The Journal of Animal and Plant Sciences, 22(2 Suppl.): 2012, Page: 108-112
ISSN: 1018-7081

EFFECT OF AGE ON MILK FATTY ACIDS IN DAIRY BUFFFALOES

M. S. Qureshi*, S. Jan, A. Mushtaq, I.U. Rahman[1], M. Jan and Ikramullah

Faculty of Animal Husbandry and Veterinary Sciences, Khyber Pukhtunkhwa Agricultural University, Peshawar-25120, Pakistan; [1]Pakistan Council for Scientific and Industrial Research Laboratories, University Road, Peshawar.
*Corresponding author: drmsqureshi@aup.edu.pk

ABSTRACT

Milk quality has been an issue of public health concern and changes occur in milk composition with the changing physiological and management states. An experiment was conducted to investigate changes in milk yield and fatty acids profile with advancing age and lactation stage in dairy buffaloes. A total of 12 multiparous lactating dairy buffaloes were divided into two groups younger (1-4 lactation number) and mature (8-10 lactation number) having six animals in each one, which were further subdivided into early (1-3 months) and late (7-10 months) lactation stage. All the buffaloes were stall fed, green fodders were provided *ad libitum* and concentrate mixture at the scale of 1 kg/2 lit of milk production. The experiment continued for six weeks starting from November upto mid of December 2009. Milk samples were weekly collected for analysis of fatty acids profile. Saturated FAs (SFAs) showed the highest concentration out of the total milk fatty acids and averaged 70.41 g/100 g ranging from 64.96 to 78.83 g/100 g. The concentration of C14:1, C16:1 and C18:3 was significantly ($P<0.05$) higher in younger buffaloes while C18:1cis, medium chain fatty acids (MCFA) and long chain fatty acids (LCFA) were higher in older ones ($P <0.05$). The ratio of Δ9 desaturase activity was significantly ($P <0.05$) higher in younger animals (0.11) compared to older (0.07) while it was not affected by lactation stage. It was concluded that the milk fatty acids quality was better in younger animals at early lactation.

Key words*:* Lactation stage, unsaturated fatty acids, desaturase activity, buffaloes, milk.

INTRODUCTION

Buffaloes are the major dairy animals of Pakistan with a population of about 29 million heads and securing second position in the world (Anwar *et al*, 2008). Milk is as primordial as mankind itself, as it is the substance created to feed the mammalian infant. In Pakistan the demand for milk is increasing day by day [and is mostly fulfilled through buffalo's production (Economic Survey, 2009).

Bovine milk fat contains about 98% triglycerides and the remaining 2% includes free FAs, diglycerides, phospholipids, and traces of fat soluble vitamins (Jenness, 1988). Milk fat is most variable component and affected through various physiological and environmental factors (Doreau *et al*., 1999; Lock and Shingfield, 2004). According to Grummer (1991), that milk fatty acids (FAs) of cows have 70% of saturated FAs (SFAs), 25% monounsaturated FAs (MUFAs) and 5% is polyunsaturated fatty acids (PUFAs). The FA with the highest concentration in milk is C16:0 and C18:1 9-cis (Collomb *et al*., 2002).

In bovine milk FAs the short chain FAs (SCFA) and medium chain FAs (MCFAs) (4:0 to 14:0) synthesized within the mammary gland are known as de novo fatty acids Short and medium chain FAs are about 45% of the total milk FAs, while 55% is synthesized through dietary and adipose tissues reserves (Moore and Christie, 1979). The beneficial fatty acids to human health includes CLA, C18:1trans and C18:2 and C20:1 (Bauman, 2007). CLA is present in a very small amount in the milk. But it has a very high effectiveness and prevents cancer; it is also effective against diabetes, good for growth and health and supportive for the development of immune system (Anwar and Romziah, 2008). In dairy products CLA (C18:2 9-cis, 11-trans) is most important isomer and accounts about 75-90% of the total CLA in milk (Bauman *et al*., 1999) and having anti-carcinogenic characteristics (Parodi, 1999).

Fats from animal origin increased risk of cardiovascular diseases due to higher consumption of SFAs (Bonanome and Grundy 1988; Grummer, 1991). The three fatty acids like lauric, myristic and palmitic acids (C12:0, C14:0 and C16:0, respectively) are considered to be hypercholesterolemic fatty acids (HCFA) leading to cardiovascular disease (Baumam, D. E. (2007). The proportion of HCFA in milk is almost 44% of the total milk fatty acids. However, Clandinin *et al*. (2000) recommended that palmitic acid (C16:0) may not show harmful effects if the availability of C18:2 n−6 is fulfilled; stearic acid (C18:0) is largely neutral, while oleic, linoleic and α-linolenic acids are considered cardioprotective (Djoussé *et al*., 2001; Bemelmans, Brore, and feskens, 2002).

