EFFECT OF PHYSIOLOGICAL STATES ON MILK FATTY ACIDS PROFILE IN DAIRY COWS AND BUFFALOES

A dissertation submitted to the N.W.F.P Agricultural University, Peshawar in partial fulfillment of the requirement for the Degree of

DOCTOR OF PHILOSOPHY IN LIVESTOCK MANAGEMENT

BY

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Department of Livestock Management, Faculty of Animal Husbandry and Veterinary Sciences, N.W.F.P AGRICULTURAL UNIVERSITY, PESHAWAR, PAKISTAN. DECEMBER, 2009
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ABSTRACT

Milk composition is the result of various biological reactions, affected by feed intake and physiological status of animal. Physiological status, in turn, is affected by environment modulated through hypothalamus and pituitary. Milk composition and quality are important characteristics that determine the nutritive value and consumer acceptability. The following three studies were conducted to investigate relationship of lactation stage and body condition with milk yield and composition with special reference to fatty acids in crossbred dairy cows and buffaloes (Bubalus bubalis) under tropical conditions.

Study I - Variation in milk fatty acids composition with body condition: A total of 24 Nili-Ravi buffaloes within 60 days after parturition, were selected from a private dairy farm at district Peshawar. All the animals consumed the same diet during the experimental period. A total of 576 raw milk samples were collected for laboratory analysis. The study continued up to 6 months during the year 2008. Body condition score (BCS), milk yield and composition were recorded once a week. Means for milk fatty acids profile were compared for various levels of BCS. The mean milk yield and fat contents were 9.28 kg/d and 5.36 %, respectively. The total saturated fatty acids (SFAs) were 64.22 g/100g of total fatty acids and the unsaturated fatty acids (UFAs) were 35.79 g/100g of total fatty acids. Out of the SFAs, highest amount was recorded for C16:0, followed by C18:0 and C14:0. The total sum of hypercholesterolemic fatty acids (HCFAs, C12:0, C14:0 and C16:0) were 43.33 g/100g of total fatty acids. The concentrations of UFAs were greater with the moderate (2.5) BCS followed by poor (1.5) and highest one (3.5) while the SFAs showed an opposite trend. The correlation analysis showed that milk yield was negatively affected by BCS and milk fat positively though, non-significantly. The present study suggests that Nili-Ravi dairy buffaloes produce milk, with the highest concentration of C18:1 cis-9. Two HCFAs (C12:0 and C14:0) were associated with higher body condition. Buffaloes with moderate body condition yielded milk containing healthier fatty acids.

Study II - Changes in milk fatty acid profile with advancing lactation: This study was conducted to determine the effect of lactation advancement on milk yield and its fatty acid composition in crossbred cows during the initial 16 weeks of lactation. A total of 28 F1 crossbred cows (HF x Sahiwal) within 1st week after parturition were selected from a large state farm. The animals were maintained under uniform management conditions in a well-ventilated shed. The animals were milked twice and milk samples were collected from each cow once a week during 1, 4, 8, 12 and 16 weeks of lactation. BCS and milk yield were recorded on the day of sample collection. Means for milk fatty acids composition were compared for five weeks (1, 4, 8, 12 and 16) of lactation. The
total amount of saturated fatty acids (SFAs) was on the average 67.88 g/100 g of total fatty acids and the unsaturated fatty acids (UFA) were 32.39 g/100 g of total fatty acids. In the SFAs the highest amount was of palmitic acid (23.09 g/100 g of total fatty acids). The highest monounsaturated fatty acids (MUFAs) level was of oleic acid (C\textsubscript{18:1} cis-9, 24.68 g/100 g of total fatty acids). Mean concentration of polyunsaturated fatty acids (PUFAs) was 3.95 g/100 g of total fatty acids. The total sum of medium chain fatty acids C\textsubscript{12:0}, C\textsubscript{14:0} and C\textsubscript{16:0} identified as hypercholesterolemic fatty acids (HCFAs) was 38.40 g/100 g of total fatty acids. The correlation analysis showed a significantly positive relationship between BCS and milk fat percent. The present study suggests that concentrations of UFA were higher in earlier weeks and declined during mid lactation. With advancement of lactation, from wk 1 to 16 of lactation, the proportion of both de novo fatty acids and PUFAs increased and pre-formed fatty acids (specifically C\textsubscript{18:0} and C\textsubscript{18:1} cis\textsubscript{9}) decreased. The two hypercholesterolemic fatty acids (C\textsubscript{12:0} and C\textsubscript{14:0}) increased with advancing lactation and the cows in early lactation yielded milk containing healthier fatty acids.

**Study III - Body condition score as an indicator of milk yield and composition:** This study was undertaken to evaluate the role of body condition score (BCS) as an indicator of milk yield and composition in Nili-Ravi buffaloes under subtropical conditions. A total of 36 buffaloes within 1\textsuperscript{st} week of parturition were selected from a private peri-urban dairy farm at district Peshawar. All the animals were offered green fodders ad libitum and concentrate at the rate of 1 kg per 2 kg of milk produced. Milk yield (kg/d) and BCS (scale 1-5) were recorded weekly and milk samples (n = 1008) were collected for analysis of fat, protein and lactose contents. The study continued for 7 months, starting from November 2007 to May 2008. BCS significantly affected milk yield and fat and protein contents. Lactose was least affected with changes in BCS during lactation. Highest yield was recorded with moderate BCS in buffaloes. BCS correlated positively with milk fat and protein and negatively with milk yield. Milk yield decreased while BCS increased with advancing lactation. The negative relationship may be due to mobilization of body reserves, indicating better genetic potential of buffalo as a dairy breed. The results indicated that BCS may be used as an indicator for maintaining milk yield and composition in dairy buffaloes.

**Keywords:** Dairy buffalo, crossbred cows, milk composition, milk yield, lactation stage, body condition score, saturated fatty acids, unsaturated fatty acids, hypercholesterolemic fatty acids
Dedication

This humble effort is
Dedicated to
My loving parents,
Husband and children
Who always
Pray for my success.
ACKNOWLEDGEMENTS

I would like to express my deepest sense of gratitude to Almighty Allah, most gracious and the most beneficent, who enabled me to complete this task. As a preface to the research presented in this dissertation, I have many, many people to acknowledge for their advice, assistance and support during my study at NWFP Agricultural University Peshawar. First, I am very grateful to Prof. Dr. M. Subhan Qureshi for serving as my dissertation advisor and providing support, guidance, suggestion and encouragement throughout the course of my Ph.D project. I am also thankful to Dr. Sarzamin for his useful discussion, moral support and serving as co-advisor.

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I will also acknowledge the support of my family especially my mother, father, brothers and sisters. Finally, I will appreciate the support and encouragement of my husband and my little kids Saad and Ihsun as their unconditional love has meant so much to me. I am especially thankful to them for their patience, encouragement, advice, laughter, prayers and support and for sharing these years with me. I can’t imagine having done this without their support.

Anila Mushtaq
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I. INTRODUCTION

Milk is the primary product of livestock sector and has placed Pakistan on the 3rd position in global ranking (International Dairy Federation, 2008). The cattle herds include a variety of purebred animals such as Sahiwal, Red Sindhi, Tharparker and majority of crossbred animals while Kundi and Nili-Ravi dominate the buffalo herds. Out of the present cattle population, 25% are crossbred animals while the remaining 75% are local cows. All the way through crossbreeding of cattle in NWFP milk production increased by 73% and age at puberty reduced about 82% (Syed et al., 1994). Qureshi et al. (2001) have reported various reproductive and productive problems linked with crossbreeding in cattle. Generally the mean milk yield is very low in Pakistan in comparison to developed countries e.g. one New Zealand cow produces as much milk as three dairy cows in Pakistan, while average milk produced by seven Pakistani cows is equivalent to one American cow’s production (Garcia et al., 2003). In Pakistan milk production, processing and marketing are still in primitive stage and needs a lot of research and development support to meet consumer’s demand.

Milk composition is the outcome of various biological reactions, affected by feed intake and physiological status of animals. Physiological status, in turn, is affected by environment modulated through hypothalamus and pituitary. Milk physical, chemical and bacteriological characteristics will determine its nutritional significance plus acceptability by consumers. Milk composition is the outcome of the flow of substrates from the blood circulation to the mammary tissues. According to Neville et al. (2001), the tight junctions (TJ) have an important role in the partitioning of substrates for milk synthesis.

Body condition scores (BCS) has been considered an effective tool in monitoring the energy condition of cows and herds (Jeffrey and James, 1989), indicating the energy intake and utilization in the form of growth and milk production. Therefore, it is routinely practiced in the farm management for evaluation of nutritional status of animals. BCS is a subjective, visual or physical assessment of the quantity of metabolizable energy deposited in the fat and muscle of a live animal.

To assess changes in body reserves as a consequence of negative energy balance BCS has been recommended as a practical monitoring tool (Berry et al., 2002). In spite of its
subjectivity, BCS gives an accurate evaluation of a live animal's energy reserves. It has been confirmed that body reserves are better reflected by BCS than by live weight change (Grainger et al., 1982; Johnson, 1984; Ducker et al., 1985).

The mobilization of body energy reserves is the major capability of the dairy cow. Mobilization of reserves is indispensable for maintaining high milk yield following parturition. Like the other lactating animals, dairy cattle are in negative energy status at the start of lactation (Nielsen, 1999). The energy intake of high yielding Holstein cows during initial lactation is almost lower than half of the energy necessary for production purposes (Veerkamp et al., 1995). The change in BCS in the first few weeks of lactation point towards the level of metabolic load as the shortfall of energy to milk production is considered to be accomplished by mobilizing body reserves (Pryce and Løvendahl, 1999). The obese cows at calving time may probably yield less milk along with increase in reproductive and health disorders (Morrow, 1976; Gearhart et al., 1990).

Energy shortfall in early lactation enhances, but energy intake does not maintain pace with continuously increasing milk yield, creating a competitive situation among milk yield, fertility and health status of the dairy cow as all these traits are interlinked with energy (Staufenbiel et al., 1992). As a result, reduced reproductive performance with higher milk yield is observed (Spalding et al., 1975; Faust et al., 1988). High-yielding herds usually get very limited dry period, suggesting that the drop in fertility can be compensated by proper management (Laben et al., 1982; Nebel and McGilliard, 1993). According to Várhegyi (1999), from the time of parturition until the peak of lactation stage energy and protein needs raised by four to ten times.

Reynolds and Beever (1995) observed that at optimum production level body tissue mobilization may normally maintain up to 7 kg of milk per day. High yielding dairy cows needs a lot of glucose, mostly to synthesize milk lactose, fat and maintenance of the nervous system. A cow whose milk production is about 30-50 kg per day will demand for about 2.7-4.0 kg glucose on daily basis (Tóth and Schmidt, 2004). Also, Flachowsky and Lebzien (1997) observed that considering the diet, not greater than 0.5-1.0 kg glucose could be absorbed from the small intestine because microbes also utilized the carbohydrates during fermentation in the rumen. Furthermore, 520-540 g glucose can be reserved in the liver and blood plasma while some 1.2-1.3 kg glucose needs can be met
through gluconeogenesis in a cow with a production level of 30-50 kg milk/day (Schmidt et al., 2006).

Most research work conducted on BCS in relation to milk production are from countries with temperate environment, predominantly on their specific breeds of cows like Holstein Friesian (Treacher et al., 1986; Garnsworthy and Jones, 1987) plus the ration provided to animals is mainly in the mixed form (Domecq et al., 1997). In local dairy buffaloes, relationship of BCS with productivity and fertility has been extensively studied by our group (Qureshi et al., 2002a). BCS was significantly affected by the period of calving and season of the year and in turn it affected fat-corrected milk yield ($r=0.26$, $P<0.05$). Animals calving during the normal breeding season had higher BCS. The post parturition estrus interval ($r=-0.20$) negatively correlated with BCS. Buffaloes resuming estrus activity was having continually higher BCS compare to those showing no estrus activity.

With the increase in awareness regarding quality of milk, consumers are now demanding milk with higher concentration of unsaturated fatty acids (UFAs), which are essential for good health. The three SFAs, C$_{12:0}$, C$_{14:0}$ and C$_{16:0}$ are considered to be hypercholesterolemic (Williams, 2000) and comprise almost 44% of the total milk fatty acids. However, Clandinin et al., (2000) suggests that palmitic acid (C$_{16:0}$) may not harmfully effect if the availability of C$_{18:2}$ n−6 is fulfilled; stearic acid (C$_{18:0}$) is largely neutral, while oleic, linoleic and $\alpha$-linolenic acids are considered cardioprotective (Djoussé et al., 2001; Bemelmans et al., 2002). Conjugated linoleic acid (CLA), vaccinic (C$_{18:1}$ trans11), linolenic (C$_{18:3}$) and particularly rumenic acid (C$_{18:2}$ cis-9, trans11) have shown positive health effect like preventing mammary gland and skin cancer in experimental animals (Ha et al., 1990; Ip et al., 1994). Vaccinic acid has been linked with anti-carcinogenic characteristics owing to its conversion into rumenic acid (Turpeinen et al., 2002; Corl et al., 2003).

With initiation of adipose tissues mobilization the percentage of stearic, oleic as well as short chain fatty acids increases speedily (Laasko et al., 1996). The difference was independent of seasonal or nutritional effect and was attributed to the physiological incapability of cows at the start of lactation to adjust to higher dry matter consumption in order to accomplish the energy needs. Throughout the early stage of lactation the
synthesis of C₄₀ to C₁₂₀ increased initially and then decreased while lipolysis of fatty acids from adipose tissues increased (Palmquist et al., 1993).

Cattle and buffalo milk has been a major item of human diet in the south East Asia and the Mediterranean region. Little information is available about the milk fatty acids profile in relation to management and physiological states of the local dairy animals. BCS may play a role in regulating appetite and feed intake thereby affecting milk production and its composition, especially the fatty acids profile in dairy animals under subtropical conditions. Therefore, the recent study was conducted with the following objectives:

**Objectives:**

1. To study variation in milk fatty acids profile in relation to body condition in dairy buffaloes.
2. To examine changes in milk fatty acid profile with lactation advancement in dairy cows.
3. To investigate relationship of body condition with milk yield and composition in buffaloes.
II. REVIEW OF LITERATURE

Milk production and its composition are the outcome of metabolism and the transportation of substrates to the mammary tissues through blood circulation. The transportation of substrates is controlled by various physiological and management factors affecting the homeostasis and nutritional partitioning in the animals’ body. BCS is an intermediate factor between the feed intake and the productivity status of the animal. The extra energy consumed by the animal is deposited in the form of adipose tissues reserves, which transmits chemical signals for influencing the nutrients intake, milk yield and composition.

The information generated so far on this subject, has been reviewed under the following subtitles.

2.1 Body condition score

BCS is a subjective, visual or physical assessment of the quantity of metabolizable energy deposited in the fat and muscle of a live animal. It has been considered an effective tool in monitoring the energy intake of cows and herds (Jeffrey and James, 1989). BCS has been recommended to assess alterations in body reserves due to negative energy balance (Berry et al., 2002). The condition scoring at calving is a direct measurement of the nutritional status before parturition and reproduction can be enhanced in beef herds by evaluating BCS as a criterion for determining nutritional status (Spitzer, 1986). The author also recommended sorting the cows by condition, 90 to 100 days before calving and feeding the animals to maintain a BCS of 5 to 7 to maximize reproductive performance and minimize supplemental feeding.

Until 1970s, no mechanism was available to determine the cow’s energy reserves. Although, the animal body weight (BW) recording was a usual practice but it did not provide reliable estimate of the energy reserves, as the reserves vary about 40% in cows with same body weight (Gibb et al., 1992; Andrew et al., 1994). Therefore, assessment of body condition scoring is suggested for accurate determination of energy reserves (Macdonald and Roche, 2004). Wright and Russel (1984) found a significantly positive correlation ($r^2 = 0.86$) between a live animal visual evaluation of body condition and the
actual dissected fat in Friesian cows. The findings suggested that BCS is a practical evaluation of body fat reserves, reducing the frame size and gut fill effects. Moreover, BCS is a very simple, least expensive and applicable technique to be practiced on farms, increasing the evaluation of large numbers of animals at a time and hence enhancing the numerical power in research and revealing meaningful relations with production, reproduction and health status of the herds of animals.

The body condition is usually judged through a 5-point scale, with 1 equivalent to an extremely lean cow, while 5 to a cow having excessive fat reserves (Peters and Ball, 1987). Body reserves are better reflected by BCS than by live weight change (Grainger et al., 1982; Johnson, 1984; Ducker et al., 1985). Coffey et al. (2003) reported that changes in energy balance are reflected in subsequent changes in the BCS of cow during the entire lactation period. In high yielding Holstein-Friesian cows (Yamada et al., 1994) the first insemination conception rate was significantly lower in cows with a markedly decreased body condition score during early to mid lactation period. These results indicated that monitoring the BCS during a period from drying to early and mid lactation might be a useful tool in the prevention of peri-parturient disease and infertility after parturition.

From the above review it is cleared that body condition scoring is a useful and reliable monitoring tool to be used in routine farm practices. The technique is very simple and its applicability will enhance production efficiency.

2.2 Postpartum BCS loss

The mobilization of body energy reserves is the major capability of the dairy cow. The mobilization of reserves is indispensable for maintaining high milk yield following parturition. Like the other lactating animals, the dairy cows are normally in negative energy balance in the start of lactation (Nielsen, 1999). The genetic correlations between milk yield and dry matter consumption showed that energy intake of higher milk producing Holstein cows during initial lactation is almost half of the energy required for production purposes (Veerkamp et al., 1995). The change in BCS at the start of lactation point towards the level of metabolic load as the shortfall of energy to milk production is considered to be met through mobilizing body reserves (Pryce and Løvendahl, 1999). The
over conditioned cows at calving time may probably yield less milk along with increased in reproductive and health disorders (Morrow, 1976; Gearhart et al., 1990).

