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FACTORS AFFECTING FATTY-ACID PROFILE OF LACTIC LIPIDS IN THE WATER BUFFALO (*Bubalus bubalis*) – A REVIEW

SUMMARY

The aim of this review was to study how lactic fatty acids (FA) are affected by different factors in the different pathways in the buffalo – a species differing from cattle with its response to feeding strategies, metabolism and specific functional composition of milk. It underlines the role of management to manipulate enzymatic desaturation and bacterial synthesis and that of ripening and pasteurization in dairy technology. The review presents some proofs of effect of season on FAs, but it should be correctly discriminated from the effect of lactation stage, showing improved unsaturation in advanced lactation. Farming system has major impact, and it can include feeding strategies (rumen-inert fats, seeds, bioactive compounds, etc.) to affect rumen biohydrogenation, but it is implied that the doze is important. In fact, the best control over bubaline lactic FAs is via natural grazing, improving omega ratio, conjugated linolenic (CLA) and *trans*-vaccenic acid (TVA). This is not only because of plants' composition of soluble sugars, vitamins, polyphenols and proteins, but mostly because of the stimulated bacterial synthesis and Δ9-desaturase activity, responsible for the *de novo* synthesis. The results about the transformations in the beneficial fatty acids in the yoghurt production are controversial explaining the predominantly negative impact mostly with pasteurization. Cheese processing generally alters the individual isomers but not the groups of beneficial fatty acids (CLA) as a whole chiefly due to ripening and pasteurization (only if the temperature is high), but not due to renneting.

Key words: *Bubalus bubalis*, milk, fatty acids, grazing, supplementation, dairy processing

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INTRODUCTION

Buffalo milk is a delicacy product characterized by high density – associated not only with its higher dry matter, but also with the high proportion of high-melting triglycerides (Ramamurthy and Narayanan, 1971; Khan, T.I. et al., 2019) – and by specific, appealing odor, attributed to some volatile organic compounds (Moio et al.,1993)and to the high concentrations of short-chain (SCFA) fatty acids (Naydenova, 2005; Güler et al., 2005). In buffalo milk fat content is double higher but cholesterol is lower, and the lipid globules are larger, as compared to cow milk (Zicarelli, 2004; Islam et al., 2014). Noteworthy is also the presence of gangliosides, which are not found in cow milk and which have anti-inflammatory and anti-toxin effects (Ahmad et al., 2013).

Buffalo milk is rich in whey proteins, calcium and in particular colloidal Ca (Nguyen et al., 2014; Islam et al., 2014). According to Chandan et al. (2006), during dairy processing all these components enhance the effect of probiotic bacteria, which inhibit the absorption of cholesterol and hence significantly reduce blood pressure.

Bulgarian yoghurt (germinated with *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*) is a specific type of fermented dairy product that has gained recognition on the world market. Such probiotic microflora has synergistic effects that result in specific texture, composition and sensory properties of that dairy product (Ebringer et al., 2008). Buffalo yoghurt is characterized by microstructure that causes higher syneresis – interrupted by large fat globules, binding less protein and featuring more serum pores (Nguyen et al., 2014; Abesinghe et al., 2020), as well as by higher titratable acidity associated with firmer and smoother coagulum (Naydenova, 2005). Both yoghurt and cheese are natural carriers of probiotics with essential effects on the immune system and gastrointestinal health (Abesinghe et al., 2020).

Casein in buffalo milk is roughly 80% of total protein (20% whey protein) and almost all of it is in the form of larger more numerous micels, as compared to bovine milk (Ahmad et al., 2013). The particular role of high k-casein and superb proportions amongst protein, whey protein, types of casein and fats are important for the outstanding rennet coagulation, gelling and firming properties in the production of curd (Ariota et al., 2007; Abesinghe et al., 2020; Islam et al., 2014).

Moreover, the higher fat content of buffalo milk is essential as it leads to higher ratio with protein, also contributing to the superior elastic property of the curd (Ariota et al., 2007). In association with the higher fat content and dry matter, during ripening buffalo cheese is characterized by lower intensity of physical and chemical changes and lower lipolytic activity, as concluded by Ivanov et al. (2016).

Compared to cow, buffalo milk is characterized by higher saturated fatty acids, mainly palmitic acid, *trans* fatty acids, and conjugated linolenic acid (Ménard, et al., 2010; Penchev et al., 2016).

