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Nutritional and Management Support to Reproduction in Dairy Buffaloes Under Tropical Conditions

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Abstract.- Dairy buffaloes contribute 86.57 million tons of milk out of which India's contribution is the highest (59.21%), followed by Pakistan, China and Egypt (20.37, 2.90 and 2.30%, respectively). The South-Asian region supports 93.38% of the world buffalo population with a growth rate of 12.91% during the last decade as compared to 26.01% and 35.06%, in Pakistan and Italy, where special focus has been made on buffalo research and development. The buffalo production system of the rural and peri-urban types expose them to a variety of management, feeding and environmental stresses. Reproductive cyclicity and a normal pregnancy will only continue when there is no thermal, nutritional or productivity stress. Buffalo reproduction is characterized by delayed puberty, silent estrus, long post-partum ovarian inactivity, and, on the whole, poor fertility. In peri-urban dairy farms in Pakistan during 150 days after calving, 68.63% buffaloes were found in estrus. Postpartum ovulation interval was 59.37±4.76 days. A decline in calving interval from 42 to 36 month during the period from 1975 to 2005 has been reported in Italian buffaloes. The digestibility of crude protein is higher in buffaloes than high-yielding cows and they possess 5% higher efficiency of utilization of metabolizable energy for milk production. Purine derivatives in buffalo urine are 50% of the other species showing better nitrogen utilization due to lower glomerular filtration rate leaving more time for recycling to the rumen and metabolized by bacteria. Response of buffalo is low and inconsistent to multiple ovulation and embryo transfer (MOET) treatments, impeding the application of various biotechnologies aimed to enhance genetic progress through the maternal contribution. The viable embryo production has increased significantly from less than 1 per flushing to 2.5-3.0 in general and over 4 in isolated cases and conception rate following embryo transfer improved from about 10% to about 30-40%. Ovarian inactivity associated with poor nutrition could be an important cause of low reproductive rates in swamp buffalo cows, and that the condition could be prevented by adequate feed intakes. Seasonality of reproduction is evident from the shortest postpartum ovulation interval noted during autumn with lowest incidence of silent ovulations. It coincided with the minimum intake of crude protein and maximum intake of metabolizable energy (ME). Photoperiod has a marked influence on buffalo reproduction in certain areas of the world, however in some tropical areas nearest to the equator the light seems to have a minimal or no effect on the reproductive cues however the nutrition and heat stress measured throughout temperature-humidity-indexes play an important role in the reproductive functions of buffaloes. Body condition score (BCS) has been used as a good indicator of energy status and the animals receiving more ME above requirements during prepartum period were able to maintain a relatively good BCS despite mobilization of body reserves. Concentrates supplementation raised milk progesterone levels (MPL) in high and low yielding buffaloes. Growth and development of follicles during periods of negative energy balance lead to impaired development of the CL and a reduction in progesterone secretion. MPL showed a pattern opposite to atmospheric temperature. It may be concluded that nutrition intervenes in reproductive functions at almost all stages and it also interacts with other environmental parameters. A proper strategy is needed to keep the animal productive, healthy and fertile with minimum input costs.

Keywords: dairy buffaloes, reproduction, nutrition, body condition, pregnancy, progesterone, management.

INTRODUCTION

Dairy buffalo has been used as an important tool for meeting food requirements of human population in the rural and urban areas. This animal contributes 86.57 million tons of milk out of which India's contribution is the highest (59.21%), followed by Pakistan, China and Egypt (20.37, 2.90 and 2.30%, respectively). The South-Asian region supports 93.38% of the world buffalo population of 177.25 million heads (FAO, 2009). Growth rate of buffalo population in the region has been 12.91% during the last decade as compared to 26.01% and 35.06%, in Pakistan and Italy, where special focus has been made on buffalo research and development.

The Mediterranean region, including European and the Near East countries, support 3.4% of world buffalo population. Italy has recorded a growth rate in buffalo population of about 142% during the last two decades while dairy cattle and

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horses have decreased in number. This phenomenon has been supported by the increasing demand for mozzarella cheese both on the national and international markets and the denomination of Controlled Origin (DOP) as "Mozzarella de Bufala Compana" for this cheese registered in Italy and Brussels for the European Union (Borghese and Mazzi, 2005).

Peri-urban dairy farming has emerged around bigger cities and towns in Pakistan and elsewhere in the Indo-Pakistan sub-continent, lying in the tropical and subtropical regions. However, these farms are run on non-scientific lines and there is no tradition of consulting animal health, reproduction or nutritional experts in identifying or addressing the relevant problems. The socio-economic status of the peri-urban dairy farmers is usually low, getting negligible inputs from livestock, financial or marketing institutions or experts. This state of affairs has led to heavy calf losses, irregular breeding, imbalanced feeding, ungainly loans and a hostile marketing system; causing heavy economic losses (Qureshi *et al*., 2002). However, buffalo has sustained various impeding factors and exhibited a fair population growth rate in various regions of the world, especially Italy followed by Pakistan and Iran (35.06, 26.01, 25.01, Table I).

Artificial insemination has been introduced in buffaloes since 1950s in Indo-Pakistan region along with cattle, however it could not compete with the later due to problems in estrus detection and low conception rates. Natural service is preferred over artificial insemination for physiological and management reasons. This has resulted in use of non-selected sires for natural service which leads to deterioration in the genetic quality of buffalo population. The delayed postpartum breeding for fear of decrease in milk yield under field conditions, is another cause of genetic deterioration. This paper reviews the reproductive performance of buffaloes and the factors affecting it, with special focus on nutritional management.