The composition of milk FAs is dependent on several factors, such as lactation stage, breed, genetic variation, age, health and feed composition (Murphy *et al*., 1995; Garnsworthy, Masson, Lock, and Mottram, 2006; Qureshi *et al*, 2007). Lactation stage is one of the major factor influencing milk production and

concentration of milk and its FAs in buffaloes. Milk yield declined during the late lactation stage, but there is increase in milk FAs (Şekerden, 1999). During mid or late lactation milk fat content did increase with type of fat supplement provided to animal (Chilliard *et al*., 2003, 2007). Auldist *et al*. (1998) stated that there is an increase in saturated FAs with the advancement of lactation from early to late (7.9 mg/g to 9.7mg/g).

Very limited work has been carried out in this part of the world to investigate the changes in milk FAs profile in relation to advancing age and lactation. Therefore, the present study was conducted in order to evaluate milk FAs composition with changes in physiological condition of animals.

MATERIALS AND METHODS

Study Area: An experiment was conducted at Military Dairy Farm, Peshawar to investigate milk FAs composition at various physiological stages in dairy buffaloes. Duration of the experiment was six weeks starting from 1st November upto mid of December (2009). Milk samples were analyzed for milk FAs profile in Molecular and Biochemical Laboratory Institute of Animal Breeding and Genetics of Khyber Pukhtunkhwa Agricultural University Peshawar and Analytical Laboratory of Pakistan Council for Scientific and Industrial Research (PCSIR).

Selection of animals and management: In the present study 12 lactating multiparous Nili-Ravi buffaloes of nearly same body weight (450 to 550kg) were selected from Military Dairy Farm Peshawar. The animals were divided into two groups younger (1 to 4 lactation number) and older (8 to 10 lactation number) having six animals in each. Each group was further subdivided on the basis of lactation stage into early (1 to 3 months postpartum) and late (7 to 10 months postpartum) with three animals in each subgroup. The animals were kept in uniform management conditions. All the buffaloes were stall fed and green fodders were provided ad libitum while concentrate mixture at the scale of 1 kg/2L of milk production as recommended by Ranjhan (1994) for lactating buffaloes under tropical conditions. Drinking water was provided from the adjacent tank two times daily.

Milk sampling and FA analysis: The evening milk samples (30ml each) were collected in bottles from all of the buffaloes at weekly interval. Milk samples were stored in freezer in at –20ºC until analyzed. The milk fat separation method of Feng *et al*. (2004) was performed. A 20 ml of milk in a 50 ml conical plastic tube was centrifuged for 30 min at 12,000 rpm on 4 °C. 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. Another centrifugation at 13,000 rpm for 20 min at room temperature was carried out using micro centrifuge. After centrifugation, the fat was 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.

About 1.5ml of 0.5 M methanolic NaOH was added to 25mg lipid sample in a glass tube and was capped and heated at 100°C for 5 minute. After cooling 2.50ml of BF3 in Methanol was mixed with it and then heated at 100°C for 30 min. The mixture was cooled and 1 ml of isooctane was added, shaked vigorously for 30 seconds. Immediately 5ml saturated NaCl was added and agitate it carefully. Then the mixture was cooled to room temperature. Isooctane (hexane) layer was separated from aqueous lower phase, and it was transferred to a clean glass tube and was capped tightly.

The FA methyl esters (FAMEs) were determined using Shimadzu gas chromatograph mass spectrometer (model GCMS- QP 2010 plus, Japan) with electron impact (EI) detector. The separation and quantification of FAMES was performed with capillary column of 0.32 mm diameter and 0.25µ thickness (TRB-FFAP). Helium was used as carrier gas. The column temperature was held at 84ºC for 4 min, was ramped to 175ºC at the rate of 15ºC /min for 15 minutes and finally increased to 220ºC at the rate of 2.5ºC /min and was held for 25ºC /min. The injector and ion source temperature was maintained at 250ºC respectively. The inter face temperature was kept at 240ºC on scan mode with M/2 from 85 to 380. The peaks were identified by 37 components FAME standard mix (Accustandard, Inc USA) accompanied by MS library. The quantification was carried out by Area Normalization method.