The essential nutritional values of milk and dairy products are indisputable but still there is criticism against their mass consumption, because of the saturated nature of the lactic lipids (Givens and Shingfield, 2006). But, as ruminant products, they are a dominant provider of the beneficial conjugated linoleic acid (CLA), trans-vaccenic acid (TVA) and other monounsaturated (MUFA) acids, and even some saturated fatty acids (SFA) like butyric and stearic (German, 1999; Lawson et al., 2001; Vargas-Bello-Pérez and Garnsworthy, 2013). Buffalo milk was also established to reduce cancer risks in a trial of Ramirez et al. (2013). Thus, milk and its derivative foodstuffs have been proved to have anti-cardiovascular, anti-mineral fertilizing was applied. carcinogenic, anti-atherogenic, anti-obesity, anti-diabetic effects and stimulate immune system (Belury, 2002; Parodi, 2004; Dilzer and Park, 2012).

In addition, fatty acids are indicative of the quality of the different types of cheese, as they are strongly responsible for the formation of flavor and they are precursors for other volatile aromatic compounds (Khalid and Marth, 1996).

There are two main sources of fatty acids in milk – originally formed, unchanged FAs and such synthesized *de novo* in the mammary gland (Chilliard et al., 2000). The *de novo* FAs are synthesized from acetate, butyrate, and volatile FA which are a product of fermentation of cellulose and hemicellulose by specific bacteria in the rumen. In the mammary gland, the acetate and β-hydroxybutyrate are precursors of the fatty acids with 4 to 12 carbon atoms and great parts of the myristic acid and palmitic acids (Shingfield et al., 2013). The rest part of the latter two FAs originates from circulating lipoproteins rich in triacylglycerols and also from intestinally absorbed lipids and such from body fat mobilization (Bauman and Griinari, 2003; Shingfield et al., 2010).

Long-chain fatty acids (LCFA) are some of the FAs deriving from elongation of diet FAs or such deposited in the udder, and in particular long-chain saturated fatty acids (LCSFA) like C20:0, C22:0 and C24:0 may derive both from diet and body fat mobilization (Correddu et al., 2017). Odd- and branched-chain (BCFA) and other valuable FAs come via completely different pathway. Though rumen microorganisms apply biohydrogenation to polyunsaturated fatty acids (PUFA) which converts them to SFA (Jenkins et al., 2008), in this process some bacterial genera produce wide range of intermediates with high benefits, like rumenic acid (Palmquist et al., 2005).

An important feature of milk and dairy products is how their fatty-acid profile is affected by environmental, physiological and especially managemental factors and how it can be manipulated through the different pathways to produce more beneficial foodstuffs. There are plethora of studies and experiments on dairy cows, but such regarding animal effects, environmental and managemental factors, rumen-inert supplementation, and dairy processing specifications on the fatty acids of buffalo lactic lipids (in milk and dairy products) are limited. Because, though appearing similar, the body response to the complex synthesis of these metabolites in the two species should be considered with different respect, as suggested in their response to roughage shift in our previous study (Penchev et al., 2016) and to the metabolic pressure during the transition period (Pegolo et al., 2017).

MATERIAL AND METHODS

A profound comprehensive search of scientific literature was conducted in order to study the problem in focus. In the present review information from publications obtained on the Internet – exclusively Google Scholar but mostly Research Gate, PubMed, Scopus and Web of Science – as well as journal editions worldwide and also editions in Bulgaria were used.

More specifically, the expertise and research experience in fatty-acid analysis of buffalo production and the experimental results of our animal breeding team with regard to diet supplementation were also taken in consideration as a foundation for this literature review.

RESULTS AND DISCUSSION

The current technology for fatty-acid analysis of milk allows identification of a great number of molecules from the groups of BCFA, *cis* and *trans* isomers of 18:1, 18:2 and 18:3, which are involved in the processes in the rumen, *de novo* synthesized in the mammary gland, and other FA deriving from elongation of diet FA or such deposited in the udder, including LCFA (Correddu et al., 2017).