REPRODUCTIVE PERFORMANCE

Reproductive performance of buffaloes is influenced by various factors. The buffalo production system of the rural and peri-urban types expose them to a variety of management, feeding and environmental conditions. Hypothalamus is the organ controlling the reproductive system, along-with growth, productivity and response to stress conditions. Therefore, reproductive axis is influenced by the other two axes. Reproductive cyclicity and a normal pregnancy will only continue when there is no thermal, nutritional or productivity stress. So, we can say that reproduction is the last priority of animals' body. The use of systems which reduce the effect of stress, especially high temperature stress, and mainly the use of systems which protect the buffalo from direct sunlight, improves reproductive performance (Campanile and Balestrieri, 2002). In fact, buffaloes benefit from the possibility of dipping in water, as showed by an improvement in their reproductive activity (Di Palo *et al*., 2001).

Buffalo has been blamed for poor reproductive performance reflected by prolonged calving interval and silent estrus. Artificial insemination has got poor response from the farming community due to poor estrus detection status and lower conception rate as compared to cattle (Baruselli *et al*., 2000). Delayed onset of puberty has been reported in buffaloes as compared to cattle (Jainudeen and Hafez, 1993). Buffalo reproduction is characterized by delayed puberty, silent oestrus, long post-partum ovarian inactivity, and, on the whole, poor fertility (Singh, 1988; Madan *et al*., 1994; Singla *et al*., 1996).

A total of 84.2% of the Nili-Ravi dairy buffaloes studied had resumed normal ovarian cyclic activity within 100 days postpartum (Shah, 2007). The remaining 15.8% had abnormal progesterone profiles: 13 % with true anoestrus (no ovarian activity), 2 % with cystic ovaries and 4 % with persistent corpora lutea. Among the animals which had calved in spring and summer a significantly higher incidence of true anoestrus occurred.

In Nili-Ravi dairy buffaloes located in the north-west frontier province of Pakistan, postpartum uterine involution completed during 34.30±1.33 days, ranging from 21 to 74 days (Qureshi and Ahmad, 2008). Uterine involution was completed within 35 days in 55.0% buffaloes, 50 days in 85.0%, while within 74 days in all buffaloes. As

Table I.- Buffalo population in various regions of the world during 2007.

Region	Population			Milk production	
	1997	2007	Growth (%)	Production (MT)	% world
India	89.92	98.70	8.90	59.21	68.40
Pakistan	20.84	28.17	26.01	20.37	23.53
China	21.73	22.72	4.36	2.90	3.35
Egypt	3.10	3.98	22.15	2.30	2.66
Nepal	3.36	4.37	23.00	0.96	1.11
Iran	0.47	0.62	25.00	0.24	0.28
Myanmar	2.30	2.84	19.16	0.22	0.25
Italy	0.15	0.23	35.06	0.20	0.23
South Asia	116.17	133.38	12.91	80.84	93.38
World	159.19	177.25	10.19	86.57	100.01

Source: FAO, 2009

very few animals (15%), completed uterine involution beyond 50 days postpartum; the mean fell at 34.30 days postpartum. Uterine involution was completed within two months after calving in all animals except two animals; one had dystocia and the other suffered from retained placenta with subsequent metritis. During 150 days after calving, 68.63% buffaloes were found in estrus, while the remaining 31.37% animals remained anestrous. Mean postpartum ovulation interval (POI), was 59.37±4.76 days, ranging from 24 to 150 days. The overall mean (postpartum estrous interval) PEI was 69.03±6.03 days, ranging from 21 to 147. Based on the observable estrus signs and milk progesterone levels, three types of estrus events were observed, that is, ovulatory estrus (43.9%), anovulatory estrus (4.6%) and silent estrus (51.5%). BCS in buffaloes resuming oestrus was constantly higher during pre and postpartum periods than those failing to resume oestrous activity. BCS correlates negatively with placenta expulsion time and postpartum estrus interval.

THE INFERTILITY

Zicarelli (2007) reviewed whether buffalo is a non-precocious and hypo-fertile species. He reported a decline in calving interval from 42 to 36 month during the period from 1975 to 2005. It was suggested that the delayed age at first calving was not a characteristic of the species, but it depended on the management of the growing animals. In an earlier report (Zicarelli *et al*., 2005) the lower efficiency in growing rate in buffaloes in the period that should be of compensatory growth was attributed to the characteristics of the dry matter utilized, that is inadequate for replacing the maternal milk and/or the mixed diet (maternal milk and forage) that buffalo assumes during natural weaning. It was observed that accumulation of adipose tissues in subjects with delayed growth, implied higher values of the feed conversion index. It accounted for the necessity to utilize diets more concentrated in energy compared to bovine, for anticipating the age at first calving in buffalo.

A review of reproductive acyclicity and anestrus in dairy buffaloes revealed that first ovulation as detected by rectal palpation and progesterone analysis between 28–71 and 24–55 days, respectively, after calving (El-Wishy, 2007). Postpartum estrus in the same studies occurred between 44 and 87 days. Reports concerned with data compiled from breeding records of research stations, breeding farms and small holders where estrus is a subjective measure, gave much longer periods. Also data from Egypt, India and Pakistan indicated that only 34–49% of buffaloes showed estrus during the first 90 days after calving and 31–42% remained anestrus for more than 150 days. In swamp buffaloes both postpartum ovulation and estrus are more delayed than in dairy buffaloes.

UTILIZATION OF NUTRIENTS

Buffaloes have been shown to convert poor quality roughage into milk and meat in a better way.

Their digestibility of crude protein is higher than high-yielding cows and they possess 5% higher efficiency of utilization of metabolizable energy for milk production (Mudgal, 1988). Food proteins are hydrolyzed to peptides and amino acids by rumen microorganisms but some amino acids are degraded to organic acids, ammonia and carbon dioxide (McDonald *et al.*, 1984). Rumen-degradable proteins are either converted by ruminal microflora, to microbial protein or digested in the small intestine; or they are hydrolyzed to ammonia and excreted as urea. Buffaloes showed higher total digestibility of crude protein fed sugar cane silage treated with *Lactobacillus buchneri* than cattle (Maeda *et al*., 2007). Wora-anu *et al*. (2006) found that ruminal cellulolytic, proteolytic and amylolytic bacteria of swamp buffaloes were higher than those found in cattle fed similar diets.

