo calves feed (Kakkar et al 1997). 20% concentrate could be replaced by about 650g of UMMB without effecting the quality and quantity of milk produced and body weights in lactating buffaloes (Chauhan et al 1997) when the basic diet included 30-35 kg green fodder. Five to eight percent increase in milk production, with 400-500g daily intake of UMMB in dairy animals was recorded by Makkar and Saijpal (1996), and Malik et al (1997). Vu et al (1999) observed 11% increase in milk yield with 3-5% increase in its fat content in dairy cattle supplemented with UMMB. Moreover, UMMB feeding shortened the interval from calving to onset of ovarian activity (94 Vs 112 days), calving to first oestrus (110 Vs 135 days), calving to conception (121Vs 152 days) and the inter-calving interval (13.4 Vs 14.4 months). UMMB supplemented crossbred cows required less services per conception than controls (Srinivas and Gupta 1996, Srinivas and Verma 2000). Prepartum supplementary feeding of urea and molasses for 42 days reduced the average postpartum interval to first oestrus by 30 days in dairy cows than in the unsupplemented controls (Fordyce et al 1997).

## **2.3 Physical, biochemical and endocrinological changes during peripartum period**

### **Peripartal body weight changes**

Onset of milk production after parturition requires lot of energy, which usually exceeds the energy inputs in dairy cattle. The animal has to mobilize its energy reserves from body to make up the difference between energy inputs and energy out put (Roche et al 2000). This mobilization leads to decrease in the body weight and body condition scores of the animals. The extent and duration of body weight loss varied with species, parity, season, level of feeding and milk yield (Bhalaru et al 1986, Bar-Peled et al 1995, Khatkar et al 1997). Changes in body weight during early lactation had bearing on and could be used as index of postpartum fertility in dairy cows and buffaloes (Bhalaru et al 1981, Khatkar et al 1997). In buffaloes, the average loss of body weight during first five months postpartum was 20.2 Kg with maximum loss during rainy season and minimum during winter season. Large sized buffaloes (body weight >550 kg) lost more weight and for longer duration (>5months) compared to small and medium sized buffaloes perhaps due to their proportionately extra nutritional requirements before and after calving (Bhalaru et al 1981). Most cows in first lactation started regaining weight by third month postpartum. However, 48% of these could not regain the lost weight even by the end of 6<sup>th</sup> month (Khatkar et al 1997). The cows losing more weight had higher milk yield and longer service period. Conversely the cows losing less weight had lesser milk yield and had shorter service period. Buffaloes losing more weight and for longer duration had lower first and the

overall conception rate and required more services per conception, though post partum oestrus interval remained unaffected (Bhalaru et al 1981). Body weight loss during first 2-4 weeks postpartum were more critical determinant of ovarian activity. Staples et al (1990) recorded loss in body weight of cows up to 8 weeks post partum. Cows losing more weight during first two weeks had delayed onset of ovarian activity than those losing less weight in first two weeks (> 60 Vs <40 days). The loss in body weight in two groups after third week of parturition was similar. Agyemang et al (1991) observed, short calving intervals (582 Vs 652 days) in cows that had started gaining weight within 4 months postpartum. The animals gaining weight at the time of breeding had better reproductive performance (Takkar and Chaudhary 1994). Ten percent fall in live weight of cows lowered their fertility (McClure 1970). In crossbred cows >15 kg lose in body weight at parturition and its delayed gain lowered the reproductive performance of animals (Takkar and Chaudhary 1994). LH pulse frequency was reduced in ovariectomized heifers made to lose 20% body weight (Butler and Smith 1989). Greater serum concentrations of LH in response to GnRH on day 3 postpartum were observed in heifers heavier during pregnancy and at calving (Leers-Sucheta et al 1994).

#### **Peripartal changes in body condition score (BCS)**

Body condition scoring, though a subjective method, is very helpful to assess the metabolizable energy stored as fat and muscles (Edmonson et al 1989) and hence the productive and reproductive prospects of an animal. Body condition scoring in visual way is a rapid and easy method of assessing the

condition of cattle without the use of scales and is relatively unaffected by body size. It has been done using different scales viz. 0-5 point scale (Mulvany 1981), 1-5 point scale (Edmonson et al 1989), 8 grade scale (Earle D F 1976), 1-9 scale (Leers-Sucheta et al 1994) or ten point system (Grainger C and McGowan A A 1982). However, 1-5 point scale pertaining mostly to hind quarters of the animal is most commonly used. This method originally derived for Holstein cows has been successfully employed in buffaloes also (Vale 2000). At 1-5 scale, the normal BCS values are expected to be as 3 to 3.75 at calving, 2.25 to 2.75 at peak yield, 3.0 to 3.5 at 150-200 days postpartum and 3.0 to 3.75 at dry off (Chrnetzki 1999). The animals should maintain this BCS after drying off. The gain in BCS after drying off was helpful for postpartum reproduction provided the animals were not over conditioned (Domecq et al 1997). Overconditioned cows with BCS 4 - 5 were predisposed to various metabolic diseases including ketosis, fatty liver disease and foot problems (Gearhart et al 1990, Studder 1998). However, Butler and Smith (1989) observed no adverse affect in over conditioning of cows with BCS of 4 – 5 at calving. The reasons of these differences remain to be determined, but could relate to types of rations fed, degree of over conditioning at calving, prepartum adjustment to the lactating cow ration, or postpartum nutritional status and body condition losses.

Cows losing BCS <0.5, 0.5-1.0 or >1.0 during first five weeks postpartum took 27, 31 and 42 days, respectively, to first ovulation postpartum (Butler and Smith 1989). Cows that lost one point of BCS in the first month of lactation were 1.5 times less likely to conceive than were the cows that did not lose one point of

BCS (Domecq et al 1997). After parturition the animals should not lose more than 1.0 BCS (Studer 1998). With similar body condition scores the different genotypes may behave differently. European cows at BCS > 5.0 (1-9 scale) took significantly less time for the first rise in progesterone levels postpartum compared to cows at 3 - 3.5 BCS. However, Zebu cows with BCS 3 – 3.5 or >5.0 took almost similar time for the first rise in progesterone levels post partum. Changes in BCS after parturition had significant effect on days open and days to first rise in progesterone level postpartum in all the genotypes (Lamothe et al 2000). BCS was critical in determining pregnancy rate in dairy cattle in subtropical Florida. Higher number of animals were pregnant with higher BCS than at lower BCS (97% Vs 43%; Warnick et al 2000).

### **Peripartal biochemical changes in blood**

There are significant fluctuations in the blood composition of dairy cows associated with the interaction between the effects of season, milk yield and stage of lactation (Singh 1993, Marai et al 1999).

### **Changes in circulatory concentrations of urea**

There is a direct relationship between the protein intake and the blood urea concentration. Low concentrations of urea indicated low protein intake and an early warning of low protein status of animals. Lower urea concentration indicated the shortage of ruminally degradable proteins, which were associated with poor reproduction (Khan 2000). Increase in ruminally degradable fraction of protein in diet increased blood urea nitrogen, which resulted in high urea levels in

vaginal mucus also. When crude protein increased from 13 to 20%, the urea level in vagina mucus increased from 7.6 to 19 mg percent (Chrnetzki 1999). Blood urea nitrogen levels more than 20mg/dl on the day of insemination decreased the conception rates (Ferguson et al 1988) because it altered the uterine ph and reduced fertility in dairy cows (Butler 1998). To avoid it, crude protein intake should be restricted to 18% with its ruminally undegradable fraction 38-40% and degradable fraction 25-35%. There should be readily available source of energy in the rumen so that whatever ammonia is produced is utilized for synthesis of microbial protein (Studer 1998). Feeding 250g urea daily for 21 days increased the levels of urea in milk and plasma but had no adverse effect on reproduction in cows (Biggadike et al 2000). Improvement in grazing increased the plasma urea levels, body weight and body condition score of animals but had no effect on levels of nonesterified fatty acids (Haddada et al 2000).

#### **Changes in circulatory concentrations of total plasma proteins**

Low plane of nutrition due to sub-optimal intake is responsible for delayed onset of postpartum ovarian activity (Roberts 1971). Significantly higher level of total serum protein (7.75 Vs 6.28g/dl) were observed in normal cycling buffaloes as compared to suboestrus buffaloes, suggesting hypoproteinemia to be responsible for poor expression of oestrus (Chauhan 1980). Delayed onset of ovarian activity in summer calved animals was related to total protein deficiency in buffaloes in summer months (Singh 1993). Some other studies, however, could not correlate the total protein levels to the reproductive efficiency of

animals (Dhoble 1978, Gentile et al 1978). Low level of albumin and haemoglobin were indicative of long standing low protein status. Supplementary feeding in addition to normal diet increased plasma albumin and total proteins levels in cows (Filho et al 2000). Creatine is found mostly in skeletal muscle and is normally excreted as creatinine. Increased levels of circulatory and excretory creatinine are indicative of muscular dystrophy (Blood et al 1983).

### **Changes in circulatory concentrations of blood glucose**

The blood glucose concentrations appear to be associated with reproduction in dairy animals. Significantly higher concentrations of blood glucose were observed in cycling buffaloes than in anoestrus buffaloes (Dhoble and Gupta 1979, Chauhan et al 1981, Shanker et al 1981, Derashri et al 1984, Singh 1993, Mohammed 1997). Srivastava et al (1986) and Chandolia and Verma (1987) could not find significant difference between cycling and acyclic buffaloes.

Blood glucose levels increase towards parturition, reach peak at parturition, decrease in early lactation and slowly recover afterwards (Blood et al 1993). Blood glucose levels were significantly higher during pregnancy than in oestrus and anoestrus buffaloes (Derashri et al 1984). Quayam et al (1990) however reported that blood glucose levels started declining after 60 days before parturition ( $59.34 \pm 0.91$  mg/dl), to be lowest at parturition ( $35.57 \pm 0.35$ mg/dl). It further increased to be  $43.70 \pm 0.98$  mg/dl at 60 days postpartum.

Blood glucose concentrations may be an unreliable index of the adequacy of energy intake in situations of marginal and/or transient energy imbalance. A

number of factors can cause short-term changes in blood glucose. Stage of lactation, time of milking, excitement, environmental temperature, chemical nature of carbohydrates, physical form and roughage content of feed alter the levels of blood glucose. The major cause of variation in blood glucose concentration may be the fluctuations in daily feed intake (Blood et al 1993). Blood glucose levels around parturition are not entirely reflective of energy intake, but are also governed by complex hormonal interplay occurring at that time.

### **Concentrations of certain minerals and their role in dairy animal reproduction**

Minerals are needed for normal reproduction due to their role in cellular metabolism, maintenance and growth. Normal circulatory concentrations of calcium, copper and zinc in cattle have been reported to be 8-10 mg/dl,  $1.26 \pm 0.31$   $\mu\text{g/ml}$  and 0.80-1.20  $\mu\text{g/ml}$  (Blood et al 1983), while the critical concentrations were 8 mg/dl, 0.65  $\mu\text{g/ml}$  and 0.60  $\mu\text{g/ml}$ , respectively (McDowell et al 1993). Buffaloes maintained higher concentration of calcium, magnesium, copper and cobalt in plasma in comparison to crossbred cows kept under similar feeding practices. Further, the concentrations of copper (20.30  $\mu\text{mol/l}$ ), Iron (69.20  $\mu\text{mol/l}$ ) and Zinc (95.50  $\mu\text{mol/l}$ ) were significantly higher in buffaloes kept at organized farms than in those kept at unorganized farms (Randhawa 1999).

Plasma copper concentrations were found to be significantly lower in anoestrus buffaloes than normal cycling or repeat breeders (Osman et al 1985, Dabas et al 1987, Jain 1994). However, Chauhan (1980) and Mohammed (1997) recorded higher concentration of serum copper in buffaloes with unobserved oestrus or in true anoestrus respectively. Kumar (1993) recorded no difference in circulatory copper concentrations of normal cycling and anoestrus buffaloes. Average plasma copper concentration in different age groups of cattle and buffaloes of unorganized farm were significantly lower and the prevalence rate was higher in comparison to the buffaloes at the organized farm (Randhawa 1999). Molybdenum and sulphur interact with copper to form copper-thio-molybdate complex and make it unavailable to the sites of its utilization (McDowell et al 1993). Hence, copper : molybdenum ratio has been considered as a better indicator of its bio-availability. Further copper concentration in hairs rather than in plasma was more reliable indicator of molybdenum induced copper deficiency (Randhawa 1999).

Zinc deficiency adversely effects various phases of reproductive process in male and female ruminants (McDowell et al 1993). Zinc concentrations were higher in buffaloes with normal oestrus than with unobserved oestrus (Chauhan 1980). Zinc levels were also higher in anoestrus buffaloes, which responded to hormonal therapy than in non - responders (Mohammed 1997). Winter calved buffaloes had higher levels of zinc than summer calved buffaloes (Kumar 1993).

As a component of hemoglobin, myoglobin and cytochrome, iron plays a vital role in cellular respiration. Iron deficiency seldom occurs in adult livestock

unless there is considerable loss due to parasites or disease. Intake of soiled grasses during grazing facilitates iron intake that alleviates iron deficiency in grazing ruminants (McDowell et al 1993). Plasma zinc and iron concentration in crossbred cows and buffaloes were above normal levels in different feeding systems in Punjab (Randhawa 1999). However, Osman et al (1985) reported significantly lower iron levels in anoestrus than in cycling buffaloes.

Most of the cows and buffaloes of organized as well unorganized farms of Punjab had subnormal calcium levels (Randhawa 1999). Several workers (Bansal et al 1978, Shankar et al 1983, Dhoble and Gupta 1986) reported lower plasma phosphorus and calcium in anoestrus than in cycling buffaloes. Chandolia et al (1987), Dabas et al (1987), Kumar et al (1992) and Patel et al (1994) revealed non-significant differences between serum calcium values of cyclic and acyclic buffaloes.

### **Prepartum supplementary feeding and its effect on reproduction**

Prepartum nutrition, as reflected in body condition, is an important determinant of postpartum ovarian activity in cattle (Leers Sucheta et al 1994). In early lactation, energy derived from the intake of feed is less than required for maintenance and milk yield in high producing dairy cows and may put them to negative energy balance (Butler and Smith 1989). Accordingly, these cows must calve with adequate tissue reserves for mobilization to help meet the energy and protein requirement of peak milk production during early lactation (Gearhart et al 1990). Feeding during mid and late lactation must be adequate to maintain milk

production and allow the cow to restore body tissue reserves or condition in anticipation of the subsequent lactation (Domeq et al 1997). Maximum negative energy balance occurs during 1st to 2<sup>nd</sup> week of lactation and recovers thereafter at a variable rate. First oestrus and ovulation occurred about 10 days after the negative energy balance started diminishing, which occurs near to the peak lactation yield. Ovulations started occurring while energy balance was still negative but was diminishing towards zero (Butler et al 1981, Butler and Smith 1989). The negative energy balance, and hence, postpartum ovarian activity was related to dry matter intake postpartum. By day 25 postpartum, the cycling cows were consuming dry matter 3% of their body weight, which the anoestrus cows started consuming two weeks beyond that (Staples et al 1990). Prepartum supplementary feeding increased dry matter intake in peripartal cows and buffaloes (Sharma 1989, Singh 1998). Prepartum supplementary feeding resulted in better reproduction postpartum. The interval to first oestrus was significantly reduced in cows (46.2 Vs 53.5 and 66.6 Vs 87.1 days; Singh 1998, Bellows and Short 1978) by supplementary prepartum feeding. High plane of nutrition during prepartum period followed by moderate or low level of nutrition post partum resulted in better reproduction than higher level of nutrition during pre- and postpartum period (Ducker et al 1985). The supplementation of transitional ration should be introduced 3-4 weeks before calving to allow proper ruminal adjustment to the post calving diet (Goff and Horst 1997).

The mechanism by which undernutrition impaired postpartum reproductive function involved the regulation of LH secretion (Leers-Sucheta 1994). Feeding

beef cows a diet deficient in crude protein before parturition reduced pituitary content of gonadotropin and responsiveness to exogenous GnRH (Nolan et al 1988). Similarly, reducing dietary energy during gestation decreased pituitary response to oestradiol (Echternkamp et al 1982) and GnRH (Killen et al 1989) in postpartum heifers.

### **Relationship of milk yield with reproduction**

Reproductive performance was found to be positively correlated to milk production in cows (Staples et al 1990, Domeq et al 1997). Butler and Smith (1989) and Nebel and McGilliard (1993) however found negative correlation between milk yield and reproductive performance postpartum. High producing cows might be more reproductively sound because they are healthier than low producing cows (Shanks et al 1979). On the contrary higher milk yield may increase the intensity and duration of negative energy balance and hence may adversely effect reproduction (Butler and Smith 1989). The correlation between milk yield and days to first ovulation became significant only after 40 days of parturition when most of the cows had already ovulated (Butler et al 1981). Due to these contradictory arguments in favor or against, the early postpartum milk yield cannot be considered as an ideal index of ensuing fertility in dairy animals. Energy status of an animal has been considered important than milk yield in determining the days to first breeding (Staples et al 1990).

## **Chapter III**

### **Materials and Methods**

#### **3.1 Incidence of anoestrus in buffaloes**

Data on eight hundred and fifteen buffaloes, belonging to 211 marginal farmers and 15 nomadic farmers were included in the study. Marginal farmers belonged to villages Talwandi Malian, Kishanpura and Diya Kalan of Distt. Moga in agro-climatic zone IV and villages Zandawalla and Balahar Vinzu of Distt. Bathinda in agro climatic zone V of Punjab. Nomadic farmers, which keep changing places from time to time were living in the periphery of Ludhiana at the time of this survey. Information regarding reproductive status of animals, feeding practices and general management was collected from these farmers on a questionnaire (annexure) in the month of June 1999. Buffaloes belonging to agro-climatic zones IV and V and to nomads were classified as groups I, II, or III respectively for further analysis. Different agro-climatic zones had differences in type of soil, rainfall and climatic temperature (Singh and Chaudhary 1996). The information thus gathered was subjected to appropriate statistical analysis.

#### **3.2 Preparation of Urea Molasses Multi-nutrient Blocks (UMMB):**

Five different formulations enlisted below involving locally available ingredients were designed and tested for preparation of blocks both by hot as well as cold processes (Table 1)

**Hot process of making UMMB:**

Urea and molasses were mixed together in an iron pan and heated slowly for half an hour. While still hot, it was mixed properly with other ingredients. Blocks were prepared by pressing the contents with a hydraulic press (Fig. 1).

**Cold process of making UMMB:**

Urea and molasses were mixed together and kept for 24 hours before they were mixed with other ingredients on a polythene sheet or in an iron pan. Blocks were prepared with iron frame under the foot pressure of one person. The blocks were air dried before feeding (Fig. 2).

**Table 1: Proportions of various ingredients in different formulations of UMMB**

Ingredients (%)	Formulations				
	I	II	III	IV	V
Molasses	40	40	35	35	35
Urea	10	10	10	10	10
De-oiled rice bran	-	26	-	33	17
Oiled rice bran	26	-	33	-	16
Ground-nut cake	10	10	10	10	10
Common salt	4	4	2	2	2
Cement	10	10	10	10	10

**Fig. 1 'Hot method' for preparation of UMMB (a) heating of urea and molasses for 30 minutes, (b) mixing of other ingredients with hot 'urea and molasses', (c) hydraulic press for making blocks.**



**Fig. 2 'Cold method' for preparation of UMMB (a) urea, molasses and other ingredients being mixed in an iron pan (b) iron case for making blocks, (c) making blocks under pressure of one man.**



### **UMMB acceptability trials**

The hot process was a cumbersome procedure involving extra time, resources and labor. The I and II formulations of UMMB did not result into blocks of acceptable texture. Accordingly, making of UMMB by the hot process and the formulations I and II were abandoned. Blocks (1 or 2 kg.) prepared by cold process using the rest three formulations were fed to ten animals belonging to marginal dairy farmers in village Issewal and at P.A.U. Dairy farm. Acceptance of the blocks by the animals was recorded. Based on these observations, 2 kg. blocks from formulation V were used for further studies.

### **3.3 Effect of pre partum UMMB supplementary feeding on post partum reproduction:**

The impact of pre-partum UMMB feeding was assessed in buffaloes at two different locations in two different seasons as explained below:

#### **3.3.1 Experiment I: Supplementary feeding in prepartum buffaloes due for calving in winter at organized dairy farm:**

Fifteen pleuriparous buffaloes (2<sup>nd</sup>–5<sup>th</sup> gestation) and ensuing to parturate in months of September and October 2000 at P.A.U. dairy farm were selected for the study. Ten buffaloes were subjected to UMMB supplementary feeding in addition to normal diet in the last trimester of their gestation up to parturition. A two kilogram block of UMMB was offered daily to each animal for 24 hours and individual intake was calculated from the leftover. Basic diet included 30-40 kg.

pearl millet, sorghum or machchari along with 2 kg. appropriate concentrates. Observations as given below were made from the start of the UMMB supplementation up to 60 days after parturition. The buffaloes were weighed at weekly intervals before morning feeding with an animal weighing machine (range 0-1000 kg.). Body condition score (BCS; 1-5 point scale; Edmonson et al 1989) and heart girth (using Weigh Band; Dalton Supplies Ltd. Nettlebed, Henley-on-Thames, Oxon) were recorded weekly. Venous blood samples were collected at weekly intervals in (a) acid washed heparinised glass tubes for various biochemical and progesterone estimations and (b) in glass tube having sodium fluoride (approx.10.0 mg/ml of the blood collected) for measuring blood glucose concentrations. The buffaloes were observed visually and by a teaser bull for oestrus activity during their postpartum period. Daily milk yield of these animals was recorded for two months.

