EFFECT OF AGE AND LACATION ON MILK FATTY ACID PROFILE IN DAIRY BUFFFALOES

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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 acid 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, had access to green fodders ad libitum and received concentrate mixture at the rate of 1 kg/2 lit of milk production. The experiment continued for six weeks starting from November until mid December 2009. Milk samples were collected weekly for analysis of the fatty acid profile. Saturated FAs (SFAs) had the highest concentration out of the total milk fatty acids and averaged about 70.41 g/100 g ranging from 64.96 to 78.83 g/100 g. The concentrations of C14:1, C16:1 and C18:3 were 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 acid quality was better in younger animals at early lactation.

Keywords: 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 head (Economic Survey, 2009) and securing them thte second position in the world (Bilal *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 (Hussian *et al*., 2010) and is mostly fulfilled through buffalos' 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 by

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various physiological and environmental factors (Doreau *et al*., 1999; Lock and Shingfield, 2004). According to Grummer (1991), milk fatty acids (FAs) of cows has 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 FAs. Short and medium chain FAs are about 45% of the total milk FAs, while the remaining 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, accounting for about 75-90% of the total CLA in milk (Bauman *et al*., 1999), and has anti-carcinogenic characteristics (Parodi, 1999).

Fats of animal origin increased risk of cardiovascular diseases due to higher consumption of SFAs (Bonanome and Grundy 1988; Grummer, 1991). The three fatty acids lauric, myristic and palmitic acids (C12:0, C14:0 and C16:0, 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) stated 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). Lactation stage is one of the major factors influencing milk production and concentration of milk and its FAs in buffaloes. Milk yield declined during the late lactation stage, but there is an increase in milk FAs (Şekerden, 1999). During mid or late lactation milk fat content did increase with type of fat supplement provided to the 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 9.7 mg/g).

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

MATERIALS AND METHODS

An experiment was conducted at Military Dairy Farm, Peshawar to investigate milk FA composition at various physiological stages in dairy buffaloes. Duration of the experiment was six weeks starting from 1st November to mid of December (2009). Milk samples were analyzed for milk FA profile in the 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 550 kg) 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 under uniform management conditions. All the buffaloes were stall fed and had access to green fodders ad libitum while concentrate mixture were provides at the rate of 1 kg/2 L 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 analysis

Evening milk samples (30 ml each) were collected in bottles from all of the buffaloes at weekly intervals. Milk samples were stored in a freezer in at -20ºC until analyzed.

Determination of milk FAs

Fat extraction

The milk fat separation method of Feng *et al*. (2004) was performed. A 20 ml sample of milk in a 50 ml conical plastic tube was centrifuged for 30 minutes 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 minutes until the fat cake melted. Another centrifugation at 13,000 rpm for 20 minutes at room temperature was carried out using a micro centrifuge. After centrifugation, the fat was separated into three layers: the top layer of lipid; the middle layer of protein, fat and the water insoluble solids and the bottom layer of water.

Preparation of methyl esters

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

Gas chromatography

The FA methyl esters (FAMEs) were determined using a Shimadzu gas chromatograph mass spectrometer (model GCMS- QP 2010 plus, Japan) with an electron impact (EI) detector. The separation and quantification of FAMES was performed with a capillary column of 0.32 mm diameter and 0.25 µ thickness (TRB-FFAP). Helium was used as the carrier gas. The column temperature was held at 84ºC for 4 minutes, was ramped to 175ºC at the rate of 15ºC /minute for 15 minutes and finally increased to 220ºC at the rate of 2.5ºC /minute and was held for 25ºC/minute. 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 the Area Normalization method.

Statistical analysis

The data was statistically analyzed by the standard procedures of analysis of variance using 2x2 factorial design, as described by Steel and Torrie, (1981). The independent variables were lactation number and lactation stage, whereas the dependent variables were blood metabolites, milk yield and milk FAs profile. The means were compared for significance of differences with the LSD procedure. A statistical package (SAS, 1997) was used to perform the above analysis on a computer.