Purine derivatives excreted in urine, have been used to measure microbial protein production in the rumen. Digestion of microbial nucleic acid in the intestine is associated with microbial protein production. Purine derivatives in buffalo urine are 50% of the other species (Thanh *et al*., 2004). Thanh and Ørskov (2006) reported that glomerular filtration rate may be lower in buffaloes than cattle leaving more time in the blood thus more time for recycling to the rumen and metabolized by bacteria or the permeability from the blood to the rumen is greater in buffaloes than cattle.

In a study initial values of blood metabolites were homogeneous in both intensive fed (IF) and pasture fed (PF) groups of buffalo heifers (Terzano *et al*., 2007). Live weight increased in both treatment groups with IF heifers gaining more weight than PS ones (0.87 kg/d vs 0.61 kg/d of average daily gain, respectively). At day 7 from the start of the trial, significant changes occurred regarding some energy metabolism parameters: particularly a decrease of plasma glucose and triglycerides and an increase of plasma NEFA levels was found in PS group. These changes could be related to a stress condition consequent on the drastic change of rearing system and on the different diet energy level (0.88 MFU/kg DM vs 0.49 ± 0.11 MFU/kg DM, respectively in IF and PS group). Afterwards the main differences were related to glucose level (always significant lower over time in PS group) with the consequent significant increase of triglycerides level. Plasma urea concentration, a nutritional indicator of the nitrogen balance (Slobodianik *et al.,* 1999); was mainly affected by feeding system more than total protein and albumin level; it rose in PS group immediately after the start of the trial and continued to be highly significant all over the time due to higher nitrogen availability in forages.

Orskov (2007) reported biochemical adaptation of buffaloes to different climatic conditions. It was reported that when ruminants are exposed to fasting either due to lack of available food or illness, the beta hydroxybutyrate in the blood increases rapidly due to glucose deficiency. When this occurs, the ruminants derive some glucose precursors from turn over in the animal's body but this means loss of leans tissues as this would normally be re-synthesized. To avoid such protein loss, the ruminants may be fed at about one third of energy maintenance. He reported that in the buffalo purine derivatives were recycled in the rumen to further supplement a very efficient recycling of urea nitrogen. This is an advantage that buffaloes need a less nitrogen in the poor quality roughage they consume.

FOLLICULOGENESIS AND OVULATION

Sufficient number of primordial follicles and appropriate concentration of hormones are required for ovulation and embryo production in buffaloes. The total number of primordial follicles in riverine buffalo heifers were reported to vary between 12000-19000 (Samad and Nasseri, 1979) and 1222-40327 (Danell, 1987). In swamp buffaloes, detailed studies on the number of primordial follicles at different age indicated that the population decreased very fast from puberty onwards and subsequently the decrease appeared to decline with age (Smith, 1990).

In majority of cattle (~75%) 3 follicular waves have been observed whereas 63.3% of buffalo exhibit 2 waves at an interval of about 11 days during the estrous cycle (Baruselli *et al*., 1997). In each follicular wave a cohort (~5-7) of gonadotropin responsive antral follicles (~3-5 mm) begin to develop. From this group, finally a dominant follicle emerges which continues to grow (> 10 mm) and secrets elevated levels of estradiol 17β, inhibin and several other factors leading to atresia of remaining subordinate follicles. Blood In-A (inhibin-A) levels have been found in ruminants, to influence ovarian follicular dynamics (Medan *et al*., 2003), and being potential predictor of the responses to the super-ovulatory treatments (Gonzalez-Bulnes *et al*., 2004). A study on Nili-Ravi buffaloes reported that (1) the majority of buffaloes had a two wave pattern of follicular growth and emergence of a third wave was associated with a longer luteal phase, and (2) follicular dynamics during the 3 days before oestrus were similar in buffaloes undergoing spontaneous and $PGF_{2\alpha}$-induced luteolysis (Warriach and Ahmad, 2007).

EMBRYO PRODUCTION CAPACITY

Response of buffalo is low and inconsistent to multiple ovulation and embryo transfer (MOET) treatments, impeding the application of various biotechnologies aimed to enhance genetic progress through the maternal contribution (Zicarelli, 1997). In addition to the limitations, in terms of embryo output, of MOET in this species, one of the major factors that impair reproductive development in artificially inseminated herds is the failure in estrus detection. The correct management of estrus detection requires continuous observation of the herd and qualified, responsible and knowledgeable labour. The authors reported that OPU (ovum pickup) can be performed on a wider typology of donors such as non-cyclic animals, pregnant cows, subjects with patent oviducts or genital tract infections, and animals that are not responsive to hormonal stimulation, the last representing a high proportion in buffalo.

A study was conducted on 10 juvenile buffaloes, concluding that: (1) low follicle numbers compared with cattle are already present in juvenile buffaloes; (2) most of the largest healthy follicles in juvenile buffaloes are refractory to exogenous gonadotrophins; and (3) as a consequence, the ability of juvenile buffaloes to superovulate is even lower than in adults (Ty *et al*., 1994). According to Misra and Tyagi (2007) the viable embryo production has increased significantly from less than 1 per flushing to 2.5-3.0 in general and over 4 in isolated cases and conception rate following embryo transfer improved from about 10% to about 30-40%. However, response to super-ovulatory treatments and recovery of viable embryos following super-ovulation is still low compared to cattle due to various factors.

Poor response to superovulation and low embryo recovery is attributed to low primordial follicle pool of 20 (in swamp) to 30% (in riverine) in buffaloes as compared to cattle and high rate of follicular atresia. As a consequence, 10% buffalo failed to respond (0-2 CL) and nearly half responded poorly (0-5 CL) to superovulation treatment (Misra, 1996).