Although, the feed intake is increasing in early lactation but the tissues reserves are also mobilized at that time, the extent of body tissue loss may be overlapped by gut fill, thus body weight changes may not indicate changes in bio-energetically vital tissues (National Research Council, 2001). Veerkamp and Brotherstone (1997) concluded that the selection for milk yield increases the space between energy consumption and expenditure at the initial stage of lactation, as in achieving the breeding objective the dry matter intake (DMI) has been overlooked. Since energy intake does not maintain its pace with continuously increasing milk yield. The energy shortfall in early lactation enhances, creating a competitive conditions among milk yield, fertility and health status of the dairy cow as all these traits are interlinked with energy (Staufenbiel et al., 1992). Qureshi et al. (2007) reported that buffalo does not produce at the cost of its own body reserves under tropical conditions, showing an earlier post conception decline in milk yield.

It can be concluded from the above discussion that energy balance is not properly maintained during lactation as a result productivity of the animal is affected. Lactation is an alternating cycle of lipolysis and lipogenesis in body stores that allow the cow to meet her energy requirements for milk secretion. BCS may be a useful tool to indicate the energy status of the animal and to compensate the shortfall immediately.

### 2.3 Postpartum BCS and metabolic status

Adipose tissue experiences coordinated metabolic adaptations during pregnancy and lactation. These adaptations comprising of mid pregnancy anabolism then a major shift to catabolism in late pregnancy and a remarkable catabolic shift in early lactation (Kalkhoff et al., 1978; McNamara et al., 1985) after achieving peak of lactation, anabolism again take over (McNamara and Hillers, 1986). The sympathetic nervous system (SNS) innervates (Wirsen, 1965) and applies a tonic regulation on adipose tissue mobilization (Havel and Goldfien, 1959). The SNS action as measured by net energy liberates from nerve endings is changed during exercise (Pequignot et al., 1980), fasting and fatness (Rath et al., 1973). Restriction of SNS activity by blockers of net energy synthesis, storage and transmission consequently increased withholding of fat in adipose tissue.
(Dulloo et al., 1985). Probably the SNS and the tissue adrenergic receptor in coordination with the insulin and lipid synthesis system regulate the direction and magnitude of adipose tissue fat metabolic activity during lactation.

According to Várhegyi (1999), from the calving until the attainment of peak milk yield the energy and protein needs raised by four to ten times. Reynolds and Beever (1995) observed that at optimum production level body tissue mobilization may normally keep up about 7 kg of milk per day. High yielding dairy cows need a lot of glucose, mostly to synthesize milk lactose and fat along with maintenance of the nervous system. A cow whose milk production is 30-50 kg per day will require about 2.7-4.0 kg glucose on daily basis (Tóth and Schmidt, 2004). Also, Flachowsky and Lebzien (1997) reported that considering the diet, not greater than 0.5-1.0 kg glucose could be absorbed from the intestine owing to the consumption of carbohydrates in the rumen during microbial fermentation. Furthermore, 520-540 g glucose can be reserved in the liver and blood plasma while some 1.2-1.3 kg glucose needs can be attained through gluconeogenesis in a cow with a production level of 30-50 kg milk/day (Schmidt et al., 2006).

The amount and rate of tissue mobilization possibly depends upon several factors such as the body condition, stage of lactation, milk production level, hormonal treatment with bovine somatotropin and diet composition (Chilliard et al., 1991; McGuffey et al., 1991: McNamara et al., 1995). Lower energy mobilization is an attribute of cows with lower milk production (Hart et al., 1979). According to Madhav et al. (1998) that high producing dairy cows mobilize both body fat and to a further limited quantity, body protein in early period of lactation. A large amount of energy is mobilized during the earlier weeks after parturition and accounts for about 30% of prepartum energy deposits.

The mammary cell is a highly organized factory, whose rate of secretion is very high. The udder utilizes about 80% of total glucose, acetate and amino acids available in blood (Bath et al., 1985). Broster and Broster (1998) reviewed that the majority of experiments have shown a drop in dry matter intake (DMI) with the increase in BCS at calving, thorough utilization of body reserves leads to a high plasma non-esterified fatty acids (NEFA) levels that depress cow’s appetite as well as DMI (Overton and Waldron, 2004). Cows in higher body condition eat less and will probably in a more negative energy balance compared with cows having lower body condition score, offered the same diet
(Grummer et al., 2004). Garnsworthy (1988) reported an inconsistent relationship between BCS at parturition (PBCS) and milk yield and cows with higher BCS at calving lose more body condition during lactation, which may lower milk yield. The cows that are over conditioned at calving may encounter increased reproductive and health problems along with lower production (Fronk et al., 1980; Boisclair et al., 1986; Gearhart et al., 1990).

2.4 BCS affects milk yield and composition

Pedron et al. (1993) observed that change in BCS affected the peak yield and the whole lactation curve. In contrast, Waltner et al. (1993) suggested that pre-partum BCS and a change in BCS during lactation were associated to total yield of 3.5% fat corrected milk (FCM) at 90 day of lactation. Wildman et al., (1982) reported that dairy merit was decreased by more than 50 % when there was increased in body condition scores. The over conditioned dairy cow is inefficient for milk production whether due to stage of lactation or solely conditioning. Higher genetic merit cows having higher milk production potential have greater BCS loss in early lactation compare to lower genetic merit cows (Gainger et al., 1985; Veerkamp et al., 1994).

Jílek et al. (2008) reported that cows with moderate BCS showed the highest milk yield in the first 5 months of lactation period. Roche et al. (2007) reported that optimal level of BCS at calving with regard to milk production was around 3.5 (1 to 5 scale). Although, a non-significant improvement in milk yield was recorded beyond a BCS of 3.0.

The high genetic merit dairy cattle have a higher tendency for mobilization of body fat reserves to cover milk production demands (Pryce et al., 2002). This was demonstrated in cows chosen for higher milk production (Berry et al., 2003). These cows had lower BCS during the lactation period and the changes in BCS immediately after calving were higher than the lower producing cows (Horan et al., 2005). Thus, mobilization of body fat reserves and milk production are closely related (Pryce et al., 2002). BCS and milk yield are in a negative correlation (Veerkamp and Brotherstone, 1997) and high yielding dairy cows generally have a lower BCS (Pryce et al., 2001). Cows that are genetically inclined to lose more BCS in early lactation tend to have higher
yields of milk, fat and protein (Dechow et al., 2002). Enhancing the feeding intensity before parturition resulted in improvement in milk production (Broster and Thomas, 1981). It was attributed to the improved mammogenesis and lactogenesis in later stage of pregnancy and earlier weeks of lactation to enhance digestive adjustment, plus better body condition of the cow. Obese cows normally consumed less but produce the similar quantity of milk compared to average weight cows, probably due to body fat mobilization (Garnsworthy, 1988).

It may be suggested on the basis of the above referred studies that BCS reflects the nutritional status of an animal and its loss is associated with negative energy balance. Prepartum BCS and changes in postpartum BCS are good indicators of milk production during the entire length of lactation in cattle and buffaloes.

### 2.5 Lactation stage affects milk yield

The efficiency of milk synthesis depends upon the number alveoli (mammary secreting cells) and the maintenance of the metabolic action of each and every alveolus. The final stage of mammary gland development specifically the alveoli growth is accelerated in the last trimester of pregnancy (Bath et al., 1985). It is directed by estrogens, progesterone, prolactin and growth hormones etc. The abundant milk secretion is encouraged by the high level of prolactin and adrenal steroids, immediately after decline in progesterone with the onset of lactation (Forsyth, 1983).

The advancement of lactation is associated with changes in hormonal concentration and in lactation efficiency of dairy animals. The normal lactation curve for cattle and buffaloes shows a rapidly increasing trend during the initial 3 to 6 weeks after parturition and a gradual decline afterwards (Bath et al., 1985). Huth (1995) has showed in his experiments that variation in cow milk yield can be seen in the following day after feed deficiency. The maximum variation in milk yield and fat content was observed at the beginning of lactation (first four weeks of lactation) and at the end (weeks 39-42) while the middle period of lactation was relatively stable (weeks 19-22). Aziz et al. (2003) observed six lactation records and documented the values of the early milk yield (a) varying between 30.30-44.38 kg, rate of increase to attain peak yield (b) ranged between 0.17-0.27 kg and the rate of decrease after achieving peak production (c) were between
0.02-0.03 kg. Gondal (1985) worked on Pakistani buffaloes for upto 39 wks of lactation. Peak yield was reported on 8th wk postpartum. The values for a, b and c were 37.45, 300 and 0.037, respectively.

The advancement of lactation is associated with changes in hormonal concentration well as in lactation efficiency of dairy animals. Peak milk production and maximum variation is observed during early lactation period. Therefore, great attention is required during that specific period in order to achieve maximum productivity.

2.6 Lactation stage affects milk composition

The hormonal changes during lactation result in changes in the composition of milk. An inverse relationship between milk yield with its protein and fat contents exists (Eckles et al., 1973; Banerjee, 1985). As yield increases, the percentage composition of fat and protein decreases. Lactose shows a little decline while ash contents slightly increases with advancing lactation. Egbowon (2004) reported that milk protein and fat percentages are inversely related to milk yield. The fat and protein are at moderate level in initial stage, decreased to the minimum level during peak lactation and gradually increased towards the end of lactation. Harris and Bachman (2008) suggested that with the onset of lactation, solids-not fats (SNF) content is relatively high in the first month, drops to a low in the second and further increases as lactation progresses. Pavic et al. (2002) stated that in the middle and at the end of the lactation period the contents of total solids, fat, protein and pH value were higher (P< 0.01) than at the beginning.

Madsen (1975) reported that very high milk yield at the beginning of lactation (steep lactation curve) puts a high physiological stress on cows, which often leads to reproductive disorders. Attaining peak milk yield earlier in lactation would indicate that positive energy balance is being accomplished at an earlier stage in lactation. Malau-Aduli et al. (2001) reported that breed, age, stage of lactation, season and plane of nutrition significantly affect milk yield and composition in goats. Boros (1986) observed that lactose was fairly constant over the lactation period showing no considerable changes.

The percentage of fatty acids synthesized de novo increases while the proportion of all C18 fatty acids decreases at the start of lactation (Karijord et al., 1982; Lynch et al.,
Aludist et al. (1998) evaluated the relationship of lactation stage with conjugated linoleic acid (CLA) on pasture based systems throughout the length of lactation and observed a slight increasing trend (7.9 to 9.7 mg/g of fatty acids) in advanced lactation.

Milk yield and composition are inversely related and change in one parameter will ultimately leads to change in other. Therefore, both should be simultaneously observed during lactation period for achieving higher milk quality.

2.7 The pregnancy affect

Milk requires various substrates for its synthesis and with the advancement of pregnancy to the third trimester more nutrient substrates are diverted towards development of fetus, placenta and fluids. Shah et al. (2009) reported that the increasing fertility status in dairy animals was associated with an increase in blood glucose levels from 6.0 to 9.5 mg/dl in dairy animals while the milk lactose level decreased from 3.9 to 3.4 % at the same time. Bachman et al. (1988) and Akers (2002) have attributed the post conception decline in milk yield of dairy cows to hormonal changes. Apart from hormonal effect, yield losses might be due to the nutritive requirements of the fetus.

Freetly and Ferrell (1998) reported that the energy needs during pregnancy comprises of the energy deposited in the fetus, plus the energy utilized for the fetus metabolism and maternal tissues to carry the fetus. Qureshi et al. (2007) suggested that fertility and milk yield have genetic relationship leading to production of animals with higher yield and improved fertility for selected animals. In the later study they observed that fat-corrected milk (FCM) was 14.50±0.20 kg/d, varying from 2 to 35 kg/d; and was higher in the estrus group, associated positively with post parturition ovulation interval (r = 0.31, P < 0.01 (Qureshi and Ahmad, 2008). BCS was constantly higher in buffaloes resuming estrus than those failing to restart ovarian cyclicity. Fulkerson (1984) and Moate and Harris (1983) also showed a positive relationship between milk production and reproduction in pasture-based systems.

Pryce and Veerkamp (2001) concluded that about 50% of the total progress in milk yield in the UK can be ascribed to genetic improvement only. However, an antagonistic association between milk production and several fertility traits was observed (Hoekstra et al., 1994; Beam and Butler, 1999; Darwash et al., 1999; Royal et al., 2000). Raheja et al.
(1989) have found no relationship between milk production and reproduction. Wanner (1991) suggested that high yielding cows are subjected to fertility problems but often human fault causes disturbances, especially in feeding. It was argued that higher milk yield and good fertility are not mutually exclusive, provided that optimal rumen function can be achieved by feeding high quality feeds correctly and by supplying essential nutrients according to requirements.

Early and late conception relation with the milk yield was reported in buffaloes (Qureshi et al., 2007). An earlier decline in post-conception milk yield was observed in the buffaloes conceived in earlier weeks of lactation, than in those conceived later on. An obvious decline in milk yield was observed within 3rd, 5th and 6th month of gestation in the animals conceived at initial, mid or advanced lactation stage. In pregnant animals the milk yield increases up to 8 weeks following conception and then decreases steadily. In a later study on dairy buffaloes, these authors (Khan et al., 2009) suggested that there was a coherent declining pattern in milk yield with delaying conception, associated with increasing calving interval. Animals conceived at advanced stage of lactation demonstrated a decline in economic return of 24 to 27% in comparison to those conceiving earlier.

Ehrhardt et al. (2001) concluded that the plasma leptin hormone concentration increases in the first trimester of pregnancy in ewes while in the advanced pregnancy in cows. The level of leptin decreases immediately before calving and stay low during lactation in both ewes and cows. Holtenious et al. (2003) and Liefers et al. (2003) stated that leptin hormone is produced by adipose tissue and controlling the adipose tissue mass by discouraging appetite and enhancing energy expenditure. In ewes and cows circulating leptin concentrations were positively related to the energy consumption daily in the short term and the level of adiposity and nutrition in the long term.

Interaction of pregnancy with milk composition was investigated by Shah et al. (2009) in dairy buffaloes. A significant effect of pregnancy and weeks post-conception (PC) on milk yield was observed. Pregnancy and weeks PC affected SNF, protein and lactose (%). Milk fats contents of non-pregnant buffaloes were higher than pregnant buffaloes. Pregnant animals with supplemented ration showed an increased concentration of milk fats and lactose. Milk fats increased linearly with the PC weeks in all the three
production groups. Milk protein declined with PC weeks.

Mather and Lal (1969) and Wilcox et al. (1959) noted pregnancy effects on fat, protein and SNF percentages after 4 month of pregnancy for mature cows and slightly later for heifers. Loganathan and Thompson (1967) reported significant effects of stage of pregnancy on fat yield and SNF percentages, but pregnancy contributed only less than 0.5% of the total variance in each. Sharma et al. (1990) concluded that milk yield declined and constituent percentages increased after 3 months of lactation.

2.8 The progesterone effect

Earlier findings have attributed the post conception decline in milk yield of dairy cows to hormonal changes (Bachman et al., 1988; Akers, 2002). Our previous study (Khan et al., 2009) concluded that post conception milk yield decline increases with the increase in progesterone concentration over 6.44 ng/ml.

Qureshi et al. (2000) observed poor reproductive performance in buffaloes after parturation in low breeding season. This was attributed to the inadequate production of progesterone by the corpus luteum compare to normal breeding season. El-Wishy (2007), studied hormonal alteration and uterine involution immediately after parturition in buffaloes and reported the various trends of estrogens and progesterone in the advanced pregnant stage and throughout parturition time. An increase in progesterone level after parturition was observed in buffaloes (Qureshi and Ahmad, 2008). A little increase during the first month followed by a swift increase in the second; a plateau up to fourth and an immediate increase in concentration afterwards.

Knight and Wilde (1993) observed a decrease in mammary cells number with the subsequent decrease in milk yield after achieving the peak. Also, Forsyth (1999) linked the decrease in milk production with the commencement of pregnancy accompanied by increase in progesterone level and decrease in milk synthesis and secretion.

It was concluded from the above discussion that progesterone is the major hormone required for maintaining pregnancy and its altered level with advancement of lactation may affect milk yield and its composition.
2.9 The age and heritability factors

The increasing age results in alteration in cellular functions leading to an initial increase and a later decrease in milk production efficiency. This has been confirmed by Khan and Qureshi (2009) in a series of studies on dairy buffaloes. The mature cows produce about 25% more milk than 2 years old heifer. The increase in milk yield with the increasing age was partially (5%) attributed to higher body weight, whereas the remaining (20%) is the result of increased development of the udder during recurring pregnancies (Bath et al., 1985). Similarly, Tahir et al. (1989), Sardar et al. (1997) and Afzal et al. (2007) also reported significant effect of parity on milk production and found more milk in cows with greater parities than those with lesser parities. Bajwa et al. (2004) and Lee and Kim (2006) reported increase in milk yield towards 5th parity and decline thereafter.

The heritability of fat, protein and solids-not-fat contents (0.50) is higher than milk yield (0.30, Bath et al., 1985), leaving little room for non-genetic factors to affect milk composition. Agnihotri and Rajkumar (2007) reported significant effect of parity on milk yield while the changes in total solids, fat, SNF, ash casein were not significant in goat’s milk. Pal et al. (1996) also did not observe the effect of parity on various milk constituents except casein, which was affected due to dam’s parity. DePeters et al. (1995) studied the fatty acid composition of milk in Holstein, Jersey and Brown Swiss. The percentage of short and medium chain fatty acids as well as milk fat content was genetically positively correlated (Karijord et al., 1982). Stull and Brown (1964) and Beaulieu and Palmique (1995) reported that C6 to C14 of the total milk fat content were about 8 to 42% high in Jersey compare to Holsteins and the difference was independent of feed effect. In Jersey C18:0 was 13% higher and oleic acid 15% lower. The steaeryl CoA desaturase (SCD) activity is also reported by many authors within and among breeds and thus the proportion of C18:0 and C18:1 also varies. Aludist et al. (2004) showed that Freisian cows had more CLA concentration than Jersey milk fat, suggesting less active SCD activity in Jersey. Whitelock et al. (2002) reported the diet vs. breed interaction in Brown Swiss and Holstein cows. White et al. (2001) have studied the various feeding managements in Holstein and Jersey cows and reported slightly higher CLA in Holsteins.
2.10 The nutritional intake and partitioning

In lactating animals, mammary tissues metabolic activity is encouraged by galactopoietics hormones and above all somatotropin (BST) have a vital role. The organization of extra-mammary metabolic activity to make sure the prioritization of the mammary tissues for nutrients uptake is also associated with BST (Bauman and Currie, 1980). The main effect of BST is to activate the mammary gland through activation of somatomedin production and reduction of glucose as well as amino acid oxidation, at the cost of adipose tissue long-chain fatty acids. In early lactating animals insulin secretion and tissues reaction to insulin decreases while glucagon secretion is retained or enhanced. Consequently, glucose production in liver and lipolysis from adipose tissue reserves increases while the glucose and amino acid uptake decreases in adipose and muscular tissues (Chilliard, 1987). During early lactation a decline in the thyroid hormones release was observed, possibly discouraging the basal energy expenses and protein yield (Aceves et al., 1985). Nutrient consumption efficiency of milk yield depends upon the balance between the various nutrients of glucose, fats and protein coming from the digestive system and the requirements of the mammary tissues for each individual nutrient (Preston and Leng, 1986).