Wide range of fatty acids, including low-concentration FAs precious for the consumer, was analyzed by Pegolo et al. (2017) for the milk of Italian Mediterranean buffaloes from six farms using one and the same ration from January to May (from cold to warm season). Lactation phase was found to have significant effect. The dynamics show that after increase in early lactation, the medium-chain fatty acids (MCFA) decrease generally throughout the period, while for LCFA it is the opposite – a general increase after initial decrease. MUFA were also found to increase generally, also after an initial decrease until day 120. These changes in the trend of the dynamics of the lactic fatty acids from fourth month on might be associated with the end of the period of negative energy balance, though in this species it is slightly expressed (Pegolo et al., 2017), or with overcoming the period of highest productivity, the peak being established usually in the fifth week postpartum in the Italian buffaloes (Borghese et al., 2013). Total PUFA were also found to increase generally after an initial decrease until day 120, and the increase in the rumenic acid in particular was straightforward.

The observations of these Italian authors about PUFA (on as many as 272 heads) are supported by the results of Zotos and Bampidis (2014) who monitored the fatty-acid changes in smaller number of dairy Greek buffaloes (also Mediterranean type, n= 40) from September to February (from warm to cold season – opposite to Pegolo et al., 2017), finding an increase in the concentrations of the beneficial PUFA and CLA. Similar dynamics in PUFA with the advance of lactation in dairy buffaloes was observed also by Sharma et al. (2000), while Verdurico et al. (2012) did not find any significant changes as it was in our study on the Bulgarian Murrah, especially in buffaloes under intensive farming (Ilieva et al., 2020). On the other hand, Zotos and Bampidis (2014) did not establish significant alterations in butyric, vaccenic and rumenic acid which is different from the Italian study.

There are two sources of fatty acids in milk. Except for the circulation of preformed FA, there is also *de novo* synthesis in the wall of the mammary gland

(Chilliard et al., 2000). One such FA synthesized *de novo* is the butyric acid with the mediation of acetyl-CoA carboxylase and FA synthase enzymes (Chilliard et al., 2000), and another is the *trans*-vaccenic acid (C18:1*trans*-9) and it depends on the activity of Δ9-desaturase (stearoyl-CoA desaturase), being a catalysator of the desaturation of C18:0. In a study to characterize the fatty acid profile of buffalo milk, Correddu et al. (2017) pointed out that desaturation is involved in the production of most of the *cis* and *trans* isomers of C18:1 and CLA *cis*-9,*trans*-11.

In correspondence with the study of Pegolo et al. (2017) about PUFA, the buffaloes from the Mediterranean breed in Brazil were also found to slightly but steadily increase this value in their milk throughout lactation (Verdurico et al., 2012), and the Murrah breed in India to have highest PUFA in the most advanced stage of lactation (Sharma et al., 2000).

Nevertheless, unlike the above cited studies, Qureshi et al. (2012) came to the conclusion that the higher lipid value of milk is in early lactation stage.

While in the above quoted studies variability of fatty acids is attributed to the animal factor lactation stage, the research of Talpur et al. (2008) provides evidence of the actual impact of the environmental factor season of test day in buffaloes in Pakistan, as well as in other ruminants. This was associated with the availability of fresh grass during summer and hence with the better biohydrogenation. Substantial difference was found in CLA, showing higher summer level by 33% compared to winter, and especially in rumenic acid in particular – by 72%. Respectively, total PUFA (by 15.7%) and in particular 18:3n-3 (by 58%) were also higher in summer season; MUFA and TVA – by 12 and 42% respectively. Total saturation of buffalo milk dropped from winter to summer by 7%, including the decrease in C18:0 by 22%. All this was confirmed in the dairy cows, ewes and goats within the same study.

In contrast, Nie et al. (2022) did not find seasonal alterations in Chinese crossbred buffaloes in the main FA groups (SFA, MUFA, PUFA) and also in butyric, TVA and rumenic acids. Nevertheless, the concentrations of some SFAs (C8:0, C10:0, C18:0) were significantly lower in summer milk, while other SFAs (C15:0, C15:1, C16:0, C17:0, C17:1, C15:0) were higher; C18:3(ω-6), C18:3(ω-3), C20:0, C20:2, and C20:4 were also higher. More importantly, in agreement with the previous citation, total PUFAn-3 were significantly higher in summer, and the ratio n-6/n-3 was lower; atherogenicity and thrombogenicity indices were also favorable in the summer. SCFA were lower in summer, while MCFA and LCFA did not vary between seasons.

In total disagreement, for buffaloes in the conditions of India Saroj et al. (2017) established opposite tendency – all the FAs valuable for health were higher in the winter season. Though the ration of the studied dairy animals was principally the same in the two seasons, the source of green roughage was different, transferring the “resposibility” for the changes upon the factor feeding/management. Similarly, in Iranian buffaloes, Chashnidel et al. (2007) found higher PUFA and CLA in summer due to forage resources.