### **3.3.2 Experiment II: Supplementary feeding in prepartum buffaloes due for calving in summer at unorganized dairy farm:**

Eighteen pleuriparous buffaloes, in 2<sup>nd</sup>- 4<sup>th</sup> gestation and due to parturate in months from April through June 2001 in village Dapai of agroclimatic zone III in Distt. Ludhiana, were selected for the study. Eleven buffaloes were exposed to 2.0 kg. UMMB supplementary feeding daily in addition to conventional diet in their last trimester of gestation up to parturition. Seven buffaloes remained as unsupplemented control. The diet included 20-30 kg. mature barseem, sorghum or pearl millet. However, since the period of the study also covered a two month

“ fodder lean period”, (May-June; Pandey 1995), each animal had to be exposed to a basic diet only of about 2kg wheat straw with concentrates for variable period of 1-2 months during the study. UMMB intake, body condition score and girth of buffaloes were recorded at weekly intervals as in experiment 1. Blood samples for various biochemical parameters were collected every fortnight from the start of the study up to 90 days postpartum. The oestrus activity postpartum, as observed by the farmers was recorded.

### **3.3.3 Supplementary UMMB feeding of nonpregnant, anoestrus buffaloes at unorganized dairy farms- Effect on reproduction:**

Fifty-four buffaloes belonging to marginal farmers and in anoestrus for more than six months were selected from three villages in agro climatic zones III, IV, and V of Punjab. The buffaloes were taken on the basis of history with their reproductive status confirmed through per rectal examination. Buffaloes belonging to villages Dapai (Agroclimatic zone III), Talwandi Malian (Agroclimatic zone IV) and Jandawala (Agroclimatic zone V) were grouped into groups I, II and III, respectively. Herd status, feeding and managerial practices adopted by individual farmers were recorded. Forty buffaloes were provided with limited blocks (1-2 blocks per farmer) i.e. 250-500g per animal and advised exposure UMMB for limited hours when the animals were under direct observation of farmer i.e. 4-6 hours daily for one month while 14 served as control. BCS and girth were recorded at weekly intervals. Glucose, total proteins, albumen, urea, and minerals in plasma were estimated at the beginning and at end of the UMMB

feeding to assess the nutritional status of these animals. The UMMB intake and its effect on reproductive status were assessed from the daily observations made by the farmers.

### **3.4 Biochemical analysis of plasma samples**

Blood samples were collected in mineral free (dipped overnight in 2N hydrochloric acid) heparinised glass vials within 4-6 hours after feeding. Blood collected was centrifuged, plasma harvested and stored in plastic vials at  $-20^{\circ}\text{C}$  for pending biochemical estimations. For glucose estimation, 1-2 ml blood was collected in glass vials having sodium fluoride @ approx.10.0 mg/ml of blood collected. Glucose was estimated within one day of blood collection by GOD/POD method. Total plasma proteins, albumin, urea and creatinine were estimated by biuret, BCG, urease and picrate methods, respectively using kits supplied by Bayer Diagnostics, Baroda India.

For mineral estimations, two ml plasma was digested with 10 ml distilled concentrated nitric acid. Digestates were diluted to 10 ml volume with double distilled water. Various minerals, copper, zinc, calcium and Iron were estimated by Inductively Coupled Argon Plasma Atomic Emission Spectroscopy (ICP-AES, Model Labtam-8440, Australia).

Progesterone estimations were done by liquid phase radioimmunoassay (RIA) procedure (Kamboj and Prakash 1993) with modifications (Kumar 2001).

The data from the experiments was subjected to appropriate statistical analysis (Singh et al 1991)

# RESULTS AND DISCUSSION

## 4.1 Incidence of anoestrus in buffaloes

The buffaloes tended to pass harsh months of summer in their pregnancy. Overall analysis of the survey revealed that sixty seven to seventy-six percent buffaloes were pregnant during the month of June 1999. Three to thirteen percent buffaloes were in early postpartum period at the time of survey (Table 2). Majority of the nonpregnant buffaloes (63-83%) were in anoestrus during summer months suggesting seasonal breeding trends in buffaloes (Table 3). Earlier studies have also reported that most buffaloes passed the summer months in their pregnancy while majority of the rest nonpregnant buffaloes stopped cycling in summer months (Singh et al 1989). Only few buffaloes (7%) calved in summer months (Rao and Rao 1996). The buffaloes with nomads were either pregnant (76%) or anoestrus (83% of the nonpregnant), these proportions being significantly higher ( $p < 0.05$ ) than the corresponding values in group I (Table 1 and 2). This depicted strict seasonal breeding in nomadic herds as compared to the buffaloes owned by marginal farmers. Although the proportions of pregnant buffaloes in group 2 and 3 were similar (74.30 and 75.78%) the reproduction in group 3 buffaloes only appeared to be strongly governed by seasonality. The fact that higher proportion of buffaloes in group 2 (26% VS 17%; Table 3) were cycling in summer, forms the basis for above interpretation. The

nomadic animals depended for their nutrition on grazing, availability of which is highly seasonal. Further, the frequency and time of wallowing depended on the proximity of these flocks to canals/ponds. Minimum nutrition coupled with poor management might have been responsible for the higher seasonal trends of reproduction in nomadic herds. Reverse comparison also suggested that breeding seasonality in buffaloes could be reduced to some extent by better nutrition and management. This is in comparison with observations made by Raizada et al (1969) and Vale (1990).

Postpartum anoestrus remained to be the major reproductive disorder in buffaloes leading to long calving to conception intervals (Table 4). Accordingly intercalving intervals of forty-eight to sixty-six percent buffaloes would be more than 14 months. Perera (2000) and Vale (2000) reviewed that variability in duration of postpartum anoestrus depended upon the type of management. Buffaloes grazed on lush pastures experienced fertile oestrus as early as 30-60 days postpartum. Intercalving intervals extended beyond 1230 days in buffaloes exposed to nutritional, climatic and suckling stressors. More than 20% animals had more than one year of calving to conception intervals. These animals continued to be in anoestrus in all the four seasons of the year suggesting that not the season alone, other factors such as poor nutrition and management might also have been responsible for prolonged anoestrus. Higher number of animals conceived within four months of parturition at location 2 compared to location 1.

The incidence of other reproductive problems was very less (Table 5). Higher incidence of abortion observed in location 3 was due to abortion in a complete herd. Lower incidence of repeat breeding in present study (3.0-4.0 %) compared to earlier studies at PAU dairy farm (23.29%; Singh et al 1983) could be attributed to practice of natural service in the village buffaloes and artificial insemination at PAU.

**Table 2. Reproductive status of buffaloes during the month of June 1999 at different locations**

	Reproductive status of buffaloes (% animals)			
	Pregnant	Recently calved/aborted (<60 days)	Anoestrus	Repeat breeder
Group 1 (n=337)	67.35 a	10.38	21.06	1.18
Group 2 (n=288)	74.30	13.88	11.80	-
Group 3 (n=190)	75.78 b	3.68	20.52	-

**Table 3. Incidence of anoestrus in non - pregnant buffaloes in summer months at different locations**

	Anoestrus (% animals)	Evidence of cyclicity (% animals)
Group 1 (n=113)	62.83 <sup>a</sup>	37.16
Group 2 (n=46)	73.91	26.08
Group 3 (n=47)	82.97 <sup>b</sup>	17.02

- Values with different superscripts are significantly different (p<0.05)

**Table 4. Postpartum calving to conception interval in buffaloes at different farms in different locations**

	<4 Months (% animals)	4-12 Months (% animals)	>1Year (% animals)
Group 1 (n=240)	34.28	37.14	28.57
Group 2 (n=178)	51.68	22.47	25.84
Group 3 (n=190)	49.28	36.42	14.28

**Table 5. Incidence of major reproductive disorders (other than anoestrus) in buffaloes in year 1998-1999 at different locations**

	Repeat Breeder (% animals)	Abortion (% animals)	Prolapse of genitalia (% animals)
Group 1 (n=337)	4.15	1.18	0.59
Group 2 (n=288)	3.12	2.77	3.12
Group 3 (n=190)	-	10.52	1.57

#### **4.1.2 Conventional feeding and managemental practices in Punjab**

In general, the animals were fed with green fodder, wheat/rice straw as a staple diet with few people supplementing concentrates. However, there are two distinct fodder lean periods in Northern India (May-June and October-November),

exposing the animals to varying degree of green fodder deficiency (Pandey 1995). The present study assessed the extent of this deficiency and the farmer's efforts to alleviate its effects through replacement feeding/supplement.

The survey revealed that all the farmers involved at location 1 and location 2 grew green fodder viz. barseem, oats available in plenty in late November through early April and sorgum, maize and pearl millet available for feeding in June through September. Eleven percent farmers grew Napier grass available round the year. The amount of green fodder available to individual buffalo however, could not be obtained. Accordingly, the farmers with lack of round the year green fodder were categorized as deficient in green fodder. The survey revealed that 89%, 100%, 100% farms were deficient in green fodder at locations 1,2 and 3 respectively (Table 6). Supplementary feeding of mineral mixture was very rare (7% in group 1 only). All the marginal farmers enrolled in the study (group 1 and 2) were feeding varying proportion of wheat straw as an integral part of diet to their animals through most of the year. Nomadic buffaloes were grazed for 3-5 hours daily in empty fields and on roadsides. In addition, they were continuously fed with rice straw and with off and on provision of varying amount of green fodder especially to newly calved buffaloes. Twenty percent of the nomadic farmers were feeding commercially available concentrates to lactating buffaloes. None of the farmers was feeding urea based rations to their animals.

Majority of farmers were not giving any supplements prepartum (Table 7) as 34, 54 and 40 % farmers in group 1, 2 and 3 respectively were giving sarson

oil, Ghee, oil seeds 15-60 days during late pregnancy on the top of conventional feeding. Most farmers increased diet of buffaloes after parturition (Table 7). While relatively higher proportion of farmers (54%) fed supplements to prepartum buffaloes at location 2, more farmers at location 1 (100%) supplement diets of postpartum buffaloes. This variation appeared to be due to area – based traditions. Relatively, higher number of animals conceiving within four months of parturition at location 2 (Table 4) could be related to higher frequency of prepartum supplementary feed of buffaloes, which is in accordance with observations by (Bellows and Short 1978).

One to three times daily cooling of buffaloes during summer either by bathing (91 % at location 1) or by wallowing (97 % at location 2 and 89 % at location 3) was recorded as a routine practice with most of the farmers. Availability of ponds /canals in the vicinity of a farm determined the type of cooling procedure adopted. Wallowing and bathing are the most common ways to overcome the heat stress of summer months in buffaloes (Perera 2000). Improved reproductive efficiency has been reported in buffaloes exposed to wallowing or bathing (Roy et al 1968, Srivastiva et al 1978).

Detection of heat is one of the major hindrances to optimal buffalo reproduction. Buffaloes have weak symptoms of oestrus characterized by poor homosexual behavior, little mucus discharge and short duration of oestrus especially during summer months (Singh et al 1984, Perera 1999). Hence the methods of heat detection employed on a farm would have great bearing on the efficiency of heat detection and hence of reproduction in an animal. In the

present study, more farmers were dependent on doka as a sign of ensuing oestrus rather than mucus discharge or overt signs of oestrus (Table 8). Doka has been recorded as the most consistent feature associated with oestrus in buffaloes. Doka is a vernacular term used for retention of milk leading to engorgement of teats even after milking (Bawa 1968). Animals had doka for a variable period of 3-4 days before showing other signs of oestrus. Continuation of doka for 4-7 days after impregnation was considered as a symptom of pregnancy. Higher proportion of buffaloes was showing overt signs of oestrus at location 2 than at location 1. Genetic, nutritional or managemental factors like presence of male animals in the herd for some part of the day are the speculated possibilities for different number of animals showing overt signs of oestrus at different locations.

**Table 6 Extent of green fodder, straw and supplements fed by farmers (%) to buffaloes in Punjab**

	Green fodder round the year	Green Fodder Defi.	Wheat Straw	Urea based diet	Min Mix.	Commercial Concentrates
Group I (n=107)	11	89.43	100	0	7	16
Group II (n=104)	0	100	100	0	0	04
Group III (n=15)	0	100	100	0	0	20

**Table 7. Proportion of farmers at different locations giving supplementary feeding to their buffaloes in late pregnancy and/or early postpartum period at different locations**

Location	Supplementary feeding pre-partum (% farmers)	Supplementary feeding post-partum (% farmers)
Location 1 (n=107)	33.64 <sup>a</sup>	100.00 <sup>a</sup>
Location 2 (n=104)	53.84 <sup>b</sup>	77.88 <sup>b</sup>
Location 3 (n=15)	40	80

- Values with different superscripts in columns are significantly different (p<0.05)

**Table 8. Commonly observed symptoms of oestrus in buffaloes in different locations**

	Doka (% animals)	Mucus (% animals)	Overt Signs (% animals)
Location 1 (n=337)	77.57	53.27	53.27 <sup>a</sup>
Location 2 (n=288)	90.38	66.34	93.26 <sup>b</sup>
Location 3 (n=190)	100	0	46.66

- Values with different superscripts in columns are significantly different (p<0.05)

## **4.2 Formulation and Preparation of UMMB**

In the past, UMMB had been prepared by 'hot process' (Malik et al 1993) or by cold process (Tripathi 1997). In the present study, both methods were tried using five different formulations (Table 1).

### **UMMB preparation by 'hot process':**

This process was undertaken to form the blocks in the initial stages. However, it required heating of the ingredients, more labor, longer time and equipment such as a hydrolic press. Above all it required high initial costs to procure the equipment required. The hot process was therefore abandoned owing to its costly, mechanical and cumbersome procedures. The blocks so produced have been reported to have poor shelf life as compared to blocks prepared by cold process (Tripathi 1997).

### **UMMB preparation by 'cold process':**

This process required lesser time, labor and equipment as compared to the 'hot process'. Being convient for use in the field, this process was adopted for further studies. Blocks of 1 and 2 kg weight were prepared.

The physical characteristics of blocks prepared by cold process using different formulations have been enlisted in Table 9.

**Table 9. Physical characteristics of the UMMB blocks**

Characteristics	Formulations				
	I	II	III	IV	V*
Hardness	+	+	+++	+++	+++
Days to Dry	-	-	8-10	2-4	3-6
Brittleness	-	-	+	++	+
Cost (Rs./Kg)	-	-	4.22	4.02	4.11
Acceptability by the animals	Not tried	Not tried	100%	100%	100%

\* Easy to prepare as does not stick to pans

Formulations I and II with 40% molasses could not be converted into enough hard blocks fit for licking. The higher proportion of molasses used by Srinivas and Verma (2000) could be due to the quality of molasses and/or other ingredient used by them. The blocks prepared by III, IV and V formulations were hard enough to be licked by the animals after drying. Variable number of days were required to dry the blocks to desired hardness depending on the weather. Blocks of all the formulations were liked by the animals. Based on observations in this and in Experiments 1 and 2, the daily average UMMB consumption by each animal was calculated to be  $627 \pm 34$  to  $674 \pm 16$  g (Tables 10 and 31). The use of 33% de-oiled rice bran made the blocks more brittle, while the blocks prepared by using 33% oiled rice bran took more time to dry and were difficult to prepare due to the sticky nature of the material. Formulation V with 16% oiled and 17% de-oiled rice bran was relatively easier to prepare, lesser brittle and

required moderate duration (3-5 days) to dry. One kg. block had more tendency to break than 2 kg. block.

The proximate analysis of different UMMB formulations conducted immediately after preparation and after 14 months of preservation, is given in Table 10.

**Table 10. Proximate analysis of fresh and preserved UMMB formulations III, IV and V (%Dry Matter)**

Components	Freshly prepared UMMB			UMMB preserved for 14 Months		
	III	IV	V	III	IV	V
Dry Matter (%)	85.00	84.00	86.50	84.44	81.80	83.53
CP	42.38	42.95	41.84	40.86	40.78	41.30
NDF	26.70	25.90	26.50	26.00	26.00	27.00
ADF	-	-	-	21.00	14.50	17.50
Cellulose	3.60	6.35	4.10	5.00	7.00	5.50
EE	1.35	0.45	0.75	2.50	0.50	1.00
Ash	28.32	26.35	27.47	35.05	22.75	26.75

Basically, the dry matter contents and their components in blocks of each formulation were similar and did change only marginally after 14 months which could be due to absorption of moisture as the blocks had no waterproof covering during storage. Malik et al (1993) had advised polythene covering of blocks to avoid such changes and also other kinds of deterioration. Marginal changes observed between components of blocks of various formulations could be the

effect of difference in quantity of their ingredients (Kakkar and Makkar 1995, Chauhan et al 1997). None of the components underwent remarkable change during preservation. Further no deterioration of color, odor, texture or any apparent contamination with fungus was recorded during this period of preservation. With similar chemical characteristics and better physical characteristics formulation V in 2 kg. blocks prepared by cold process was selected for further use in this study.

#### **4.3 To assess the effect of pre-partum UMMB feeding on post partum reproduction in buffaloes.**

Post-partum period is an important period to affect the inter-calving interval. It is vulnerable to various environmental factors of which nutrition, climatic stress and diseases are most important. After parturition, milk production is on priority in dairy cattle. Body reserves are mobilized if the energy required is not properly met by feed intake. The excessive loss of body reserves and hence of body condition and weight adversely affect reproduction. To prevent it, therefore, the animal should have enough body reserves before parturition and enough feed intake after parturition to meet the energy demand of body (Gearhart et al 1990, Staples et al 1990). Immediately after parturition, animals are unable to increase dry matter intake owing to limited rumen capacity and delayed microfloral adjustment to new energy rich diet fed conventionally (Goff and Horst 1997, Roche et al 2000). Hence, supplementary prepartum feeding is needed to develop sufficient body reserves and for timely adjustment of ruminal

microflora prepartum to the postpartum diet (Domeq et al 1997, Goff and Horst 1997). UMMB being good source of energy and protein is considered to improve ruminal microflora and increase dry matter intake in ruminants (Malik et al 1993, Tripathi 1997). The present study (Experiment 1 and 2) was therefore, undertaken to assess the effect of pre partum supplementary UMMB feeding on post partum reproduction in buffaloes.

#### **4.3.1 Experiment I: Supplementary feeding in prepartum buffaloes due for calving in winter at organized dairy farm:**

This experiment was conducted at organized dairy farm of Punjab Agricultural University, Ludhiana to have close observations on UMMB supplementation to prepartum buffaloes due to parturate in the months of September to October 2000. The average daily in-take of UMMB recorded in this study (674 g; Table 11) is comparable to other reports wherein UMMB was prepared by using bentonite as binder (Malik et al 1997, Kakkar et al 1997). The observations of this study do not approve the apprehended reduced intake of blocks bound with cement (Kakkar and Makkar 1995).