OVULATION AND NUTRITIONAL STATUS

Rasby *et al*. (1992) reported that nutrition restriction has a negative influence on LH release. Animals in anestrus showed decrease in diameter of the dominant follicle and in ovulation rate to the GnRH treatment. Nutritional status of an animal is reflected by the BCS. Several studies also demonstrate the negative effect of low BCS on ovarian cyclicity and pregnancy rates in beef cows (D'occhio *et al*., 1990; Viscarra *et al*., 1998). Furthermore, investigations on postpartum reproduction indicate that BCS is a useful indicator of energy status and rebreeding potential (DeRouen *et al*., 1994). It was suggested that buffaloes may have to present BCS $\geq$ 3.5 for a satisfactory response to the treatment with GnRH and prostaglandins for fixed time artificial insemination (FTAI).

Ovarian activity, oestrus and conception were recorded in a total of 75 swamp buffalo cows after oestrous synchronization at two mating seasons and at two levels of nutrition. It was concluded that ovarian inactivity associated with poor nutrition could be an important cause of low reproductive rates in swamp buffalo cows, and that the condition could be prevented or corrected by adequate feed intakes. In peri-urban dairy farmers in north-western Pakistan, shortest postpartum ovulation interval was noted during autumn (August to October) and the incidence of silent ovulations was lowest (Qureshi *et al*., 1999a). It coincided with the minimum intake of crude protein (CPI) and maximum intake of metabolizable energy (MEI, $p<0.01$). It was also associated with higher calcium and zinc intake and lower phosphorus, copper and magnesium intake.

In another study Qureshi *et al*. (2002) reported that BCS and postpartum ovulation interval were correlated with ME intake ($p<0.01$). Prepartum ME intake was higher in oestrous as compared to anoestrous animals ($p<0.05$). Higher and lower ME intakes were associated with anoestrus, while a moderate energy intake was associated with a postpartum estrus interval (PEI) of less than 75 days. BCS was negatively correlated with PEI ($p<0.01$) and was higher in oestrous buffaloes than anestrus. It was concluded that excess intake of crude protein, associated with higher serum urea levels and low energy intake, associated with poor body condition, are the key factors for low reproductive efficiency.

SEASONALITY OF REPRODUCTION

Buffalo is a photoperiodic species and like sheep, it has to be considered a "short day" species. They have heats throughout the year but are more fertile when daylight hours decrease. According to Zicarelli (1995), this characteristic is due to their tropical origins; in fact, in these areas the availability of forage coincides with the period in which dark hours increase. Therefore, it has been supposed that animals which calve in the most suitable period for survival of the offspring were selected. It seems that they have retained this characteristic even when transferred to places where forage is always available. In countries like Italy, where market demand requires a concentration of deliveries in the spring-summer period (not corresponding to buffalo reproductive activity) the out-of-season technique is widely applied. As a result, buffaloes which are less sensitive to photoperiodic effects have been selected. When the out-of-season technique has been applied for long periods a lower loss of fertility was observed (15% vs. 30%) compared to the farms in which it has been adopted for shorter periods (Campanile, 1997).

It was suggested that that photoperiod has a marked influence on buffalo reproduction in certain areas of the world, however in some tropical areas like in Brazil, mainly in the Amazon valley and areas nearest of the equator the light seems to have a minimal effect or no effect on the reproductive cues however the nutrition and heat stress measured throughout temperature/humidity indexes (THI) play an important role in the reproductive functions of buffaloes (Vale, 2007). It was also suggested that THI >75 has a negative effect on reproductive performance of buffaloes.

Seasonal changes affect reproductive performance through altered pattern of ambient temperature, photoperiod and availability of green fodders. This has led to a seasonal pattern of breeding in dairy buffaloes. Opposite trend of breeding has been reported in buffaloes and calves which has been noted as a blessings for countries like Pakistan (Shah *et al*., 1989). Qureshi *et al*. (1999a) reported that the buffaloes calving during the normal breeding season (NBS, August to January) ($p<0.01$) showed postpartum estrus interval of 55.95 days versus 91.15 days in those calving during the low breeding season (LBS, February to July). Milk progesterone levels (MPL) in the LBS remained lower than the NBS ($p<0.01$). Shortest postpartum ovulation interval was noted during autumn (August to October), followed by winter (November to January), summer (May to July) and spring (February to April). The incidence of silent ovulations was higher during LBS than NBS (70.6% versus 29.4%). In a concurrent study (Qureshi *et al*., 1999b, 2002, Table II), milk progesterone levels in buffaloes showed a pattern opposite to atmospheric temperature. In NBS calvers serum glucose levels were higher ($p<0.01$) and magnesium levels were lower ($p<0.01$) than performance in buffaloes calving in the LBS coincided with a low BCS ($p<0.01$). Fat corrected milk production (FCM) was higher in NBS than LBS ($p<0.01$) calvers.

Sousa *et al*. (2006) in the Ribeira Valley of Brazil, state (Latitude 14 to 33^{o} South) found that 86 % of female buffaloes did not express any seasonal anoestrus in a long photoperiod season. Furthermore

Table II.- Effect of calving period and season on protein intake and serum urea concentration (least square means*±standard error).