Controversial are also the results of Naydenova (2005), finding higher SFA in the buffalo milk produced in spring/summer for one farm and the opposite for another. The same concerns in particular the *de novo* synthesized SFAs with 6 to

12 atoms, and also those of mixed origin from C14:0 to C16:0. This author also shares the opinion that all changes are associated mostly with forage resources than with seasonality of FA synthesis, hydrogenation, microflora development, etc.

Buffalo milk produced in summer should be considered more beneficial than that in winter not only because of the effect of season and the related forage resources as associated with the high activity of Δ9-desaturase and acetyl-CoA carboxylase rather than to the actual component composition of the diet, namely the botanical properties of the plants.

As Pegolo et al. (2017) also found, parity did not have significant effect on most of the fatty acids, but it affected BCFA and also MUFA, in particular oleic acid (n-9), which is the Δ9-desaturase major product. The study of Qureshi et al. (2012), though, established effect of parity on the concentrations of C14:1, C16:1 and C18:3, showing higher values in younger buffaloes. In similarity with Pegolo et al. (2017), C18:1*cis* was also affected, together with MCFA and LCFA which were found in higher concentrations in older buffaloes. The Δ9-desaturase activity was significantly higher in younger animals, but it was not affected by lactation phase. To support this, colostrum exhibited lower levels of saturation and n-6/n-3 ratio compared to mature milk (Zhigao et al., 2024).

Effect of parity was observed also in other studies on bovine (Nogalski et al., 2012; Samková et al., 2012) and bubaline cows (Qureshi et al., 2012), mainly based on the emphasized effect of the earlier months postpartum in lactating cattle (Stoop et al., 2009; Wang et al., 2013) and buffaloes (Sharma et al. 2000; Verdurico et al., 2012).

Qureshi et al. (2010) reported that with the increase of body condition score (BCS) in dairy Nili-Ravi buffaloes the SCFA/MCFA ratio increased, which applies also to the concentration of C18:0. Total unsaturated fatty acids (UFA) and C18:2*cis*-9, -12 and C18:3*cis*-9, -12, -15 in particular, and also C18:1*cis*-9 have highest concentrations in the lactating animals with moderate BCS.

As implied above, in its broader sense, the effect of season in most cases suggest an effect of feeding and farming system. Ilieva et al. (2020) found substantial beneficial effect of pasture on all important fatty acids in milk – especially in the *trans*-FA, like rumenic and vaccenic, omega-6/omega-3 ratio, total MUFA and PUFA, and to a smaller extent in atherogenicity and thrombogenicity index. In the same time, there was a decrease of the levels of some FAs (TVA, GLA, CLA, total PUFA) in milk with the advance of the pasture season which is to be attributed to the botanical and phenological changes of the grassland (Ferlay et al., 2008; Gorlier et al., 2012) and its protein content (Elgersma et al., 2003).

In two other grazing herds of Bulgarian Murrah, the results of Naydenova (2005) showed that the most atherogenic C14:0 and the C12:0 are higher but C16:0 is lower, MUFA is higher and PUFA is definitely lower in comparation to that study of Ilieva et al. (2020). In Colombia (Bustamante et al., 2017) and Brazil (Gagliostro et al., 2015), the beneficial FAs in milk from pasture were generally higher, and in Romania (Vidu et al., 2015) – lower.

The effect of grazing is associated with stimulation of Δ9-desaturase (Shingfield et al., 2005; Kalač and Samková, 2010), responsible for the synthesisis of *trans* isomers (Bauman et al. 2006) by adding a *cis*-9-double bond on the FA chain (Shingfield et al., 2008). On one hand, 25% of TVA (*trans*-11-18:1) that is synthesized in the rumen is *trans*formed by mammary Δ9d to rumenic acid (*cis*-9,*trans*-11-18:2) (Mosley et al., 2006), so being responsible for more than 70% of that CLA in milk (Shingfield et al., 2013). On the other hand, pasture farming has an impact on ruminal biohydrogenation of unsaturated fatty acids (UFA) and on the growth of specific bacteria in the rumen on the basis of higher concentrations of soluble sugars in fresh plants compared with conserved roughage (Kelly et al., 1998; French et al., 2000). This stimulates Δ9d, respectively CLA, via mammary pathway and prevents the reduction of vaccenic to stearic acid (Nudda et al., 2005), and also enhances synthesis of PUFAn-3 (Chilliard et al., 2001; Dewhurst et al., 2006) via rumen bacteriome. In buffalo, mammary Δ9-desaturase enzyme activity was established greater in pasture farming explaining the higher CLA (Fernandes et al. 2007).