Perry and Stalling (1993) reported that milk fat concentration increased from 3.36% to 3.69% by increasing fiber from 17% to 25%. Egbowon (2004) reported that under-feeding causes lower proportion of milk components. The major end products of microbial fermentation of the substrates in the rumen are volatile fatty acids (VFA’s), various gases and microbial protein. The proportions and concentration of VFA’s depends on the type of substrates and microbial species in the rumen. Propionic acids predominate the concentrate based diet while, acetic acid on forage based diets (Shah, 1994).

According to Sarwar et al. (2002), the majority of nutrient requirements of dairy animals are met through feeding forages in Pakistan in contrast to advanced countries, where they are fed liberal amounts of grains. Harris and Bachman (2008) reported that nutrition of the cow has visible effect on milk composition, specifically the fat content of milk. The SNF content of milk also varies with changes in the ration but to a lesser extent than the fat content. Feeding extra energy to high producing cows may improve the SNF
by about 0.2 percentage units. While reducing the energy fed to high producing cows below requirements may decrease SNF as much as 0.2-0.5 percentage units. Forage quality and quantity affect milk SNF. Increasing roughage intake usually reduces SNF and milk production. The decline is mainly due to reduction in energy or dry matter intake. Improving energy or dry matter intake will restore the SNF to its optimal level. With intake of good quality hay SNF increases, but with poor quality both intake and SNF contents will be lower. Adding more roughage to the ration has negligible effect on SNF. However, a minimum amount of roughage is needed for normal milk fat percent and health maintenance of the cow.

It can be reviewed from the above discussion that feed provides various substrates for milk synthesis and the homeostatic control in animal body channelizes the metabolites towards growth, lactation and reproductive cyclicity. With changes in physiological status the body requirements also vary and consequently affecting the nutritional partitioning.

2.11 Dietary sources of milk fatty acids

Fatty acids of milk are contributed by various sources comprising, dietary intake, lipolysis, desaturation and de novo synthesis. In the rumen the cellulose and hemicellulose in the animals feed are converted to acetate, propionate and butyrate by the microbial fermentation within the rumen. These precursors are released into the blood stream and while butyrate is converted to hydroxybutyrate inside rumen wall. Majority of fatty acids coming from plants sources in ruminants’ diets are LCFA and are highly unsaturated ones but the rumen microbes quickly convert them into SFAs, that is why there is a higher concentration of SFAs in cow’s milk (Bath et al. 1985).

The ration contributes precursors of fatty acid to the metabolic pool through rumen fermentation and digestion in the small intestine. Dietary source of fatty acids have been reported by Bauman and Currie (1980) during lactation in cattle. The decline in adipose tissues lipoprotein lipase activity together with the energy demands of milk production directed nutrients towards milk synthesis and production. This resulted in little dietary lipids make their way to the adipocyte for deposition. A research work in lactating beef (Bottger et al., 2002) as well as dairy (McNamara et al., 1995) cows have identified food
derived lipids as nutritional partitioning nutraceuticals. Nutraceuticals were defined as nutrients having physiological effects external to their commonly accepted functions (Williams and Stanko, 2000).

Milk fat is largely hypercholesterolemic in nature in contrast to polyunsaturated oils (Noakes et al., 1996). Lauric, Myristic and Palmitic acids are mainly responsible for this hypercholesterolemic characteristic. Now it is a great challenge for the dairy industry to increase the proportion of UFAs instead of SFAs.

Beam et al. (2000) observed during an in vitro study of rumen contents that addition of C_{18:2} in soybean oil reduced the rate of lipolysis. A higher proportion of C_{18:2} within oils resulted in the drop of biohydrogenation rate. Murphy et al. (1995) offered soybean or rapeseed oil to grazing dairy cows and reported an increase in concentration of long chain fatty acids and decrease in short and medium chain fatty acids. Crocker et al. (1998) reported that lactating dairy cows needs dietary ingredients high in energy like starch, but processing of corn (steam flaking and dry rolling) is required to improve starch digestibility. Milk yield and composition was almost same for both types and changes in fatty acid composition apart from C_{18:1} and C_{18:2}. Bayourthe et al. (2000) evaluated various types of canola oils and canola protein on milk composition and physical characteristics of butter. Canola oil was having 58% C_{18:1}, 20% C_{18:2} and only 6% of C_{16:0}. With the intake of canola oil the percentage of C_{12:0} to C_{16:0} decreased while C_{18:1} increased.

Ashes et al. (1997) analyzed the potential of supplemental fats in relation to milk fat content and composition. Pelleted or prilled fats and starch and vegetable-blended fats, yellow grease, oil protected by formaldehyde-treated protein and protein supplements and oleamides were used. The supplements increased the energy concentration of feed and alter the fatty acids composition of milk. Avila et al. (2000) found that the animal requires energy in their ration in order to increase milk production but the fiber allowance should not be ignored. Supplemental fats are fulfilling the energy requirements like tallow and yellow grease can be acquired at reasonable rate. When both of the energy sources were offered to Holstein cows, the supplementation resulted in enhanced milk yield, fat and vaccenic acid (11t-18:1).
Rumen-protected fats were procured through treatment of oil seeds and pure fats to escape ruminal digestion (Mansbridge and Blake, 1997). Noakes et al. (1996) observed a slight decline in LDL-cholesterol; 4.49 to 4.25 mmol/L by adding modified milk fat in the human diets. An unsaturated milk fatty acids were obtained by the inclusion of protected oils denatured by formaldehyde action. Wright et al. (1998a, 1998b) offered a non-degradable protein supplement having fishmeal which contains 22:6 n-3 that is very important for the newborns. The concentration enhanced almost 0.15 to 0.33% of the total LCFAs in milk.

Aigster et al. (2000) offered calcium salts of sunflower oil to dairy cows. Since oleic acid is the major fatty acid in sunflower oil and its concentration increased from 26.3 to 40.2% in the original and treated one, respectively. Also the sum of hypercholesterolemic fatty acids decreased from 40 to 33%. Mansbridge and Blake (1997) recommended that in order to avoid the rumen biohydrogenation, the dietary oil may be protected by its conversion into calcium. A further method used for avoiding ruminal disturbance is the formation of fatty acyl amides and butylamine with the soybean oil (Jenkins et al., 1996). The proportion of linoleic acid increased while lauric acid and myristic also increased in relation to soybean oil but comparatively decreased with control. The concentration of \( C_{18:1-t} \), \( C_{18:1} \) and protein also reduced. Although, Jenkins (1998) reported almost similar findings but \( C_{18:1-t} \) contents were not investigated in their study.

It may be concluded that dietary sources provide fatty acids through rumen degradation of nutrients and enzymatic digestion in the intestine. Also, a major source providing precursors for milk fatty acids synthesis. Therefore, research work is required to explore the balance of dietary fatty acids that support the desirable proportion of LDL and HDL concentration in blood in terms of human health.

### 2.12 Lipolytic source of fatty acids

Approximately 50% of milk fat is derived directly from the dietary long chain fatty acids (LCFA) or from body stores. Lake et al. (2006) studied the fatty acid profile of blood plasma, adipose tissues and milk fatty acid in lactating (Angus × Gelbvieh) beef cows. They suggested that moderate BCS will provide greater percentage of \( C_{18:0} \) and \( C_{18:1 \ trans-10} \) in milk which was attributed to the dietary source. In adipose tissue, \( C_{16:0} \),
C\textsubscript{18:0} and C\textsubscript{18:1 cis-9}, account for nearly 90% of fatty acids in molar proportions (Christie, 1981) and body fat mobilization would probably increase direct accumulation of these fatty acids into milk fat. Desaturation of stearic acid occurs in the intestinal epithelium and mammary tissues (Enoch et al., 1976). Some 40–50% of C\textsubscript{18:1 cis-9} in milk is derived from the desaturation of C\textsubscript{18:0} through the activity of desaturase enzyme within the mammary tissues (Chillard et al., 2000).

A lower BCS cows will largely dependent on the dietary sources for milk fatty acids synthesis rather than on the adipose tissues reserves (Pedron et al., 1993). Acetate is the major precursor for synthesis of short-chain fatty acids whereas, body fat or ingested lipids are associated with long-chain fatty acids (Payne et al., 1979).

High genetic merit dairy cattle have a higher predisposition for mobilization of body fat reserves to cover milk production demands (Veerkamp, 1998; Pryce et al., 2002). Veerkamp and Brotherstone (1997) found that BCS and milk yield are in a negative correlation. Higher genetic merit cows in regard to milk production have consistently higher rates of lipolysis and hormone sensitive lipase activity (McNamara et al., 1987; Smith and McNamara, 1990). Garnworthy and Topps (1982) reported that dairy animals maintained a physiological target for BCS during lactation.

From the above review it may be concluded that lipolysis or body reserves mobilization may be an indicator for milk production as well as for its composition. Thus, optimum level of BCS should be explored in order to attain maximum productivity and quality milk.

2.13 De novo synthesis and desaturation

Milk fat may either derived directly dietary source as preformed fatty acids or from de novo synthesis inside the mammary cells. De novo synthesis of most of the C\textsubscript{4:0} to C\textsubscript{14:0} and partly C\textsubscript{16:0} occurs in the mammary cells from VFA’s (acetate and β-hydroxybutyrate which resulted from the microbial fermentation inside the rumen (Mansbridge and Blake, 1997). Fatty acids synthesis essentially involves the conversion of acetyl-CoA to malonyl-CoA that is further used in a stepwise chain elongation process leading to a series of short and medium chain SFAs (Hawke and Taylor, 1995). The ruminal biohydrogenation is a main hindrance in the incorporation of UFAs present in the
dietary sources into milk fat (Jenkins, 1993).

Neville and Picciano (1997) reported that mammary gland development is parallel with the speed of fat synthesis in lactating mammals and reduces with the malnutrition in ruminants and rodents except seals and hibernating bears that are normally on fasting during lactation. Whereby, non-esterified fatty acids, insulin and prolactin play there role in its regulation. The food having trans-fatty acids may decelerate the milk fat synthesis under certain situations by affecting the action of acetyl coenzyme-A.

Higher percentages of C_{12:0} and C_{14:0} in milk from higher BCS beef cows was reported by Lake et al. (2006) advocating more de novo synthesis of fatty acids inside the mammary tissues. The de novo synthesis resulted in the short and medium chain and partly C_{16:0} from the mammary gland utilization of actate and β-hydroxybutyrate (Bath et al., 1985). Fatty acid synthase accelerates the conversion of acetyl-Co A and malonyl-Co A into various types of fatty acids up to C_{16:0} (Yin et al., 2001). According to Palmquist et al. (1993) the fatty acids synthesized de novo in the mammary cells are from C_{6:0} to C_{14:0} and partially C_{16:0}.

The milk from early lactation has less C_{4:0} to C_{12:0} than the mid and late lactation cow’s milk in New Zealand (Auldist et al., 1998). The changes observed was not due to any effect of season or feed but was associated with the physiological incapability of cows at the start of lactation to adjust to higher feed intake to fulfill the energy demands. In early lactation the synthesis of C_{4:0} to C_{12:0} increases and then decreases while lipolysis from adipocytes increases (Palmquist et al., 1993). Rowney and Christian (1996) examined diet and lactation stage effects on milk. It was concluded that the diet quality had the major influence on milk quality.

Diets having Rapessd, Soybean and Linseed are higher in oleic, linoleic and linolenic acid respectively and generally resulting in increased concentration of these fatty acids in milk. Fish oil has been used to supply the LCFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and can stimulate the elevated level of CLA (Chillard et al., 2000). LaCount et al. (1994) infused fatty acids from canola or sunflower oil in to the abomasums of lactating cattle. The concentration of oleic acid increased in milk and all other C_{18:0} remained unchanged. Jenkins (1998) reported an increase of 48% oleic acid of the total milk fatty acids by offering oleamide as a rumen protected fat. Dhiman et al.
(1999) showed higher CLA content with cattle grazing pasture than when fed pasture plus grain.

In Mediterranean dairy buffaloes activity of \( \Delta^9 \)-desaturase enzyme was studied (Fernandes et al., 2007). The farm maintained on pasture and corn silage showed higher ratios of UFA/SFA indicating greater \( \Delta^9 \)-desaturase activity. The stearoyl-coenzyme A (CoA) desaturase accelerates the start of a double bond in the \( \Delta^9 \) point, between 9 and 10 carbon of a variety of fatty acyl CoA substrates. The desired substrate is \( \text{C}_{18:0} \), which is converted to \( \text{C}_{18:1} \). Thus oleic acid in milk fat is in part a consequence of stearoyl CoA desaturase (Banks, 1987).

### 2.14 Public health importance

With the increase in awareness regarding quality of milk, consumers are now demanding milk with high concentration of unsaturated fatty acids (UFA), which are considered to be healthier. The three SFA mainly \( \text{C}_{12:0} \), \( \text{C}_{14:0} \) and \( \text{C}_{16:0} \) are hypercholesterolemic (Williams, 2000) and comprise almost 44% of the total milk fatty acids. However, Clandinin et al., (2000) suggests that palmitic acid (\( \text{C}_{16:0} \)) may not harmfully effect if the availability of \( \text{C}_{18:2} \) n-6 is fulfilled; stearic acid (\( \text{C}_{18:0} \)) is largely neutral, while oleic, linoleic and \( \alpha \)-linolenic acids are considered cardioprotective (Djoussé et al., 2001; Bemelmans et al., 2002). Conjugated linoleic acid (CLA), vaccinic, linolenic and particularly rumenic acid have shown positive health effect like preventing mammary gland and skin cancer in experimental animals (Ha et al., 1990; Ip et al., 1994). Vaccinic acid has been linked with anti-carcinogenic properties owing to its conversion into rumenic acid (Turpeinen et al., 2002; Corl et al., 2003).

Milk fat components like CLA, butyric acid, branched chain fatty acids and the fat-soluble vitamins have showed anti-carcinogenic properties in experimental animals (Parodi, 1999, 2008). In addition to milk fats the protein components also exhibited anticancer activity (Parodi, 2007). Parodi (2009) put forward five biological reasons to make clear any association between dairy product utilization and the risk of prostate cancer in humans. Calcium lowers the calcitriol synthesis; existence of insulin-like growth factor-1; concentration of fat and SFAs; branched-chain fatty acids and estrogens.

Butyric acid is present naturally in milk fat and is involved in metabolic activity
(Sibel et al., 2006). It affects the cell growth and make demarcation broadly in large number of cancer cell lines including breast and colon cancer, where it encourage apoptosis and may avoid metastases to the liver (Parodi, 1999). At the molecular level, it promotes histone acetylation that may benefit DNA repair and stimulates expression of tumor suppressor genes (Parodi, 1996, 1997).

Review of research studies revealed that omega 3 fatty acids have favorable cardiovascular and anti-inflammatory characteristics but their consumption is usually inadequate (Williams, 2000). The CLA arises as a metabolic by-product of rumen hydrogenation and is mostly associated with the animal origin diets and its health promoting role is usually monitored in the experimental animals. But the beneficial effect of CLA on human health has yet to be examined. Linoleic and alpha-linolenic acids derived from plant material are the precursors for essential fatty acids (EFA): arachidonic acid (AA), EPA and DHA acids (Tapiero et al., 2002).

Compounds that can be converted into AA or various sources AA are required in order to synthesize EFA or prostaglandins (PGs) by a cyclooxygenase (COX) enzyme. Experiments on pregnant rats concluded that the dietary or genetic modulation of serum, very low-density lipoproteins-triglycerides furnish specific fatty acids that may stimulate phosphatidylcholine synthesis (Ryan et al., 2002).

There are about 20 isomers of CLA in milk but the major one is cis-9, trans-11 consist almost 75–90% of total CLA (Lock and Bauman, 2004). Various research works on animal models revealed that cis-9, trans-11 isomer of CLA has anti-carcinogenic and anti-atherogenic characteristics. Cis-9, trans-11-CLA is produced as an intermediate during the biohydrogenation of linoleic acid in the rumen. However, it is only a temporary intermediate and the main pathway of CLA synthesis is endogenous one. It was suggested that concentrations of CLA, EPA and DHA can be improved by dietary manipulation and feeding management of dairy cows.

Several fatty acids are considered to be main inflammatory mediators like the eicosanoids, prostaglandin E2 and leukotriene B4 derived from the n26 polyunsaturated fatty acids and arachidonic acid (Handerson et al., 1987). A number of other important ones are the cytokines, interleukin-1b and tumor necrosis factor a (TNF-a), and there is strong facts about the connection of TNF-a in the joint pathology of rheumatoid arthritis.
The n26 fats are consumed more than n23 from the typical western countries diets (Simopoulos, 1991). The dominance of n26 fat might be due to the large quantity of linoleic acid (C_{18:2 \text{ n-6}}) presence in the diet, which is usually in higher amount in soy, corn, safflower and sunflower oils. LA and ALA are crucial ones but synthesis did not occur inside the animal body it must be provided through dietary source.