Statistical analysis: The data was statistically analyzed by the standard procedures of analysis of variance using 2 x 2 factorial designs, as described by Steel and Torrie, (1981). The independent variables were lactation number and lactation stage, where as the dependent variables were blood metabolites, milk yield and milk FAs profile. The means were compared for significance of differences with the LSD procedure. The statistical package (SAS, 1997) was used to perform the above analysis on computer.

RESULTS AND DISCUSSION

Milk Fatty Acids concentration in buffaloes: Fatty acids profile and desaturase activity in buffaloes is presented in Table 1. Saturated FAs (SFAs) has the highest concentration out of the total milk FAs and was averaged about 70.41 g/100g ranging from 64.96 to 78.83 g/100g. Within SFAs the highest level was of C16:0 (31.24 g/100g) followed by C14:0 (12.02 g/100g) and C18:0 (11.43 g/100g). The sum of three hypercholestremic FAs (C12:0, C14:0 and C16:0) was

45.79 g/100g. The average concentration of unsaturated FAs (UFAs) was 35.04 g/100g varying from 21.17 to 29.59 g/100g. In UFAs the highest concentration was of C18:1 cis 21.41 g/100g varying from 14.58 to 27.17 g/100g. The sum of short chain FAs (SCFAs) was about 4.92 g/100g. The concentration of medium chain FAs (MCFAs) on average basis was 17.79 g/100g, long chain FAs (LCFAs) was 45.06 g/100g, monounsaturated FAs (MUFAs) was 23.91 g/100g and polyunsaturated FAs (PUFAs) was about 3.85 g/100g in total milk FAs. The ratio of Δ9 desaturase activity on average basis was 0.07.

The concentration of SFAs, UFAs and PUFAs and HCFAs in the present study was related with the findings of Mihaylova and Peeva, (2007) in Bulgarian Murrah buffalo where the SFAs varied from 64.92% to 77.60%, UFAs 19.56% to 31.42%, PUFAs 2.63% to 3.81% of the total FAs and HCFAs were about 43.62%. Talpur *et al*. (2007) reported concentration of short and medium chain FAs in Kundi 23.53g/100g and Nili-Ravi 25.95g/100g, long chain FAs for Kundi and Nili-Ravi was about 46.28 g/100g and 41.79 g/100g, respectively. The concentration of MUFAs (31.68%) and PUFAs (3.28%) reported by Fernandes *et al*. (2007) in Murrah-crossbred buffaloes was closely related with our findings. Varricchio *et al*. (2007) reported similar values of SFAs (65.5%), MUFAs (27.0%) and PUFAs (4.5%) in buffaloes when fed different feeding rations. Stoop *et al*. (2009) reported the values of Short-chain FAs (C4 and C6–12) averaged about 14%, medium chain FAs 44%, long-chain FAs 30%, SFAs 71% and UFAs 26% in Holstein-Friesian cows.

Δ9 desaturase is an enzyme that long chain SFAs to UFAs in the mammary gland which is beneficial to human health. Fernandes *et al*. (2007) reported Δ9 desaturase activity in Murrah-crossbred buffaloes in four farms, fed different rations and the ratio of desaturase activity was 0.064, 0.065, 0.062 and 0.065 for farm 1, 2, 3 and 4. Our findings of the desaturase activity were closely related with Lock and Garnsworthy (2003) where a ratio of 0.062 was reported.

Table 1. Descriptive statistics for milk yield, FAs profile (g/100g) and Δ9 desaturase activity in buffaloes (mean ± SE, n=72).