In these processes, it has an important role especially in the desaturation of C18:0 to TVA (Ntambi & Miyazaki, 2004; Bernard et al., 2013), with established higher activity in grazing buffaloes as compared to such bred intensively (Fernandes et al., 2007).

In this way, the degree of biohydrogenation can be judged by the level of stearic acid, as Nielsen et al. (2006) suggest. The tendency for diminishing biohydrogenation as a result of lower pH (Talpur, 2008) in response to silage feeding (Jalč et al., 2013), is confirmed in the buffaloes from the study of Penchev et al. (2016) where MUFA, PUFA, linoleic acid and n-6/n-3 ratio tended to improve with the consumption of alfalfa hay after replacing silage in the diet. This change in the roughage source presumably appears as a stress factor resembling the phenomenon of negative energy balance, regardless of the lactation stage causing mobilization of LCFA from body fat reserves. To our personal practical knowledge, Bulgarian Murrah buffaloes would the sooner compromise their milk production than change their lipid metabolism, which explains the steady pattern of alteration of FA profile of milk during the period of roughage shift. In addition, as a species, the buffalo is also rather unpretentious to forage and such diet shift would not lead to any major misbalance.

In this context, organic buffalo milk and mozzarella showed significantly higher CLA*cis*-9,*trans*-11, TVA and antioxidants than conventional production in a study of Bergamo et al. (2003) in the Italian Mediterranean breed. Similarly, Uzun et al. (2018) found higher PUFA in lactating buffaloes from the same breed fed fresh sorghum roughage, which was preserved in the mozzarella dairy processing.

The fatty-acid profile of most forages, feeds (including some oilseeds), and some by-products used in ruminants' feeding contain UFA with 18 carbon atoms, predominantly C18:2*cis*-9, -12 and C18:3*cis*-9, -12, and -15. Some oilseed supplements have high levels of MUFA, especially C18:1*cis*-9 (Morales and Ungerfeld, 2015; Dewanckele et al., 2020). The best examples for biohydrogenation is the isomerization of 18:2n-6 to CLA*cis*-9,*trans*-11, then hydrogenated to TVA (18:1*trans*-11) and finally to stearic acid (18:0) (Bauman & Griinari, 2003).

The scientific literature shows that ruminal bacteria proliferation and activity can be improved by nutritional manipulation reducing UFA biohydrogenation which can affect milk fatty-acid quality. The effects of supplementing diet with rumen-inert fats on the productivity are well presented for dairy cows, but such reports for the buffalo are limited, especially with regard to fatty acids. The enzymatic activity of Δ9-desaturase is definitely affected by the lipid content of the diet (Ntambi and Miyazaki, 2004; Bernard et al., 2013).

Varricchio et al. (2007) observed that protected fats (calcium salts of fatty acids) did not have positive results in the subjected Mediterranean lactating buffaloes, increasing the level of C16:0. Nevertheless, the supplemented whole cottonseeds from the same publication had beneficial effect on the fatty acid profile, namely n-6/n-3 ratio, PUFA, atherogenicity index and CLA. In Murrah buffaloes in India, Shelke et al. (2012) also found that supplementing rumen protected fat did have effect on milk fatty-acid profile, expressed in a 36% increase in UFA.

In their 21-day experiment on 12 adult Nili Ravi buffaloes, Hifzulrahman et al. (2019) tested the effects of supplementation of rapeseed oil, calcium salts of palm oil and high palmitic acid on dry matter intake, milk yield, milk composition and fatty-acid profile. The authors found increased MCFA in the calcium and palmitic groups, while rapeseed supplementation increased LCFA.