Due to the harmful effects of some SFAs on human health, milk fat has a bad reputation, because it consists of 65–75% of SFAs (Debry, 2001). Denke and Grundy (1992) compared the cholesterolemic effects of C_{12:0} with C_{16:0} and C_{18:1}. Compared with C_{16:0}, the C_{12:0} lowered total and LDL-cholesterol concentrations and have shown no effect on HDL-cholesterol concentrations while C_{16:0} was more hypercholesterolemic. On the contrary, when compared with C_{18:1}, C_{12:0} was hypercholesterolemic, elevating both total and LDL-cholesterol concentrations. However, non-significant effect on HDL-cholesterol concentrations was observed. Zock et al. (1994) compared C_{14:0} with C_{16:0} and C_{18:1} and observed that C_{14:0} notably increased both total and LDL-cholesterol concentrations, showing a much larger cholesterol-raising result when substituted for C_{18:1} than for C_{16:0}. However, Tholstrup et al. (1994) reported no increase in total or LDL cholesterol when C_{14:0} was substituted for C_{16:0}. Also, C_{16:0} was less hypercholesterolemic than a combination of C_{12:0} and C_{14:0}.

The cholesterolemic effects of C_{16:0} and C_{18:0} was compared by Tholstrup et al. (1994) and Bonanome and Grundy (1988). Both studies showed that when C_{16:0} is substituted for C_{18:0}, C_{16:0} increases the concentrations of plasma total, LDL and HDL cholesterol. Cholesterol-raising effects of C_{16:0} compared with C_{18:0} was 14-28% increase in total cholesterol, 28 to 36% increase in LDL cholesterol and 6 to 15% increase in HDL-cholesterol concentrations. Kris-Etherton et al. (1993) and Howard et al. (1995) observed a greater total-cholesterol-lowering effect of C_{18:2} compared with C_{18:1} but McDonald et al. (1989) stated similar effects of C_{18:2} and C_{18:1} on plasma total and lipoprotein cholesterol concentrations.

Poly-unsaturated fatty acids (PUFAs) reduce the cholesterol content more effectively than monounsaturated fatty acids (Williams et al., 2000). Oleic acid and linolenic acid of the n-3 family have anticancer and anti-atherogenic properties (Haug et
In addition to its effect on cholesterol level, linoleic acid, the most vital in the \( \omega-6 \) family, enhances the sensibility to insulin and reduces the incidences of type 2 diabetes (Hu et al., 2001). Western diets are known to be deficient in n-3 and excessive in n-6. This disequilibrium promotes numerous diseases, like cardiovascular diseases, cancer, and inflammatory diseases (Simopoulos, 2002). This ratio is usually higher than 12 in developed countries.

Current dietary recommendation suggest dietary n-6:n-3 lower than 5 to reduce the risk of cardiovascular diseases, cancer, autoimmune disorders, allergies, obesity, and various mental disorders (Sabikhi, 2004). Excess of n-6 can lead to disruption of the biosynthesis of prostaglandins and consequently lead to inflammation, fatness, high blood pressure, disturbance in digestive tract, and depressed immune function. Deficiency in n-3 can also lead to other physiologic disorders, such as asthma and heart diseases (Sabikhi, 2004). This ratio is naturally low in milk products (1.6; Haug et al., 2007). Dairy and meat products are rich in conjugated linoleic acid (2.5–18.0 mg/g of fat in bovine milk), which is a mixture of positional and geometric isomers of \( \text{C}_{18:2} \) \text{cis-9}, \text{cis-12}. The most important isomers are the rumenic acid which represents about 75 to 90% of the total CLA and \( \text{C}_{18:2} \) \text{trans-10}, \text{cis-12}. According to several animal models, CLA exhibits antiatherogenic, antiobesity, and anticarcinogenic proprieties (Corl et al., 2001; MacDonald, 2000; McGuire and McGuire, 2000; Parodi, 1997). Moreover, CLA are capable to modulate the immune response and bone growth to support cell growth, etc. (Keating et al., 2005; Lock and Bauman, 2004; MacDonald, 2000; Tanaka, 2005; Whale et al., 2004).

Arnould and Soyeurt (2009) reported the greatest breed differences in fatty acids composition between Holstein and Jersey milk. Milk fat of the latter breed contains higher proportion of SFAs, particularly short-chain fatty acids. The variation of the delta-9 desaturase activity estimated from specific fatty acids ratios could explain partially these breed differences. The selection of a specific breed appears to be an option to improve the nutritional quality of milk fat. Normally, the proportions of fatty acids in milk are more heritable than their proportions in milk fat. The occurrence of some single nucleotide polymorphisms probably explains the observed individual genetic variability. The polymorphisms detected on SCD1 and DGAT1 genes influence the milk fatty acid
composition. The SCD1 V allele increases the unsaturation of C16 and C18. The DGAT1 A allele is related to the unsaturation of C18. Therefore, a combination of the molecular and quantitative approaches should be used to develop tools helping farmers in the selection of their animals to improve the nutritional quality of the milk fat.

It was concluded from the discussion above that lipids have been targeted in a number of human diseases, like cancer and cardiovascular disease, both in beneficial or detrimental manner. In nutshell, scientists are aware of the significance of this important organic compound and are working to modify the feeding and breeding practices to achieve the ideal milk fatty acid profile with respect to human health.
III. Variation in milk fatty acids composition with body condition in dairy buffaloes (*Bubalus bubalis*)

3.1 ABSTRACT

Buffaloes usually maintain higher body condition and do not produce milk at the cost of their own body reserves under tropical conditions. The mobilization of body reserves for fulfilling the demands of lactation has been extensively studied in dairy cows while limited work is available on this aspect in dairy buffaloes. Therefore, the present study was conducted to examine variations in milk fatty acid profiles in Nili-Ravi buffaloes with the body condition. A total of 24 Nili-Ravi buffaloes within 60 days after parturition, were selected from a private dairy farm at district Peshawar. All the animals consumed the same diet during the experimental period. A total of 576 raw milk samples were collected for laboratory analysis. The study continued up to 6 months during the year 2008. Body condition score (BCS), milk yield and composition were recorded once a week. Means for milk fatty acid profile were compared for various levels of BCS. The mean milk yield and fat contents were 9.28 kg/d and 5.36%, respectively. Mean concentration of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) were 64.22 g/100g and 35.79 g/100g of total fatty acids, respectively. Out of SFAs, the highest amount was recorded for C16:0, followed by C18:0 and C14:0. The total sum of hypercholesterolemic fatty acids (HCFAs) C12:0, C14:0 and C16:0 were 43.33 g/100g of total fatty acids. The concentrations of UFAs were greater with the moderate (2.5) BCS followed by poor (1.5) and highest one (3.5) while SFAs showed an opposite trend. The correlation analysis showed that milk yield was negatively affected by BCS and milk fat positively, though non-significantly. The present study suggests that Nili-Ravi dairy buffaloes produce milk almost similar to dairy cows, regarding availability of cardioprotective fatty acids, with the highest concentration of C18:1 cis-9. Two HCFA (C12:0 and C14:0) were associated with higher (3.5) body condition. Buffaloes with moderate (2.5) body condition yielded milk containing healthier fatty acids.

**Keywords:** body condition, saturated fatty acids, unsaturated fatty acids, hypercholesterolemic fatty acids, dairy buffaloes.
3.2 INTRODUCTION

Bovine milk fat is an essential part of human diet. The milk of all ruminants contains lipid but the concentrations usually differ among species from 2 to 8 % (Belitz and Grosch, 1999). Milk primarily consists of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFA). Normally the cow’s milk fat consists of 70% SFAs, 25% MUFAs and 5% PUFAs (Grummer, 1991; Lock and Shingfield, 2004). Considering the beneficial effects of unsaturated fatty acids in milk on human health, the ideal amount of these fatty acids would be some 60% of MUFA (Pascal, 1996), 30% of SFAs and 10% of PUFAs in milk fat (Hayes and Khosla, 1992).

Not all of the SFAs increase blood cholesterol in humans. Only three lauric, myristic and palmitic acids (C_{12:0}, C_{14:0} and C_{16:0}, respectively) are considered to be hypercholesterolemic fatty acids (HCFA, Williams, 2000) leading to cardiovascular disease. The proportion of HCFA in milk is almost 44% of the total milk fatty acids. However, Clandinin et al., (2000) recommended that palmitic acid (C_{16:0}) may not show harmful effect if the availability of C_{18:2 \, n-6} is fulfilled; stearic acid (C_{18:0}) is largely neutral, while oleic, linoleic and α-linolenic acids are considered cardioprotective (Djoussé et al., 2001; Bemelmans et al., 2002). The conjugated linoleic acid (CLA), vaccinic (C_{18:1 \, trans11}), linolenic (C_{18:3}) and rumenic acid (C_{18:2 \, cis-9, \, trans11}) have shown positive health effect like preventing mammary gland and skin cancer in experimental animals (Ha et al., 1990; Ip et al., 1994). Vaccinic acid has been linked with anti-carcinogenic effect following its conversion into C_{18:2 \, cis-9, \, trans11} (Turpeinen et al., 2002; Corl et al., 2003).

Various sources of milk fatty acids may be categorized into dietary intake, adipose tissues mobilization and de novo synthesis within mammary tissues. To fulfill the requirements of lactation, mobilization of adipose tissues reserves is triggered when the energy balance is negative. During fat metabolism, desaturation of stearic acid occurs in the intestinal epithelium and mammary tissues by the desaturase enzymes (Enoch et al., 1976). Hence, the fatty acids from dietary and adipose tissue mobilization results in greater quantities of long chain SFAs and UFAs. The lower BCS cows will depend mostly on the exogenous sources of fatty acids other than adipose tissue stores for milk fat synthesis (Pedron et al., 1993).
The forthcoming challenge to the dairy industry is provision of healthy food to consumers. In order, to meet this challenge the beneficial fatty acids must be increased and the critical ones must be reduced in milk. Buffaloes maintain higher body condition and do not produce milk at the cost of its own body reserves (Qureshi et al., 2007). In order to provide precursors for milk synthesis, the process of lipolysis has been identified as a good dairy characteristic in cattle while buffaloes do not qualify this criterion by maintaining higher BCS during lactation. Therefore, the present study was conducted to observe the changes in milk fatty acid contents with variation in body condition under tropical conditions.

3.3 MATERIALS AND METHODS

The present study was conducted at district Peshawar located in the northwest frontier province of Pakistan. A total of 24 Nili-Ravi multiparous (2-3rd parity) buffaloes with body weight ranging from 400 to 550 kg having milk production within range of 10 to 12 liters/d and within 60 days after parturition were selected out of 50 animals at the private dairy farm situated at village Palosi close to university campus. The BCS at the time of selection ranged from 1.5 to 3.5. Selected animals were ear-tagged, de-wormed and vaccinated. The buffaloes were reared under intensive farming system with higher input cost but little support in farm development, scientific management and marketing. The experimental animals were offered green fodders (Egyptian clover and whole crop maize, 50 % each) ad libitum and concentrate, consisting of 18% crude protein plus 72% total digestible nutrients. Concentrates ingredients comprised maize oil cake, cotton seed cake, wheat bran, molasses and macrominerals. The ingredients were mixed and offered to the experimental animals at the rate of 1 kg/2 kg of milk produced as per prevailing practices. All the animals consumed the same diet in the experimental period. Twice a day milking with 12 hours interval was in practice. A total of 576 raw milk samples were collected from buffaloes, for laboratory analysis. The animals were stall-fed and chopped fodders were provided at 1000 and 1400 hrs. Water shower was made available to animals two times a day, throughout the warm conditions.

During the experimental period, all the animals were confined in the shed with access to drinking water availability throughout the day from an adjacent tank. The study
continued up to 6 months during the year 2008.

3.3.1 Body Condition Scoring

The body condition score (BCS) of all the cows was recorded weekly, using the method described by Peters and Ball (1987) observed by the same person throughout the experimental period. According to this method the thickness of fat over the lumber and tail head area was estimated and was assigned a score from 1 (very weak) to 5 (very fat). 1, spine prominent and transverse processes feel sharp with little fat cover; 2, transverse processes can be felt but are rounded with a thin covering of fat; 3, individual transverse vertebral processes can only be felt by firm pressure; 4, the transverse processes cannot be felt; and 5, the transverse processes covered with a thick layer of fat. The experimental animals fell within the range of 1.5, 2.5 and 3.5 and were categorized as poor, moderate and high, respectively.

3.3.2 Milk yield (MY), sampling and analysis

The milk yield (kg/day) and milk composition were recorded once a week for up to 24 weeks period. Evening milk samples (200 ml each) were taken in bottles and were further transported in an icebox to dairy production laboratory. The samples were stored in refrigerator at –20°C until analyzed. The fat, protein and lactose contents were determined using ultrasonic milk analyzer (model Ekomilk Total Ultrasonic Milk Analyzer, Bullteh 2000, Stara Zarqora, Bulgharia) according to manufacturer’s instructions.

3.3.3 Determination of fatty acids

3.3.3.1 Fat extraction

The milk fat separation was performed by the procedure of Feng et al. (2004). A 20 ml of fresh milk in a 50 ml conical plastic tube was centrifuged at 4 °C for 30 min at 12,000 rpm. A portion (1.0 g) of the fat cake layer was transferred to 1.5 ml micro-tube and left at room temperature for approximately 20 min until the fat cake melted. This was again centrifuged at 13,000 rpm for 20 min at room temperature by microcentrifuge.
After centrifugation, the fat had separated into 3 layers: the top layer of lipid; the middle layer of protein, fat and the water insoluble solids and the bottom layer of water.

3.3.3.2 Preparation of methyl esters

The lipids were transesterified with sodium methoxide as described by Christie (1982) and modified by Chouinard et al. (1999). Hexane (2mL) was transferred into 40 mg of oil and then 40 μL of methyl acetate was mixed with it. When the mixture was vortexed, 40 μL of methylation reagent (1.75 mL methanol: 0.4 mL of 5.4 mol/L sodium methylate) was added. After vortexing the solution was kept for 10 min and after that 60 μL of termination reagent (1 g oxalic acid/30 mL diethyl ether) was added. The centrifugation was performed for 5 min at 2400 x g at 5°C leaving a clear layer of hexane; a portion of the hexane was transferred to gas chromatography vials and kept at –20 °C.

3.3.3.3 Gas chromatography

Quantification of the fatty acid methyl esters (FAME) was carried out by Perkin-Elmer chromatograph (model Clarus 500, Beaconsfield, UK) having flame ionization detector. The separation of FAME was carried out with 100-m fused silica capillary column (i.d. 0.25 mm) coated with 0.2 μm film of cyanopropylpolysiloxane (CP-SIL 88; Varain, Netherlands). Hydrogen was used as carrier gas. The FAME of 1 μL was identified as the appropriate quantity and hence injected manually. Column temperature was increased to 70°C and held for 4 min, increased to 110°C (8°C/min), increased to 170°C (5°C/min), held at 170°C for 10 min and ramped to 240°C (8°C/min) and held for 7 minutes. Injector and detector temperatures were kept at 225 and 250°C respectively. Peaks were identified by pure methyl ester standards (GLC-10 and GLC-30 FAME mixtures; Materya Inc. Pleasant Gap, PA. USA). A butter oil reference standard (CRM 164; Bureau of References, Belgium) was used to determine recoveries and correction factors for individual fatty acids.

3.3.4 Statistical analysis

Statistics were performed by SPSS 10.0 (1999) for Windows XP. The fatty acid
composition data obtained were subjected to analysis of variance for means comparison using the general linear model (GLM) procedures. Means for fatty acid profiles of milk were compared for various levels of BCS (poor; moderate and high). Means were subsequently ranked using Duncan’s Multiple Range Test (DMRT) as described by Steel and Torrie (1980). Number of observations, means and standard deviations were recorded for the groups. A bivariate analysis with Pearson’s correlations (p < 0.05 level, 2-tailed) was performed to determine the relationship between BCS, individual fatty acids, fat content and milk yield.

3.4 RESULTS

The mean milk yield in Nili-Ravi buffaloes recorded during the study was 9.28 kg/d ranging from 4.77 to 16.00 kg/d (Table 3.1). The average fat percentage was 5.36, ranging from 3.00 to 8.23. The concentration of saturated fatty acids (SFAs) was on the average 64.22 g/100g, varying from 59.01 to 67.65 g/100g and the unsaturated fatty acids (UFAs) were 35.79 g/100g, ranging from 32.35 to 40.99 g/100g of total fatty acids. Out of the SFAs the highest amount was of palmitic acid (C_{16:0}, 30.06 g/100g), followed by stearic acid (C_{18:0}, 14.70 g/100g) and myristic acid (C_{14:0}, 10.84 g/100g). The highest monounsaturated fatty acids (MUFA) level was of oleic acid (C_{18:1 \text{ cis-9}}, 29.47 g/100g of total fatty acids). The amount of polyunsaturated fatty acids (PUFAs) was 4.91 g/100g of total fatty acids. The three fatty acids like, C_{12:0}, C_{14:0} and C_{16:0} are critical fatty acids, considered as hypercholesterolemic (HCFAs) associated with cardiovascular disease in human population. The total sum of these three fatty acids was 43.33 g/100g of total fatty acids.

3.4.1 Effect of BCS on milk fatty acids profile

The fatty acid composition of buffalo’s milk as influenced by BCS is presented in table 3.2. The fatty acids were significantly (p< 0.05) influenced by the body condition. The amount of unsaturated fatty acids (UFAs) were greater with the moderate BCS followed by poor and highest one (38.16±1.92, 37.39±2.09 and 35.15±1.77 g/100g of
total fatty acids respectively, P< 0.05) whereas, the SFAs showed an opposite trend (Figure 3.1).

The amount of short and medium chain fatty acids as well as C\textsubscript{18:0} were greater with the increase in BCS, while C\textsubscript{18:1 cis-9}, C\textsubscript{18:2 cis-9, cis-12} and C\textsubscript{18:3 cis-9, cis-12, cis-15} were greater with moderate BCS and lowest with higher BCS (Figure 3.2). The two hypercholesterolemic fatty acids (HCFAs C\textsubscript{12:0} and C\textsubscript{14:0}) increased with BCS whereas, the C\textsubscript{16:0} was higher with the moderate BCS (Figure 3.3).