Fatty acids	Min	Max	Mean	SE	Age of animals		P-Value
					Younger[1]	Mature[2]	
C: 8	0.60	3.36	1.57	0.56	1.53±0.12	1.61±0.12	NS
C:10	1.11	3.98	2.72	0.68	2.58±0.09	2.88±0.09	NS
C12:0	1.07	3.85	2.53	0.64	2.50±0.08	2.58±0.08	NS
C14:0	8.15	15.44	12.02	1.68	11.74±0.21	12.30±0.21	NS
C15:0B	0.69	1.63	1.06	0.19			
C15:0	1.60	3.00	2.17	0.31			
C16:0	24.00	39.00	31.24	2.78	31.25±0.58	31.25±0.58	NS
C17:0	0.82	1.95	1.15	0.21			
C18:0	11.35	14.08	11.43	2.06	11.43±0.35	11.42±0.35	NS
C20:0	0.03	0.74	0.39	0.16			
C22:0	0.51	0.99	0.72	0.02			
C14:1	0.36	3.36	1.05	0.58	1.23±0.08	0.87±0.08	*
C16:1	0.68	3.33	1.56	0.58	1.92±0.04	1.22±0.04	***
C17:1	0.09	0.78	0.30	0.13			
C18:1 cis	14.58	27.17	21.41	2.66	20.59±0.24	21.41±0.24	*
C18:1 trans	0.75	3.00	2.12	0.55	2.07±0.12	2.11±0.12	NS
C18:2 cis	0.48	2.70	1.32	0.49	1.33±0.06	1.30±0.06	NS
C18:2 trans	0.02	0.96	0.18	0.14	0.19±0.01	0.17±0.01	NS
C18:3n3	0.04	1.44	0.34	0.25	0.39±0.03	0.31±0.03	**
SFAs[a]	64.96	78.83	70.41	3.66	68.99±0.26	71.02±0.26	NS
UFAs[b]	21.17	29.59	35.04	3.59	29.61±0.35	28.98±0.35	NS
SCFAs[c]	1.71	7.31	4.92	0.12	4.11±0.15	4.49±0.15	NS
MCFAs[d]	14.12	21.58	17.79	0.22	17.42±0.22	18.17±0.22	*
LCFAs[e]	34.53	51.86	45.06	0.35	44.91±0.45	47.13±0.45	*
MUFAs[f]	16.47	30.86	23.91	0.37	26.13±0.30	25.6±0.30	NS
PUFAs[g]	2.21	6.14	3.85	0.10	1.92±0.07	1.78±0.07	NS
Desaturation[h]	0.02	0.32	0.07	0.002	0.11±0.01	0.07±0.01	*

[a]SFAs (saturated FAs), [b]UFAs (Unsaturated FAs), [c]SCFAs (short chain FAs), [d]MCFAs (medium chain FAs), [e]LCFAs (long chain FAs), [f]MUFAs (monounsaturated FAs) and [g]PUFAs (poly unsaturated FAs); [h]Δ^9 desaturase, enzyme that converts medium and long chain FA to polyunsaturated FA in the mammary epithelium and it is calculated by an indirect method (C14:1c9 /C14:0); [1]Younger Animals, (1-4) Lactation Number; [2]Mature Animals, (8-10) Lactation Number.

Effect of age on milk fatty acids profile: The means of milk fatty acids profile as affected by age is presented in Table 2. The concentration of C14:1, C16:1 and C18:3 was significantly (P<0.05) higher in younger buffaloes while C18:1cis, MCFA and LCFA were higher in older ones (P <0.05). Although, SFA and UFA were not affected by age but the data trend revealed a little higher SFA in older animals compared to younger ones and vice versa for UFA. The ratio of Δ9 desaturase activity was significantly (P <0.05) higher in younger animals (0.11) compared to older (0.07).

The concentration of MCFA and LCFA significantly increased with advancing age. This may be due to higher triglycerides level in blood which leads to more production of LCFA in milk. Milk FAs production depends on diet composition, feed quantity, rumen fermentation, liver metabolism, body reserves mobilization and mammary gland absorption (Garnsworthy *et al*., 2006). Half of milk fat is derived directly from the dietary LCFAs or from body reserves. FAs synthesis involves conversion of acetyl-CoA to malonyl-CoA that is further used in chain elongation process leading to a series of short and medium SFAs (Howke and Taylor, 1995).

Our results are in support with the findings of De La Fuente *et al*. (2009), reported that with the advancing age the concentration of SCFAs and MCFAs increased. Kelsey *et al*. (2003) reported a significant effect of lactation number in dairy cattle. As lactation progresses the concentration of de novo FAs such as caprylic acid, capric acid, lauric acid, myristic acid and palmitic acid increased. In animals blood triglyceride is prepared from glycerol-3 phosphate which is taken from glucose and body reserves. The long chain FA is synthesized from dietary source or blood lipid source (Grummer, 1991).

UFAs like C14:1, C16:1 and C18:3 concentrations increased in younger animals while C18:1 was higher in older animals. De La Fuente *et al*. (2009) reported in ewes that MUFAs and PUFAs were decreased as ewes age increased. In adipose tissue, 18:1cis 9, 16:0, and 18:0 was about 90% of FAs in roughly equal molar proportions (Christie, 1981). The concentration of longer chain FAs arise from either dietary sources or body reserves (Bauman and Davis, 1974). The main preformed FA from blood lipid source is C16:0, C18: O and C18: 1 (Duncan and Garton, 1963), most of FA is shifted to mammary gland by LDL and VLDL or chylomicrons which are about 10-15, 60 or 85 % triacylglycerol respectively. Though, HDL account for approximately 90% of blood lipids (Christie, 1981). Uptake of FA into the mammary gland from HDL is poor (Brumby *et al*., 1972) and this may explain the low levels of PUFA in milk.