**Table 11. UMMB intake in pre-partum buffaloes at P.A.U. dairy farm**

Animal No.	Total days of UMMB feeding	Average intake /day (g)	Total intake (Kg)
1	59	759.54±23.21 <sup>b</sup>	37.97
2	69	686.14±25.72	39.11
3	80	662.64±28.57	45.06
4	73	746.40±22.47 <sup>b</sup>	46.27
5	64	597.16±39.15 <sup>a</sup>	34.04
6	70	626.36±34.60	37.58
7	70	697.10±30.42	40.43
8	84	656.31±28.29	47.91
9	88	624.15±30.89	48.04
10	60	685.52±38.61	39.07
Average	71.70±3.08	674.13±16.49	41.54±01.54

- Values with different superscripts in columns are significantly different ( $p < 0.05$ )

#### **4.3.1.1 Effect of prepartum UMMB supplementary feeding on body weight, body condition score and heart girth changes before and after parturition**

##### **Changes in body weight**

All buffaloes gained weight during prepartum period and lost it at and after parturition (Table 12; Fig 3). The weight gain in the buffaloes during prepartum period, though statistically non-significant, was higher in supplemented than in

the controls. A fall in body weight observed at parturition in both the groups, continued for next two months. Cumulative body weight loss was lesser during first month postpartum in the supplemented buffaloes (Fig 4). However, the total weight loss during first two months postpartum was more in the supplemented group ( $p>0.05$ ). Similar trends in body weight changes following supplementary feeding prepartum have also been reported in cattle (Singh 1998) and in buffaloes (Sharma et al 1993). However Sharma et al (1993) further reported that the supplemented buffaloes started gaining weight earlier so that the overall body weight gain was higher than in the controls in the first 90 days postpartum. In the present study however, the animals were losing weight even at the end of 2<sup>nd</sup> month. This is probably due to the fact that unlike in the studies by Sharma et al (1993), the supplementary feeding in the present study was restricted only in the prepartum period. The body weight loss at parturition is due to expulsion of fetus, placenta and fetal fluids. The loss thereafter is due to mobilization of body reserves for production of milk (Touckberry and Batra 1976, Wood et al 1980, Khatkar et al 1997, Bar et al 1995). The extent of body weight loss depended upon a number of factors including parity, body size, milk production and nutritional status of an animal and the season of calving (Bhalaru et al 1986, Sharma et al 1993). Another critical observation that could be derived from this and from earlier studies on buffaloes (Bhalaru et al 1981) is that the buffaloes keep losing body weight for longer duration postpartum than the cows kept under similar environmental conditions (Khatkar et al 1997). Prepartum supplementary

feeding however gave the advantage of lesser losses during the first month postpartum (This study)

### **Changes in Body Condition Score (BCS)**

The overall changes observed in BCS paralleled the corresponding changes observed in body weight in the control and the supplemented buffaloes. The average BCS at the start of UMMB supplementary to prepartum buffaloes was 3.50 in both the groups. (Table13). The average BCS at calving in the supplemented and the control buffaloes was 3.80 and 3.75 respectively. The prepartum BCS gain by supplemented buffaloes was marginally better (0.30) than by the control buffaloes (0.25 points). This is probably due to the fact that the buffaloes under study belonged to an organized dairy farm being kept on a well balanced diet. Out of 15, nine buffaloes parturated with BCS <4.0 and six with BCS  $\geq$ 4.0. None of the animals in either group suffered from any clinical metabolic disease after parturition. This is contrary to the finding that the cows gaining BCS during dry period were predisposed to metabolic diseases (Gearhart et al 1990, Grummer 1993, Roche et al 2000). The observations of present study are consistent with Domeq et al (1997) suggesting no adverse effect of gaining BCS in prepartum dry period provided animals are not over conditioned. Smith and Butler (1989) did not observe any adverse effect in cows parturated even at BCS between 4 to 5.

A significant loss in BCS was observed at parturition in all the buffaloes, which continued through next two months (Table 12; Fig 5), which is in line with observations on cows (Butler and Smith 1989, Studer 1998). However, this fall in

BCS was lesser in the supplemented than in the control group during the first month postpartum (Fig 6). A significant relationship had been reported between the body weight and the BCS in cows. With each decrease in BCS by 1.0 point, 53.0 kg body lipid was lost (Wright et al 1987).

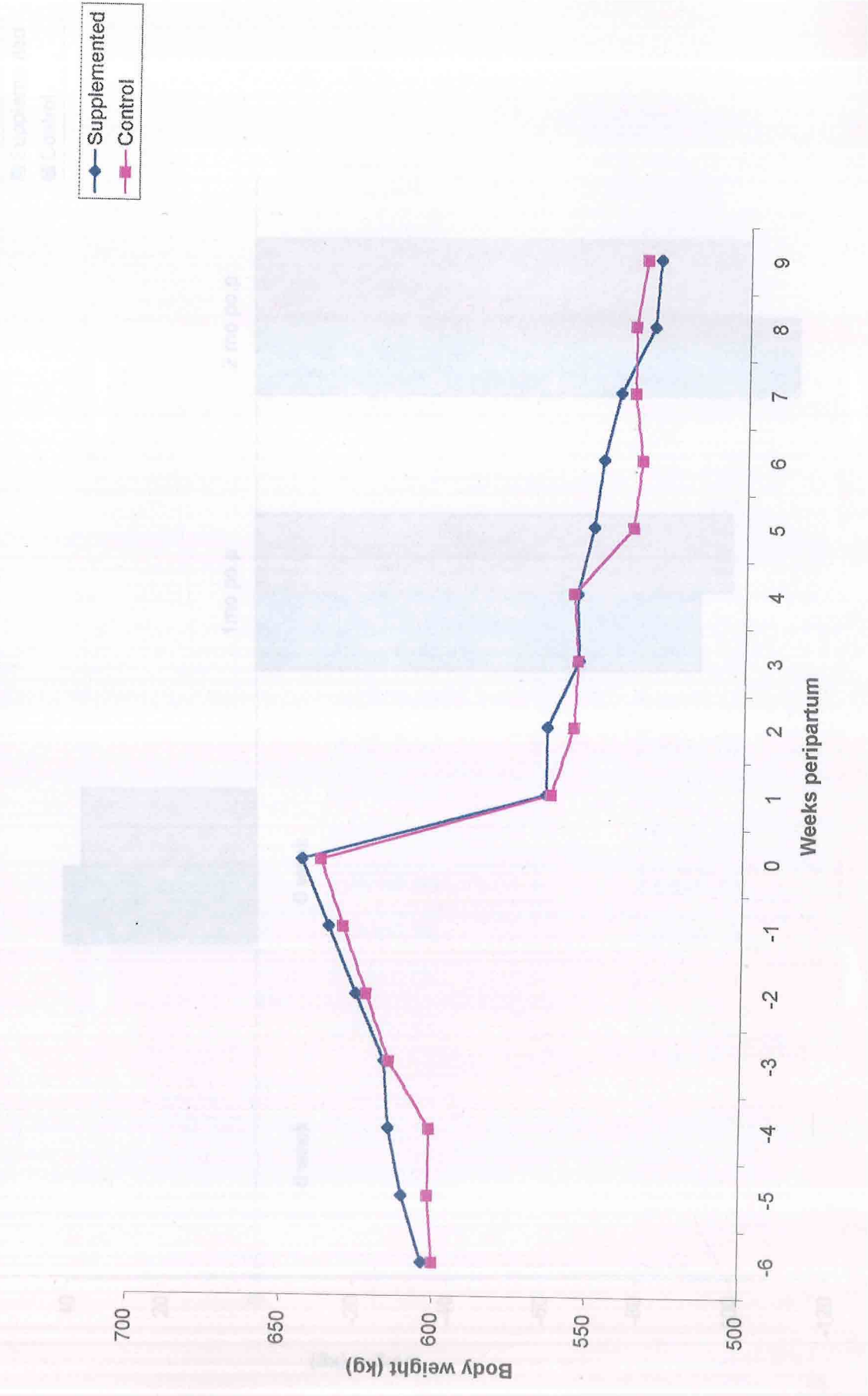
### **Changes in heart girth**

Changes in girth had similar trend as in body weight and in BCS during pre and postpartum period (Table 14; Fig 7 and 8). Animals gained girth towards parturition and lost thereafter through next two months. However, difference of girth changes in supplemented and control buffaloes were not statistically significant ( $p > 0.05$ ). Girth measurements have been used as an estimate of body weight in cows (Otte et al 1992) and buffaloes (Manik et al 1981). It was however, observed that the posture of an animal led to greater variations in the girth measurements adding to huge changes in the calculation of body weight.

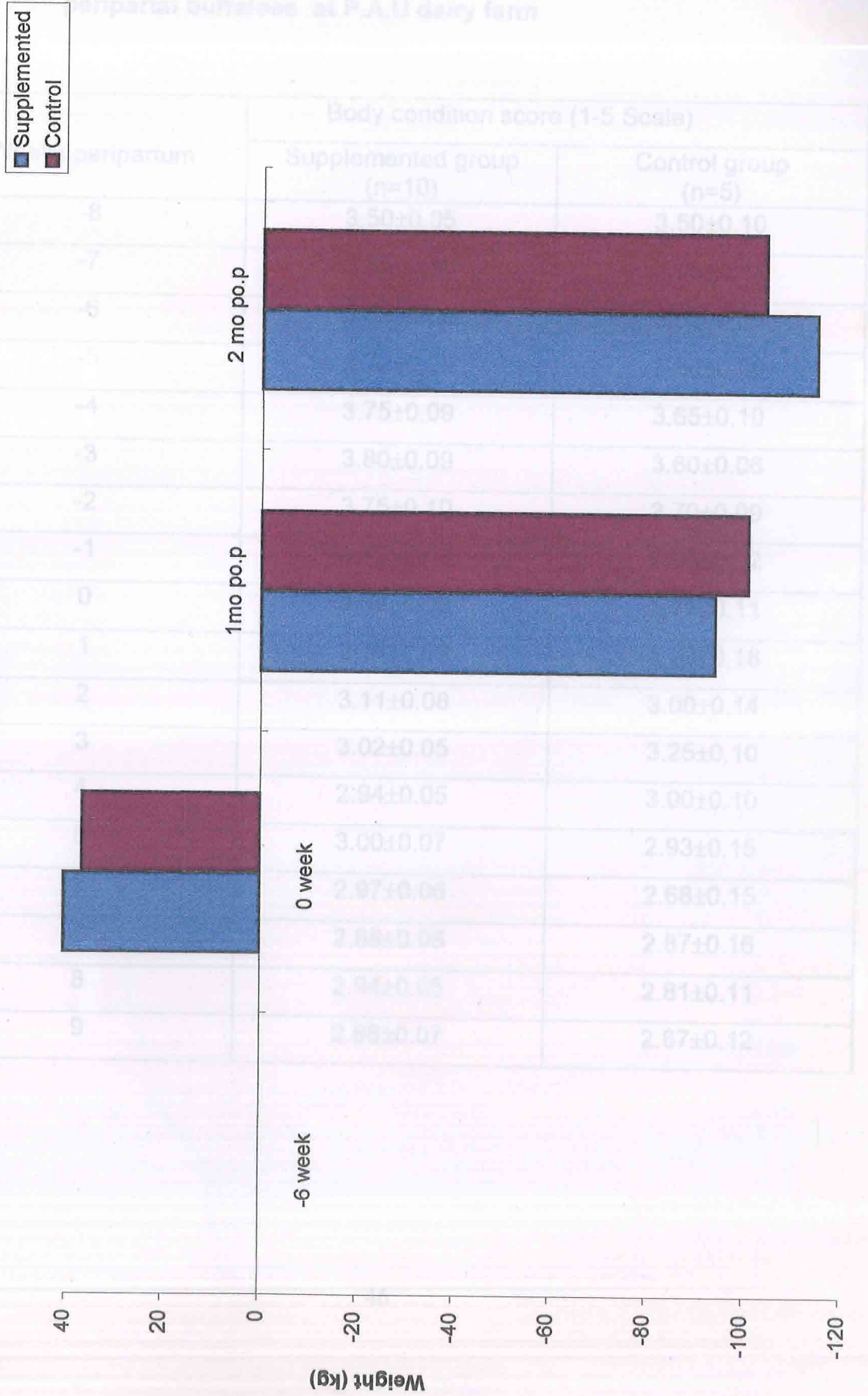
**Table 12. Body weight (kg; Mean $\pm$ SE ) of UMMB supplemented peripartal buffaloes at P.A.U dairy farm**

Weeks peripartum	Body weight (kg.)	
	Supplemented group (n=10)	Control group (n=5)
-6	603.50 $\pm$ 19.37	600.00 $\pm$ 54.33
-5	610.40 $\pm$ 18.97	601.80 $\pm$ 55.41
-4	614.90 $\pm$ 18.90	601.40 $\pm$ 55.73
-3	616.30 $\pm$ 18.14	614.80 $\pm$ 54.88
-2	625.80 $\pm$ 18.48	622.40 $\pm$ 56.23
-1	634.90 $\pm$ 18.93	630.20 $\pm$ 56.46
0	644.00 $\pm$ 18.59	637.80 $\pm$ 51.86
1	564.30 $\pm$ 19.49	562.80 $\pm$ 47.70
2	564.30 $\pm$ 17.79	555.60 $\pm$ 47.36
3	554.10 $\pm$ 17.09	554.40 $\pm$ 45.54
4	554.90 $\pm$ 17.25	556.00 $\pm$ 48.81
5	550.00 $\pm$ 17.45	537.00 $\pm$ 34.75
6	547.00 $\pm$ 17.79	534.40 $\pm$ 36.76
7	541.60 $\pm$ 17.60	537.00 $\pm$ 41.64
8	530.90 $\pm$ 17.62	537.20 $\pm$ 39.04
9	529.20 $\pm$ 17.44	533.60 $\pm$ 36.32

Fig 3 Body weight of UMMB supplemented peripartal buffaloes (P.A.U. dairy farm)



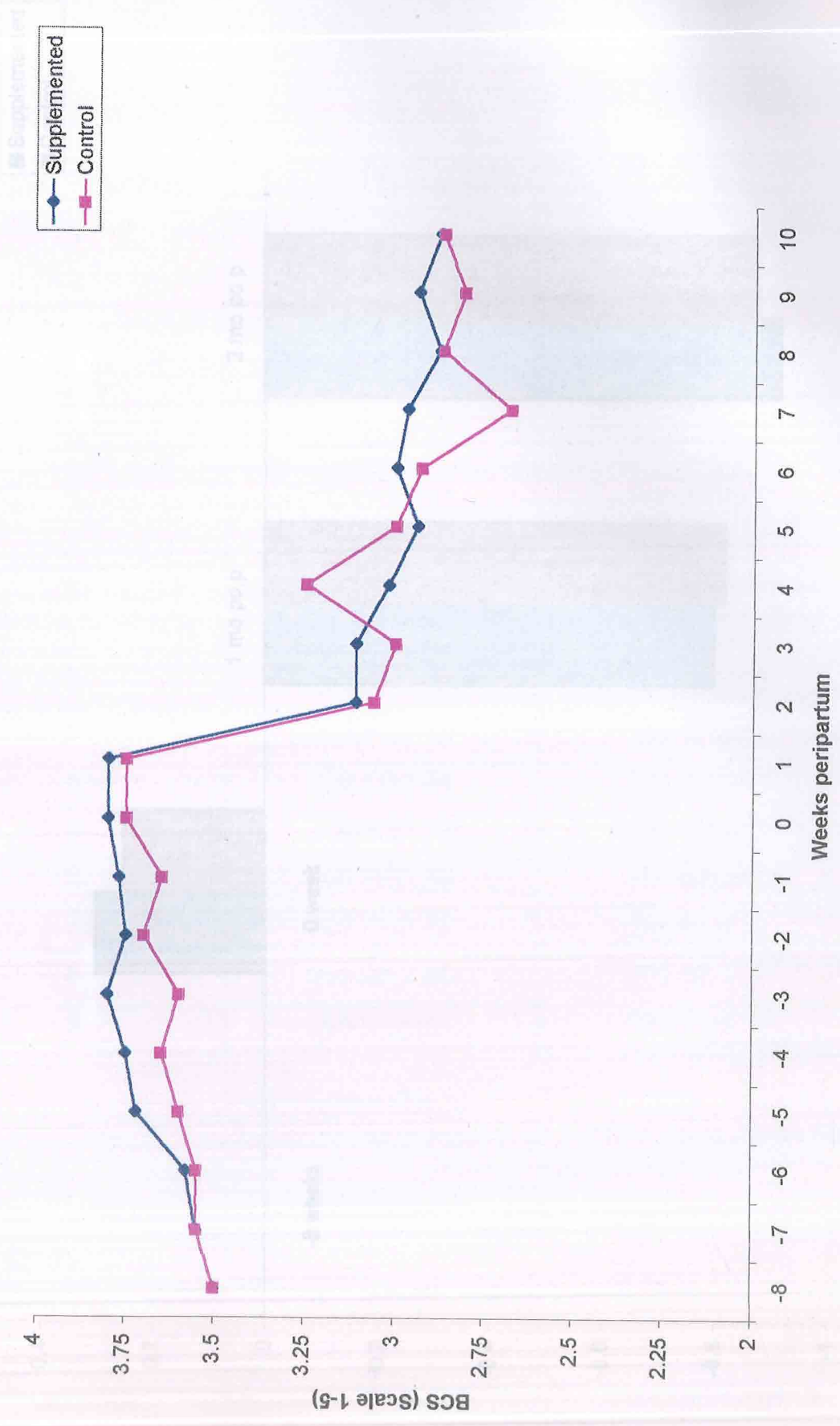
**Fig. 4 Peripartal body weight changes in UMMB supplemented buffaloes (P.A.U. dairy farm)**



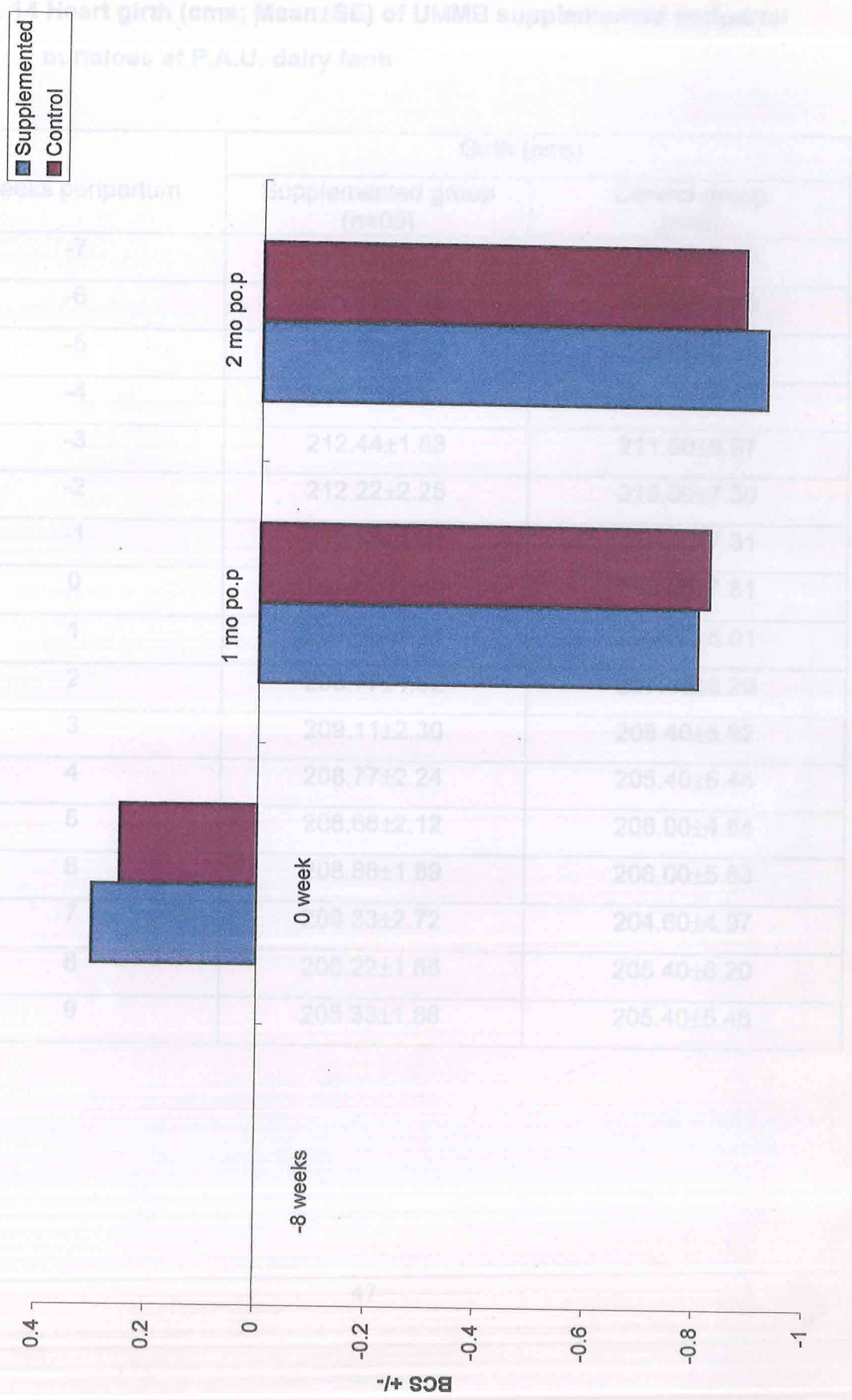
**Table13 Body condition score (Mean  $\pm$ SE) of UMMB supplemented peripartal buffaloes at P.A.U dairy farm**

Weeks peripartum	Body condition score (1-5 Scale)	
	Supplemented group (n=10)	Control group (n=5)
-8	3.50 $\pm$ 0.05	3.50 $\pm$ 0.10
-7	3.55 $\pm$ 0.10	3.55 $\pm$ 0.14
-6	3.58 $\pm$ 0.09	3.55 $\pm$ 0.12
-5	3.72 $\pm$ 0.09	3.60 $\pm$ 0.06
-4	3.75 $\pm$ 0.09	3.65 $\pm$ 0.10
-3	3.80 $\pm$ 0.09	3.60 $\pm$ 0.06
-2	3.75 $\pm$ 0.10	3.70 $\pm$ 0.09
-1	3.77 $\pm$ 0.09	3.65 $\pm$ 0.12
0	3.80 $\pm$ 0.09	3.75 $\pm$ 0.11
1	3.11 $\pm$ 0.09	3.06 $\pm$ 0.18
2	3.11 $\pm$ 0.08	3.00 $\pm$ 0.14
3	3.02 $\pm$ 0.05	3.25 $\pm$ 0.10
4	2.94 $\pm$ 0.05	3.00 $\pm$ 0.10
5	3.00 $\pm$ 0.07	2.93 $\pm$ 0.15
6	2.97 $\pm$ 0.06	2.68 $\pm$ 0.15
7	2.88 $\pm$ 0.06	2.87 $\pm$ 0.16
8	2.94 $\pm$ 0.05	2.81 $\pm$ 0.11
9	2.88 $\pm$ 0.07	2.87 $\pm$ 0.12