3.4.2 Correlation of fatty acids

The correlation analysis showed that milk yield was negatively affected by BCS and milk fat positively though non-significantly (Table 3.3). The short and medium chain fatty acids as well as C\textsubscript{18:0} correlated positively while the monounsaturated (MUFAs), polyunsaturated fatty acids (PUFAs) and C\textsubscript{16:0} negatively with body condition. All the SFAs (short, medium and long chain fatty acids) except C\textsubscript{16:0}, correlated positively with each other and negatively with UFAs. Similarly, the UFAs showed a mutual positive correlation. Also C\textsubscript{18:0} correlated negatively but with increasing intensity with C\textsubscript{18:1 cis-9}, C\textsubscript{18:2 cis, cis-9, 12} and C\textsubscript{18:3 cis-9,12,15} (r = -0.168, -0.519 and -0.899, respectively, P< 0.05).

The figure 3.1 shows that moderate BCS was associated with the highest concentration of UFAs and lowest SFAs.

3.5 DISCUSSION

The HCFA (C\textsubscript{12:0}, C\textsubscript{14:0} and C\textsubscript{16:0}) found in this study on Nili-Ravi buffaloes were considerably lower and cardioprotective fatty acids (C\textsubscript{18:1} and C\textsubscript{18:2} and C\textsubscript{18:3}) level were higher than the Bulgharian Murrah buffaloes as reported by Mihaylova and Peeva (2007). They found that total amount of SFAs were 72.15 % (ranging from 64.92 to 77.60%), PUFAs 3.15% and the HCFAs were 43.62 %. Our results are similar with the findings of Fernandes et al. (2007) that the total SFAs, MUFAs and PUFAs in Murrah buffaloes in Brazil were 65.04%, 31.68% and 3.28% respectively and the HCFAs varied from 32.48 to 42.90%. Our values for dairy buffaloes are not much different from dairy cows where the SFAs varied from 60 to 65 % and UFAs 35 to 40 % of the total fatty acids (Lock and
Talpur et al. (2008) compared fatty acid composition of Nili-Ravi and Kundi buffaloes in Sindh province. The average SFAs were; 66.96 g/100g and 69.09 g/100; MUFAs 27.62 and 25.20 g/100g; PUFAs 2.77 and 2.76 g/100g and HCFAs 42.8 and 46.54 g/100g of total fatty acids for Kundi and Nili-Ravi breed respectively. It appears that the cardioprotective quality of milk from Nili-Ravi buffaloes is almost similar to dairy cows and Brazilian and better than Bulgharian Murrah buffaloes.

3.5.1 Effect of BCS on milk fatty acids profile

Concentration of unsaturated fatty acids (UFAs) was highest with the moderate BCS followed by poor and highest one. Our findings confirm a previous report on lactating Angus × Gelbvieh beef cows (Lake et al., 2006) suggesting that moderate BCS will provide greater percentage of C$_{18:0}$ and C$_{18:1}$ trans-10 in milk which was attributed to the dietary source. In the current study increasing body condition was reflected by an increase in C$_{18:1}$ cis-9 and decreased in C$_{18:0}$ concentrations, showing an improvement in the milk quality. Further increase in BCS up to higher level was associated with a reverse pattern of the two fatty acids.

The opposite pattern of BCS and UFAs concentration was probably due to lipolysis. In adipose tissue, C$_{18:1}$ cis-9, C$_{16:0}$ and C$_{18:0}$ account for nearly 90% of fatty acids in molar proportions (Christie, 1981) and body fat mobilization would probably increase direct accumulation of these fatty acids into milk fat. In addition, desaturation of stearic acid occurs in the intestinal epithelium and mammary tissues (Enoch et al., 1976). Some 40–50% of C$_{18:1}$ cis-9 in milk fat is formed from C$_{18:0}$ in the mammary cell through desaturase (Chillard et al., 2000). The net outcome of all these processes is the higher level of UFAs and more specifically the C$_{18:1}$ concentration, which has been confirmed through this study.

In Mediterranean dairy buffaloes activity of $\Delta^9$-desaturase enzyme was studied (Fernandes et al., 2007). The farm maintained on pasture and corn silage showed higher ratios of UFAs/SFAs indicating greater $\Delta^9$-desaturase activity. The stearoyl-coenzyme A (CoA) desaturase catalyzes the start of a double bond in the $\Delta^9$ position of a variety of fatty acyl CoA substrates. The desired substrate is C$_{18:0}$, which is converted to C$_{18:1}$. Thus, C$_{18:1}$ in milk fat is in part a consequence of stearoyl CoA desaturase (Banks, 1987).
As a result, milk fatty acids tend to be higher in UFAs. The higher BCS was associated with higher concentrations of SFAs in this study. Similarly, higher percentages of C_{12:0} and C_{14:0} in milk from higher BCS beef cows was reported by Lake et al. (2006) advocating increased de novo synthesis of fatty acids within the mammary glands. Our findings of higher C_{12:0} and C_{14:0} associated with higher BCS confirms their results. The fatty acids synthesized de novo resulted in 4 to 16 carbons from the mammary gland utilization of acetate and β-hydroxybutyrate (Bath et al., 1985). Fatty acid synthase accelerates the conversion of acetyl-Co A and malonyl-Co A into various fatty acids up to C_{16:0} (Yin et al., 2001). According to Palmquist et al. (1993), the fatty acids synthesized de novo in the mammary cell are C_{6:0} to C_{14:0} and partially C_{16:0}.

Lower BCS in this study was associated with a milk quality better than higher and lower than moderate BCS in respect of concentration of HCFAs, UFAs and SFAs. In previous studies, cows with a poor BCS depend largely on dietary sources for fatty acid synthesis rather than looking for adipose tissue reserves to synthesize milk fats (Pedron et al., 1993). The major precursor for synthesis of short chain fatty acids is acetate while body fat and ingested lipids contributes largely to long-chain fatty acids (Payne et al., 1979).

In buffaloes the milk yield was negatively correlated with BCS probably due to mobilization of body reserves, revealing their better genetic potential to be used as dairy animal under tropical conditions. Dairy cows with higher genetic merit have a higher predisposition for mobilization of body fat reserves to cover milk production demands (Veerkamp, 1998; Pryce et al., 2002). These findings were supported by a study of Veerkamp and Brotherstone (1997) that BCS and milk yield are in a negative correlation. Higher genetic merit cows for milk production have consistently higher rates of lipolysis and hormone sensitive lipase activity (McNamara et al., 1987; Smith and McNamara 1990). It has been observed that dairy animals maintained a physiological target for BCS during lactation (Garnworthy and Topps, 1982). These physiological targets may get reduced with the genetic improvement directed towards increased milk yield. As the dairy buffalo is not an improved breed, it may have set a higher physiological target for BCS as compared with Holstein Friesian. This phenomenon may be confirmed in
buffaloes and Holstein Friesian cows maintained under the same management in the field conditions; where the lactating buffaloes may be seen with the higher BCS under tropical environment.

3.6 CONCLUSION

The present study suggests that Nili-Ravi dairy buffaloes produces milk almost similar to dairy cows regarding availability of cardioprotective fatty acids, with the highest concentration of oleic acid (C\textsubscript{18:1cis-9}, 29.47 g/100g of total fatty acids). Buffaloes with moderate body condition yielded greater concentrations of these fatty acids followed by poor and highest ones. Two hypercholeolemic fatty acids (C\textsubscript{12:0} and C\textsubscript{14:0}) were associated with higher body condition.

3.7 ACKNOWLEDGMENT

The authors acknowledge the continuous support of the livestock management department, faculty of animal husbandry and veterinary sciences and the NWFP, agricultural university Peshawar, for extended all support in completion of this study. Centralized resource laboratory, University of Peshawar very kindly extended their analytical facility for the completion of this task. Moreover, the authors acknowledge the support of the public and private sector dairy farms in providing the animals and facilitating in data collection.
Table 3.1: Descriptive statistics for milk yield, fat contents and fatty acids profiles (g/100g, n=576).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>^1BCS</td>
<td>3.20</td>
<td>0.58</td>
<td>1.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Fat %</td>
<td>5.35</td>
<td>1.18</td>
<td>3.00</td>
<td>8.23</td>
</tr>
<tr>
<td>^2MY (kg/d)</td>
<td>9.27</td>
<td>3.26</td>
<td>4.77</td>
<td>16.00</td>
</tr>
<tr>
<td>C8</td>
<td>1.37</td>
<td>0.30</td>
<td>1.15</td>
<td>2.00</td>
</tr>
<tr>
<td>C10</td>
<td>2.23</td>
<td>0.44</td>
<td>1.20</td>
<td>2.82</td>
</tr>
<tr>
<td>C12</td>
<td>2.43</td>
<td>0.52</td>
<td>1.74</td>
<td>3.50</td>
</tr>
<tr>
<td>C14:0</td>
<td>10.84</td>
<td>0.91</td>
<td>8.40</td>
<td>11.65</td>
</tr>
<tr>
<td>C16:0</td>
<td>30.06</td>
<td>1.68</td>
<td>28.00</td>
<td>32.88</td>
</tr>
<tr>
<td>C18:0</td>
<td>14.75</td>
<td>1.62</td>
<td>11.50</td>
<td>16.91</td>
</tr>
<tr>
<td>C18:1^ cis-9</td>
<td>29.47</td>
<td>1.43</td>
<td>28.00</td>
<td>32.32</td>
</tr>
<tr>
<td>C18:2^ cis-9, 12</td>
<td>2.45</td>
<td>0.53</td>
<td>1.60</td>
<td>3.24</td>
</tr>
<tr>
<td>C18:3^ cis-9, 12, 15</td>
<td>2.46</td>
<td>0.80</td>
<td>1.65</td>
<td>3.45</td>
</tr>
<tr>
<td>^3SFAs%</td>
<td>64.20</td>
<td>2.16</td>
<td>59.01</td>
<td>67.65</td>
</tr>
<tr>
<td>^4UFAs%</td>
<td>35.80</td>
<td>2.16</td>
<td>32.35</td>
<td>40.99</td>
</tr>
</tbody>
</table>

^1Body condition score; ^2Milk yield; ^3SFAs = saturated fatty acids; ^4UFAs = Unsaturated fatty acids
Table 3.2: Mean and standard deviation for milk fatty acids (g/100 g) as influenced by body condition score in dairy buffaloes (n = 576).

<table>
<thead>
<tr>
<th>BCS</th>
<th>C8:0</th>
<th>C10:0</th>
<th>C12:0</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C18:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>(1.16^{b}\pm0.16)</td>
<td>(1.69^{b}\pm0.39)</td>
<td>(1.85^{b}\pm0.53)</td>
<td>(10.28^{b}\pm1.08)</td>
<td>(31.32^{b}\pm0.29)</td>
<td>(12.93^{b}\pm0.67)</td>
</tr>
<tr>
<td>2.5</td>
<td>(1.17^{b}\pm0.17)</td>
<td>(1.75^{b}\pm0.37)</td>
<td>(1.87^{b}\pm0.22)</td>
<td>(10.04^{b}\pm1.16)</td>
<td>(31.49^{b}\pm1.71)</td>
<td>(12.80^{b}\pm0.63)</td>
</tr>
<tr>
<td>3.5</td>
<td>(1.44^{a}\pm0.33)</td>
<td>(2.38^{a}\pm0.35)</td>
<td>(2.56^{a}\pm0.48)</td>
<td>(11.04^{a}\pm0.73)</td>
<td>(29.64^{a}\pm0.34)</td>
<td>(15.30^{a}\pm1.40)</td>
</tr>
</tbody>
</table>

P-value: \(P < 0.05\) for all comparisons.

\(^{1}\)BCS: Body condition score (scale 1 to 5); \(^{2}\)SFAs = saturated fatty acids; \(^{3}\)UFAs = Unsaturated fatty acid; \(^{a,b,c}\) Mean in the same column having different superscripts are significantly different \((P<0.05)\).
Table 3.3: Correlation of milk fatty acids (g/100 g) with BCS, fat and milk yield in dairy buffaloes.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlating parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCS</td>
</tr>
<tr>
<td>Fat</td>
<td>0.074ns</td>
</tr>
<tr>
<td>1^MY</td>
<td>-0.349**</td>
</tr>
<tr>
<td>C-8:0</td>
<td>0.588**</td>
</tr>
<tr>
<td>C-10:0</td>
<td>0.557**</td>
</tr>
<tr>
<td>C-12:0</td>
<td>0.388**</td>
</tr>
<tr>
<td>C-14:0</td>
<td>-0.411**</td>
</tr>
<tr>
<td>C-16:0</td>
<td>0.597**</td>
</tr>
<tr>
<td>C-18:0</td>
<td>-0.168**</td>
</tr>
<tr>
<td>C-18:2</td>
<td>-0.254**</td>
</tr>
<tr>
<td>C-18:3</td>
<td>-0.548**</td>
</tr>
<tr>
<td>2^SFAs</td>
<td>0.435**</td>
</tr>
<tr>
<td>2^UFA</td>
<td>-0.414**</td>
</tr>
<tr>
<td>C16:0</td>
<td>0.081ns</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.081ns</td>
</tr>
<tr>
<td>C18:1</td>
<td>0.740**</td>
</tr>
<tr>
<td>C18:2</td>
<td>-0.014ns</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.307**</td>
</tr>
</tbody>
</table>

**P< 0.01; * P< 0.05; ns Non-Significant difference; 1^MY=Milk yield; 2^SFAs= saturated fatty acids; 2^UFA= Unsaturated fatty acid
Figure 3.1: Changes in concentration of saturated (SFAs, dotted area) and un-saturated fatty acids (UFAs, vertical lines) with body condition score (BCS) in dairy buffaloes. Both types of the fatty acids were significantly different across various levels of BCS (P<0.05). The SFAs and UFAs correlated negatively and significantly with one another (r=-0.614, P<0.01).
Figure 3.2: Changes in oleic acid (C_{18:1}cis-9, ■), mono-unsaturated fatty acids (MUFA) and stearic acid (C_{18:0}, ▲) with body condition score (BCS) in Nili-Ravi buffaloes. The two fatty acids showed a negative correlation (r=-0.168, P<0.01).
Figure 3.3: Changes in hypercholesterolemic (C_{12:0}, □; C_{14:0}, ■ and C_{16:0}, ▲) fatty acids with body condition score (BCS) in Nili-Ravi Buffaloes. The three types of fatty acids showed a significant correlation with the BCS (r=0.557, 0.388 and -0.411 respectively, P<0.01)
3.8 REFERENCES


IV. Changes in milk fatty acid profile with advancing lactation in crossbred dairy cows under subtropical conditions

4.1 ABSTRACT

Milk fatty acid composition is governed by unique rumen and tissues derived fatty acids that vary with stage of lactation and among individual cows. The factors affecting the availability of the fatty acids have been the area of current research. The most dramatic changes in milk yield and composition occur during earlier weeks of lactation. The present study was conducted to determine effect of lactation advancement on milk yield and its fatty acid composition in crossbred cows during the initial 16 weeks after parturition. A total of 28 F1 crossbred cows (HF x Sahiwal) within 1st week after parturition were selected from a large state farm. The animals were maintained under uniform management conditions in a well-ventilated shed. The animals were milked twice a day and milk samples were collected from each cow once a week during 1, 4, 8, 12 and 16 weeks of lactation. BCS and milk yield were recorded on the day of sampling. The saturated fatty acids (SFA) averaged 67.88g/100g of total fatty acids and the unsaturated fatty acids (UFA) were 32.39 g/100g of total fatty acids. Out of the SFAs the highest amount was of palmitic acid (C16:0, 23.09 g/100g of total fatty acids). The highest monounsaturated fatty acids (MUFA) level was of oleic acid (C18:1 cis-9, 24.68 g/100g of total fatty acids). Mean concentration of polyunsaturated fatty acids (PUFA) was 3.95 g/100g of total fatty acids. The total sum of medium chain fatty acids C12:0, C14:0 and C16:0 identified as hypercholestrolemic fatty acids was 38.40 g/100g of total fatty acids. The correlation analysis showed a significantly positive relationship between BCS and milk fat percent. The present study suggests that the concentrations of unsaturated fatty acids were higher in earlier weeks and declined during mid lactation. With advancement of lactation, from wk 1 to 16 of lactation, the proportion of both de novo fatty acids and poly-unsaturated fatty acids increased and pre-formed fatty acids (specifically C18:0 and C18:1 cis-9) decreased. The two hypercholestolemic fatty acids (C12:0, and C14:0) increased with advancing lactation and the cows in early lactation yielded milk containing healthier fatty acids.
Keywords: Lactation week, crossbred cows, saturated fatty acids, unsaturated fatty acids, hypercholestrolemic fatty acids, body condition

4.2 INTRODUCTION

Milk fatty acid composition is important for both milk processing and human health. Bovine milk lipids represent roughly 95% triacylglycerols and the majority consists of C_{4:0} to C_{18:0} fatty acids (Jensen, 2002). Milk fatty acids originate either directly from dietary source or it comes as a result of mobilization of body reserves, de novo synthesis or desaturation within the mammary tissues (Enoch et al., 1976; Bath et al., 1985). Approximately 50% of milk fat comprised of LCFAs by the utilization of dietary lipids or through lipolysis of the body fat deposits. The remaining 50% are synthesized in the mammary secretary cells from volatile fatty acids (acetates and \( \beta \)-hydroxy butyrate) resulted from the microbial fermentation inside the rumen (Bath et al., 1985). Many unsaturated fatty acids (UFAs) become hydrogenated with in the rumen, which largely explains the greater proportion of saturated fatty acid (SFAs) in ruminant’s milk. Normally the cow’s milk fat consists of 70% SFAs, 25% MUFAs and 5% PUFAs (Grummer, 1991; Lock and Shingfield, 2004). The fat is the major variable constituent of milk and the composition of its fatty acids is influenced by various physiological and management factors (Aludist et al., 1998).