Conclusions: Saturated FAs (SFAs) has the highest concentration out of the total milk FAs. The concentration of C14:1, C16:1 and C18:3 were higher in younger buffaloes while C18:1cis, MCFA and LCFA were higher in older ones. The ratio of Δ9 desaturase activity was significantly higher in younger compared to older animals. The findings suggested that the milk FA quality was better in younger animals at early lactation stage. In order to get more and better quality of milk the animals should be carefully managed during lactation.

Acknowledgement: This study was sponsored by the Pakistan Council for Science and Technology, Islamabad and lab was provided by PCSIR Peshawar.

REFERENCES

Anwar, M., and Romziah, S. (2008). Effect of linoleic acid agent induction in complete feed on bioconversion of milk butter fat and FA of milk product proceedings. *The 15th Congress of FAVA 27-30 October, Joint Symposium on Emerging Diseases. Bangkok, Thailand.*

Auldist, M. J., Walsh, B. J., and Thomson, N. A. (1998). Seasonal and lactation influences on bovine milk composition in New Zealand. *Journal of Dairy Research,* 65, 401-411.

Baumam, D. E. (2007). The enrichment of milk fat with FA of importance in human health maintenance and disease prevention. *Advances in Animal Nutrition.* 1-18.

Bauman, D. E., and Davis, C. L. (1974). Biosynthesis of milk fat. *Page 31 in Lactation-A Comprehensive Treatise. Vol. 2. B. L. Larson and V. R. Smith, edition. Academic Press, New York, NY.*

Bauman, D. E., L. H. Baumgard, B. A. Corl, and J. M. Griinari, (1999). Biosynthesis of conjugated linoleic acid in ruminants. *Proceedings of American Society of Animal Sciences,*1-12.

Bemelmans, W. J. E., J. Brore and E. J. M. Feskens, (2002). Effect of an increased intake of linoleic acid and group nutritional education on cardiovascular risk factors. The Mediterranean Alpha-linlenic Enriched Gorningen Dietary Intervation (MARGARIN) study. *American Journal of clinical Nutrition.* 75, 221-227.

Bonanome, A., and Grundy, S. M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoproteins. *New England Journal of Medicine,* 318,1244-1248.

Brumby, P. E., Stony, J. E., and Sutton, J. D. (1972). Metabolism of cod-liver oil in relation to milk fat secretion. *Journal of Dairy Science.* 39, 167-182.

Chilliard, Y., Ferlay, A., Rouel, J., and Lamberet, G. (2003). A review of nutritional physiological factors affecting goat milk lipid synthesis and lipolysis. *Journal of Dairy Science.* 86, 751-1770.

Chilliard, Y., F. Glasser, A. Ferlay, L. Bernard, J. Rouel, and M. Doreau, (2007). Diet, rumen biohydrogenation

and nutritional quality of cow and goat milk fat. *Europian Journal of Lipid Science and Technology.* (EJLST) 109, 828-855.

Christie, W. W. (1981). *Lipid Metabolism in Ruminant Animals. Oxford: Pergamon Press.*

Clandinin, M. T., S. L. Cook, S. D. Konrad and M. A. French, (2000). The effect of Palmitic acid on lipoprotein cholesterol levels. *International Journal of Food Science and Nutrition.* 51,61-71.

Collomb, M., H. Eyer and R. Sieber, (2002). Chemical structure and composition of FA in milk fat Agrarforschung. *Journal of Animal Science*. 9, 240-245.

De La Fuente, L. F., E. Barbosa, J. A. Carriedo, C. Gonzalo, R. Arenas, J. M. Fresno, and F. San Primitivo (2009). Factors influencing variation of fatty acid content in ovine milk. *Journal of Dairy Science.* 92, 3791–3799

Djousse, L., J. S. Pankow, and J. H. Eckfeldt, (2001). Relation between dietary linolenic acid and coronary artery disease in the national heart, lung and blood institute. Family heart study. *American Journal of clinical Nutrition.* 74,612-619.

Doreau, Y., H. Chilliard, H. Rulquin and D. I. Demeyer, (1999). Manipulation of milk fat in dairy cows. Pages 8 1-109 *in* Recent Advances in Animal Nutrition – 1999. P.C. Garnsworthy and J. Wiseman, ed. Nottingham University Press, Nottingham, UK.