Group	Crude protein (kg/day)	Degradable protein (kg/day)	CP/ME (g/MJ)	Serum urea (mg/100 ml)
		Calving periods		
NBS	1.81±0.02	1.32±0.02	12.2±0.07a	31.7±1.43b
LBS	1.83±0.02	1.34±0.02	11.8±0.10b	39.4±21.6a
Probability	NS	NS	p<0.01	p<0.01
		Seasons		
Autumn	1.79±0.02c	1.26±0.02c	11.5±0.10c	36.4±2.92ab
Winter	1.68±0.02d	1.22±0.02c	12.0±0.09b	29.9±1.96b
Spring	1.89±0.03b	1.42±0.02b	12.2±0.09b	38.6±2.95a
Summer	2.08±0.05a	1.55±0.04a	12.7±0.19a	40.1±2.53a
Probability	p<0.01	p<0.01	p<0.01	p<0.05

* Means within a group in the same column with different letters differ significantly.
NBS: Normal breeding season, LBS: Low breeding season, NS: Non-significant.

the same author used melatonin implants in a group of animals in the same environmental conditions and could not observe any improvement in the animals cycle activity and the fertility did not show any statistic significant difference ($p<0.05$).

The calving distribution in the Amazon valley is completely different of the other Brazilians regions and do not have any influence of the photoperiod as reported by Vale *et al*. (1990), Zicarelli and Vale (2002). Ribeiro (2002) found the yearly calving distribution in the buffalo production system near of the Equator line, The same results were also observed by Vale *et al*. (1996) with a little difference concerning the peak of calving which has occurred between November and December.

Nutritional effects on seasonality of reproduction is more evident in countries where buffaloes calve during the most favorable periods (Campanile, 1997). Negative phenomena may be observed in countries where the calving calendar is modified by applying the out of breeding season mating technique (Zicarelli, 1997). This technique is applied, in the Mediterranean region where there is a requirement for mating programs in buffaloes to be conducted during the spring-summer period (unfavorable for the reproductive activity since buffalo is a short-day species), so that calving coincides with the yearly peak in demand for buffalo milk. This creates a potential conflict between the seasonal nadir in reproduction and the need to establish pregnancies, because the increase of day-length determines a seasonal decline in reproductive activity, which is manifested by a reduced incidence of estrous behavior, a decrease in the proportion of females that undergo regular estrous cycles and generally lower conception rates.

It was observed that embryonic loss in animals mated by artificial insemination is 20-40% during seasons characterized by high number of light hours (Campanile *et al*., 2005), whereas values of around 7% were recorded in Brazil during decreasing light days (Baruselli *et al*., 1997). In contrast to the previous work, an embryonic mortality rate of 20% was reported for buffaloes close to the equator (Vale *et al*., 1989). In any case, embryo mortality in buffalo occurs later than in bovine, usually between 25 and 40 days from AI (Campanile *et al*., 2005). In buffaloes naturally mated (Vecchio *et al*., 2007), independently from the conception period, 8.8% and 13.4% showed respectively embryonic mortality between 28-45 days (embryonic mortality-EM) days and between 46-90 days (foetal mortality – FM) of pregnancy. Companile and Neglia (2007) found no differences between the incidence of EM in relation to the conception period, while a high incidence ($P<0.01$) of FM was found during a period of increasing daylight length (transitional period: December-March) compared to the April-July period.

BODY CONDITION AND LEPTIN SIGNALING

Body condition score (BSC) reflects the nutritional status of a dairy animal in relation to milk production. Buffaloes submitted to an under-nutrition management acquired a low BCS (Vale, 2004). Female buffaloes which calved with a. BSC 2 of 2.5 (in a scale of 1-5) presented delayed ovulation and service period and more service per conception rate compared with buffaloes with a BSC between 3 -4. As in the Amazon region and other parts of Brazil and Latin America most of the production system use extensive management it could be predict that the nutrition play a big role inside the buffalo production systems.

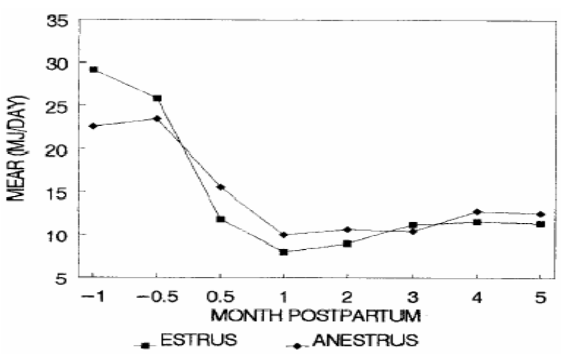

Fig. 1. Metabolizable energy intake above requirement (MEAR) in estrous and anestrous buffaloes (Qureshi *et al*., 2002).

In Nili-Ravi dairy buffaloes maintained under peri-urban farming system in Peshawar, Pakistan, the increasing energy intake increased BCS (r=0.16) and duration of expulsion of placenta (r=0.19) and discharge of lochia (r=0.24) but decreased postpartum ovulation interval (r=-0.27, p<0.01; Qureshi *et al*., 2002; Table III). The authors suggested that the animals receiving more ME above requirements during prepartum period were able to maintain a relatively good BCS despite mobilization of body reserves (Fig. 1). Higher MEAR during prepartum period were accompanied by a higher BCS in animals which came into oestrus (Fig. 2). Figure 3 shows that non of the buffaloes in the NBS calving group had poor BCS (1.0 to 2.5). Conversely, none of the LBS calving buffaloes had good BCS (BCS>3.0), which is consistent with the higher intake of metabolizable energy (p<0.01) during summer and autumn. It has been reported in buffaloes that BCS affects their fertility as pregnancy rate improved from 39.7, 53.9 and 56.7% with the improvement in BCS from 3.0 to 3.5 and 4.0, respectively (Baruselli *et al*., 1999).