Milk fatty acid composition is governed by unique rumen derived fatty acids that vary with lactation stage and among individual cows (Palmquist et al., 1993). Like other lactating animals, dairy cows are in negative energy status at the start of lactation (Nielsen, 1999). With the initiation of adipose tissues mobilization the percentage of stearic, oleic as well as short chain fatty acids increases speedily (Laasko et al., 1996). The difference was independent of seasonal or nutritional effect and was attributed to the physiological incapability of cows at the start of lactation to adjust to higher dry matter consumption in order to accomplish the energy needs.

Homeorhetic mechanisms controlling the coordinated variation in tissue metabolism that occur with the beginning of lactation may also partition a greater proportion of dietary and tissue derived nutrients toward milk synthesis. These variations may affect the relative percentage of preformed and de novo fatty acids in milk.
With the increase in awareness regarding quality of milk, consumers now preferred milk with high proportion of UFAs. The unsaturated fatty acids are generally called ‘healthy fats’, mainly for their impact on the level of cholesterol in blood (Ward et al., 1998; Haug et al., 2007). The three SFAs (C_{12:0}, C_{14:0} and C_{16:0}) are considered hypercholesterolemic fatty acids (HCFAs, Williams, 2000). However, Clandinin et al. (2000) recommended that palmitic acid (C_{16:0}) may not show harmful effect if the availability of C_{18:2 \text{ n-6}} is fulfilled; stearic acid (C_{18:0}) is largely neutral, while oleic, linoleic and α-linolenic acids are considered cardioprotective (Bemelmans et al., 2002).

The daily milk production showed a characteristic rise for 3-6 weeks after parturition, followed by a gradual decline until near the end of lactation (Darkeley and White, 1928) while fat content varies inversely with milk production (Brown et al., 1962). However, very little is known concerning variability of the fatty acids of milk with advancing lactation in crossbred cows in our local management practices. The crossbreeding of cattle has been initiated in Pakistan from the last five decades, with the influx of artificial insemination. According to national livestock policy, the local cows in plain-irrigated areas are crossed with exotic Holstein-Frisian breed while the cows in hilly and dry areas are crossed with Jersey breed (Rollinson, 1978). All the way through crossbreeding of cattle in NWFP milk production increased by 73 % and age at puberty declined by 82 % (Syed et al., 1994). The crossbreeding, as a result of genetic recombination has resulted in various physiological based disorders in fertility and productivity (Qureshi, 2004). The crossbred cattle could not get sufficient attention of the dairy scientist in the country because of their focus on dairy buffaloes (Qureshi, 2009; Pasha et al., 2009). However, contributions of the crossbred cattle in the national economy call for intensive exploration of these animals regarding their productivity and products quality.

Factors affecting the availability of useful fatty acids have been the area of current research and most dramatic changes in milk yield and composition occur during early weeks of lactation (Kay et al., 2005; Lake et al., 2006). Therefore, the present study was conducted to examine the association of lactation advancement with milk yield and its fatty acid profile in crossbred cows specifically considering the initial 16 weeks of lactation.
4.3 MATERIALS AND METHODS

4.3.1 Selection and management of animals

The present study was conducted at Peshawar, in the central valley of the North-West Frontier Province (NWFP) of Pakistan, which is located at the latitude of 31-37° north and longitude of 65-74° east. A total of 28 F1 crossbred multiparous cows (HF x Sahiwal) within 1st week after parturition were selected from a large state farm.

The animals were maintained under uniform management conditions in a well-ventilated shed. The animals were stall-fed and chopped fodder was provided at 1000 and 1400 hrs. Animal sheds were washed daily at morning. The cows were hand milked two times per day at 0400 and 1600 hrs. Before milking, udder was washed by using warm water and dried with damp cloth.

During the experimental period, all the animals were confined in the shed and were only taken out for water 3 to 4 times to an adjacent tank in an open barn. The study continued from January to April 2008.

4.3.2 Feeding regime

All the animals were offered identical ration consisting of green fodders and concentrates. The green fodders (Egyptian clover) was fed ad libitum and commercial concentrates; consisting of 18% crude protein plus 72% total digestible nutrients was offered at a scale of 1 kg per 3 kg milk production as per routine practices at the farm. In addition, wheat straw 2.27 kg/animal/d were fed along with concentrate mixture.

4.3.3 Body condition scoring

The body condition score (BCS) of all cows was documented once during wk 1, 4, 8, 12 and 16 of lactation, using the method described by Peters and Ball (1987). The thickness of fat over the lumber and tail head area was estimated and was assigned a score from 1 (very weak) to 5 (very fat). The BCS was categorized as: 1, spine prominent and transverse process feel sharp with little fat cover; 2, transverse processes can be felt but are rounded with a thin covering of fat; 3, individual transverse vertebral processes can only be felt by firm pressure; 4, the transverse processes cannot be felt; and 5, the
transverse processes covered with a thick layer of fat.

4.3.4 Milk yield and laboratory analysis of milk sample

Cows were milked twice a day at 12-h intervals. Milk samples were collected at both milking times once a week on the day of recording of BCS and milk yield. The composite milk samples from both morning and evening milking were analyzed for fat contents using ultrasonic milk analyzer (Ekomilk Total Ultrasonic Milk Analyzer, Bullteh 2000, Stara Zarqora, Bulgharia) according to manufacturer’s instructions. The milk samples were further stored at −20°C.

4.3.5 Determination of Fatty Acids

4.3.5.1 Fat extraction

Fat extraction was performed as mentioned previously in section 3.3.3.1.

4.3.5.2 Preparation of methyl esters

Methyl esters were prepared as described in section 3.3.3.2.

4.3.5.3 Gas chromatography

The details of gas chromatography protocol have been given earlier in section 3.3.3.3.

4.3.6 Statistical Analysis

The data were analyzed by SPSS 10.0 (1999) for Windows XP. The milk fatty acid composition data were subjected to analysis of variance by using the general linear model (GLM) procedures. Means for fatty acids profile were compared for five weeks (1, 4, 8, 12 and 16) of lactation. Means were subsequently ranked using Duncan’s Multiple Range Test (DMRT) as described by Steel and Torrie (1980). A bivariate analysis with Pearson’s correlations (p < 0.05 level, 2-tailed) was applied to determine the relationship between BCS, individual fatty acids, fat content and milk yield.
4.4 RESULTS

The total amount of saturated fatty acids (SFAs) averaged 67.88g/100g varying from 63.34 to 70.31 g/100g and the mean unsaturated fatty acids (UFAs) were 32.39 g/100g ranging from 30.0 to 36.66 g/100g of total fatty acids (Table 4.1). Out of the SFAs the highest amount was of palmitic acid (C16:0, 23.09 g/100g of total fatty acids), followed by stearic acid (C18:0, 16.88 g/100g of total fatty acids) and myristic acid (C14:0, 11.09 g/100g of total fatty acids). The highest monounsaturated fatty acids (MUFAs) level was of oleic acid (C18:1 cis-9, 24.68 g/100g of total fatty acids). The amount of polyunsaturated fatty acids (PUFAs) was 3.95 g/100g of total fatty acids. The total sum of three medium chain fatty acids C12:0, C14:0 and C16:0 identified as hypercholesterolemic fatty acids (HCFAs) was 38.40 g/100g of total fatty acids.

The mean milk yield in crossbred cows recorded during the study was 10.67 kg/d ranging from 7.0 to 16.0 kg/d. (Table 4.1). The average fat percentage was 3.54, varying from 3.00 to 4.86. The correlation analysis showed a significantly positive relationship between BCS and milk fat percent (r=0.278, Figure 4.1).

4.4.1 Changes in unsaturated fatty acids

The fatty acid composition of crossbred cow’s milk as affected by lactation week is presented in table 4.2. Week of lactation significantly (p< 0.05) affected the individual fatty acids. The amount of UFAs were higher during 1st and 4th week and linearly declined during 8th to 12th week and later on remained unchanged (32.99±1.52, 33.41±1.82, 32.11±1.40, 30.90±0.87 and 30.90±0.81 g/100g, respectively, Figure 4.2).

C18:1 cis-9 decreased from wk 1 to 16 of lactation (P < 0.01, Figure 4.3) while polyunsaturated fatty acids (C18:2 cis, cis-9, 12 and C18:3 cis-9, cis-12, cis-15) increased. C18:1 cis-9, correlated negatively with C18:2 cis, cis-9, 12 and C18:3 cis-9, 12, 15 (r = -0.318 and -0.235, respectively, P< 0.05) and short and medium chain fatty acids (C4 to C14). Also, C18:2 cis, cis-9, 12 correlated positively with C16 (r = 0.184).

4.4.2 Changes in saturated fatty acids

The saturated fatty acids showed an increasing pattern, opposite to the trend showed by UFAs during the same period (Figure 4.2). As lactation progressed, the proportion of
de novo (C_4 \text{ to } C_{14}) fatty acids increased (P < 0.01, Figure 4.3). The two hypercholesterolemic fatty acids (HCFAs, C_{12:0} \text{ and } C_{14:0}) increased with advancing lactation, while C_{16:0} was higher in the first week then gradually declined up to 8\textsuperscript{th} week, and resumed increasing trend with advancing lactation.

The short and medium chain fatty acids showed a mutual positive correlation and also with poly unsaturated fatty acids except C_{12}, which correlated negatively with C_{16}. All de novo synthesized fatty acids (C_8 \text{ to } C_{14}) correlated negatively with C_{18:1 \text{ cis}-9}. The SFA correlated positively with de novo synthesized fatty acids while negatively with C_{18:1 \text{ cis}-9}. In contrast to SFAs the UFAs correlated negatively with de novo synthesized fatty acids and positively with C_{18:1 \text{ cis}-9}.

4.5 DISCUSSION

The HCFAs and SFAs and UFAs found in this study is in conformity with the findings of Lock and Garnsworthy (2003) in Holstein Friesian dairy cows where the SFAs varied from 60.0 to 65.0\% and UFA 32.0 to 36.0 \% of the total fatty acids and the HCFAs ranging from 32.6 to 36.0\%. White et al. (2001) reported values close to our findings in Jersey (66.64 g/100g) and Holsteins (63.38 g/100g) when fed pastures and concentrates. Talpur et al. (2006) reported a lower proportion of SFAs content in White Thari and Red Sindhi cows of Pakistan (60.58 vs. 55.53 g/100g, respectively) and the UFAs was reported as 31.61 in Thari vs. 35.02 g/100g in Red Sindhi while HCFAs were 37.34 and 35.84 g/100g when fed total mixed ration. It appears that the crossbred cows have higher concentration of saturated fatty acids and HCFAs but the cardioprotective quality of milk is better than Nili-Ravi buffaloes’ milk as observed in our parallel study (38.40 in crossbred cows vs. 43.33 g/100g of HCFAs in buffaloes).

4.5.1 Changes in unsaturated fatty acids

In the current study, concentrations of preformed fatty acids (sum of 18 and 18:1) were higher in earlier weeks of lactation and lower later on. As expected, owing to increased adipose tissue mobilization immediately after calving, higher concentration of preformed fatty acid were recorded in earlier lactation (wk 1 and 4). Stearic and oleic
acids concentration also showed the same pattern. The major fatty acid in adipocytes is oleic acid and is also the main fatty acid released from adipocytes during lipolysis (Rukkwamsuk et al., 2000; Gillis et al., 2004). The fatty acids like C\textsubscript{16:0}, C\textsubscript{18:0} and C\textsubscript{18:1 cis-9} account for nearly 90% in adipose tissues (Christie, 1981) and body fat mobilization would presumably the transfer of these fatty acids into milk. Also, desaturation of stearic acid occurs in the intestinal epithelium and mammary tissues (Enoch et al., 1976). Some 40–50% of C\textsubscript{18:1 cis-9} in milk fat resulted from C\textsubscript{18:0} in the mammary gland via desaturase, the stearoyl-coenzyme A (CoA) which activates the initiation of a double bond in the \(\Delta^9\) position, between carbons 9 and 10 of a variety of fatty acyl CoA substrates (Chillard et al., 2000). The desired substrate for this is stearic acid, which is converted to oleic acid. Thus oleic acid content of milk fat is in part an outcome of stearoyl CoA desaturase (Banks, 1987). These observations explain that unsaturated fatty acids tend to be higher in early lactation.

At the beginning of lactation, cows experience negative energy balance, resulting in mobilization of adipose tissues reserves and insertion of these long chains fatty acids from adipose tissues into milk fat (Belyea and Adams, 1990). In addition, high uptake of long-chain fatty acids inhibits de novo synthesis of short-chain fatty acids by mammary tissue (Bauman and Davis, 1974).

It is suggested that the cardioprotective quality of milk is better in early lactation probably due to lipolysis of body reserves because of lower feed intake. During advancing lactation, although the polyunsaturated fatty acids increase, but the higher levels of hypercholesterolemic fatty acids make such milk undesirable for the senior citizens.

4.5.2 Changes in saturated fatty acids

Generally, de novo fatty acids comprise approximately 40% by weight over the entire lactation (Bauman and Davis, 1974) while preformed fatty acids normally contribute a larger portion of the total fatty acids in early lactation. The contribution from de novo synthesized fatty acids increases with advancing lactation (Palmquist et al., 1993). The proportion of short chains fatty acids (de novo synthesis), were low at the start of lactation and these fatty acids increases, reaching >90% of maximal proportions by 8 to
10 wk of lactation. This is consistent with the release of inhibition by adipose tissues mobilization, which is mostly completed by 4 to 6 wk of lactation (Garnsworthy and Huggett, 1992). Similarly, Kay et al. (2005) observed a rise in de novo synthesized fatty acids and a decline in preformed fatty acids predominantly stearic and oleic acids with progressing lactation.

In this present study the opposite pattern of BCS and preformed UFAs concentration was probably due to higher contribution of lipolysis to UFAs rather than SFAs. As adipose tissue largely contains C_{16:0}, C_{18:1} cis-9 and C_{18:0}, lipolysis would logically lead to increase accumulation of these fatty acids into milk fat. Whereas, BCS was positively correlated with de novo synthesized SFAs in the current study. With the decrease in reliance over adipose tissue mobilization after achieving positive energy balance within 4 to 8 weeks postpartum, the increase dietary intake is associated with the enhanced rates of de novo synthesis of fatty acids. Bath et al. (1985) reported that the fatty acids synthesized de novo (4 to 16 carbons) mainly resulted from the acetate and β-hydroxybutyrate in mammary gland. The conversion of acetyl-Co A and malonyl-Co A into various fatty acids up to C_{16:0} is catalyzed by the fatty acids synthase (Yin et al., 2001). Similarly, Palmquist et al. (1993) reported that the fatty acids synthesized de novo in the mammary gland are C_{6:0} to C_{14:0} and partially C_{16:0}.

In the present study the higher concentration of SFAs in milk after 8 weeks of lactation presumably resulted from de novo synthesis of fatty acids in mammary gland arises from acetate and butyrate availability from dietary source.

4.5.3 Changes in the sources of fatty acids production

Fatty acids of milk are contributed by various sources comprising, dietary intake, lipolysis, desaturation and de novo synthesis. With advancing lactation the dietary intake shows a gradual increase from the day of parturition onward. On the other hand, milk yield goes on increasing rapidly demanding for higher substrates supply. So, immediately postpartum, the mobilization of body reserves is higher resulting into availability of fatty acids in the milk. Later on, this role is taken up by the higher dietary intake. With the advancing lactation stage, there are changes in hormones from hypothalamus and
pituitary, which regulate various body functions including the production of various types of fatty acids.

Changes in the sources of fatty acids during lactation lead to alteration in fatty acid composition during early weeks of lactation adipose tissue mobilization contributed mainly to the total fatty acid pool, that lead to higher concentration of preformed lipolytic sources fatty acids specifically $C_{18:1}$ and $C_{18:0}$ in milk. Also there is inter-conversion of these long chain fatty acids to $C_{18:2}$ and its trans-isomers and CLA contents (Bauman et al., 1999) but milk have limited amount of these PUFAs at early lactation in the present study and may be due to limited dietary contribution. Dietary sources are mainly responsible for these polyunsaturated fatty acids. According to Bauman et al. (1999) the forages consists mostly of glycolipids and phospholipids while fatty acids are largely the unsaturated ones ($C_{18:2}$ and $C_{18:3}$).

With advancing lactation when mobilization is inhibited and replaced by dietary sources which lead to unique rumen derived, de novo synthesized fatty acids as well as preformed polyunsaturated fatty acids. The net outcome is the higher concentration of higher UFAs with lipolysis and higher SFAs with lipogenesis with progressing lactation. Acetate is the main precursor for the synthesis of short-chain fatty acids while body fat or ingested lipids served as a source for long-chain fatty acids (Payne et al., 1979). Higher proportion of $C_{12:0}$ and $C_{14:0}$ in milk with higher BCS beef cows was reported by Lake et al. (2006) advocating that more de novo synthesis of fatty acids occurs within the mammary glands.

It is suggested that the hypercholesterolemic fatty acids increases with advancing lactation which is probably due to inhibition of lipolysis and increase synthesis of de novo fatty acids from dietary sources. The higher concentrations of these acids are associated with cardiovascular diseases which needs proper attention in dietary formulation for the senior citizens.

**4.5.4 Correlation of individual fatty acids**

Our findings of positive correlation of fatty acid with fat percentage and short chain fatty acids and negative for long-chain fatty acids are supported by Karijord et al. (1982) who reported that genetic correlations with fat percentage were consistently positive for
the proportion of short-chain fatty acids and negative for long-chain fatty acids. Strong positive correlations both phenotypic and genetic were observed among proportions of various short-chain fatty acids (C_{6:0} to C_{14:0}) and also among the various unsaturated fatty acids. The correlations between a short-chain fatty acids and an unsaturated C_{18:0} fatty acids were negative in all cases in the present study.