**Fig 5 Body condition score of UMMB supplemented peripartal buffaloes (PAU dairy farm)**



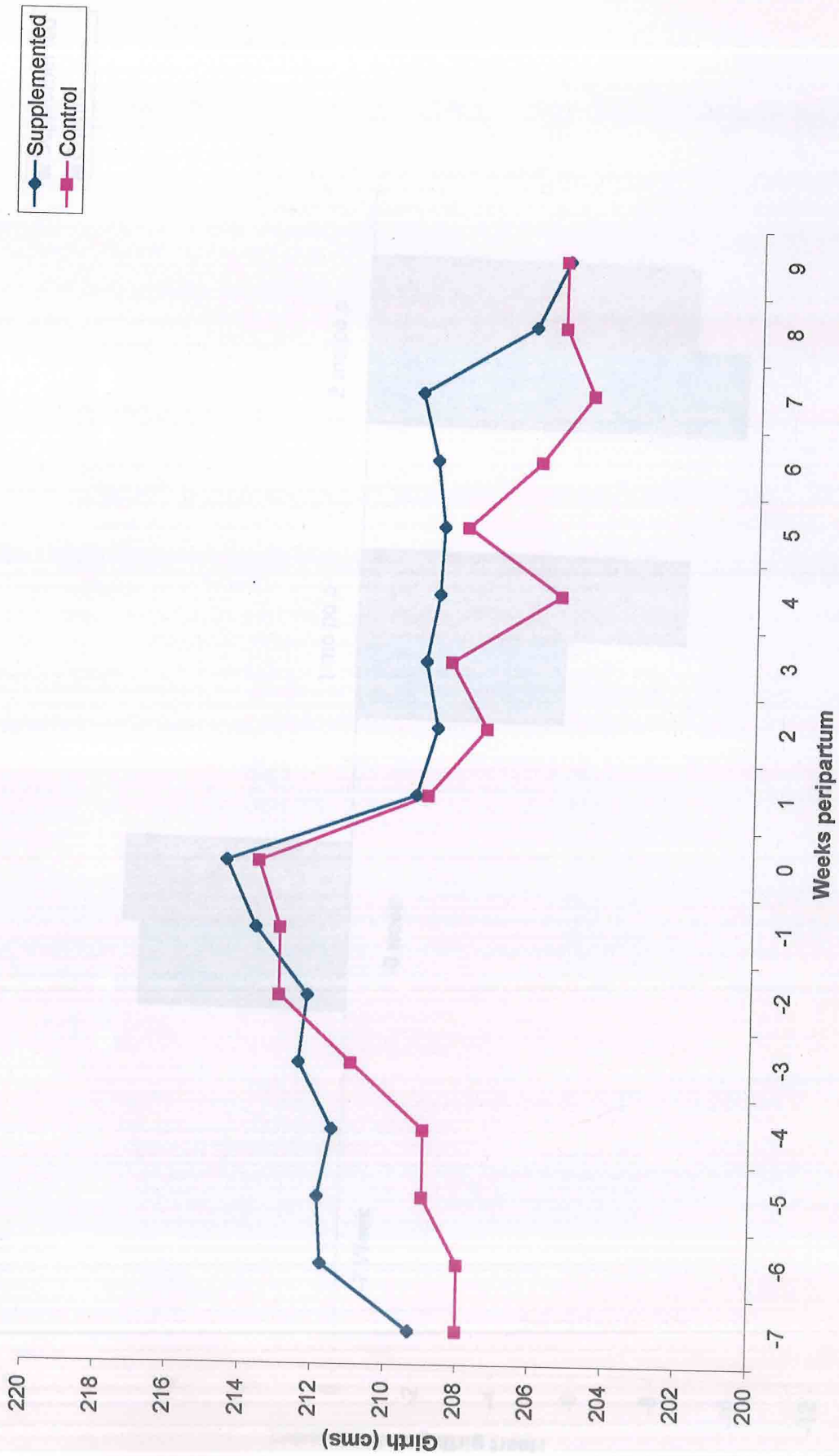
**Fig 6 Peripartal body condition score changes in UMMB supplemented buffaloes (P.A.U. dairy farm)**



**Table. 14 Heart girth (cms; Mean±SE) of UMMB supplemented peripartal buffaloes at P.A.U. dairy farm**

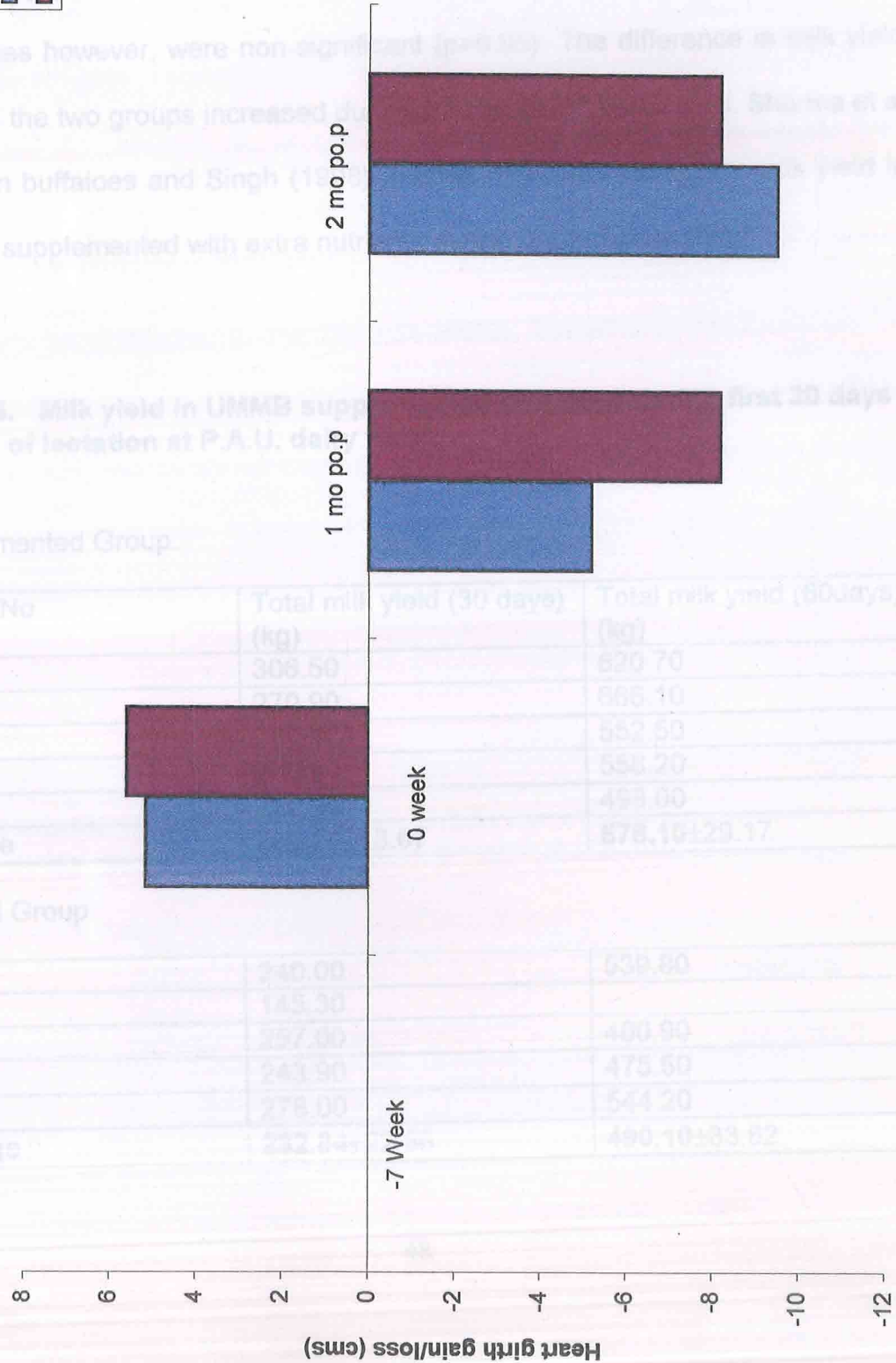
Weeks peripartum	Girth (cms)	
	Supplemented group (n=09)	Control group (n=5)
-7	209.40±2.25	208.00±8.10
-6	211.77±2.15	208.00±7.79
-5	211.88±2.33	209.00±8.19
-4	211.51±2.16	209.80±8.18
-3	212.44±1.83	211.00±6.97
-2	212.22±2.25	213.00±7.30
-1	213.66±1.91	213.00±7.31
0	214.50±1.80	213.60±7.81
1	209.33±2.21	209.00±6.01
2	208.77±1.92	207.40±6.29
3	209.11±2.30	208.40±5.92
4	208.77±2.24	205.40±6.44
5	208.66±2.12	208.00±4.84
6	208.88±1.89	206.00±5.83
7	209.33±2.72	204.60±4.97
8	206.22±1.86	205.40±6.20
9	205.33±1.86	205.40±5.46

Fig. 7 Heart girth of UMMB supplemented peripartal buffaloes (P.A.U. dairy farm)



**Fig 8 Peripartal heart girth changes in UMMB supplemented buffaloes (P.A.U. dairy farm)**

■ Supplemented  
■ Control



#### 4.3.1.2 Postpartum milk yield in buffaloes supplemented with UMMB prepartum at PAU dairy farm

Average milk production in first 60 days by UMMB supplemented buffaloes was more than produced by corresponding control buffaloes having same parity (2<sup>nd</sup> and 3<sup>rd</sup>) and similar previous lactation yield (Table 15). These differences however, were non-significant ( $p>0.05$ ). The difference in milk yield between the two groups increased during 2<sup>nd</sup> month (30 Vs 88 Kg.). Sharma et al (1993) in buffaloes and Singh (1998) in cows also reported higher milk yield in animals supplemented with extra nutrients during prepartum period.

**Table15. Milk yield in UMMB supplemented buffaloes during first 30 days of lactation at P.A.U. dairy farm**

Supplemented Group.

Animal No.	Total milk yield (30 days) (kg)	Total milk yield (60days) (kg)
1680	306.50	620.70
1794	270.90	666.10
1710	249.80	552.50
1768	260.90	558.20
1678	222.90	498.00
Average	<b>262.20±13.67</b>	<b>578.10±29.17</b>

Control Group

1795	240.00	539.80
1776	145.30	
1714	257.00	400.90
1822	243.90	475.50
1763	278.00	544.20
Average	<b>232.84±22.86</b>	<b>490.10±33.62</b>

#### 4.3.1.3 Effect of prepartum UMMB feeding on postpartum reproduction

All observations on reproductive aspects in UMMB supplemented buffaloes were within the physiological limits, although the supplemented buffaloes tended to have nonsignificantly heavier placenta that was shed earlier than in the controls (Tables 16 and 17). These along with earlier observations on fetal weight by Sharma et al (1993) refuted the conventional belief that supplementary prepartum feeding enlarges fetus leading to dystocia. Higher proportion of buffaloes (20%) had clear mucus discharge at first oestrus postpartum as compared to the controls (60%). Turbidity in mucus, if any in supplemented group was less intense (+) than in controls (++ to ++++) and higher proportion (80 Vs 60%) of supplemented animals became fit for artificial insemination within first 60 days of parturition than in the controls. Also more animals conceived within 70 days of parturition in the supplemented group (30 Vs 0%).

**Table 16 Postpartum observations on certain reproductive parameters in buffaloes supplemented UMMB prepartum at PAU dairy farm**

Group	Gestation period (days)	Calf Weight. (kg)	Placenta Wt. (kg)	Time to shed placenta (hrs.)
Supplemented (n=10)	317.00	31.50±1.36	4.65±0.37	5.04±0.20
Control (n=5)	314.00	31.60±1.86	3.60±0.24	5.54±0.65

**Table 16 (Contd.)**

Groups	Proportion of buffaloes		
	Turbid mucus at 1 <sup>st</sup> oestrus	Fit for insemination within 60 days	Conception rate (up to 70 days)
Supplemented (n=10)	20% (+)	80%	30%
Control (n=5)	60% (+-++++)	60%	0%

(+) denotes intensity of turbidity on (+ to +++) scale

Days to first observed heat did not vary between the two groups (Table 17), because probably the first heat is not related to the nutritional status but to the estrogens clearance in an otherwise normally fed animal (Beam and Butler 1997, Beam and Butler 1998, Butler 2000). All buffaloes in control as well as in the supplemented groups exhibited behavioral oestrus on an average 24.40 (range= 15-40) days and on 24.00 (range=15-45) days after calving. As evidenced by plasma progesterone concentrations >1.0 ng/ml, 4/5 (80%) and 9/10 (90%) buffaloes in the respective group came into ovulatory heat on 48 (range, 34-57) days and on 34 (range, 23-49) days postpartum, respectively (Table 16). First heat in three (75%) and one (11%) buffaloes in the respective groups was silent. One buffalo in each group (20 and 10% respectively) remained acyclic during the postpartum period of study. The days taken to first ovulatory heat (34 Vs 48 days) and the proportion of silent heat (11 Vs 75%) was noticeably lower in the supplemented than in the control group of buffaloes.

**Table 17 Postpartum observations related to onset of oestrus in buffaloes supplemented with UMMB prepartum at PAU dairy farm**

Group	Days to 1 <sup>st</sup> observed heat	Days to first ovulatory heat	Incidence of silent estrus	Anoestrus up to 60 days
Supplemented (n=10)	24.00 ±3.17 (n=10)	34.33±2.56 (n=09)	11% (n=01)	10% (n=01)
Control (n=5)	24.40 ±5.67 (n=05)	48.25±5.25 (n=04)	75% (n=03)	20% (n=01)

#### **4.3.1.4 Interrelationship between body weight, body condition score, productive and reproductive performance of buffaloes**

Body weight, BCS and girth had similar pattern of changes before and after parturition both in supplemented and un-supplemented buffaloes. Of the three methods, the BCS appeared to be more reliable and convenient method to assess body condition of an animal, as unlike body weight, it did not fluctuate inconsistently due to changes in feed intake and unlike heart girth, it did not change due to changes in posture of an animal. Further, it did not involve any equipment and handling of animals to study the BCS.

Buffaloes in both the groups under study exhibited behavioral oestrus within 45 days of parturition (average 24 days). However, first ovulation in both the groups occurred much later (average 34 days and 48 days in control and supplemented, respectively). Beam and Butler (1998) recorded a follicular wave

in cows as early as 2<sup>nd</sup> week postpartum although ovulations at that time occurred in only 50% of the animals. First follicular wave emerged regardless of the energy balance of the animals and IGF-1 circulatory concentrations appeared to have direct bearing on the ovulatory process in postpartum cows (Beam and Butler 1997).

In the present study, the supplemented buffaloes reduced lesser weight during 1<sup>st</sup> postpartum month than their controls counterparts. This probably accounted for relatively early ovulations in supplemented buffaloes (34 Vs 48 days). The supplemented buffaloes lost relatively more weight during 2 months than the control which however did not appear to have affected ovulation. Similar observations by Staples et al (1990) also supported that reproduction in cows losing more weight during early postpartum period (first two weeks) was badly affected than those losing less during this period.

The buffaloes in the present study lost less than 1.0 point BCS during two months after parturition and the average BCS in 15/16 buffaloes remained >2.70 through the postpartum period under study. Earlier studies in dairy cows have found that ideal BCS values at parturition should be within 3.0-4.0 and should not fall by more than 1.0 point at parturition and should remain above 2.25 during early postpartum period/ peak lactation (Butler and Smith 1989, Domeq et al 1997, Studer 1998, Charnetzki 1999). One buffalo from the supplemented group had 3.0 BCS at parturition and lost 1.0 point at parturition and during postpartum period. However, its postpartum reproductive performance was normal and did not appear to be affected by the inordinate changes in BCS. Lack of relationship

between her reproductive performance postpartum and BCS remained unexplained. The extent of loss and the absolute BCS value of the buffaloes in the present study remained within desired range, which may explain the overall normal performance of these buffaloes.

The supplemented buffaloes gave higher amount of milk (578 vs 490 kg; Table 15) during 60 days postpartum than in the controls. Their reproductive performance postpartum was also better than their unsupplemented counterparts (Table 16 and 17). Reproductive performance was found to be positively correlated to productive performance in cows (Staples et al 1990, Domeq et al 1997). Butler and Smith (1989) and Nebel and McGilliard (1993) however found negative correlation between milk yield and reproductive performance postpartum. High producing cows might be more reproductively sound because they are healthier than low producing cows (Shanks et al 1979). On the contrary higher milk yield may increase the intensity and duration of negative energy balance and hence may adversely effect reproduction (Butler and Smith 1989). Due to these contradictory arguments in favor or against, the early postpartum milk yield cannot be considered as an ideal index of ensuing fertility in dairy animals. Energy status of an animal has been considered important than milk yield in determining the days to first breeding (Staples et al 1990). Maximum negative energy balance occurred during first two weeks of lactation (Butler et al 1981). First oestrus and ovulations were recorded about 10 days later when the negative energy balance was approaching zero. The correlation of milk yield and days to first ovulation becomes significant at about 60 days postpartum (Butler et

al 1981). Most of the animals in their as well as in the present study had already ovulated before this time. This is further strengthened by the observations that body weight or body condition losses are more important during first month postpartum than afterwards. Staples et al (1990) reported that cows losing more weight during first two weeks of lactation had delayed ovarian activity. Loss in weight between 3<sup>rd</sup> and 9<sup>th</sup> week was similar in animals having early or delayed ovarian activity. Cows that lost 0.80 points of BCS during the first month of lactation were 1.36 times less likely to conceive than the cows losing less than that (Domeq et al 1997). The lesser loss of body weight and of BCS in first month of lactation in the present study might have favored early ovulatory heat and conception in the supplemented group. Fordyce et al (1997) reported that Bos indicus-cross cows supplemented with urea molasses during late pregnancy had shorter service period although no apparent changes occurred in BCS and body weight peripartum. Due to wide individual variation in the milk production in buffaloes, leading to high SE, conclusions drawn out of this study cannot be statistically authoritative. However, a closer look on the performance of individual animal, revealed that individual variations existed in relationship of milk production with postpartum duration to ovulations. One buffalo which, yielded minimum daily milk (4.84 kg) ovulated at the earliest (28 days). Further, the only buffalo (No 1872) with silent heat have had consumed minimum amount of UMMB within the supplemented group.

The forgoing observations, overall and on individual buffaloes, revealed that prepartum UMMB supplementation helped in improving production and reproduction in postpartum buffaloes.

#### **4.3.1.5 Biochemical changes in plasma in UMMB supplemented buffaloes at PAU dairy farm and their relationship with reproduction.**

Total plasma protein, albumin, urea, creatinine and glucose concentrations are given in Tables 18 to 27. The values of these parameters remained in the normal physiological range. The levels of total proteins were high in supplemented group compared to control group during prepartum period. After parturition levels were similar in two groups. Extra supplementation of feed increased total plasma proteins in earlier studies (Sharma 1989, Fiho et al 2000) Ovarian activity may (Chauhan 1980, Singh 1993) or may not be (Dhoble 1978, Gentile et al 1978) related to levels of total plasma proteins. In the present study levels of total proteins were never lower enough to hinder the ovarian activity. Albumin levels in the supplemented group were lower suggesting the high level of total plasma protein to be due globulin/fibrinogen levels in supplemented group.

Urea levels indicated the intake of crude protein specifically the ruminally degradable fraction (Khan 2000). Higher plasma urea levels in supplemented group than in control group in this study were indicative of higher crude protein intake in form of UMMB or through increased feed intake. Concentration of blood urea nitrogen more than 20mg/dl was reported to change the uterine

environment and effect reproduction in cows (Butler 2000). No adverse effect of UMMB feeding was recorded in supplemented buffaloes in present study, as all had normal parturition. Creatinine levels were lower in the supplemented group after parturition indicating lower muscle wastage.