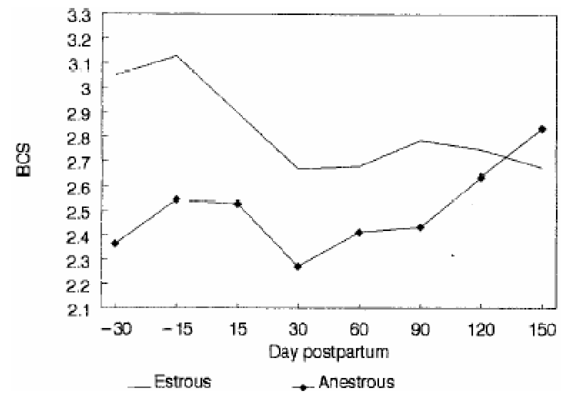

Fig. 2. Postpartum body condition score (BCS) of estrous and anestrous buffaloes (Qureshi *et al*., 2002).

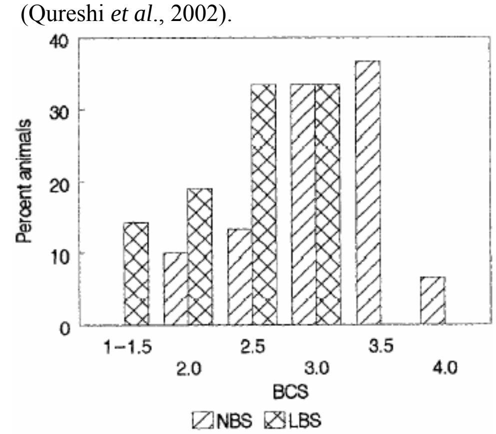

Fig. 3. Distribution of buffaloes (%) on the basis of body condition score (BCS) during normal (NBS) and low breeding season (LBS) (Qureshi *et al*., 2002).

Leptin is a hormone produced mainly by the adipose cells regulating body weight through BCS signaling of hypothalamic centers (Casabiell *et al*., 2001). It interacts with other endocrine systems acting as a permissive factor that allows the triggering of energy-demanding situations, as the onset of puberty and the reproduction, only when the size of the fuel reserve is large enough to guarantee its success. It plays a role in pregnancy

Table III.- Correlation of various reproductive performance in buffaloes (Pearson's correlation coefficients[1]).

Variate	Placenta xpulsion	Lochia discharge	Ovulation	Oestrus
Crude protein (CP)	0.21**	-0.14	0.21**	0.08*
Degradable protein	0.10*	0.22**	0.01	0.31**
CP above equirements	0.37**	0.06	0.03	0.10**
Metabolizable energy ME)	0.19*	0.28**	0.03	0.03
ME above requirements	0.19*	0.24**	0.27**	0.04
Serum urea	0.16	0.18	0.30**	0.28**
Body condition score	0.17	0.01	0.06	0.20**

[1] The Pearson's product-movement correlation determined through correlation analysis; * Significant at p<0.05; ** Significant at p<0.01.

and lactation, as it is produced by the placenta and is present in maternal milk. Leptin expression of Egyptian water buffalo, cow, and one-humped camel tissues were examined (Bartha *et al*., 2005). The mammary gland produced leptin in each species. The local hormone production contributed to milk leptin and most probably maintained lactation. Tissues participating in production have an autoregulative mechanism through which tissues can be relatively independent of the plasma leptin levels in order to maintain the desired function.

Circulating leptin increased during pubertal development in rodents, human females and heifers leptin stimulated the release of GnRH from rat and porcine hypothalamic explants (Zieba *et al*., 2005). Moreover, leptin increased the release of LH in rats and from adenohypophyseal explants and/or cells from full-fed rats and pigs. Previously, an overall metabolic control was attributed to the leptin hormone, however, leptin gene expression was demonstrated in several additional peripheral tissues. Sayed *et al*. (2003) studied localization of leptin and its receptor mRNA transcripts in Egyptian dairy buffaloes. It was revealed that leptin and its receptor transcripts are expressed specifically in the alveolar epithelial cells of the mammary gland. These morphological data support that leptin could also act as an autocrine and paracrine mediator for mammary gland metabolism and as a facilitator of alveolar epithelial cell activity during lactation.

MILK PROGESTERONE LEVELS

Our group reported that concentrates supplementation raises milk progesterone levels (MPL) in high and low yielders (Khan *et al*, 2008; Figs. 4, 5). The animals with traditional ration exhibited a sharp decline in milk yield with the increasing progesterone levels, in an un-interrupted trend. However, the concentrate supplemented animals showed the decline in two phases. With the increasing progesterone concentrations from 2.0 to 5.84 ng/ml the decrease in milk yield was zero. Further increase in progesterone concentrations resulted in a decreasing milk yield with a comparatively slower rate than the buffaloes on traditional ration. All the high, moderate and low milk yielding buffaloes exhibited a decline in milk yield with the increasing progesterone levels (MPL) beyond 6 ng/ml. However, this decline was faster in higher yielders followed by moderate and low yielders.

In another study, cyclic underfed cattle had progressively smaller and less estrogenic dominant follicles before they succumb to anestrous (Bossis *et al*., 1999). The smaller dominant follicles gave rise to smaller corpora lutea. Steroidogenic capacity of luteal cells is also dependent on hormones such as somatotropin, insulin and IGF-I that are controlled by the nutrition of the cow (Lucy, 2000). Association of progesterone levels with milk yield acting via nutritional status has been suggested (Lucy, 2001). Under nutrition or negative energy balance may compromise pregnancy through its effects on the corpus luteum. High producing dairy cows had lower blood concentrations of progesterone and the lower blood progesterone concentration were attributed to infertility. Mechanism of action of nutritional stress associated

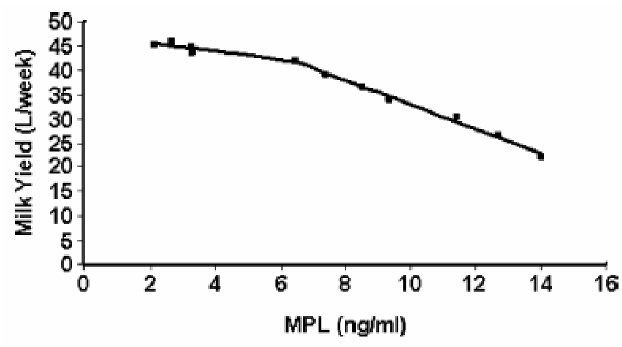

Fig. 4. Changes in milk yield with the increasing milk progesterone levels in dairy buffaloes. (Y=47.41937 - 0.88148 P - 1.59907 P'; For MPL < 6.44: P'=0; R12=0.761, Y=-0.8237X + 46.763; and for MPL > 6.44: P'=P - 6.44; R2=0.9875; Y=-3.3822 X + 43.328; Joining point of the two lines is 6.44, the critical point defining the drastic decline in milk yield) (Khan *et al*., 2008).