4.6 CONCLUSION

The present study suggests that the concentrations of unsaturated fatty acids were higher in earlier weeks and declined during mid lactation. With progressing lactation, proportion of de novo fatty acids as well as poly-unsaturated fatty acids increased whereas, pre-formed fatty acids (specifically C_{18:0} and C_{18:1 \text{ cis-9}}) decreased from wk 1 to 16 of lactation. The two hypercholesterolemic fatty acids (C_{12:0} and C_{14:0}) increased with advancing lactation and crossbred cows in early lactation yielded milk containing healthier fatty acids.
Table 4.1: Descriptive statistics for milk yield, fat contents and fatty acids profile (g/100 g, n=140).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS</td>
<td>2.0</td>
<td>3.5</td>
<td>2.77</td>
<td>0.47</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.00</td>
<td>4.86</td>
<td>3.54</td>
<td>0.46</td>
</tr>
<tr>
<td>^1MY (kg/d)</td>
<td>7.00</td>
<td>16.00</td>
<td>10.67</td>
<td>2.08</td>
</tr>
<tr>
<td>C8:0</td>
<td>1.40</td>
<td>2.17</td>
<td>1.74</td>
<td>0.16</td>
</tr>
<tr>
<td>C10:0</td>
<td>3.20</td>
<td>4.00</td>
<td>3.65</td>
<td>0.21</td>
</tr>
<tr>
<td>C12:0</td>
<td>3.10</td>
<td>4.92</td>
<td>4.22</td>
<td>0.38</td>
</tr>
<tr>
<td>C14:0</td>
<td>9.00</td>
<td>15.00</td>
<td>11.09</td>
<td>1.50</td>
</tr>
<tr>
<td>C16:0</td>
<td>18.00</td>
<td>26.00</td>
<td>23.09</td>
<td>1.62</td>
</tr>
<tr>
<td>C18:0</td>
<td>14.12</td>
<td>19.00</td>
<td>16.88</td>
<td>1.25</td>
</tr>
<tr>
<td>C18:1 cis-9</td>
<td>21.00</td>
<td>29.00</td>
<td>24.68</td>
<td>1.75</td>
</tr>
<tr>
<td>C18:2 cis-9,12</td>
<td>2.10</td>
<td>4.78</td>
<td>2.97</td>
<td>0.63</td>
</tr>
<tr>
<td>C18:3 cis 9,12,15</td>
<td>0.60</td>
<td>2.00</td>
<td>0.98</td>
<td>0.22</td>
</tr>
<tr>
<td>^2SFAs %</td>
<td>63.34</td>
<td>70.31</td>
<td>67.61</td>
<td>1.68</td>
</tr>
<tr>
<td>^3UFAs %</td>
<td>30.0</td>
<td>36.66</td>
<td>32.39</td>
<td>1.68</td>
</tr>
</tbody>
</table>

^1Milk yield; ^2SFAs = saturated fatty acids; ^3UFAs = Unsaturated fatty acid
Table 4.2: Mean and standard deviation for milk fatty acids (g/100 g) as influenced by lactation week in crossbred dairy cows (n = 140).

<table>
<thead>
<tr>
<th>Lactation Week</th>
<th>1</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8:0</td>
<td>1.64c±0.12</td>
<td>1.65c±0.12</td>
<td>1.72b±0.15</td>
<td>1.87a±0.12</td>
<td>1.87a±0.13</td>
<td>***</td>
</tr>
<tr>
<td>C10:0</td>
<td>3.55b±0.15</td>
<td>3.56b±0.18</td>
<td>3.60b±0.23</td>
<td>3.75a±0.19</td>
<td>3.81a±0.17</td>
<td>***</td>
</tr>
<tr>
<td>C12:0</td>
<td>3.80c±0.28</td>
<td>4.12b±0.37</td>
<td>4.25b±0.20</td>
<td>4.49a±0.31</td>
<td>4.44a±0.34</td>
<td>***</td>
</tr>
<tr>
<td>C14:0</td>
<td>9.51b±0.75</td>
<td>9.63b±0.93</td>
<td>11.89a±0.88</td>
<td>12.19a±0.60</td>
<td>12.24a±0.95</td>
<td>***</td>
</tr>
<tr>
<td>C16:0</td>
<td>24.07a±1.30</td>
<td>22.83bc±1.40</td>
<td>22.24c±1.66</td>
<td>22.90bc±1.52</td>
<td>23.40bc±1.67</td>
<td>***</td>
</tr>
<tr>
<td>C18:0</td>
<td>17.25a±1.18</td>
<td>17.16a±0.97</td>
<td>16.98a±0.74</td>
<td>16.78ab±1.53</td>
<td>16.24b±1.49</td>
<td>*</td>
</tr>
<tr>
<td>C18:1 cis-9</td>
<td>25.89a±1.25</td>
<td>25.92a±1.76</td>
<td>24.79b±1.36</td>
<td>23.39a±1.27</td>
<td>23.37a±1.03</td>
<td>***</td>
</tr>
<tr>
<td>C18:2 cis-9,12</td>
<td>2.68c±0.59</td>
<td>2.70b±0.58</td>
<td>2.97bc±0.61</td>
<td>3.18ab±0.55</td>
<td>3.36a±0.64</td>
<td>***</td>
</tr>
<tr>
<td>1SFAs%</td>
<td>67.00c±1.52</td>
<td>66.59c±1.82</td>
<td>67.89a±1.40</td>
<td>69.10a±0.87</td>
<td>69.10a±0.88</td>
<td>***</td>
</tr>
<tr>
<td>2UFAs%</td>
<td>32.99a±1.52</td>
<td>33.41a±1.82</td>
<td>32.11b±1.40</td>
<td>30.90b±0.87</td>
<td>30.90b±0.81</td>
<td>***</td>
</tr>
</tbody>
</table>

*Mean in the same row having different superscripts are significantly different (P < 0.05)

1SFAs = saturated fatty acids; 2UFAs = Unsaturated fatty acid;

* = P < 0.05; ** = P < 0.01; *** = P < 0.001
Table 4.3: Correlation of milk fatty acids (g/100 g) with BCS, fat and milk yield in dairy buffaloes.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlating parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>²BCS</td>
</tr>
<tr>
<td>Fat</td>
<td>0.278**</td>
</tr>
<tr>
<td>³MY</td>
<td>-0.022ns</td>
</tr>
<tr>
<td>C-8:0</td>
<td>-0.001ns</td>
</tr>
<tr>
<td>C-10:0</td>
<td>0.068ns</td>
</tr>
<tr>
<td>C-12:0</td>
<td>0.194†</td>
</tr>
<tr>
<td>C-14:0</td>
<td>0.102ns</td>
</tr>
<tr>
<td>C-16:0</td>
<td>-0.147</td>
</tr>
<tr>
<td>C-18:0</td>
<td>0.152</td>
</tr>
<tr>
<td>C 18:1 cis-9</td>
<td>-0.115ns</td>
</tr>
<tr>
<td>C 18:2 cis-9,12</td>
<td>-0.152ns</td>
</tr>
<tr>
<td>C 18:3 cis 9,12,15</td>
<td>-0.107ns</td>
</tr>
<tr>
<td>⁴SFA%</td>
<td>0.207†</td>
</tr>
<tr>
<td>⁴UFA%</td>
<td>-0.207†</td>
</tr>
<tr>
<td>C 16:0</td>
<td></td>
</tr>
<tr>
<td>C 18:0</td>
<td></td>
</tr>
<tr>
<td>C 18:1 cis-9</td>
<td></td>
</tr>
<tr>
<td>C 18:2 cis-9,12</td>
<td></td>
</tr>
<tr>
<td>C 18:3 cis 9,12,15</td>
<td></td>
</tr>
<tr>
<td>⁴SFA</td>
<td></td>
</tr>
<tr>
<td>⁴UFA</td>
<td></td>
</tr>
</tbody>
</table>

ns = Non-Significant difference; * = P < 0.05; ** = P < 0.01

³MY: Milk yield; ²BCS: Body condition score (scale 1 to 5); ⁴SFA= saturated fatty acids;
⁴UFA= Unsaturated fatty acid
Figure 4.1: Changes in body condition score (BCS, ■) and milk fat percent (▲) with lactation week in crossbred cows. The fat percent and BCS correlated positively ($r=0.278$, $P<0.01$).
Figure 4.2: Changes in concentration of saturated (SFAs, dotted area in the bars and the dotted trendline) and un-saturated fatty acids (UFAs, vertical lines in the bars and solid trendline) with lactation week in crossbred cows. Both types of the fatty acids significantly changed with advancing lactation (P<0.05). The two parameters showed an R² value of 0.84.
Figure 4.3: Concentration of milk fatty acids in milk fat at week 1, 4, 8, 12 and 16 of lactation in crossbred cows.
4.7 REFERENCES


V. Body condition score as an indicator of milk yield and composition in Nili-Ravi buffaloes during lactation

5.1 ABSTRACT

The production efficiency and metabolic stress of lactating cows is the dynamics of energy balance during lactation. Energy balance or imbalance is the difference between energy expenditure including yield and energy sources from intake of nutrients and mobilized body reserves. The careful management of energy in dairy animals is essential for efficient production and reproduction. BCS can be a useful monitoring tool to indicate the mobilization and deposition of body reserves during lactation. The present study was undertaken to evaluate the role of body condition score (BCS) as an indicator of milk yield and composition in Nili-Ravi buffaloes under subtropical conditions. A total of 36 buffaloes within 1st week after parturition were selected from private peri-urban dairy farm at district Peshawar. All the animals were offered green fodders ad libitum and concentrate at the rate of 1kg per 2 kg of milk produced. Milk yield (kg/d) and BCS (scale 1-5) were recorded weekly and milk samples (n = 1008) were collected for analysis of fat, protein and lactose contents. The study continued for 7 months, starting from November 2007 to May 2008. Group means were compared and correlation was worked out. BCS significantly affected milk yield, fat and protein contents. Lactose was least affected with changes in BCS during lactation. Highest milk yield was recorded with moderate BCS in the buffaloes. BCS correlated positively with fat and protein and negatively with milk yield. Milk yield decreased while BCS increased with advancing lactation. The negative relationship may be due to mobilization of body reserves, indicating their better genetic potential as dairy breeds. The results indicated that BCS may be used as an indicator for assessing milk yield and composition in dairy buffaloes.

Keywords: Dairy buffalo, BCS, milk composition, milk yield, lactation stage
5.2 INTRODUCTION

Buffalo being declared as a black gold in Pakistan with a population of 29.9 million heads, contributes 62% of the 43 million tones of milk produced in the country (GOP, 2009). Buffalo population predominantly consists of Nili-Ravi and Kundi breeds. Consumers prefer buffalo milk to cow’s milk due to its richness with higher concentration of fat, protein, lactose and lower amount of water (Ligda, 1999). Cockrill (1994) stated that of all domestic animals, Asian buffalo grab the greatest promise for milk yield. Similarly, FAO (2000) mentioned buffalo as essential but an asset underestimated.

Body condition scores (BCS) are subjective, visual or physical assessment of the amount of metabolizable energy stored in the fat and muscle in a live animal. It has been considered an effective tool in monitoring the energy intake of cows and herds (Jeffrey and James, 1989). BCS is routinely practiced in the farm management for evaluation of nutritional status of cattle. In order, to asses changes in body reserves as a consequence of negative energy balance BCS recording has been recommended (Berry et al., 2002). The body condition is usually judged through a 5-point scale, with 1 equivalent to an extremely lean cow, while 5 to a cow having excessive fat reserves (Peters and Ball, 1987). Credible research evidences confirmed that body reserves are better reflected by BCS than by live weight change (Grainger et al., 1982; Johnson, 1984; Ducker et al., 1985).

Until 1970s, no mechanism was available to determine the cow’s energy reserves. Although, the animal body weight (BW) recording was a usual practice but it did not provide reliable estimate of the energy reserves, as the reserves vary about 40 % in cows with same body weight (Gibb et al., 1992; Andrew et al., 1994). Wright and Russel (1984) found a significantly positive correlation ($r^2 = 0.86$) between a live animal visual evaluation of body condition and the actual dissected fat in Friesian cows. Although, the feed intake is increasing in early lactation but the tissues reserves are mobilized at that time, the extent of body tissue loss may be overlapped by gut fill, thus body weight changes may not indicate changes in bio-energetically vital tissues (National Research
Therefore, assessment of body condition scoring is suggested for accurate determination of energy reserves (Macdonald and Roche, 2004) in order to overcome the variability of BW. The mobilization of body energy reserves is the major capability of the dairy cow. The mobilization of reserves is indispensable for maintaining high milk yield following parturition. Like the other lactating animals, the dairy cows are normally in negative energy status at the start of lactation (Nielsen, 1999). The change in BCS in the first few weeks of lactation point towards the level of metabolic load as the shortfall of energy to milk production is considered to be met through mobilizing body reserves (Pryce and Løvendahl, 1999). Since energy intake does not maintain speed with continuously increasing milk yield, energy shortfall in early lactation enhances, creating a competitive conditions among milk yield, fertility and health status of the dairy cow as all these traits are interlinked with energy (Staufenbiel et al., 1992). Also, Várhegyi (1999) reported that from the calving until the attainment of peak milk yield the energy and protein needs raised by four to ten times.

The current milk production and nutritional quality is the result of the prevailing dairy farming practices at various farms combined with the local environmental conditions in NWFP. BCS may play a role in regulating appetite, feed intake and lactation in dairy animals under subtropical conditions. The majority of the research work conducted on the affects of BCS on milk yield is from technologically advanced countries with temperate environment, largely in Holstein Friesian cows (Treacher et al., 1986; Garnsworthy and Jones, 1987), offered mainly total mixed rations (Pedron et al., 1993; Domecq et al., 1997). Research work on the effect of BCS on milk production and its composition in the subtropical environment of Pakistan with local dairy buffaloes under the existing management practices is needed. Therefore, the present study was conducted to assess the role of BCS as an indicator of milk production and composition in Nili-Ravi dairy buffaloes under subtropical conditions.
5.3 MATERIALS AND METHODS

5.3.1 Selection and management of animals

The present study was conducted at Peshawar, situated in the central valley of the North-West Frontier Province (NWFP) of Pakistan, located at 31-37° north and 65-74° east. The temperature ranged from 25 to 48 °C during summer and 4 to 18 °C during winter. Annual precipitation was recorded as 400 millimeters. The study continued for 7 months, starting from November 2007 to May 2008. A total of 36 buffaloes within 1st week postpartum were selected from private peri-urban dairy farm situated at village Palosi close to University campus. The buffaloes were multiparous with body weight ranging from 450 to 550 kg. Selected animals were ear-tagged and vaccinated.

Peri-urban dairy farms are located close to big cities to meet the demand of urban population. The buffaloes are reared under intensive farming system with higher input cost but little scientific and marketing support. The experimental animals were offered green fodders (Egyptian clover) ad libitum and concentrate (wheat bran, cotton seed cake and maize oil cake, molasses and macrominerals) mixture at the rate of 1 kg per 2 kg of milk produced as proposed by Ranjhan (1994) for buffaloes in tropical environment. All the animals consume identical diet during the experimental period. The animals were milked twice a day at 12 hours interval. A total of 1008 raw milk samples were collected from buffaloes for laboratory analysis. The animals were stall-fed and chopped fodders were provided at 1000 and 1400 hrs. The water was made available to the animals from an adjacent tank 2 to 3 times a day.

Table 5.1: Nutrient composition of forage and concentrate used for experimental buffaloes

<table>
<thead>
<tr>
<th>Feed</th>
<th>Harvest Month</th>
<th>Cut number</th>
<th>DM (%)</th>
<th>Percent in DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Minerals</td>
</tr>
<tr>
<td>Green Fodder</td>
<td>Nov-Dec</td>
<td>1st</td>
<td>13.60</td>
<td>14.91</td>
</tr>
<tr>
<td>Egyptian Clover</td>
<td>Jan-Feb</td>
<td>2nd</td>
<td>14.31</td>
<td>10.89</td>
</tr>
<tr>
<td></td>
<td>Mar-May</td>
<td>3rd, 4th</td>
<td>15.33</td>
<td>13.00</td>
</tr>
<tr>
<td>Concentrate mixture</td>
<td></td>
<td></td>
<td>93.68</td>
<td>6.68</td>
</tr>
</tbody>
</table>
5.3.2 Body condition, milk yield, sampling and analysis

Body condition score (BCS) of all the cows was recorded weekly, by the method of Qureshi et al. (2002). Daily milk yield (kg) was recorded once a week for seven consecutive months in the buffaloes. Both morning and evening milk samples (200 ml) were collected in bottles. The bottles were transported in an icebox to the dairy technology laboratory for further analysis. Pooled milk sample from both morning and evening milking were used for analysis. Milk fat, protein and lactose contents were analyzed using ultrasonic milk analyzer (Ekomilk Total Ultrasonic Milk Analyzer, Bullteh 2000, Stara Zarqora, Bulgharia) according to manufacturer’s instructions.

5.3.3 Statistical analysis

Statistics were performed by SPSS 10.0 (1999) for Windows XP. The data collected were subjected to analysis of variance by using general linear model (GLM). Means of milk yield and composition were compared for various levels of BCS (2.5, 3.0 and 3.5; P< 0.05). Means were subsequently ranked using Duncan’s Multiple Range Test (DMRT) as described by Steel and Torrie (1980). A bivariate analysis with Pearson’s correlations (P< 0.05 level, 2-tailed) was applied to determine the relationship between BCS, milk yield and milk contents.

5.4 RESULTS

5.4.1 BCS effect on milk yield and composition

BCS significantly affected the milk yield, fat and protein contents in Nili-Ravi buffaloes (Table 5.2). Highest milk yield (9.18± 1.87 kg/day) was recorded with moderate BCS (2.5). Fat and protein contents increased with increasing BCS during lactation.

Correlation analysis of the data showed that BCS was positively associated with fat (r= 0.258, P< 0.05) and protein (r=0.194, P< 0.05) contents while negatively with milk yield (r= -0.638, P< 0.05, Table 5.3). Fat and protein were correlated positively (r= 0.58, 1 P< 0.05) with each other and negatively with lactose (r=−0.134, -0.122 respectively, P<
Figure 5.1 shows changes in milk fat, protein and lactose associated with BCS in dairy buffaloes. Figure 5.2 illustrates changes in milk yield and fat with changes in body condition score (BCS) in buffaloes. Milk yield and fat percent correlated negatively and significantly with BCS ($r = -0.417$, $P< 0.05$).