**Table 18 Average total plasma proteins concentrations (g/dl) in UMMB supplemented buffaloes before parturition at P.A.U dairy farm.**

Weeks to parturition	Supplemented Gp. (n=10)	Control Gp. (n=5)
0	9.46±0.38	9.08±0.55
-1	9.18±0.43	7.90±0.56
-2	9.23±0.33	8.60±0.43
-3	9.50±0.33	8.19±0.23
-4	9.80±0.53	9.58±0.71
-5	9.22±0.27	9.49±0.89
-6	9.50±0.59	11.35±0.34
-7	9.96±0.68	9.60±0.14
-8	9.23±0.49	8.56±0.53
-9	10.53±1.11	9.83±0.43

**Table 19 Average total plasma proteins concentrations (g/dl) in UMMB supplemented buffaloes after parturition at P.A.U dairy farm.**

Weeks after parturition	Supplemented Gp. (n=10)	Control Gp. (n=5)
0	9.46±0.38	9.08±0.55
1	8.99±0.31	9.38±0.76
2	9.75±0.47	8.65±0.26
3	9.35±0.29	9.38±0.64
4	9.70±0.38	9.89±0.40
5	9.10±0.45	9.17±0.27
6	9.52±0.28	9.42±0.50
7	9.24±0.51	9.41±0.29
8	9.52±0.56	9.39±0.40
9	10.21±0.21	10.41±0.78

**Table 20 Average plasma albumin concentrations (g/dl) in UMMB supplemented buffaloes before parturition at P.A.U dairy farm.**

Weeks to parturition	supplemented Gp. (n=10)	Control Gp. (n=5)
0	4.378±0.15	5.224±0.36
-1	4.397±0.10	5.04±0.08
-2	4.567±0.12	4.696±0.19
-3	4.773±0.17	4.826±0.28
-4	4.811±0.20	5.216±0.20
-5	4.726±0.16	4.61±0.17
-6	4.717±0.21	5.094±0.35
-7	4.667±0.34	5.115±0.14
-8	4.665±0.24	4.995±0.27
-9	4.725±0.19	4.855±0.20

**Table 21 Average plasma albumin concentrations (g/dl) in UMMB supplemented buffaloes after parturition at P.A.U dairy farm.**

Weeks after parturition	Supplemented Gp. (n=10)	Control Gp. (n=5)
0	4.378±0.15	5.224±0.36
1	4.546±0.10	5.040±0.20
2	4.324±0.13	5.402±0.34
3	4.209±0.17	5.186±0.17
4	4.234±0.19	4.736±0.19
5	4.414±0.20	4.580±0.16
6	4.681±0.14	4.870±0.22
7	4.465±0.17	4.62±0.28
8	4.501±0.09	4.308±0.28
9	4.400±0.25	4.76±0.29

**Table 22 Average plasma urea concentrations (mg/dl) in UMMB supplemented buffaloes before parturition at P.A.U dairy farm.**

Weeks to parturition	Supplemented Gp. (n=10)	Control Gp. (n=5)
0	50.28±6.26	43.4±3.22
-1	45.68±5.34	37.50±4.76
-2	61.96±3.75	43.31±2.40
-3	63.49±8.06	47.26±6.29
-4	60.66±3.11	59.99±12.30
-5	54.79±6.92	56.91±8.85
-6	59.39±5.37	50.95±3.52
-7	58.70±6.21	45.59±10.79
-8	78.23±9.36	28.23±4.99
-9	63.57±7.54	41.85±1.80

**Table 23 Average plasma urea concentrations (mg/dl) in UMMB supplemented buffaloes after parturition at P.A.U dairy farm.**

Weeks after parturition	Supplemented Gp. (n=10)	Control Gp. (n=5)
0	48.61±5.84	43.4±3.22
1	56.42±3.24	64.96±6.37
2	50.11±3.91	47.76±6.66
3	52.54±3.18	48.10±2.93
4	50.54±2.77	49.29±3.50
5	57.11±3.63	49.64±3.77
6	58.57±4.72	53.43±2.49
7	62.82±4.11	51.82±3.80
8	58.10±3.95	46.94±5.47
9	64.54±2.32	69.60±1.86

**Table 24 Average plasma creatinine concentrations (mg/dl) in UMMB supplemented buffaloes before parturition at P.A.U dairy farm.**

Weeks to parturition	Supplemented Gp. (n=10)	Control Gp. (n=5)
0	2.47±0.08	2.15±0.06
-1	2.57±0.21	2.26±0.11
-2	2.52±0.14	2.67±0.11
-3	2.64±0.08	2.45±0.07
-4	2.47±0.08	2.49±0.10
-5	2.51±0.07	2.60±0.12
-6	2.44±0.09	2.09±0.20
-7	2.35±0.10	2.44±0.15
-8	2.58±0.10	2.57±0.11
-9	2.18±0.17	1.76±0.08

**Table 25 Average plasma creatinine concentrations (mg/dl) in UMMB supplemented buffaloes after parturition at P.A.U dairy farm.**

Weeks after parturition	Supplemented Gp. (n=10)	Control Gp. (n=5)
0	2.47±0.08	2.15±0.07
1	2.40±0.07	2.40±0.04
2	2.25±0.07	2.25±0.12
3	2.23±0.10	2.40±0.13
4	2.08±0.09	2.19±0.12
5	1.76±0.18	2.05±0.08
6	1.93±0.08	2.11±0.13
7	1.89±0.15	2.04±0.08
8	1.86±0.07	2.10±0.08
9	1.53±0.12	2.05±0.04

**Table 26 Average blood glucose concentrations (mg/dl) in UMMB supplemented buffaloes before parturition at P.A.U dairy farm.**

Weeks to parturition	Supplemented Gp. (n=10)	Control Gp. (n=5)
0	71.34±4.66	43.97±5.83
-1	64.48±3.76	50.30±2.55
-2	64.16±5.74	58.43±4.63
-3	59.32±5.35	52.31±6.15
-4	47.54±4.33	47.37±1.68
-5	46.00±1.23	49.99±2.91
-6	47.36±1.37	52.10±9.09
-7	50.94±2.22	57.52±4.65
-8	49.06±2.33	52.32±1.36
-9	49.27±2.61	

**Table 27 Average blood glucose concentrations (mg/dl) in UMMB supplemented buffaloes after parturition at P.A.U dairy farm.**

Weeks after parturition	Supplemented Gp. (n=10)	Control Gp. (n=5)
0	71.34±4.60	46.61±8.35
1	59.52±3.70	65.08±8.37
2	60.65±2.80	66.89±7.72
3	57.94±2.37	65.00±3.65
4	54.91±2.12	58.37±3.18
5	55.95±2.79	58.30±3.70
6		59.66±3.37

**4.3.2 Experiment II: Supplementary feeding in prepartum buffaloes due for calving in summer at unorganized dairy farm:**

This study assessed the effect of prepartum UMMB supplementation on summer anoestrus in village buffaloes. By virtue of the traditional housing system in which they were kept, the buffaloes had access to UMMB only for limited hours (about 8-12 hours daily) while tied on mangers. The average UMMB intake by these buffaloes (Table 28) was similar to those in Experiment 1. Amount of UMMB consumed by village buffaloes within 12 hours was similar to the consumption by PAU buffaloes in 24 hours. Their UMMB consumption probably would have been higher if allowed ad lib. Higher consumption of UMMB in buffaloes with poor basic diet has also been reported by Kakkar et al (1997).

**Table 28. Average UMMB Intake by prepartum buffaloes under rural conditions**

Animal No.	Total days for UMMB feeding	Average intake /day (g)	Total intake (Kg)
1	106	443.39	47.0
2	67	477.61	32.0
3	51	627.45	32.0
4	47	723.40	34.0
5	50	680.00	34.0
6	41	731.70	30.0
7	58	655.17	38.0
8	90	488.88	44.0
9	66	765.15	50.5
10	72	715.27	51.5
11	74	594.59	44.5
<b>Average</b>	<b>65.63±5.58</b>	<b>627.51±33.96</b>	<b>39.77±2.39</b>

#### **4.3.2.1 Effect of prepartum UMMB supplementary feeding on body condition score and heart girth changes before and after parturition**

##### **Changes in body condition score**

The average BCS of prepartum buffaloes was 2.67 and 2.60 at the start of UMMB supplementation and 3.04 and 2.64 at calving in supplemented and control group buffaloes, respectively (Table 29). The BCS gain was significant (0.37 points;  $p < 0.05$ ) during this period in supplemented but not in the control group (0.04 points only). Lower overall BCS in rural buffaloes than in the buffaloes at PAU dairy farm in Experiment I could be due to better diet fed to the latter. Higher BCS gain over the controls in rural supplemented buffaloes than their PAU counterparts suggested a better effect of UMMB in already underfed animals. Contrary to observations made by Gearhart et al (1990), Grummer (1993) and Roche et al (2000) the BCS gain prepartum in Experiments I and II had no adverse affect on health of animals after parturition. Domeq et al (1997) also reported lack of any adverse effect of BCS gain in pregnancy provided the animals were not overconditioned. Buffaloes calving with better BCS (3.75 to 3.80; Experiment I) lost more BCS at parturition than those calving at lower BCS (2.67 to 3.04; Experiment II) (0.69 points Vs 0.30 points). No comparative observation on loss of BCS are available in literature. However, these are in line with finding of other workers on changes in body weight (Bhalaru et al 1981. Takkar and Chaudhary 1994). As in Experiment I, the supplemented animals in this experiment also lost higher BCS than controls by the end of 2<sup>nd</sup> month of lactation. This loss, however, was similar in both the groups during the first



month postpartum (Table 29; Fig 9 and 10). A number of factors, viz. milk yield, levels of feeding and body reserves at the time of calving govern the losses in BCS after parturition (Butler and Smith 1989).

### **Changes in heart girth**

Buffaloes in both the groups gained heart girth towards parturition and lost thereafter through next two months (Table 30; Fig 11 and 12). In supplemented group, girth gain was marginally higher than in the control group during prepartum period with relatively higher postpartum losses. High variation in girth values of individual buffaloes under field conditions than at PAU dairy farm remained unexplained. It is however, felt that these fluctuations could be due to postural changes in animals at the time of taking girth.

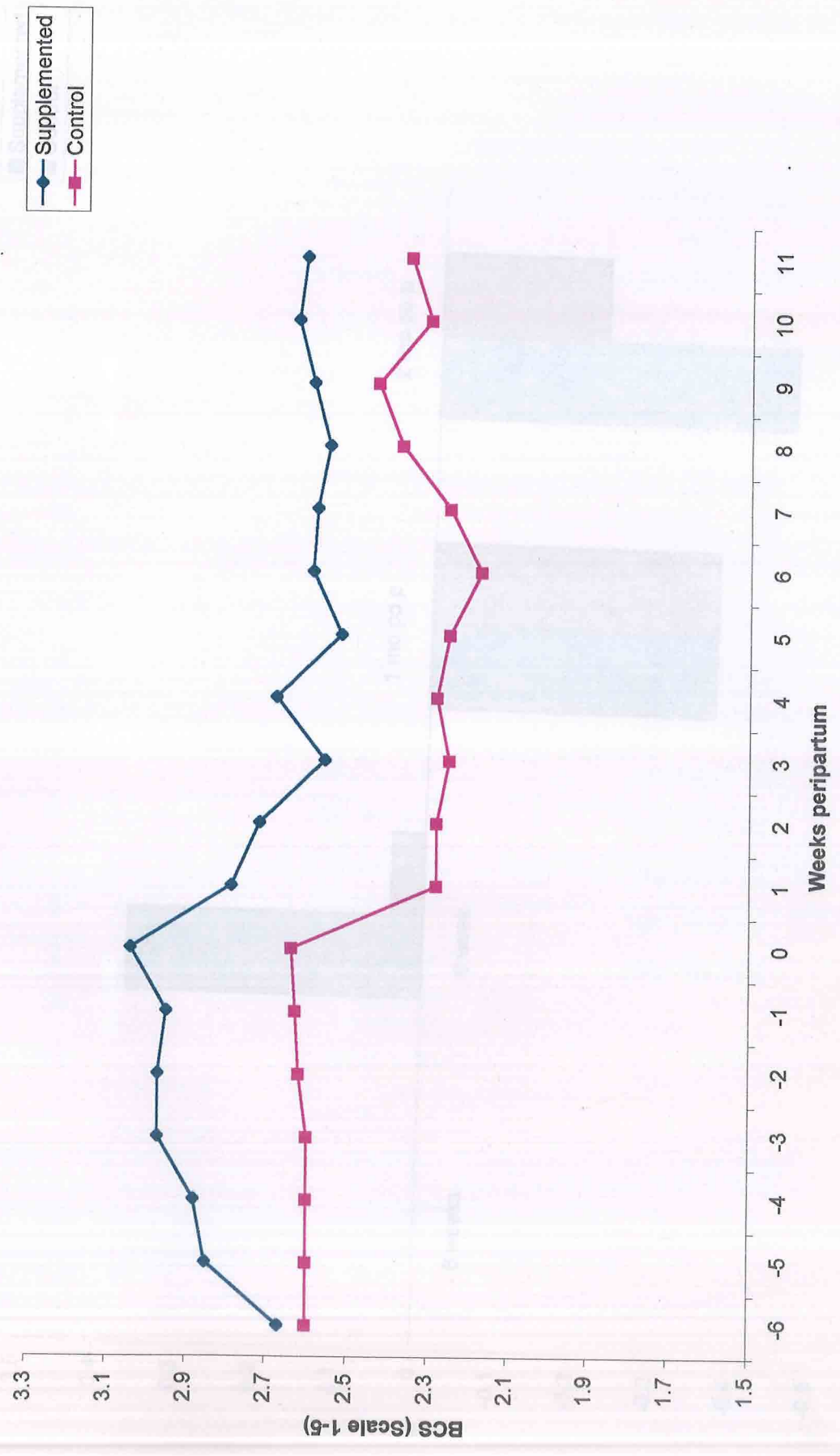
Although subjective, the feed consumption increased in all the supplemented as compared to the controls. Tripathi (1997) also reported 54% increase in intake of wheat straw based diet in dairy cows.

The concentrations of plasma total proteins, urea, creatinine and glucose remained in the normal physiological range. However, due to wide individual variation these remained inconclusive. No adverse affect of UMMB feeding was reflected in these biochemical changes.

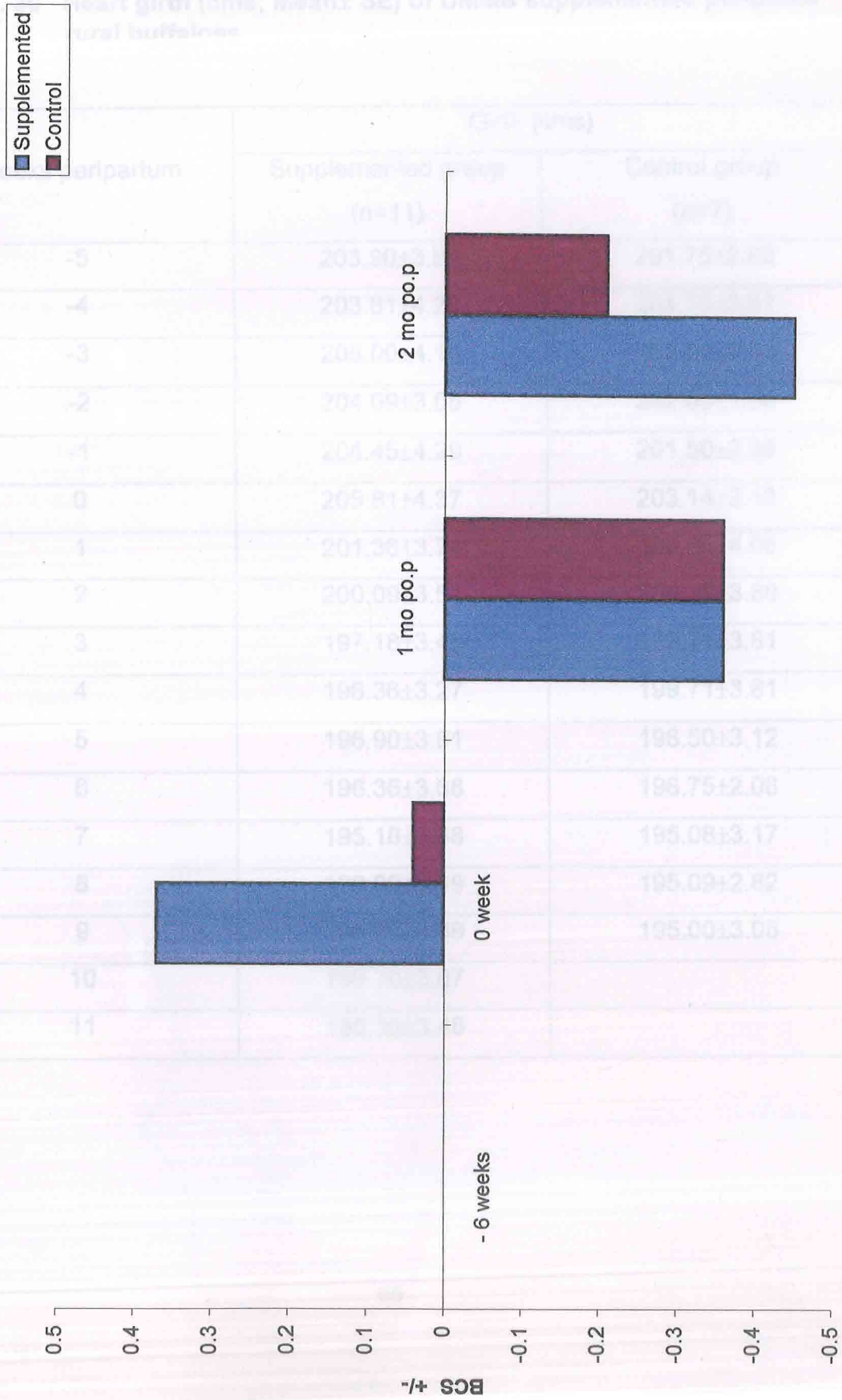
**Table. 29 Body condition score (Mean± SE) of UMMB supplemented peripartal rural buffaloes**

Weeks peripartum	Body condition score (1-5 scale)	
	Supplemented group (n=11)	Control group (n=7)
-6	2.67±0.08	2.60±0.21
-5	2.85±0.09	2.60±0.23
-4	2.88±0.06	2.60±0.25
-3	2.97±0.08	2.60±0.23
-2	2.97±0.08	2.62±0.23
-1	2.95±0.08	2.63±0.21
0	3.04±0.08	2.64±0.16
1	2.79±0.56	2.28±0.17
2	2.72±0.09	2.28±0.13
3	2.56±0.09	2.25±0.13
4	2.68±0.11	2.28±0.10
5	2.52±0.09	2.25±0.13
6	2.59±0.09	2.17±0.10
7	2.58±0.11	2.25±0.17
8	2.55±0.11	2.37±0.23
9	2.59±0.15	2.43±0.27
10	2.63±0.12	2.30±0.24
11	2.61±0.13	2.35±0.23

**Fig. 9 Body condition score of UMMB supplemented peripartal rural buffaloes**



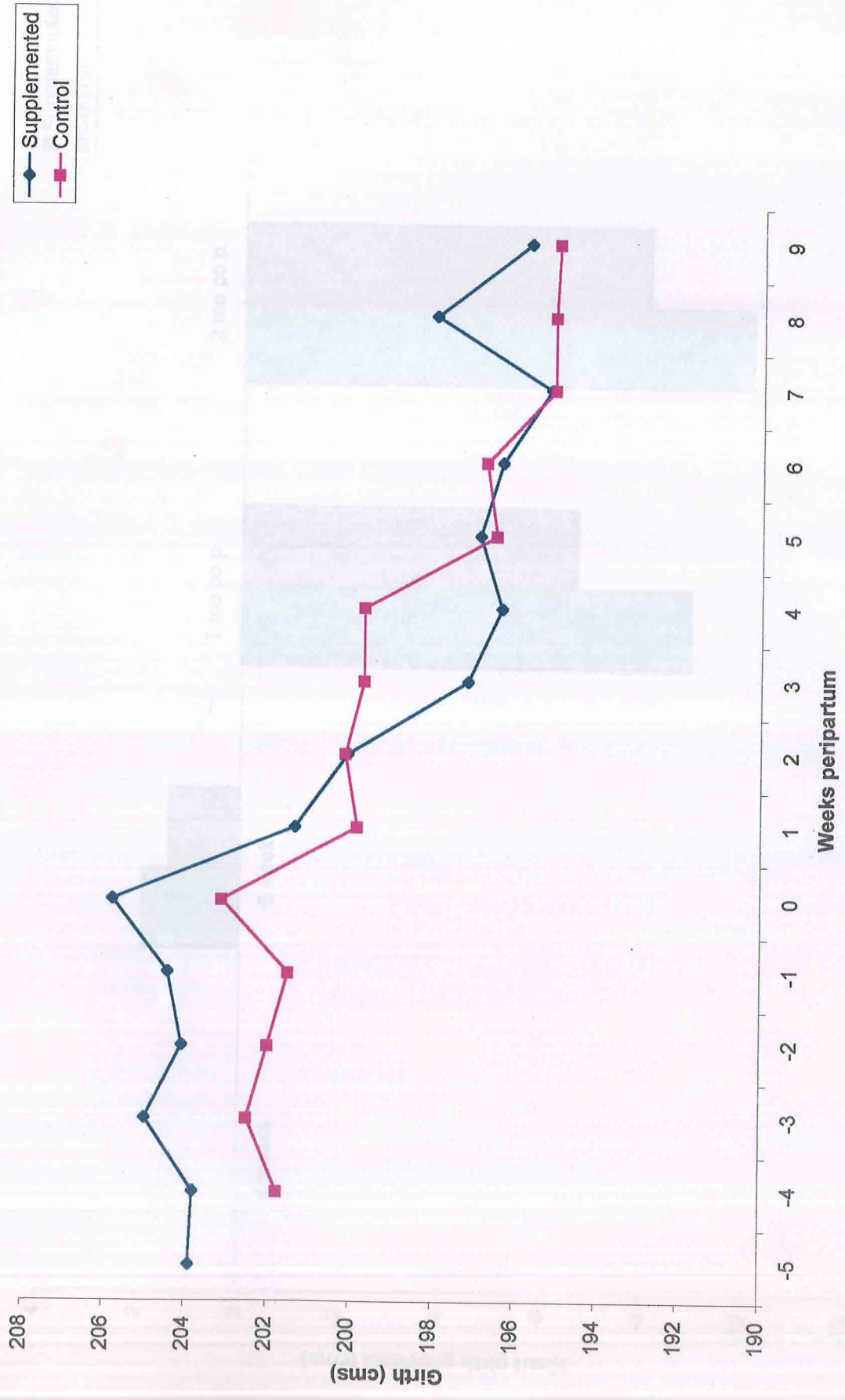
**Fig 10 Peripartal body condition score changes in UMMB supplemented rural buffaloes**