Duncan, W. R. and G. A. Garton, (1963). Plasma lipids of the cow during pregnancy and lactation. *Biochemical Journal.* 89, 414-419.

Economic Survey of Pakistan. (2009). Economic Advisor's Wing, Ministry of Finance Government of Pakistan, Islamabad, Pakistan.

Feng, S., A. L. Lock and P. C. Garnsworthy, (2004). A rapid method of determining FA composition of milk. *Journal of Dairy Science.* 87,3785-3788.

Fernandes, S. A. A., W. R. S. Mattos, S. V. Matarazzo, H. Tonhati, M. A. S. Gama and D. P. D. Lanna, (2007). Total FA in Murrah buffaloes milk on commercial farms in Brazil. *Italian Journal of Animal Science.* 6,1063-1066.

Garnsworthy, P. C., L. L. Masson, A. L. Lock, and T. T. Mottram, (2006). Variation of milk citrate with stage of lactation and de novo FA synthesis in dairy cow. *Journal of Dairy Science.* 89, 1604–1612.

Georing, H. K., and P. J. Van Soest, (1979). Forage fibre analysis. Agriculture Handbook 379. Agric. Res. Serv., USDA, Washington, DC.

Grummer, R. R. (1991). Effect of feed on the composition of milk fat. *Journal of Dairy Science.* 74, 244–3257.

Hawke, J. C., and M. W. Taylor, (1995). Influence of nutritional factors on the yield, composition and physical properties of milk fat. *In, Advanced Dairy-Chemistry 2: Lipids. 2nd edition. 37-88, Chapman and Hall, London.*

Jenness, R. G., and R. W. Clark, (1988). Lipid composition and properties. *In: Wong N.P., Keeney M. and Marth E.H., Eds. Fundamentals of dairy chemistry, 3rd Ed. New York, USA: Van Nostrand Reinhold Company.*

Kelsey, J. A., B. A. Corl, R. J. Collier, and D. E. Bauman, (2003). The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *Journal of Dairy Science.* 86, 2588-2597.

Lock, A. L., and K. J. Shingfield, (2004). Optimizing milk composition. *In: E. Kebreab, J. Mills, and D. Beever (Eds.) UK Dairying: Using science to meet consumers' needs. pp. 107-188. Nottingham University Press, Nottingham, UK.*

Mihaylova, G., and T. Peeva, (2007). Tran's FA and conjugated linoleic acid in the buffalo milk. *Italian Journal of Animal Science.* 6, 1056-1059.

Moore, J. H., and W. W. Christie, (1979). Lipid metabolism in the mammary gland of ruminant animals. Progress in. *Lipid Research.* 17, 347-395.

Murphy, J. J., J. F. Connolly and G. P. Mcneill, (1995). Effects on cow performance and milkfat composition of feeding full fat soyabeans and rapessed to dairy cows at pasture. *Livestock Production Science*. 44, 13-25.

Parodi, P. W. (1999). Congucated linoleic acid and other anticarcinogenic of milk fat. *Journal of Dairy Science.* 19, 1339-1349.

Qureshi, M. S., A. Mushtaq, S. Khan, G. Habib, Z. A. Swati, (2010). Variation in milk fatty acids composition with body condition in dairy buffaloes *(Bubalus bubalis). Asian Aust J Anim Sci,* 23: 340-345.

Ranjhan, S. K. (1994). Consultants reports on the availability and requirement of feed and fodder for Livestock and Poultry. Department of India, New Delhi.

SAS Institute, (1997). SAS User's Guide Edition. SAS Inst. Inc., Cary, NC.

Sekerden, O. (1999). Factors Affecting Milk Composition and Changes in Milk Composition with Lactation Stage in Anatolian Buffaloes. Turkish Journal of Veterinary and Animal Science. 23, 505-510.

Steel, R. G. D. and J. H. Torrie, (1980). Principals and procedures of statistics: *A biometrical approach. 2nd ed. pp. 107-109. McGraw Hill Co. Inc. New York, USA.*

Stoop, W. M., H. Bovenhuis, J. M. L. Heck and J. A. M. Van Arendonk (2009). Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *Journal of Dairy Science.* 92, 1469–1478.

Varricchio, M. L., A. Francia, F. D. Masucc, R. Romano and V. Proto, (2007). Fatty acid composition of Mediterranean buffalo milk fat. *Italian Journal of Animal Science.* 6, 509-511.