### 5.4.2 Changes in BCS during lactation

Changes in milk yield and body condition were mirror images to each other with the advancing lactation (Figure 5.3, $R^2 = 0.95, 0.88$, respectively). Milk yield showed an increasing pattern up to 4th week, associated with decrease in BCS. The BCS declined continued for another 4 weeks. Later on, the milk yield consistently decreased and BCS increased up to the last week of lactation. The BCS and milk fat contents exhibited almost parallel increasing trend with the advancing lactation. A slight decline was observed in fat contents up to 5th month postpartum and later on a gradual and consistent upward trend was recorded (Figure 5.4, $R^2 = 0.91, 0.88$, respectively).

### 5.5 DISCUSSION

#### 5.5.1 Effect of BCS on milk yield and composition

Moderate BCS supported higher milk yield in buffaloes. The milk yield was negatively correlated with BCS presumably due to mobilization of body reserves. This study confirms the previous report (Jílek et al., 2008) that cows with moderate BCS in the first month of lactation showed the highest milk yield during the first 5 months of lactation. Waltner et al. (1993) hypothesized that cows with higher BCS may display low appetite probably due to their increased catabolism of body tissues (Roche et al., 2007) and the succeeding effect of circulating free-fatty acids on feed consumption (Garnsworthy and Topps, 1982). This emphasize the importance of intermediate optimum BCS at calving for expressing higher milk yield levels. Roche et al. (2007) reported that optimum calving BCS for milk production in dairy cows was approximately 3.5 at the 5-point scale, however, there was a little increase in milk yield beyond a BCS of 3.0. The
BCS at calving remains comparatively higher and its loss during the succeeding weeks is a physiological phenomenon, leaving the score of 2.5 as an optimum, which resulted in higher yield in our study.

The dairy cow with high genetic merit, have a higher predisposition for mobilization of body fat reserves to cover milk production demands (Pryce et al., 2002). Like the other lactating animals dairy cattle generally are in negative energy balance at the start of lactation (Nielsen, 1999). Berry et al., (2003) demonstrated that cows selected for higher milk yield mobilized her lipid reserves more than low producers. Thus, compromise on her body condition is more in high producers than lower ones. The high producing cows had lower BCS during lactation and their BCS changes after calving were higher than in cows with lower genetic merit (Horan et al., 2005). Thus, mobilization of body fat reserves and milk production are closely and negative correlated (Pryce et al., 2002, Veerkamp and Brotherstone, 1997).

Garnsworthy and Topps (1982) observed a higher feed intake and milk yield in thin cows at the time of calving compare to obese cows. Whereas, cows that are over conditioned at calving may probably produce less milk along with increased reproductive and health problems (Morrow, 1976; Fronk et al., 1980; Gearhart et al., 1990). Cows that are genetically inclined to lose more BCS in early lactation tend to have higher yields of milk during entire lactation (Dechow et al., 2002). Our findings of the negative correlation of BCS with milk yield are in conformity with these reports.

Markusfeld et al. (1997) reported a significant correlation between milk yield and BCS. The decline in milk yield in cows that lost more BCS post calving is in conformity with most prior studies (Berry et al., 2007). Despite the fact that higher 120-day milk yield was reported in multiparous cows that lost more BCS in the early lactation but a non significant effect was observed in primiparous cows by Domezq et al. (1997). The environmental and genotype interactions (Falconer, 1952) may also affect the differences in the studies having dissimilar genotypes and environmental conditions. Moreover, Berry et al. (2007) reported that higher BCS at calving was correlated with higher milk fat and protein concentration in early lactation. The milk fat and protein concentration
was lower with a calving BCS of 2.75 and 3.00. This also supports our findings of linear increase in fat and protein concentration with increase in BCS in advancing lactation.

5.5.2 Changes in BCS during lactation

In early lactation, a decline and later on an increase was observed in BCS. Our results are supported by Pryce et al. (2001) who reported decline in BCS at the start of lactation and the restoration after 12 weeks of lactation. Also, Banos et al. (2004) observed a decline in BCS during the starting 2 to 3 months of lactation and an improvement later on. Coffey et al. (2003) showed that the minimum body energy level usually occur, at 3rd months after parturition, very near to the peak of daily milk yield. Cows generally return to positive energy balance between 40 and 80 days in milk (Sutter and Beever, 2000; Veerkamp et al., 2000 and Coffey et al., 2002).

A constant body condition score is related with the cow’s potential to produce milk while at the same time maintained its energy balance. It is very difficult for a cow to maintain its BCS during the whole lactation, as changes occurs constantly within daily milk yield as well as within physiology of animal that leads to variation in BCS (Coffey et al., 2003). The mobilization of reserves is indispensable for maintaining high milk yield following parturition. With advancing lactation body condition score of a cow changes probably responding to changes in her energy balance (Coffey et al., 2003). This process is correlated to the daily milk yield curve, which is quite precisely opposite to the BCS curves (Coffey et al., 2002, 2003). These results also support our findings of body condition mobilization and deposition with increase and decrease in milk production.

5.6 CONCLUSION

Higher milk yield was supported by moderate BCS in buffaloes. BCS correlated positively with fat and protein and negatively with milk yield. Milk yield increased while BCS decreased in early lactation and later on the trend was reversed. These findings suggest that BCS may be used as an indicator of milk yield and its composition in dairy animals and dairy breeders may include moderate BCS in selection if the goal is higher milk production in buffaloes under subtropical conditions.
<table>
<thead>
<tr>
<th>BCS</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Lactose (%)</th>
<th>¹MY (kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>5.01^c ± 0.81</td>
<td>3.29^b ± 0.24</td>
<td>4.21 ± 0.01</td>
<td>9.18^a ± 1.87</td>
</tr>
<tr>
<td></td>
<td>(477)</td>
<td>(477)</td>
<td>(477)</td>
<td>(477)</td>
</tr>
<tr>
<td>3.0</td>
<td>5.25^b ± 0.80</td>
<td>3.28^b ± 0.25</td>
<td>4.21 ± 0.07</td>
<td>6.78^b ± 2.24</td>
</tr>
<tr>
<td></td>
<td>(289)</td>
<td>(289)</td>
<td>(289)</td>
<td>(289)</td>
</tr>
<tr>
<td>3.5</td>
<td>5.60^a ± 1.10</td>
<td>3.43^a ± 0.25</td>
<td>4.20 ± 0.01</td>
<td>5.44^c ± 1.31</td>
</tr>
<tr>
<td></td>
<td>(242)</td>
<td>(242)</td>
<td>(242)</td>
<td>(242)</td>
</tr>
<tr>
<td>P- value</td>
<td>0.001</td>
<td>0.001</td>
<td>0.123</td>
<td>0.001</td>
</tr>
</tbody>
</table>

^a,b,c Mean in the same column having different superscripts are significantly different; ¹MY, Milk Yield.
Table 5.3: Relationship of body condition score with milk yield and composition in buffaloes (Pearson’s correlation coefficient, n=1008).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BCS</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Lactose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (%)</td>
<td>0.258**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>0.194**</td>
<td>0.581**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>-0.055 ns</td>
<td>-0.134**</td>
<td>-0.122**</td>
<td></td>
</tr>
<tr>
<td>Milk Yield (kg/d)</td>
<td>-0.638**</td>
<td>-0.417**</td>
<td>-0.285**</td>
<td>0.137**</td>
</tr>
</tbody>
</table>

** = P< 0.01; ns = non-significant difference.
Figure 5.1: Changes in milk fat (%, vertical lines), protein (%, horizontal lines) and lactose (%, dots) with BCS in Nili-Ravi buffaloes (P<0.001, P<0.001 and P>0.123, respectively).
Figure 5.2: Changes in mean milk yield (■) and fat (▲) with changes in body condition score (BCS) in buffaloes. Milk yield correlated negatively and fat percent positively with BCS (r = -0.638, 0.258, respectively).
Figure 5.3: Changes in milk yield (MY, ▲) and body condition score (BCS, ■) with advancing lactation in buffaloes. Milk yield correlated negatively and significantly with BCS (r=-0.638, P< 0.05). Equation for polynomial trend line of BCS; Y=0.00X^2-0.02X+2.72, R^2=0.88 and Milk yield; Y=-0.01X^2+0.00X+9.64, R^2=0.95.
Figure 5.4: Changes in milk fat content (▲) and body condition score (BCS, ■) with advancing lactation in buffaloes. Milk fat correlated positively and significantly with BCS (r= 0.258, P< 0.05). Equation for polynomial trend line of Fat content; \( Y=0.00X^2+0.01X+4.94, R^2=0.91 \) and BCS; \( y=0.00X^2-0.02X+2.72, R^2=0.88 \)
5.7 REFERENCES


VI. SUMMARY

Milk composition is the result of various biological reactions, affected by feed intake and physiological status of animals. Physiological status, in turn, is affected by environment modulated through hypothalamus and pituitary. Milk composition and quality are important characteristics that determine the nutritive value and consumer acceptability. During milk synthesis, substrates are taken from the blood circulation by the mammary tissues for conversion into milk constituents. An important tool in this partitioning of nutrients is the presence of tight junctions.

Cattle and buffalo milk has been a major item of human diet in the south east Asia and the Mediterranean region. Little information is available about the milk composition and the fatty acid concentrations of dairy animals and its relationship with the management and physiological states of the local animals. BCS may play a role in regulating appetite and feed intake thereby affecting milk yield and composition and especially the fatty acids profiles in dairy animals under subtropical conditions. Present series of studies were performed to investigate relationship of body condition and lactation stage on milk fatty acids composition. The objective also included variation in milk yield and composition with body condition in dairy buffaloes.

The first study was conducted to investigate the effect of body condition on milk fatty composition. A total of 24 Nili-Ravi buffaloes within 60 days after parturition were selected from a private dairy farm at district Peshawar. All the experimental animals received relatively uniform diet, consisting of green fodder and concentrates. A total of 576 raw milk samples were collected from buffaloes for laboratory analysis. The study continued up to 6 months during the year 2008. Body condition score (BCS), milk yield and composition were recorded once a week. Quantification of fatty acid methyl esters were performed by Perkin-Elmer chromatograph (model Clarus 500, Beaconsfield, UK). Identification of Peaks was carried out by using pure methyl ester standards (GLC-10 and GLC-30, FAME mixtures).

Mean concentration of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) were 64.22 g/100g and 35.79 g/100g of total fatty acids, respectively. Out of
the SFAs the highest amount was of $C_{16:0}$ followed by $C_{18:0}$ and $C_{14:0}$. The total sum of hypercholesterolemic fatty acids (HCFAs) $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ were 43.33 g/100g of total fatty acids. The concentrations of UFAs were greater with the moderate BCS followed by poor and highest one while SFAs showed an opposite trend. The correlation analysis showed that milk yield was negatively affected by BCS and milk fat positively though non-significantly. The present study suggests that Nili-Ravi buffaloes with moderate body condition yielded healthier milk fatty acid.

The present study was carried out to examine the effect of lactation advancement on milk yield and its fatty acid composition in crossbred cows during initial 16 weeks after parturition. A total of 28, F1 crossbred cows (HF x Sahiwal) within 1st week after parturition were selected from a large state farm. The animals were maintained under uniform management conditions in a well-ventilated shed. Animals were milked twice and milk samples were collected from each cow once a week during 1, 4, 8, 12 and 16 weeks of lactation. BCS and milk yield were recorded on the day of sampling.

The SFAs averaged 67.88g/100g and the UFAs 32.39 g/100g of total fatty acids. Out of the SFAs the higher concentration was of palmitic acid while the higher monounsaturated fatty acids (MUFAs) level was of oleic acid. Mean concentration of polyunsaturated fatty acids (PUFAs) was 3.95 g/100g of total fatty acids. The total sum of hypercholesterolemic fatty acids (HCFAs) were 38.40 g/100g of total fatty acids. The correlation analysis showed a significantly positive relationship between BCS and milk fat percent.

The de novo synthesized fatty acids ($C_{8:0}$ to $C_{14:0}$) correlated negatively with $C_{18:1\text{ cis}-9}$. The SFAs correlated positively with de novo synthesized fatty acids while negatively with $C_{18:1\text{ cis}-9}$. Opposite to SFAs, UFAs correlated negatively with de novo synthesized fatty acids and positively with $C_{18:1\text{ cis}-9}$. Concentrations of unsaturated fatty acids were high in earlier weeks and declined during mid lactation. With advancement of lactation, from wk 1 to 16 of lactation, the proportion of both de novo fatty acids and poly-unsaturated fatty acids increased and pre-formed fatty acids (specifically $C_{18:0}$ and $C_{18:1\text{ cis}-9}$) decreased. The two hypercholesterolemic fatty acids ($C_{12:0}$ and $C_{14:0}$) increased with advancing lactation and the cows in early lactation yielded milk containing healthier fatty acids.
The careful management of energy in dairy animals is essential for efficient production and reproduction. BCS can be a useful monitoring tool to indicate the mobilization and deposition of adipose tissues reserves within lactation. The third study was undertaken to assess the role of body condition score (BCS) as an indicator of milk yield and composition in Nili-Ravi buffaloes under subtropical conditions. A total of 36 buffaloes within 1st week after parturition were selected from a private peri-urban dairy farm near university campus.

The experimental animals were offered green fodders (Egyptian clover) ad libitum and concentrate (wheat bran, cotton seed cake and maize oil cake, molasses and macrominerals) mixture at the rate of 1 kg per 2 kg of milk produced. Animals were milked twice a day at 12 hours interval. A total of 1008 raw milk samples were collected from buffaloes for laboratory analysis. The animals were stall-fed and chopped fodders were provided at 1000 and 1400 hrs. Milk yield (kg/d) and BCS (scale 1-5) were recorded weekly and milk samples (n = 1008) were collected for analysis of fat, protein and lactose contents. The study continued for 7 months, starting from November 2007 to May 2008. Group means were compared and correlation was worked out.

BCS significantly affected milk yield, fat and protein contents. Lactose was least affected with changes in BCS during lactation. Highest milk yield was recorded with moderate BCS in the buffaloes. BCS correlated positively with fat and protein and negatively with milk yield. Milk yield decreased while BCS increased with advancing lactation. The negative relationship may be due to mobilization of body reserves, indicating their better genetic potential as dairy animal. These findings suggest that BCS may be used as an indicator for assessing milk yield and composition in dairy animals and dairy breeders may include moderate BCS in selection if the goal is higher milk production in buffaloes under subtropical conditions.
VII. CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

i. Nili-Ravi buffaloes with moderate body condition yielded greater concentrations of UFAs followed by poor and highest ones.

ii. Two hypercholesterolemic fatty acids were associated with higher body condition.

iii. SFAs and UFAs correlated negatively.

iv. The concentrations of unsaturated fatty acids were higher in earlier weeks and declined during mid lactation.

v. The proportion of short and medium chain fatty acids increased and pre-formed fatty acids decreased from wk 1 to 16 of lactation.

vi. The two hypercholesterolemic fatty acids (C_{12:0} and C_{14:0}) increased with advancing lactation.

vii. Milk yield was higher while BCS lower in early lactation and later on the trend was reversed.

viii. BCS and milk fat content exhibited positive correlation.

ix. BCS may be used as an indicator of milk yield and composition in dairy animals

7.2 RECOMMENDATIONS

For dairy farmers:

1) During selection and subsequent management the dairy buffaloes may be kept with the moderate body condition score to get higher concentrations of healthier fatty acids and higher milk yield along with preventing extra expenditures on overfeeding.

2) The postpartum drastic loss in the BCS associated with increasing milk yield, may be minimized by appropriate feeding and management strategies.
For medical practitioners:
3) The milk from crossbred dairy cows in early lactation may be preferred for consumption by the elderly people because of their higher concentrations of cardio-protective fatty acids (CPFAs).

4) HCFAs and CPFAs in milk deserve major consideration for human health rather than total SFAs and UFAs concentrations.

For researchers:
5) Further studies are recommended for identifying key biological and management elements for producing healthier milk from dairy cows and buffaloes.

6) Investigations are required for devising feeding strategies for contributing appropriate fatty acids precursors to the blood pool for producing healthier milk.

7) The desaturase enzyme in the mammary tissues and other body systems influence the availability of unsaturated fatty acids in the milk and this enzyme has been found in various types of dairy animals. This enzyme needs to be investigated and supported for improving the milk quality.
VIII. Literature Cited


Bayourthe, C., F. Enjalbert and R. Moncoulon. 2000. Effects of different forms of canola oil fatty acids plus canola meal on milk composition and physical properties


Christie, W.W. 1981. The composition, structure and function of lipids in the tissues of


Initiative, PPLPI working paper number. 3:8-25.


Khan, S.M.S. Qureshi, N. Ahmad, M. Amjad, M. Younas and A. Rahman. 2009. Feed


Mansbridge, R.J. and J.S. Blake. 1997. Nutritional factors affecting the fatty acid


resources. Perambul Books, Australia.


Ryan, A.J., J.D. Medh and D.M. McCoy. 2002. Maternal loading with very low density


genetic merit and energy intake. J. Dairy Sci. 73:772-783.


traits, food intake, live weight and condition score in Holstein Friesian cattle. Anim.
Sci. 64: 385-392.
26-32.
score to production variables in high producing Holstein dairy cattle. J. Dairy Sci.
76: 3410–3419.
Ward, R.J., M.T. Travers, S.E. Richards, R.G. Vernon, A.M. Salter, P.J. Butterly and
M.C. Barber. 1998. Stearoyl-CoA desaturase mRNA is transcribed from a single
beneficial or detrimental to health? Prog. Lipid Res. 43: 553–587.
2001. Comparison of fatty acid content of milk in Jersey and Holstein cows
consuming pasture or total mixed ration. J. Dairy Sci. 84: 2295-2301.
Ramaswamy and K.M. Kasperson. 2002. Fish oil and extruded soyabees fed in
combination increase conjugated linoleic acid in milk of dairy cows more than when
Wilcox, C.J., K.O. Pfau, R.E. Marhex and J.W. Bartlett. 1959. Genetic and
environmental influence upon solids-not-fat content of cow’s milk. J. Dairy Sci.
42:1132.
Lesch. 1982. A dairy cow body condition scoring system and its relationship to
49:165-180.
Williams, G.L. and R.L. Stanko. 2000. Dietary fats as reproductive nutraceuticals in
2009.