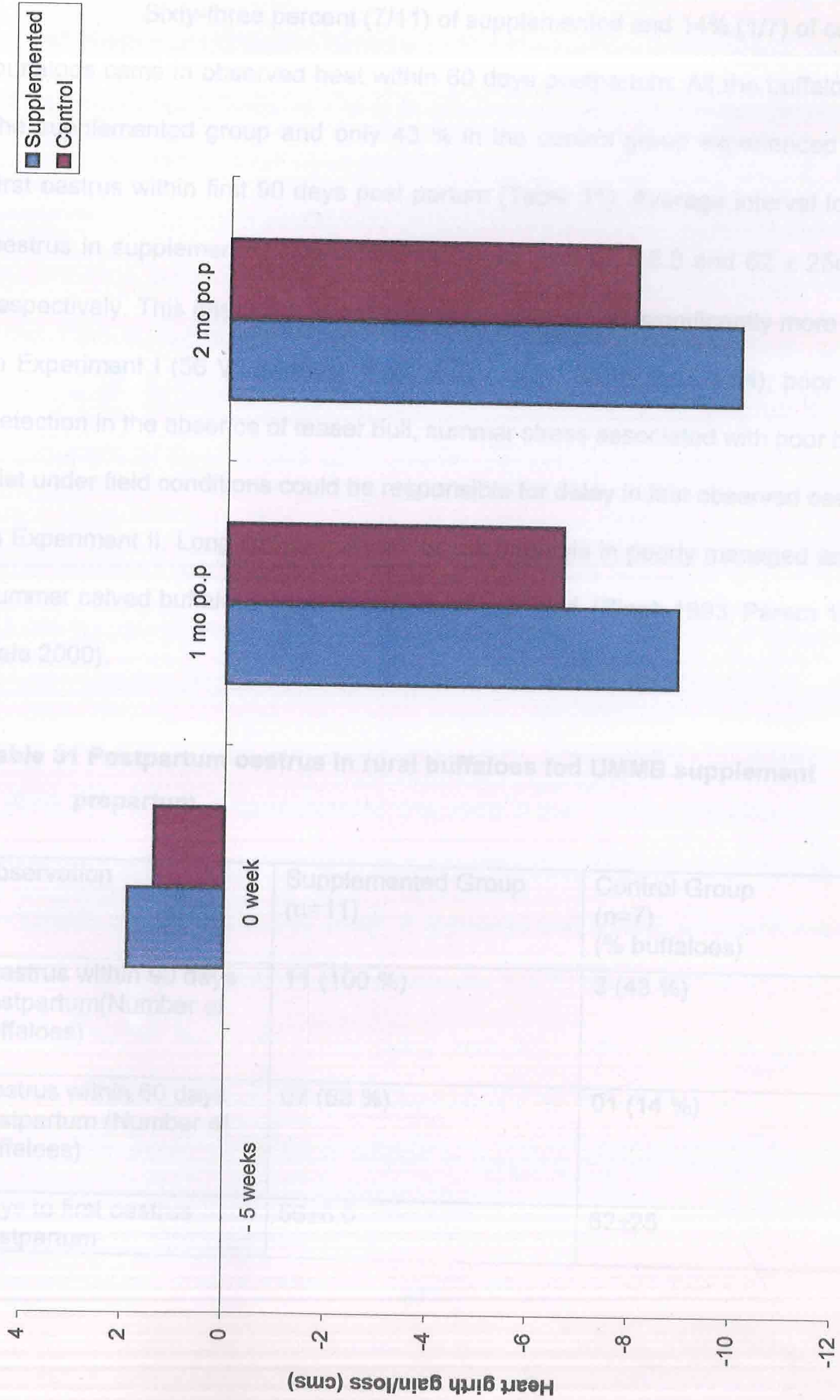
**Table. 30 Heart girth (cms; Mean± SE) of UMMB supplemented peripartal rural buffaloes**

Weeks peripartum	Girth (cms)	
	Supplemented group (n=11)	Control group (n=7)
-5	203.90±3.83	201.75±2.80
-4	203.81±4.25	201.75±3.81
-3	205.00±4.15	202.50±2.21
-2	204.09±3.05	202.00±1.08
-1	204.45±4.29	201.50±2.39
0	205.81±4.37	203.14±3.12
1	201.36±3.74	199.85±4.08
2	200.09±3.51	200.14±3.88
3	197.18±3.45	199.71±3.81
4	196.36±3.27	199.71±3.81
5	196.90±3.81	196.50±3.12
6	196.36±3.68	196.75±2.08
7	195.18±3.68	195.08±3.17
8	198.00±3.19	195.09±2.82
9	195.70±3.89	195.00±3.08
10	195.70±3.87	
11	198.36±3.48	

Fig 11 Heart girth of UMMB supplemented peripartal rural buffaloes



**Fig 12 Peripartal heart girth changes in UMMB supplemented rural buffaloes**



#### 4.3.2.2 Effect of prepartum UMMB feeding on postpartum reproduction in rural buffaloes

Sixty-three percent (7/11) of supplemented and 14% (1/7) of control buffaloes came in observed heat within 60 days postpartum. All the buffaloes in the supplemented group and only 43 % in the control group experienced their first oestrus within first 90 days post partum (Table 31). Average interval to first oestrus in supplemented and control buffaloes was  $56 \pm 6.0$  and  $62 \pm 25$  days, respectively. This interval in supplemented buffaloes was significantly more than in Experiment I (56 Vs 24 days). Poor BCS (3.75-3.80 Vs 2.67-3.04), poor heat detection in the absence of teaser bull, summer stress associated with poor basic diet under field conditions could be responsible for delay in first observed oestrus in Experiment II. Long postpartum anoestrus intervals in poorly managed and/or summer calved buffaloes have already been reported (Singh 1993, Perera 1999, Vale 2000).

**Table 31 Postpartum oestrus in rural buffaloes fed UMMB supplement prepartum.**

Observation	Supplemented Group (n=11)	Control Group (n=7) (% buffaloes)
Oestrus within 90 days postpartum (Number of buffaloes)	11 (100 %)	3 (43 %)
Oestrus within 60 days postpartum (Number of buffaloes)	07 (63 %)	01 (14 %)
Days to first oestrus Postpartum	$56 \pm 6.0$	$62 \pm 25$

These observations support Raizada et al (1969) and Vale (1990) that improved feeding could induce cyclicity in buffaloes during summer also. More buffaloes in this study came into heat in July and August. It, however, therefore, remains to be ascertained whether these animals exhibited oestrus (in off-period/summer) due to supplementary feeding or that they had already entered in to the breeding season (Singh 1993). Nevertheless, earlier onset of postpartum oestrus than in the control suggested that UMMB certainly helped in early resumption of ovarian activity postpartum. The buffaloes of supplemented group had parturated at desired BCS (3.04 points), which remained above the critical value at peak lactation. However, control buffaloes at calving had lower BCS (2.67 points) than required (3.00-3.75 points) which fell below the critical level (2.25 points) after 6 weeks of parturition (Table 29). Sixty percent supplemented and 14% control had overt signs of oestrus (Table 31). One control buffalo that came in heat had lowest milk yield (1.5 kg only). The buffaloes undertaken in this study had long days open (approx. one year) in their previous lactation. From the reproductive records of these buffaloes in the preceding lactation and from observations on controls of this study, it appeared that UMMB supplementation prepartum improved postpartum ovarian activity. From Experiment I and II it thus, could be inferred that poor reproduction was associated with poor BCS in buffaloes. Further improved prepartum feeding and hence BCS at calving and in early lactation appeared to have helped in inducing oestrus during summer. Earlier studies have also indicated better reproduction in cows with better body

condition and further improvement in reproduction with improvement in BCS (Domeq et al 1997).

#### **4.4 Supplementary UMMB feeding of nonpregnant, anoestrus buffaloes at unorganized dairy farms- Effect on reproduction:**

This study was conducted during September – October 2000 on 54 buffaloes in three different (III, IV and V) agro-climatic zones of Punjab. The buffaloes kept under similar managerial conditions were in 2nd to 7th lactation. Of these 17 had calved >12 months ago and the rest more than eight months ago. None had exhibited oestrus until this study.

Average daily UMMB intake in anoestrus buffaloes was  $337 \pm 32$  g with average per head intake of  $10.11 \pm 0.96$  kg in 30 days. The intake was lesser compared to Experiments I and II primarily due to limited daily exposure (4-6 hrs) to limited UMMB (0.5 kg). More buffaloes (46 %) had the tendency to bite and eat the blocks during 1<sup>st</sup> week of study, which reduced to only 5 % buffaloes by 4<sup>th</sup> week.

##### **4.4.1 Effects of UMMB supplementation on BCS and girth of anoestrus buffaloes**

Average BCS in control and supplemented buffaloes at the start of study was 2.25 and 2.34 respectively. A significant increase in BCS ( $p < 0.05$ ) was observed by 30 days of supplementation (Table 32; Fig. 13), which is consistent with the finding in Experiments I and II. Poor BCS (2.25) suggested malnutrition to be the possible cause of long standing anoestrus in buffaloes in this study as has been reported by (Robinson 1996). Poor BCS (2.25) recorded in these

buffaloes could be due to (a) the fact that these observations pertain to anoestrus buffaloes nutrition in which may remain ignored by the farmers; (b) that the study covered 'green fodder lean period' (September-October). UMMB supplementation for 30 days had no appreciable effect on heart girth of anoestrus buffaloes (Table 32; Fig 14).

**Table 32 Average BCS (1- 5 point scale) and heart girth (cms) of anoestrus buffaloes in different weeks of UMMB supplementation**

Parameter	Group	Weeks of UMMB supplementation				
		Wk.0	Wk.1	Wk.2	Wk.3	Wk.4
Body condition score (1-5 scale)	Supplemented (n=40)	2.34 <sup>a</sup>	2.34	2.54	2.61	2.67 <sup>b</sup>
		±0.07	±0.07	±0.09	±0.06	±0.08
	Control (n=14)	2.25	2.25	2.33	2.25	2.21
		±0.13	±0.16	±0.12	±0.13	±0.15
Heart girth (cms)	Supplemented (n=40)	189.76	191.38	193.47	192.23	192.38
		±1.63	±1.56	±2.19	±1.66	±1.50
	Control (n=14)	190.16	191.16	192.66	193.5	192.00
		±3.80	±4.36	±3.69	±3.06	±3.59

- Values with different superscripts within rows are significantly different (p<0.05)

Fig. 13 Body condition score of UMMB supplemented anoestrus rural buffaloes

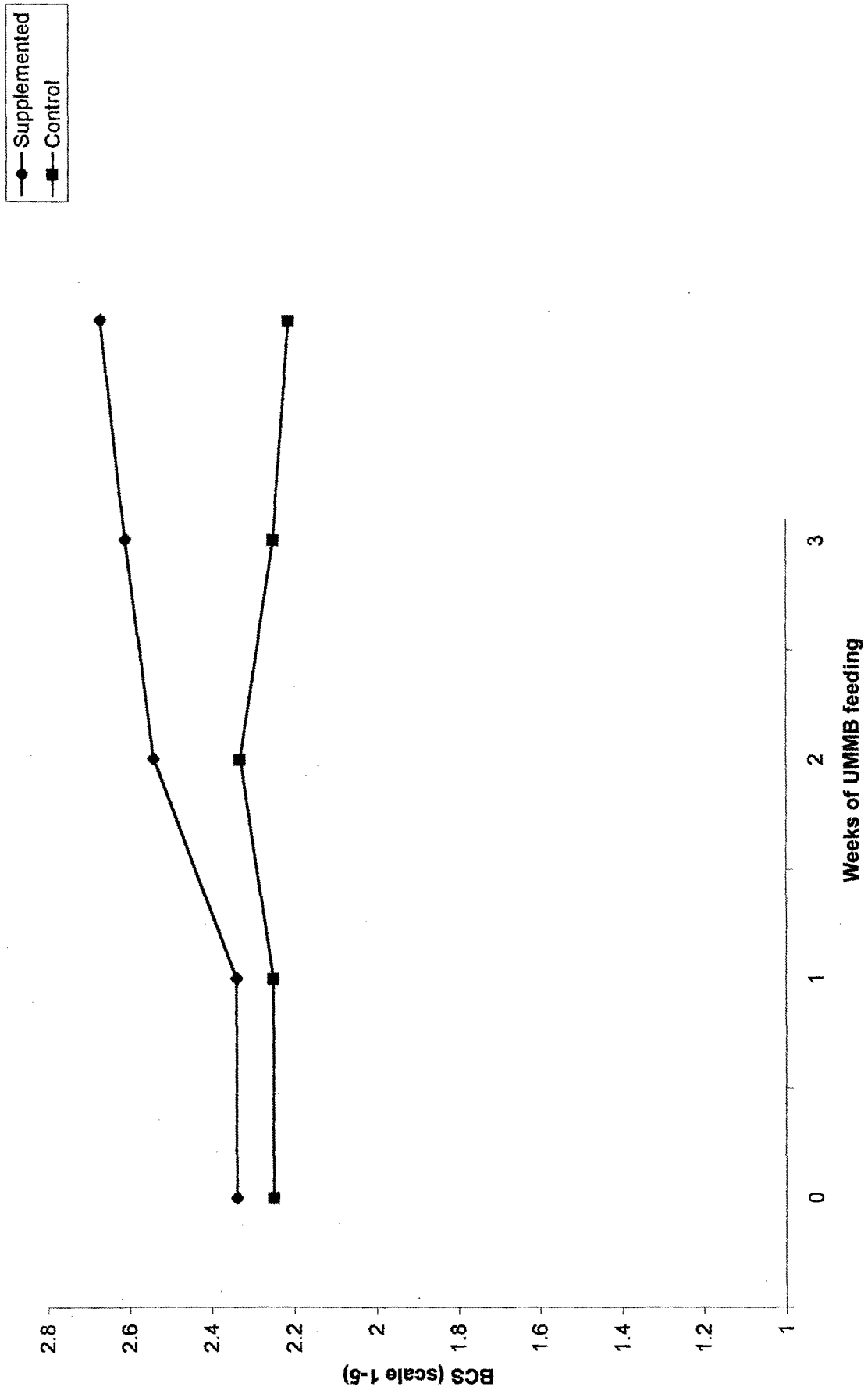
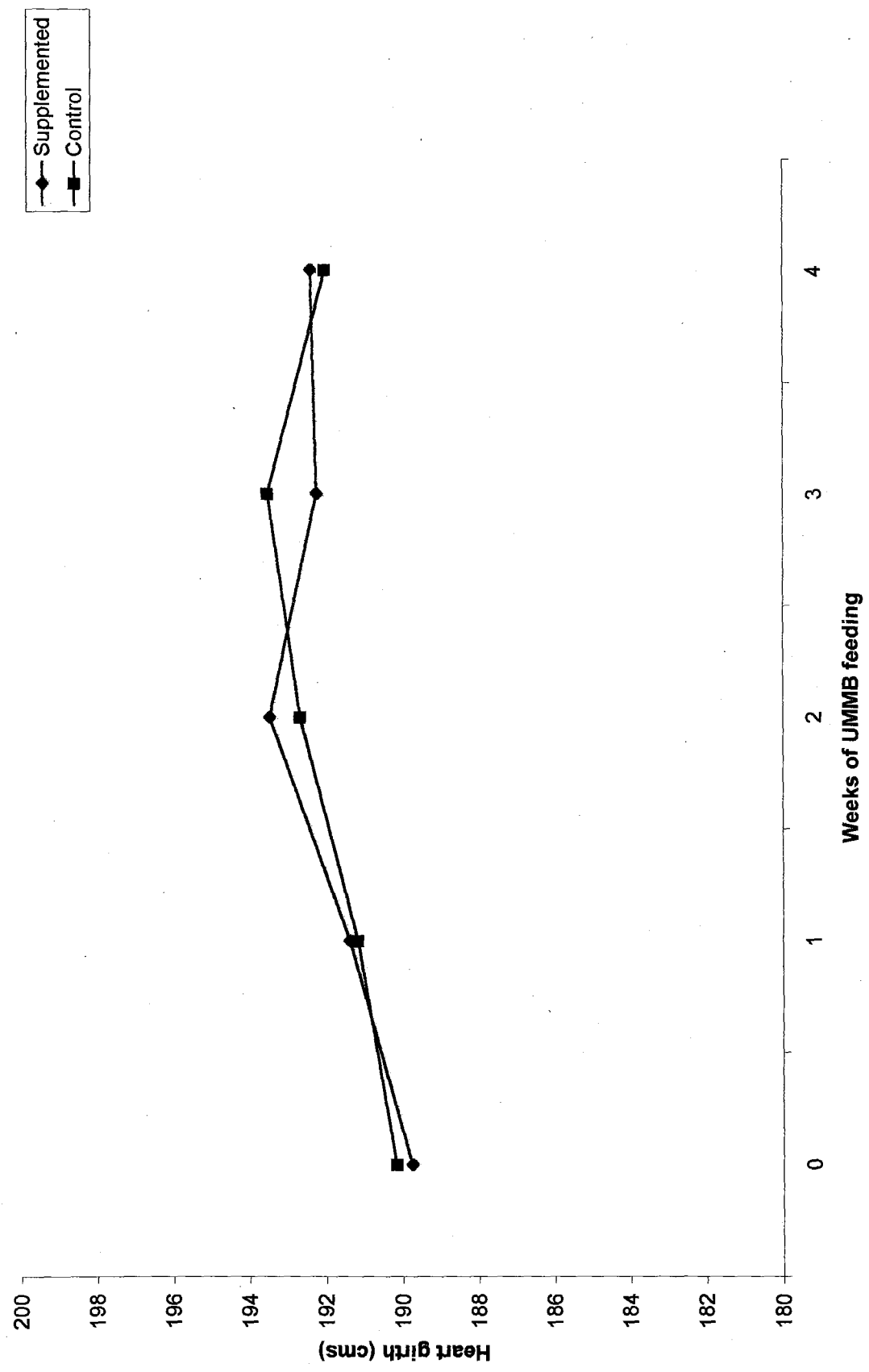


Fig. 14 Heart girth of UMMB supplemented anoestrus rural buffaloes



Forty-one percent farmers reported an increase in the feed intake of their buffaloes by about 20-25% after start of UMMB supplementation. Twenty percent farmers reported increase in milk yield and all thirteen (100%) farmers who calculated the fat percentage, reported on an average 0.5% increase in milk fat. Feed intake and increase in milk yield were the personal observations of the farmers, which could vary from individual to individual. Increase in feed intake (54 %; Tripathi 1997) and in milk yield (11 %; Vu et al 1999) following UMMB supplementation is already on record. The increased feed intake with better utilization of nutrients with UMMB supplementation improved the health of animals (Tripathi 1997) and hence its milk production. No changes in feed intake and in milk yield were seen in control buffaloes.

#### **4.4.2 Blood biochemical changes after UMMB supplementation in anoestrus buffaloes**

The levels of circulatory total plasma proteins, albumin and plasma urea were within the physiological limits and were similar before and after the UMMB supplementation and were similar to that in the controls (Table 33). Blood glucose concentrations were similar in both the groups at the beginning and at the end of the study. However, blood glucose concentration by the end of 30 days study was significantly high ( $p < 0.05$ ) in control and supplemented buffaloes. Blood glucose concentration reflects the status of preceding meals in an animal. Short term rise in glucose concentration therefore is not a reliable index of energy status of an animal (Blood et al 1993). Supplementary feeding has been

reported to increase the levels of these circulatory constituents (Sharma 1989). Lack of any change in their concentrations in the present study could be due to their drainage in increased milk yield and its fat percentage following UMMB supplementation.