RESULTS AND DISCUSSION

Milk fatty acid concentration in buffaloes

Fatty acid profile and desaturase activity in buffaloes is presented in Table 1. Saturated FAs (SFAs) had the highest concentration out of the total milk FAs and averaged about 70.41 g/100g ranging from 64.96 to 78.83 g/100 g. Within SFAs the highest level was of C16:0 (31.24 g/100g) followed by C14:0 (12.02 g/100 g) and C18:0 (11.43 g/100 g). The sum of three hypercholestremic FAs (C12:0, C14:0 and C16:0) was 45.79 g/100 g. The average concentration of unsaturated FAs (UFAs) was 35.04 g/100 g varying from 21.17 to 29.59 g/100 g. In UFAs the highest concentration was of C18:1 cis 21.41 g/100 g varying from 14.58 to 27.17 g/100 g. The sum of short chain FAs (SCFAs) was about 4.92 g/100 g. The average concentration of medium chain FAs (MCFAs) on was 17.79 g/100 g, that of long chain FAs (LCFAs) was 45.06 g/100 g, that of monounsaturated FAs (MUFAs) was 23.91 g/100 g and that of polyunsaturated FAs (PUFAs) was about 3.85 g/100 g 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 23.53 g/100 g and Nili-Ravi 25.95 g/100 g, long chain FAs for Kundi and Nili-Ravi was about 46.28 g/100 g and 41.79 g/100 g, 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 feed 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 catalyzes conversion of 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 farms 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.

Effect of age on milk fatty acids profile

The means of milk fatty acids profile as affected by age is presented are Table 2. The concentration of C14:1, C16:1 and C18:3 were significantly (P<0.05) higher in younger buffaloes while C18:1cis, MCFA and LCFA were higher in older ones (P <0.05, Figures1 and 2). Although, SFA and UFA were not affected by age, 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 triglyceride levels in the 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), who 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.

Effect of lactation stage on milk fatty acids profile

The concentration of C18:1 and PUFA was significantly (P<0.05) higher in late lactation compared to early (Table 3). Even though the SFA and UFA were not significantly affected by age, the data trend showed a little higher SFA in older animals compared to younger ones. The ratio of Δ9 desaturase activity was also non-significantly affected by lactation stage.

Many scientists have reported that dairy cows are in negative energy in early lactation stage and mobilize the body fats reserves to meet lactation demands (Garnsworthy and Topps, 1982). Bovine milk has a high proportion SFAs due to biohydrogenation of dietary FAs in rumen. The main source of SCFAs in the mammary gland is acetate and β-hydroxybutyrate.

Milk FAs of bovines with carbon chains from C4:0 to C14:0 and 50% of the C16:0 arises from de novo synthesis within the mammary gland (Bauman and Davis, 1974). According to Garnsworthy *et al*. (2006), production of milk and DMI changes with stage of lactation affected the relative proportion of individual FAs by influencing the balance between body fat mobilization and de novo synthesis of FAs in the mammary gland. Mele *et al*. (2009) found that concentration of de novo FAs increases with advancement of lactation. Kgwatalala *et al*. (2009), found that with advancing lactation the proportions of SCFAs, MCFAs, LCFAs and SFAs were higher from early to mid lactation stage.

Milk lipids are about 97-98% triglyceride. Δ9 desaturase activity controls the production of monounsaturated FAs and CLA from SFAs with in the mammary gland. Concentration of longer chain FAs, such as C18:1 comes from blood lipids or from dietary source or body reserves mobilization (Bauman and Davis, 1974). In MUFAs the concentration of C18:1, which is a major FA in adipocytes and is released from adipocytes during lipolysis (Rukkwamsuk *et al*., 2000; Gillis *et al*., 2004), decreased by 25% from wk 1 to 8 (Kay *et al*., 2005). Stoop *et al*. (2009), reported that UFAs and the unsaturation index were similar to one another and showed a minimum at mid-lactation, with UFAs being 1.5% lower in mid-lactation than in early and late lactation.

The cis-9, trans-11 CLA in milk fat originates from the incomplete biohydrogenation of polyunsaturated FAs, and diet can have a major impact on the milk fat content. Our results are closely related with Strzałkowska *et al*. (2009), in goats that with the advancement of lactation stage the concentration of MUFAs and PUFAs increased. It was reported that in MUFAs, the concentration of C18:1 also increased as lactation stage progressed and PUFAs significantly increased with advancement of lactation stage.

CONCLUSIONS

Saturated FAs (SFAs) have 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 milk, animals should be carefully managed during lactation.

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