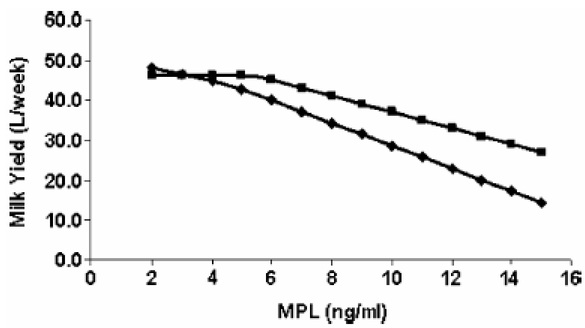

Fig. 5. Changes in milk yield with the increasing milk progesterone levels (MPL) in dairy buffaloes; PRS (■) and PRT(♦.) buffaloes (Y1=57.1677 - 2.0099P'; R-square=0.9901; Y2=51.4432 -1.5946P - 1.2549P"; R-square = 0.9910) (Khan *et al*., 2008).

with body weight loss and lower circulating progesterone concentrations has been attributed to selection for increased milk yield (Lucy and Crooker, 2001). It has been hypothesized that growth and development of follicles during periods of negative energy balance lead to impaired development of the CL and a reduction in progesterone secretion (Butler, 2000). Cows that produce more milk have smaller CL at the peak of lactation (Lucy, 2000) and CL size has been correlated positively with circulating progesterone concentration.

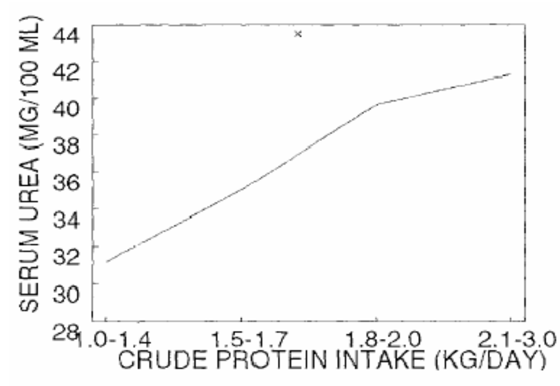

Fig. 6. Relationship of crude protein intake (CPI) and serum urea levels (SUL) in buffaloes (Qureshi *et al*., 2002).

However, circulating progesterone concentration is determined by rates of secretion and clearance. Clearance rates of progesterone increase with feed intake due, in part, to an increase in hepatic metabolism (Sangsritavong *et al*., 2002). Therefore, at least two factors (CL size and feed intake) appear to be responsible for the reduction in circulating progesterone in cows that produce more milk. The addition of fat to the rations of dairy cows resulted in an increase in the levels of progesterone in blood (Lucy *et al*., 1993).

Embryonic mortality in buffaloes are due primarily to reduced secretion of P_4 by the corpus luteum linked with a reduced capacity of the developing embryo to secrete IFNt interferon at threshold amounts necessary to prevent luteolysis (Campanile *et al*., 2005). No differences were found in P_4 plasma levels between non-pregnant and cows with embryo mortality. E_2 plasma levels did not differ between the different reproductive status considered (Spagnuolo *et al*., 2007) and that two different housing conditions did not affect plasma and follicular redox status, thus suggesting that stabulation of buffalo cows at a density of 13.3 m^2/head might be used without compromising of animal welfare and of follicular development. Furthermore, embryonic mortality seems to depend on progesterone plasma level, but not on oxidative stress.

Embryonic mortality in buffaloes are due primarily to reduced secretion of P4 by the corpus luteum linked with a reduced capacity of the developing embryo to secrete IFNt interferon at threshold amounts necessary to prevent luteolysis (Campanile *et al*., 2005). Qureshi *et al*. (2000) reported that the low postpartum reproductive performance in dairy buffaloes during low breeding season was primarily due to inadequate functioning of the *corpus luteum* in secreting optimum concentrations of progesterone, which may lead to embryonic losses.

THE STRESS FACTORS

Summer stress has been shown to lower MPL (Qureshi *et al*., 2000) in dairy buffaloes. MPL was found highest in spring (3.00±0.12 ng/ml) followed by winter (1.77±0.32), autumn (0.84±0.72 ng/ml), summer (0.25±0.04) ($P<0.01$). Summer contributed to the LBS when the MPL did not reach the optimum levels until day 84 postpartum, indicating that the corpus luteum did not function efficiently to maintain high progesterone levels required for reproductive cyclicity. Interestingly, the incidence of silent ovulation in the buffaloes was higher in LBS than in NBS (70.6 vs 29.4%, respectively).

Qureshi *et al*. (1999b) found that MPL showed a pattern opposite to atmospheric temperature. Kaur and Arora (1984) concluded that malnutrition coupled with high environmental temperature stress was responsible for long anestrous periods in buffaloes.

A study suggested that increasing suckling duration and use of oxytocin delayed POI, however, POI was shortest in buffaloes suckled for one month. The high yielding buffaloes also manifested better reproductive cyclicity; while moderate yielder showed shorter ovulation intervals and higher conception rate (Qureshi and Ahmad, 2008).