**Table 33 Blood biochemical changes of buffaloes before and after UMMB supplementation.**

Plasma Constituent	Initial		After Treatment	
	Supplemented	Control	Supplemented	Control
Total Protein (g%)	6.69±0.19 (n=32)	6.67±0.31 (n=11)	6.91±0.20 (n=32)	7.00±0.27 (n=11)
Albumin (g%)	4.11±0.08 (n=32)	3.97±0.19 (n=11)	4.00±0.13 (n=32)	4.98±0.57 (n=11)
Urea (mg%)	28.53±1.57 (n=32)	26.42±3.82 (n=11)	28.05±1.91 (n=32)	33.92±4.28 (n=11)
Glucose (mg%)	49.58±2.01 <sup>a</sup> (n=21)	47.41±1.94 <sup>a</sup> (n=08)	59.53±0.68 <sup>b</sup> (n=21)	60.15±2.01 <sup>b</sup> (n=08)

Plasma concentrations of Copper, Zinc, Calcium and Iron did not vary between controls and the supplemented buffaloes before and after UMMB supplementation. Marginally lower than normal (0.60 – 1.20 ug/ml) levels of copper and calcium were observed at different locations (Table 34). Earlier studies also indicated subnormal calcium levels in buffaloes at organized as well as unorganized farms of Punjab (Randhawa 1999) and their association with anoestrus (Chauhan 1980, Mohammad 1997).

Buffaloes of group III (AC zone V) had significantly higher ( $p < 0.05$ ) plasma zinc concentration than in groups I and II and also than normal concentrations reported earlier (0.80 – 1.20  $\mu\text{g/ml}$ ). UMMB supplementation did not change zinc concentrations in plasma at any location. Higher proportion of buffaloes showed overt signs of oestrus in agro-climatic zone V than in IV, (Table 8) could be due higher level of zinc in that area. Higher zinc levels were observed in animals showing overt signs of than in animals having unobserved oestrus (Chauhan 1980). Normal iron concentrations were found in this study. Iron deficiency seldom occurs in adult livestock unless there was considerable blood loss (McDowell et al 1993).

**Table 34 Plasma mineral status of anoestrus buffaloes before and after UMMB supplementation.**

**Copper ( $\mu\text{g/ml}$ )**

	Before UMMB supplementation		After UMMB supplementation	
	Supplemented group	Control group	Supplemented group	Control group
Group I	0.48 $\pm$ 0.06 (n=11)	0.50 $\pm$ 0.07 (n=2)	0.49 $\pm$ 0.08 (n=11)	0.49 $\pm$ 0.03 (n=2)
Group II	0.51 $\pm$ 0.04 (n=09)	0.52 $\pm$ 0.08 (n=4)	0.54 $\pm$ 0.05 (n=09)	0.49 $\pm$ 0.04 (n=4)
Group III	0.47 $\pm$ 0.04 (n=12)	0.56 $\pm$ 0.04 (n=5)	0.53 $\pm$ 0.06 (n=12)	0.46 $\pm$ 0.05 (n=5)

**Zinc ( $\mu\text{g/ml}$ )**

	Before UMMB supplementation		After UMMB supplementation	
	Supplemented group	Control group	Supplemented group	Control group
Group I	0.77 $\pm$ 0.04 <sup>a</sup> (n=11)	0.81 $\pm$ 0.05 <sup>a</sup> (n=2)	0.75 $\pm$ 0.07 <sup>a</sup> (n=11)	0.85 $\pm$ 0.06 <sup>a</sup> (n=2)
Group II	0.61 $\pm$ 0.03 <sup>a</sup> (n=09)	0.94 $\pm$ 0.34 <sup>a</sup> (n=4)	0.70 $\pm$ 0.06 <sup>a</sup> (n=09)	0.72 $\pm$ 0.10 <sup>a</sup> (n=4)
Group III	2.47 $\pm$ 0.06 <sup>b</sup> (n=12)	2.48 $\pm$ 0.18 <sup>b</sup> (n=5)	2.39 $\pm$ 0.21 <sup>b</sup> (n=12)	2.60 $\pm$ 0.20 <sup>b</sup> (n=5)

### Calcium(mg%)

	Before UMMB supplementation		After UMMB Supplementation	
	Supplemented group	Control group	Supplemented group	Control group
Group I	7.70±0.15 (n=11)	7.87±0.20 (n=2)	8.13±0.23 (n=11)	7.92±0.32 (n=2)
Group II	7.54±0.24 (n=09)	7.51±0.17 (n=4)	7.86±0.17 (n=09)	7.90±0.14 (n=4)
Group III	7.74±0.14 (n=12)	7.63±0.57 (n=5)	8.11±0.17 (n=12)	7.47±0.37 (n=5)

### Iron (µg/ml)

	Before UMMB supplementation		After UMMB Supplementation	
	Supplemented group	Control group	Supplemented group	Control group
Group I	1.98±0.56 (n=11)	2.01±0.80 (n=2)	2.67±0.92 (n=11)	2.54±0.32 (n=2)
Group II	2.25±0.33 (n=09)	2.73±0.16 (n=4)	2.19±0.28 (n=09)	2.63±0.53 (n=4)
Group III	2.97±0.41 (n=12)	2.39±0.38 (n=5)	2.55±0.18 (n=12)	2.74±0.10 (n=5)

#### 4.4.3 Effect of UMMB supplementation on reproduction in anoestrus buffaloes.

Ninety percent of supplemented buffaloes exhibited oestrus within 30 days of UMMB supplementation compared to only 28% in the control group (Table 35).

**Table 35 Effect of UMMB supplementation on reproduction in anoestrus buffaloes**

	Symptoms of oestrus (% animals)	
	Control	Supplemented
Group I	0 (0/4)	93.33 (14/15)
Group II	40 (2/5)	80.00 (8/10)
Group III	40 (2/5)	93.33 (14/15)
Overall	28.57 (4/14)	90.00 (36/40)

The present study was conducted during favorable breeding period (September – October) and on buffaloes with poor BCS. Having been in anoestrus for more than 6 months also suggested nutrition rather than season to be a cause of anoestrus in these buffaloes. Improvement in reproduction following UMMB supplementation might have been the effect of improved nutrition, which is in agreement with earlier workers.

Overall, UMMB supplementation in weak anoestrus buffaloes improved feed intake, production and reproduction during the months of September and October.

## CHAPTER V

### SUMMARY AND CONCLUSIONS

Buffalo appears to be a seasonal breeder with least breeding activity during dry summer months leading to long inter-calving intervals and huge economic losses. Poor nutrition and management have been reported to contribute to the high incidence of anoestrus in buffaloes (Perera 2000). The present study was, therefore, conducted (a) to assess the relationship, by means of survey, between breeding performance of buffaloes and feeding and managerial practices in Punjab; and (b) to develop a cost effective supplementary feeding strategy to curtail the incidence of anoestrus.

The survey covered 815 buffaloes belonging to 211 marginal and 15 nomadic farmers in IV and V agroclimatic zones of Punjab. It revealed that 63 – 83 % of the nonpregnant buffaloes at various locations were anoestrus during summer months which coincided with a wide spread shortage of green fodder. A higher proportion of buffaloes in zone V (93 %) than elsewhere (46 – 56 %) exhibited overt signs of oestrus, which coincided with better wallowing facility provided to buffaloes in that area. Occurrence of doka was observed in 77-80% buffaloes coming in oestrus. In general, the staple diet of buffaloes included varying amount of pearl millet, sorgum and maize in summer and barseem and oats in winter with wheat straw as filler, the quantity of which varied depending upon the availability of green fodder. Concentrates were fed in accordance with economic status of the farmer, and the milk yield of the animals. Supplementary prepartum feeding was reported only by 33 – 53 % farmers in different areas and

was limited mainly to inconsistent feeding of about 100 to 250 g of mustard oil or desi ghee daily mainly in the last month of gestation. Feeding of concentrates was rare and in haphazard pattern and quantity. Buffaloes with nomads were poorly managed and had strict seasonal behavior in reproduction than with village farmers.

Urea molasses multinutrient blocks (UMMB) based on locally available agro-climatic by-products were prepared by 'cold' as well as 'hot' processes, of which, the former proved to be more economical and practicable. Blocks comprised of molasses (35%), urea (10%), rice bran deoiled (17%), rice bran oiled (16%), groundnut cake (10%), salt (02%) and cement (10%) were developed and used in rest of the study.

Early postpartum dairy cattle often suffer from negative energy balance due to limited feed intake and enhanced energy demand for milk production which delays their postpartum ovarian activity. Animals calving with good body condition are less likely to undergo negative energy balance postpartum. Accordingly, the present study was undertaken on the effects of supplementary UMMB feeding to prepartum buffaloes on their postpartum reproductive performance. Ten buffaloes due to parturate in the months of September - October 2000, at PAU dairy farm were supplemented with UMMB for two to three months prepartum and five buffaloes served as unsupplemented controls. Eleven village buffaloes due to parturate in the months of April through June 2001 were also supplemented with UMMB for similar period where seven buffaloes served as unsupplemented controls. These buffaloes consumed about 650 g of UMMB

daily. All the supplemented buffaloes gained more body weight, body condition score (BCS) and heart girth during pregnancy than their unsupplemented controls. Prepartum BCS gain in supplemented buffaloes over controls (0.37 Vs 0.05) was more marked in the village buffaloes than at PAU perhaps due to relatively poor basic diet in the former. Loss of body weight, BCS and heart girth during early postpartum period was lesser in the supplemented buffaloes than their respective controls.

Milk yield during two months postpartum in UMMB supplemented PAU buffaloes was more than in controls by 90 kg/head. Higher milk yield albeit in subjective terms, was also reported in the supplemented rural buffaloes.

A high proportion of rural buffaloes supplemented with UMMB during summer (63 %) exhibited oestrus than only 14 % controls within 60 days postpartum. All PAU buffaloes, supplemented as well as controls, came into heat within 60 days postpartum. The UMMB supplementation appeared to have made better difference over controls where the animals were already on poor diet (rural buffaloes). More closer observations at PAU revealed early onset of ovulatory heat (34 Vs 48 days) in supplemented buffaloes than in controls with lesser incidence of silent oestrus (11 Vs 75%). Differences of basic diet between the villages and the PAU buffaloes together with different seasons of the two studies are suspected to have accounted for differences in their reproductive performance.

Under-nutrition is an established cause of anoestrus in animals, irrespective of season and stage of lactation. In a part of this study nutritional

status of anoestrus buffaloes was improved through UMMB supplementation and its therapeutic effect on anoestrus was studied. UMMB (335 g daily) was supplemented for 30 days to 40 anoestrus rural buffaloes in the months of September through October. In general, it improved their feed intake, body condition, productive and reproductive performance. Ninety percent supplemented anoestrus buffaloes experienced oestrus during this period. Observations on 13 supplemented buffaloes revealed that their feed intake increased by about 20-25 %, milk production by 08% and milk fat by 0.5 % during this study.

Overall, UMMB supplementary feeding during prepartum period improved postpartum production and reproduction in buffaloes kept under organized and unorganized dairy farms both in winter and summer months. UMMB supplementation induced ovarian activity in anoestrus buffaloes with poor body condition under field conditions.

Plasma concentrations of calcium, copper, zinc and iron were studied in the supplemented and control anoestrus buffaloes. The UMMB supplementation did not change their circulatory levels nor these were different in the buffaloes coming into heat than those, which remained anoestrus. Circulatory mineral concentration did not appear to reflect their deficiency as a cause of anoestrus.

Total plasma proteins, albumin, creatinine and glucose profiles were studied in all the buffaloes involved with postpartum supplemented buffaloes. UMMB supplementation did not effect plasma protein and creatinine concentrations in prepartum or postpartum period. However, the albumin fraction

significantly decreased from  $4.72 \pm 0.19$  g/dl to  $4.37 \pm 0.15$  g/dl from the start of supplementation. Urea concentration increased significantly during the period of supplementation. No marked difference between control and supplementation group were seen in glucose concentration.

From the foregoing, it is concluded that:

- 1 The survey revealed (a) that the seasonal breeding tendency does prevail in buffalo irrespective of the agro-eco zones in Punjab; (b) that malnutrition of buffaloes prevails widely; (c) that prepartum feeding practices are insignificant and haphazard where exist; (d) that incidence of anoestrus in buffaloes is very high during summer; (e) that wallowing helped in better expression of oestrus in buffaloes.
- 2 Cold process of UMMB preparation is practicable, economical and the blocks so prepared are acceptable to the farmers and animals.
- 3 Peripartal changes in the body weight, body condition score and heart girth were proportionate to each other and that observations on BCS were the easiest and more reliable index of body condition than the other two.
- 4 Prepartum supplementary feeding improved general body condition of buffaloes prepartum that improved milk production and reproduction activity postpartum.
- 5 High proportion of buffaloes supplemented during winter or summer months came into early heat (<60 days).
- 6 The quality of heat (few number of animals with silent heat and with dirty discharge) was also better in the supplemented buffaloes.

- 7 UMMB supplementation helped in inducing oestrus in a high proportion of anoestrus buffaloes.
- 8 UMMB supplementation did not reflect on plasma concentration of glucose, total proteins, albumin and the minerals (copper calcium, zinc iron) although concentration of urea increased during the course of supplementation.

## REFERENCES

- Acharya R M (1989) The buffalo: dairy, draught and meat animal of India. *Dairy Guide* **11**:19-20.
- Aggarwal S K and Purbey L N (1981) Some therapeutic measures for restoring fertility in anoestrus buffaloes under summer conditions. *Livestock Advisor* **6**: 41-44.
- Aggarwal S K and Tomar O S (1998) *Reproductive technologies in buffalo*. Indian Vet. Res. Int. Izatnagar, India.
- Agymang K, Little D A, Bah M L and Dwinger R H (1991) Effects of postpartum body weight changes on subsequent reproductive performance in N'Dama cattle maintained under traditional husbandry systems. *Anim Reprod Sci* **26**: 51-59.
- Bakshi M P S, Gupta V K and Langer P N (1986) Fermented straw as a complete basal ration for ruminants. *Agri Wastes* **16**: 37-46.
- Bansal R S, Gupta S K, Singh G B and Chauhan F S (1978) Serum levels of P and Ca in different phases of reproduction in buffaloes. *J Remount Vet Corps* **17**: 95-100.
- Bar-Peled U, Maltz E, Brunkental I, Folman Y, Kali Y, Gacitua H, Lehrer A R, Knight C H, Robinzon B and Voet H (1995) Relationship between frequent milking or suckling in early lactation and milk production of high producing dairy cows. *J Dairy Sci* **78**: 2726-36.
- Bawa M S (1968) Some observations on Dokas in the murrha buffaloes at Hissar. *Punjab Veterinarian* **VIII**: 18-24.
- Beam S W and Butler W R (1997) Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. *Biol Reprod* **56**: 133-42.
- Beam S W and Butler W R (1998) Energy balance, metabolic hormones and early postpartum follicular development in dairy cows fed prilled lipid. *J Dairy Sci* **81**: 121-31.
- Bellows R A and Short R E (1978) Effects of precalving feed level on birth weight, calving difficulty and subsequent fertility. *J Anim Sci* **46**: 1522-28.

- Bhalaru S S, Dhillon J S, and Tiwana M S (1981) Effect of body-weight at calving on body-weight changes during early lactation and post-partum reproductive performance in buffaloes. *J Agric Sci Camb* **97**: 595-97.
- Bhalaru S S, Dhillon J S, and Tiwana M S (1986) Factors affecting changes in body-weight during early lactation in buffaloes. *Indian J Dairy Sci* **39**: 51-53.
- Biggadike H J, Laven R A, Collins C A and Mansbridge R J (2000) The effect of feeding high levels of quickly degradable nitrogen to the lactating dairy cow on embryo growth. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. Vol 1, p 284. Stockholm.
- Blood D C, Radostits O M and Henderson J A (1983) *Veterinary Medicine*. Bailliere Tindall, U K.
- Borghese A and Terzano G M (2000) Postpartum anoestrus and oestrus induction in buffalo to improve reproduction efficiency. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. Vol 2, p 272. Stockholm.(Abst)
- Butler W R (2000) Nutritional interactions with reproductive performance in dairy cattle. *Anim Reprod Sci* **60-61**: 449-57.
- Butler W R (1998) Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *J Dairy Sci* **81**: 2533-39.
- Butler R W, Everett R W and Coppock C K (1981) The relationship between energy balance, milk production and ovulation in postpartum Holstein cows. *J Anim Sci* **53**: 742-48.
- Butler W R and Smith R D (1989) Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *J Dairy Sci* **72**: 767-83.
- Chandolia R K and Verma S K (1987) Studies on biochemical profiles in anoestrus buffalo heifers. *Indian Vet J* **64**: 482-84.
- Charnetzki T C (1999) Nutritional influences on reproductive function. *Proc 14<sup>th</sup> conf Indian Soc Study Anim Reprod (ISSAR)* ppVIII - XII. Ludhiana India.
- Chauhan F S (1980) *Clinical and biochemical studies on suboestrus buffaloes*. Ph.D. dissertation, Punjab Agricultural University, Ludhiana, India.
- Chauhan T R, Gupta R, Dahiya S S and Punia B S (1997) Effect of supplementing urea molasses blocks in the ration of buffaloes on nutrient utilization and milk production. *Indian J Anim Sci* **67**: 418-21.

- Chauhan F S, Takkar O P, Singh M and Tiwana M S (1981) Seasonal variations of biochemical profile and treatment of anoestrus in buffaloes. *Indian J Anim Reprod* **1**: 31-36.
- Chopra A K, Kakkar V K, Gill R S, and Kaushal J R (1974) Preparation of uromol, a urea molasses complex and its rate of breakdown in vitro. *Indian J Anim Sci* **44**: 970-72.
- Dabas Y P S, Singh S P and Sexena O P (1987) Serum concentrations of certain minerals in anoestrus cows and buffaloes. *Indian J Anim Reprod* **8**: 98.
- Derashri H J, Kavani F S, Prabhu G A and Kodagali S B (1984) Blood glucose levels in different reproductive status in Surti buffaloes. *Indian J Anim Reprod* **5**: 128.
- Dhoble R L (1978) *Some studies during anoestrus in buffaloes*. M.V.Sc, thesis Punjab Agricultural University, Ludhiana, India.
- Dhoble R L and Gupta R C (1979) A note on blood glucose level in relation to postpartum anoestrus in buffaloes *Indian J Anim Sci* **49**: 953.
- Dhoble R L and Gupta R C (1986) Serum calcium and inorganic phosphorus levels during postpartum anoestrus in buffaloes. *Indian J Anim Hlth* **25**: 123-26.
- Domecq J J, Skidmore A L, Lloyd J W and Kaneene (1997) Relationship between body condition scores and conception at first artificial insemination in a large dairy herd of high yielding Holstein cows. *J Dairy Sci* **80**: 113-20.
- Ducker M J, Rosemary A, Fisher W J, Morant S V and Bloomfield G A (1985) Nutrition and reproductive performance of dairy cattle. *Anim Prod* **41**: 1-12.
- Earle D F (1976) A guide to scoring dairy cow condition. *Aust Dep Agric J Victoria* **74**: 228. (Original not seen, Cited by Edmonson A J, Lean I J, Weaver L D, Farver T and Webster G, 1989. *J Dairy Sci* **72**: 68-78.
- Echternkamp S E, Ferrell C L and Done J D (1982) Influence of pre- and postpartum nutrition on LH secretion in suckled postpartum beef heifers. *Theriogenology* **18**: 283.
- Edmonson A J, Lean I J, Weaver L D, Farver T and Webster G (1989) A body condition scoring chart for Holstein dairy cows. *J Dairy Sci* **72**: 68-78.

- Ferguson J D Balanchard T, Galligan D T, Hoshall D C and Chalupa W (1988) Infertility in dairy cattle fed a high percentage of protein degradable in rumen. *J Am Vet Assoc* **198**: 659.
- Filho B D O, Toniollo G H, Gambarini M L and Gordo J M L (2000) Effects of the pre- and postpartum supplementation on reproductive parameters and serum levels of proteins in Canchim cows. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. Vol 1, p 277. Stockholm.
- Fordyce G, Fitzpatrick L A, Mullins T J, Cooper N J, Reid D J and Entwistle K W (1997) Prepartum supplementation effects on growth and fertility in *Bos indicus*-cross cows. *Australian J Exp Agri* **37**: 141-49. (Abstr)
- Gearhart M A, Curtis C R, Erb H N, Smith R d, Sniffen C J, Chase L E and Cooper M D (1990) Relationship of changes in condition score to cow health in Holsteins. *J Dairy Sci* **73**: 3132-40.
- Gentile G, Meretti M, Gaineri R and Glordani (1978) Corelation between some blood chemical constituents and cow fertility in Modena provence. *Anim Breed Abstr* **47** : 2845.
- Goden I (ed)(1996) *Controlled Reproduction in Cattle and Buffaloes*. pp 432-45 CAB International,UK.
- Goff J P and Horst R L (1997) Physiological changes at parturition and their relationship to metabolic disorders. *J Dairy Sci* **80**: 1260-68.
- Grainer C and McGowan A A (1982) The significance of precalving nutrition on dairy cow. pp134 – 171 in *Proc Conf Dairy Prod Pasture*, Ruakura Anim Res Stn Hamilton NZ, (Original not seen, Cited by Edmonson A J, Lean I J, Weaver L D, Farver T and Webster G, 1989. *J Dairy Sci* **72**: 68-78.
- Grummer R J (1993) Etiology of lipid related metabolic disorders in periparturient dairy cows. *J Dairy Sci* **76**: 3882-96.
- Haddada B, Ponter A A, Mezzane H, Mialot J P, Ponsart C, Humbolt P and Grimard B (2000) Various factors affecting pregnancy rate in beef cows at Pasteur in Morocco. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. Vol 1, p 294. Stockholm.
- Hassan S G, Battaway K El, Menoufy A A El, Younis M and Khattab R M (2000) Studies on postpartum anoestrus in Egyptian buffaloes. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. Vol 1,p 185-86. Stockholm.

- Heranjal D D, Sheth A R, Wadadekar K B, Desai R and Rao S S (1979) Serum gonadotropins and prolactin levels in anoestrus buffaloes. *Indian J Dairy Sci* **32**: 383-85.
- Jain G C (1994) Mineral profiles during anoestrus and repeat breeding in bovines. *Indian J Anim Sci* **9**: 241.
- Kakkar V K and Makkar G S (1995) Comparative characteristics of the available mineral block (UMMB). *Indian J Anim Nutr* **12**: 37-40.
- Kakkar V K, Malik N S and Makkar G S (1997) Uromin lick as a replacement of concentrate mixture in buffaloes. *Indian J Anim Nutr* **13**: 203-205.
- Kamboj M and Prakash B S (1993) Relationship of progesterone in plasma and whole milk of buffaloes during cyclicity and early pregnancy. *Trop Anim Hlth Prod* **25**: 185-92.
- Kaur H and Arora S P (1984) Annual pattern of plasma progesterone in normal cycling buffaloes (*Bubalis bubalis*) fed two levels of nutrition. *Anim Reprod Sci* **7**: 323-32.
- Kaur S (1993) *Comparative utilization of urea-N through gradual licking in buffalo calves*. M Sc thesis, Punjab Agricultural University, Ludhiana, India.
- Khan M A S (2000) Constraints on reproductive performance of indigenous cows under small holder village farming system in Bangladesh. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. Vol 1, p 265. Stockholm.
- Khatkar M S, Jain A K and Parmar O S (1997) Body weight changes after calving and their relationship with milk production and service period in crossbred cows. *SARAS J Livestock and Poultry Prod* **8**: 30-35.
- Killen J H, Forrest D W, Byers F M, Schelling G T and Long C R (1989) Gonadotropin-releasing hormone-induced luteinizing hormone release in heifers: Effect of nutrition during gestation. *J Anim Sci* **67**: 496-500.
- Kumar P (1993) *Postpartum ovarian activity in relation to calving season in buffaloes (bubalus bubalis)*. M Sc thesis, Punjab Agricultural University, Ludhiana, India.
- Kumar S (2001) *Studies on factors affecting the onset of follicular activity in the postpartum buffaloes*. M Sc thesis, Punjab Agricultural University, Ludhiana, India.

- Kumar H, Singh G D and Pant H C (1992) Metabolic profiles in relation to hormonal therapy for anoestrus in buffalo heifers. *Indian J Dairy Sci* **45**:278-80.
- Lamothe C, Gomez A, Figueroa G and Madej A (2000) Effects of body condition on the calving-pregnancy interval in dual purpose cows in the tropical areas of Mexico. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. Vol 1,p 257. Stockholm.
- Leers-Sucheta S, Chakraborty P K, Row K E, Turner H A and Stormshah F (1994) Gonadotropin-releasing hormone-induced secretion of luteinizing hormone in postpartum beef heifers maintained on two plans of nutrition before and after breeding. *J Anim Sci* **72**: 998-1003.
- Makkar G S and Saijpaul S (1996) Uromin-Lick ---A success story. *Progressive Farming* May, 1996: 22-23.
- Malik N S, Makkar G S and Kakkar V K (1993) Methodology of preparation and nutritive value of uromin lick. *Indian J Anim Nutr* **10**: 105-106.
- Malik N S, Makkar G S, Mehra M L and Kakkar V K (1997) Effect of uromin lick feeding to crossbred milch cows under organized farm condition with loose system. *Indian J Anim Nutr* **14**: 54-56.
- Manik R S, Jadhav K E and Nath I (1981) Predicting weight from body measurements in Murrah buffaloes. *Indian J dairy Sci* **34**: 448-50.
- Marai I F, Habeeb A A and Farghaly H M (1999) Productive, physiological and biochemical changes in imported and locally born Friesian and Holstein lactating cows under hot summer conditions of Egypt. *Trop Anim Health Prod* **31**: 233-43.
- McCLure T J (1970) An experimental study of the causes of nutritional and lactational stress infertility of pasture-fed cows associated with loss of body weight at about the time of mating. *Res Vet Sci* **11**: 247-54.
- McDowell L R, Conrad J H and Hembry F G (1993) *Minerals for Grazing Ruminants in Tropical Regions*. Animal Science Department, Center for Tropical Agriculture, University of Florida.
- Mohammed F (1997) *Studies on hormonal therapy in relation to certain biochemical profiles in anoestrus buffaloes*. M. V. Sc. thesis, submitted to Punjab Agricultural University, Ludhiana, India.
- Morrow D A (1986) *Current Therapy in Theriogenology*. W B Saunders Company, Philadelphia.

- Mulvany P (1981) Dairy cow condition scoring. Handout No. 4468. Natl. Inst. Res. Dairying, Shinfield, Reading, UK. (Original not seen, Cited by Edmonson A J, Lean I J, Weaver L D, Farver T and Webster G, 1989. *J Dairy Sci* **72**: 68-78.
- Nebel R L and McGilliard M L (1993) Interactions of high milk yield and reproductive performance in dairy cows. *J Dairy Sci* **76**: 3257.
- Nolan C J, Bull R C, Sasser R G, Ruder C A, Panlasigui P M, Schoeneman H M and Reeves (1988) Postpartum reproduction in protein restricted beef cows: effect on the hypothalamic-pituitary-ovarian axis. *J Anim Sci* **66**: 3208-17.
- Osman A M, El-Naggar M A, Farraz A A and Shehata S H M (1985) Ovarian activity among Egyptian cows and buffaloes. *Anim Breed Abstr* **54**: 2170.
- Otte M J Woods A J and Abuabara Y (1992) Liveweight estimation of cattle by scale and by tape, a method comparasion study. *Trop Anim Hlth Prod* **24**: 109-14.
- Pandey U K (1995) The livestock economy of India- a profile. *Indian J Agri Econ* **50**: 264-82.
- Pandey M D and Raizada B C (1979) Overcoming summer sterility in buffalo bulls and cows. *Indian Dairyman* **31**: 467-78.
- Patel K K, Jacob N, Kavani F S and Vadodaria V P (1994) Post-partum serum biochemical profile in Mehsana buffaloes during non-fertile and fertile oestrus. *Indian J Anim Sci* **64**: 337-39.
- Perera BMAO (1999) Reproduction in water buffalo: comparative aspects and implications for management. *J Reprod Fertility* **54**: 157-168 (Supp)
- Perera BMAO (2000) Postpartum anoestrus in buffaloes: A review of causes and possible solutions under farming systems of Sri Lanka. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. p 270. Stockholm.
- Perera BMAO, de Silva LNA, Kururvita VY and Karunaratne AM (1987) Post-partum ovarian activity, uterine involution and fertility in indigenous buffaloes at a selected village location in Sri Lanka. *Ani Repdn Sci* **14**: 115-27.
- Quayam S A, Devanathan T G and Pattabiraman S R (1990) Serum total protein and blood glucose levels during pre- peri- and postpartum Murrah buffaloes. *Indian J Anim Sci* **60**: 140-42.

- Raizada B C, Tewari R B L and Roy A (1969) Reproductive performance of buffaloes on two levels of feeding during summer months. *Indian J Anim Sci* **39**: 387-92.
- Randhawa C (1999) *Epidemiological, immunopathological and therapeutic studies in dairy animals with reference to copper and iodine*. Ph.D. thesis, Punjab Agricultural University, Ludhiana, India.
- Rao N A V and Rao R M H (1996) Calving season in Murrah buffaloes in relation to time of the day and season of the year. *Indian Vet J* **73**:1195.
- Razdan M N and Kaker M L (1980) Summer subfertility and endocrine profile of buffaloes. *Indian Dairyman* **32**: 459-63.
- Roberts S J (1971) *Veterinary Obstetrics and Genital Diseases*. Ithaca, New York.
- Robinson J J (1996) Nutrition and reproduction. *Anim Reprod Sci* **42**: 25-34.
- Roche J F, Mackey D and Diskin M D (2000) Reproductive management of postpartum cows. *Anim Reprod Sci* **60-61**: 703-12.
- Roy A, Raizada B C, Tiwari R B L, Pande M D, Yadav P G and Sengupta B P (1968) Effect of management on the fertility of buffaloes-cows bred during summer. *Indian J Vet Sci and Anim Husbandry* **38**: 554.
- Sebastian L, Mudgal V D and Nair P G (1970) Comparative efficiency of milk production by Sahiwal cattle and Murrah buffalo. *J Anim Sci* **30**: 253-56.
- Shah S N H (1988) Comparative studies of seasonal influence on breeding behaviour and conception rate of dairy buffalo and zebu cattle. *Proc 11<sup>th</sup> Int. Cong. on Anim. Reprod. and Artificial Insemination* Vol 3 p 538. Dublin, Ireland.
- Shah S N S (1990) Prolonged inter-calving intervals in the Nili Ravi buffaloes. Reijksuniversiteit Utrecht, PhD Thesis (Original not seen. Cited by Vale W G (2000) Improving the postpartum period fertility of buffaloes in Brazil. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. p 271. Stockholm).
- Shah S N H, Willemsse A H and Van de Wiel D F M (1990) Descriptive epidemiology and treatment at postpartum anoestrus in dairy buffalo under small farm conditions. *Theriogenology* **33**: 1333-45.
- Shanker V, Sharma M C, Gupta O P, Verma R P and Mishra R R (1983) Studies on biochemical constituents of blood during anoestrus, repeat breeding and cyclic buffaloes. *Livestock Advisor* **7**: 32.

- Shanks R D, Freeman A E and Berger P J (1979) Relationship of reproductive factors with interval and rate of conception. *J Dairy Sci* **62**: 74-84.
- Sharma A K (1989) *Biochemical constituents and fertility trials in buffaloes in relation to varying levels of nutrition during prepartum and postpartum period*. M. Sc thesis, Punjab Agricultural University, Ludhiana, India.
- Sharma A K, Takkar O P and Chaudhary K C (1993) Plasma prolactin and milk production in Murrah (*Bos bubalis*) buffaloes fed with elevated energy levels durinh pre- and postpartum period. *Indian J Anim Reprod* **14**: 1-4.
- Sheth A R, Wadedekar R B, Moadbidri S B, Janakiraman K and Paramesharan M (1978) Seasonal alterations in the serum prolactin and LH levels in the water buffaloes. *Current Sci* **47**: 75-77.
- Singh A and Chaudhary G (ed)(1996) *Punjab Agricultural Hand Book*. p 11. Punjab Agricultural University, Ludhiana.
- Singh G, Singh G B and Bains G S (1985) Studies on incidence of anoestrus in village buffaloes. *Indian J Anim Res* **19**: 57-60.
- Singh G, Singh G B and Dhaliwal G S (1989) Studies on reproductive status of rural buffaloes in summer. *Indian J Anim Repdn* **10**: 151-53.
- Singh G, Singh G B, Sharma S S and Sharma R D (1984) Studies on oestrus symptoms of buffalo-heifers. *Theriogenology* **21**: 849-58.
- Singh J (1998) *Effect and level of feeding during pre and postpartum periods on performance of cows*. M. Sc thesis, Punjab Agricultural University, Ludhiana, India.
- Singh J, Nanda A S, Adams G P (2000) The reproductive pattern and efficiency of female buffaloes. *Anim Reprod Sci* **60-61**: 593-04.
- Singh R (1993) *Studies on the onset of postpartum ovarian activity in relation to climatic and nutritional status of buffaloes*. M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India.
- Singh R B, Sharma R D and Singh G B (1983) Incidence of repeat breeding in cows and buffaloes. *Indian J Dairy Sci* **36**: 314-15.
- Singh R and Nanda A S (1993) Environmental variables governing seasonality in buffalo breeding. *J Anim Sci* **71**: 119 (supp 1)
- Singh S, Bansal M L, Singh T P and Kumar R (1991) *Statistical methods for research workers*. Kalayani Publishers, New Delhi.

- Srinivas B and Gupta N C (1996) Urea-molasses-mineral block licks supplementation for milk production in crossbred cattle. *Asian-Australian J Anim Sci* **8**: 47-53.
- Srinivas B and Verma N C (2000) Reproductive performance of cows on long term feeding of urea-molasses-mineral block licks. *Indian J Anim Sci* **21**: 124-25.
- Srivastava R K, Dhingra D P, Gangwar P C, Soni P L, Mehta S N, and Singh C (1978) Effect of spray cooling and wallowing on milk yield and reproductive performance of buffaloes. *J Agril Engg* **15**: 189-96.
- Srivastava H K and Kharche K G (1986) Studies on some blood constituents in normal and abnormal cycling buffaloes. *Indian J Anim Reprod* **7**: 62.
- Staples C R, Thatcher W W and Clark J H (1990) Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. *J Dairy Sci* **79**: 938-47.
- Studer E (1998) A veterinary perspective of on-farm evaluation of nutrition and reproduction. *J Dairy Sci* **81**: 872-76.
- Tahir M N, Bajwa M A, Latif M, Mustaq M and Shah M H (1981) Effects of insemination dose and season on conception rate in buffaloes. *Pakistan Vet J* **1**: 161.
- Tailor S P, Jain L S, Gupta S K and Bhatia J S (1990) Oestrus and conception rates in buffaloes under village conditions. *Indian J Anim Sci* **70**: 1020-21.
- Takkar O P and Chaudhary K C (1994) Peri-partal changes in body weight as an index of future fertility in cows and buffaloes. In: Nanda A S (ed) *Recent Advances in Animal Reproduction and Gynaecology*. pp 122-27 . USG Publishers and Distributers, Ludhiana, India.
- Touckberry R and Batra T R (1976) Body weight changes in lactating purebred and crossbred dairy cattle. *J Dairy Sci* **59**: 733-43.
- Tripathi A K (1997) New concepts in feeding. In Gupta P R(ed) *Dairy India*. pp 305-08. A-25 Priyadarshini Vihar, Delhi, India.
- Tulloch D G (1979) The water buffalo (*Bubalus bubalis*) in Australia: reproductive and parent-offspring behaviour. *Australian Wildlife Research* **6**: 265-81.
- Usmani R H, Ahmed M, Insheep E K, Dailey R A, Lewis P E and Lewis G S (1985) Uterine involution and post-partum ovarian activity in Nili Ravi buffaloes. *Theriogenology* **24**: 435-48.

- Vale W G (1990) The water buffalo in Latin America. In: Livestock reproduction in Latin America- FAO/IAEA, pp 199-200 (Original not seen. Cited by Gordon I, 1996).
- Vale W G (1997) News on boitechonogies in males. *Proc. 5th World Buffalo Congress*. pp 103-23 Coserte Italy.
- Vale W G (2000) Improving the postpartum period fertility of buffaloes in Brazil. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. p 271. Stockholm.
- Van Soest (1964) New chemical procedures for evaluation of forages. *J Anim Sci* **23**: 838-45.
- Vu D D, Cuong L X, Dung C A and Hai P H (1999) Use of urea-molasses-multinutrient block and urea-treated rice straw for improving dairy cattle productivity in Vietnam. *Prev Vet Med* **38**: 187-193.
- Warnick A C, Genho P C, Hargrove D D, Olson T A and Kunkle (2000) Factors affecting pregnancy rate in crossbred beef cows in Central Florida. *Proc 14<sup>th</sup> Int Congress on Animal Reproduction*. Vol 1,p 261. Stockholm.
- Wright I A, Rhind S M, Russel A J F, Whyte T K, McBean A J and McMillen S R (1987) Effects of body condition, food intake and temporary calf separation on the duration of the postpartum anoestrus period and associated LH, FSH and prolactin concentration in beef cows. *Anim Prod* **45**: 395-402.
- Wood P D P, King J O and Youdan P G (1980) Relationship between size, live weight change and milk production characters in early lactation in dairy cattle. *Anim Prod* **31**: 143-51.



ANNEXURE

Date : \_\_\_\_\_

Name : \_\_\_\_\_  
Village : \_\_\_\_\_  
Block : \_\_\_\_\_  
District : \_\_\_\_\_

.....  
Species : \_\_\_\_\_

1. Total No. of animals : \_\_\_\_\_

2. Pregnant : Conceived since PPFOI PPCI  
1.  
2.  
3.  
4.  
5.

3. Non-pregnant

A) Calving date B) Subsequent event  
Doka Mucus Overt signs  
1. 1.  
2. 2.  
3. 3.  
4. 4.  
5. 5.

4. Herd Status : a) Anoestrus  
b) Repeater  
c) Pregnant  
d) Recent calving

5. Anoestrus animals

1 2 3 4 5  
Age  
Parity  
Milk yield  
(Max./day)  
Anoestrus  
since

6. Management

- a) Managed by : Self / Servant / Ladies
- b) Shade / Cooler / Sprinkler / Wallowing / bath
- c) Male in the herd : Yes/No

7. Feeding Practice

- 1. Pre-partum
- 2. Post-partum

<u>Months</u>	<u>Wheat straw</u>	<u>Green</u>	<u>Conc.</u>	<u>Min. mix.</u>	<u>Any oth</u>
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
11.					
12.					

8. Specific Problems

- a) Abortions Month
- b) Prolapse
- c) Any other

## VITA

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#### **ABSTRACT**

The present study was conducted (a) to assess the relationship, by means of survey, between breeding performance of buffaloes and feeding and managerial practices in Punjab; and (b) to develop a cost - effective supplementary feeding strategy to curtail the incidence of anoestrus.

The survey covered 815 buffaloes belonging to 211 marginal and 15 nomadic farmers in IV and V agroclimatic zones of Punjab. It revealed that 63 – 83 % of the nonpregnant buffaloes at various locations were anoestrus during summer months which coincided with a wide spread shortage of green fodder. A higher proportion of buffaloes in zone V (93 %) than elsewhere (46 – 56 %) exhibited overt signs of oestrus, which coincided with better wallowing facility provided to buffaloes in that area. Occurrence of doka was observed in 77- 80% buffaloes coming in oestrus. Supplementary prepartum feeding was reported only by 33 – 53 % farmers in different areas and was limited mainly to inconsistent feeding of about 100 to 250 g of mustard oil or desi ghee daily mainly in the last month of gestation. Feeding of concentrates was rare and in haphazard pattern and quantity. Buffaloes with nomads were poorly managed and had strict seasonal pattern of reproduction than those with village farmers.

Urea molasses multinutrient blocks (UMMB) based on locally available agro-climatic by-products were prepared by 'cold' as well as 'hot' processes, of which, the former proved to be more economical and practicable.


Ten buffaloes due to parturate in the months of September - October 2000 at PAU dairy farm were supplemented with UMMB for two to three months prepartum and five buffaloes served as unsupplemented controls. Eleven village buffaloes due to parturate in the months of April through June 2001 were also supplemented with UMMB for similar period where seven buffaloes served as unsupplemented controls. These buffaloes consumed about 650 g of UMMB daily. All the supplemented buffaloes gained more body weight, body condition score (BCS) and heart girth during pregnancy than their unsupplemented controls. Prepartum BCS gain in supplemented buffaloes over controls was more marked in the village buffaloes than at PAU (0.37 Vs 0.05) perhaps due to relatively poor basic diet and poor body condition in the former. Loss of body weight, BCS and heart girth during early postpartum period were lesser in the supplemented buffaloes than in their respective controls.


Milk yield during two months postpartum in UMMB supplemented PAU buffaloes was more by 90 kg/head than in the controls. Higher milk yield, albeit in subjective terms, was also reported in the supplemented rural buffaloes.

All PAU buffaloes, supplemented as well as controls, came into heat within 60 days postpartum. A high proportion of rural buffaloes supplemented with UMMB (70 %) exhibited oestrus than only 14 % controls within 60 days postpartum. The UMMB supplementation appeared to have made better difference over controls where the animals were already on poor diet (rural buffaloes). More closer observations at PAU revealed early onset of ovulatory heat in supplemented buffaloes than in controls (34 Vs 48 days) with lesser incidence of silent oestrus (11 Vs 75%).

In another study, UMMB (335 g daily) was supplemented to 40 anoestrus rural buffaloes for 30 days in the months of September through October. Observations on 13 supplemented buffaloes revealed that their feed intake increased by about 20-25 %, milk production by 0.8 % and milk fat by 0.5 % during this study. Subjective observations in the rest also revealed that in general UMMB supplementation improved feed intake, body condition, production and reproductive performance. Ninety percent supplemented and only 28 % control buffaloes experienced oestrus during this period.

Overall, UMMB supplementary feeding during prepartum period improved postpartum production and reproduction in buffaloes kept under organized as well as unorganized dairy farms both in winter and in summer months. UMMB supplementation also induced ovarian activity in anoestrus buffaloes under field conditions.

  
Signature of Major Advisor

 24.12.2001  
Signature of Student