Deficiencies of natural protective substances or excessive exposure to stimulators of reactive oxygen species (ROS) production may result in oxidative stress, which occurs when pro-oxidants exceed the capacity of antioxidants (Halliwell and Gutteridge, 1999). Antioxidants prevent oxidative stress in healthy ovarian follicles, where the maturation and the quality of oocyte depend on the function of the granulosa cells. Cell death and atresia occur in the follicle under oxidative stress (Dharmarajan *et al*., 1999).

The metabolic oxidative stress is related to the mitochondrial activity, because O_2 consumption is not perfectly coupled to ATP production (Lopez-Luch *et al*., 2006). There is a large body of evidence reporting that caloric restriction reduces reactive oxygen species (ROS) production, health risks and delays the onset of most age related diseases by decreasing mitochondrial proton leak (Beckman and Ames, 1998). In high yielding dairy cows, metabolic drive for milk is associated with an increased production of free radicals and ROS (Stefanon *et al*., 2005), that can be exacerbated by unfavourable ambient conditions (Gabai *et al*., 2004). Sgorlon *et al*. (2007) reported that rapid modification of diet composition affects metabolic and oxidative homeostasis in lactating sheep.

A study was conducted to examine the effect of two different housing conditions on blood and follicular redox status and on reproductive performance of buffalo cows (Spagnuolo *et al*., 2007). No significant differences among the groups were found, thus suggesting that the outcome of fertilization might not depend only on the blood parameters evaluated. It was suggested that the two different housing conditions did not affect plasma and follicular redox status. Plasma levels of retinol, α-tocopherol and ascorbate, as well as titres of protein oxidation markers (N-Tyr and PC), did not differ between pregnant and non-pregnant cows, suggesting that metabolic processes and endocrine changes associated with pregnancy do not affect the indices of redox status. It has been reported that the antioxidant defence system plays a key role in preventing apoptosis and atresia, thus preserving steroidogenic function of granulosa cells (Cassano *et al*., 1999).

Embryonic loss in buffaloes mated by artificial insemination (AI) is 20-40% during seasons characterized by high number of light hours. Also in buffalo naturally mated the incidence of embryonic mortality is about 20% and a higher incidence is observed between 28-60 days of gestation in buffaloes that conceive during increasing daylight length. A reduced capacity to secrete progesterone seems to explain in part this embryonic mortality but other as yet unidentified factors contribute between 40-50% to the embryonic losses.

PROTEIN INTAKE

Under the peri-urban dairy farming in the Indo-Pakistan region, the feeding practice is not based upon requirements of the dairy animals. A same scale feeding is practiced exposing the low yielding animals to adverse affects of overfeeding. Intake of crude protein (CPI) varied between seasons and was positively correlated with serum urea levels ($r=0.22$, $p<0.01$; Qureshi *et al*., 2002). CPI was positively correlated with the duration of placenta expulsion, postpartum estrus and ovulation intervals. CPI excess to requirements was lower in animals which expressed oestrus than those remaining anoestrus ($p<0.05$, Figure 7). Figure 8 shows that the animals resuming to oestrus had a narrow and almost constant CP/ME ratio (11.9 to 12.2 g/MJ), while the anoestrus animals had a widely fluctuating ratio, ranging from 10.7 to 13.1 g/MJ. CP/ME was related positively with POI. Prepartum energy intake above requirement (MEAR) was also higher in animals returning to oestrus than the anoestrus ones ($p<0.01$).

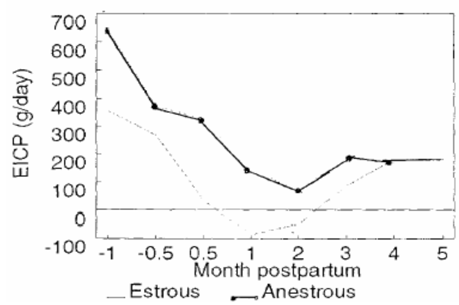

Fig. 7. Excess intake of crude protein (EICP) in estrous and anestrous buffaloes during various postpartum months (Qureshi *et al*., 2002).

A study reported production stress in high milk yielding buffaloes (Khan, *et al*., 2008) which was considered as a factor responsible for lower MPL and this lowering trend is prevented by the concentrates feed supplementation. In LMY buffaloes there seemed to be the stress of overfeeding of degradable protein (Qureshi *et al*., 2002) as they were fed at the same scale under conventional feeding regime, at par with the high and moderate yielders; and they could not utilize the excess intake of protein. In the supplemented buffaloes the animals were fed according to the feed requirements, there was no question of overfeeding which resulted in enhanced MPL. In line with our findings, Lucy (2001) has reported that under-nutrition or negative energy balance may compromise pregnancy through its effect on the corpus lutem. Higher milk yielding cows had lower progesterone concentrations, associated with infertility.

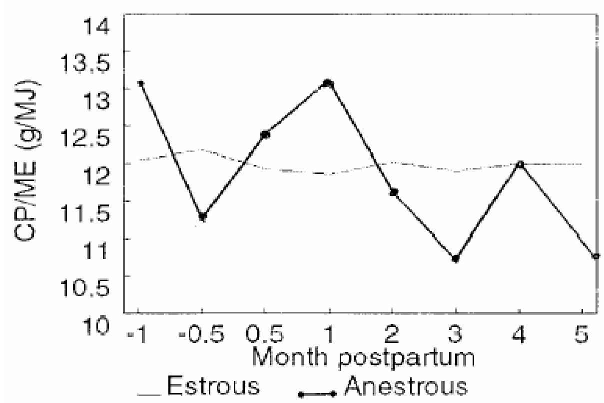

Fig. 8. Ratio of crude protein/ metabolizable energy intake (CP/ME) in estrous and anestrous buffaloes during various postpartum months (Qureshi *et al*., 2002).

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