Santillo et al. (2016) observed that buffalo supplemented higher level of flaxseed (1000 g per capita per day) had lowest concentration of SCFA, in particular C8:0 and C10:0, compared to lower flaxseed diet (500 g/d) and the protein diets. In the group with 1000 g/d flaxseed MCFA were also lowest, in that with 500 g/d – intermediate, and in the high and low protein groups the concentration was highest. In LCFA it was the opposite – highest in high supplementation, intermediate in low flaxseed, and lowest in milk produced after no flaxseed in the diet of the buffaloes. Total CLA followed the same trend as LCFA, higher by 22% in the 1000 g/d group compared to the control. Atherogenicity and thrombogenicity index and n-6/n-3 ratio were most beneficial when the flaxseed was highly presented in the diet. Protein level of the diet showed no specific effect on the fatty acids in milk, especially in combination with flaxseed supplementation, the sole effect of protein being significant on C18:0 and C18:1*trans*-11 only. The Δ9-desaturation was found to be with higher activity after feeding low-protein diet and was not affected by flaxseed supplementation.

The authors (Santillo et al., 2016) also pointed out that this supplementation might not have the same effect in different ruminant species and even different breeds of buffaloes in view of the different gene expression and hence different activity of Δ9-desaturase, on the basis of its high genetic variability observed in the bubaline species (Pauciullo et al., 2010).

In contrast to these authors’ conclusions about protein, Tyagi et al. (2007) found that feeding the high-protein berseem to buffaloes can serve as a natural diet manipulation, resulting in milk with more favorable concentrations of total CLA, rumenic acid, PUFAn-3 and n-6/n-3 ratio which was established to be preserved in the processing to three types of cheese.

Patiño et al. (2012) established that the diet supplementation of 140 ml per capita per day of fish oil resulted in constant PUFA n-6 and improved concentrations of CLA and PUFA n-3 in buffalo milk, while using lower doze of 70 ml had positive effect only on the latter.

Hassan et al. (2021) established no effects of supplementation of mixture of black pepper fruit, ginger, cinnamon, peppermint, ajwain seeds and garlic on milk performance, and more importantly on ruminal fermentation, and bacterial diversity in Murrah buffaloes in China. Nevertheless, they observed significant increase in the

in Murrah buffaloes in China. Nevertheless, they observed significant increase in the concentrations of PUFA when a lower doze of 20 g per capita per day was used. A significant decrease of SFA was found as a result and also an increase of MUFAs like C14:1, C16:1, C18:3 and C18:2n-6.

As Vlaeminck et al. (2006) points out, the latter acid is found highly presented in concentrate feeds. Ruminants with high biohydrogenation have highly presented products of C18:2n–6 in milk which is associated with intensive farming, with large use of concentrates and supplements. According to the authors, the effect of diet is associated with ruminal microorganisms, namely their growth and activity dependent on forage type, forage-to-concentrate ratio, supplementation, secondary plant metabolites and bioactive compounds. Such feeding strategies affect BCFA in milk, reported also for the buffalo in particular (Correddu et al., 2017). BCFA are mainly derivative product of bacteria that leave the rumen and are responsible for fluidity of cell membrane of rumen bacteria and their concentration in milk can be used as a diagnostic indicator of the microbial variety and abundance in the rumen (Vlaeminck et al., 2006).

The SFAs with 4 to 12 carbon atoms are synthesized by mammary gland from acetate. Great portion of myristic and palmitic acid in milk fat come through the same pathway (Shingfield et al., 2013) while the rest part of them originates from circulating lipoproteins rich in triacylglycerols and also from intestinally absorbed lipids and such from body fat mobilization (Bauman and Griinari, 2003; Shingfield et al., 2010). Odd-chain fatty acids (OCFA), BCFA and other valuable FAs come via completely different pathway.

Though rumen microorganisms apply biohydrogenation to PUFA and converts them to SFA (Jenkins et al., 2008), in this process some bacterial genera produce wide range of intermediates, like rumenic acid (Palmquist et al., 2005). Another finding of Hassan et al. (2021) was that the herbal preparation stimulated the rumen bacteria of the genera *Succinivibrionaceae*, *Butyrivibrio*, *Pseudobutyrivibrio*, and *Lachnospiraceae* improving fatty-acid profile of milk. Positive correlation was established between C18:3n-3 in milk and specific groups of bacteria showing a tendency to increase PUFA in buffalo milk.

The main source of energy for animals are volatile fatty acids (Mizrahi, 2012) which are produced by rumen bacteria via degradation of polysaccharides and cellulose (Flint et al., 2008). Other precursors of *de novo* synthesized FAs are acetate and butyrate. Buccioni et al. (2015) reported about the capacity of polyphenols to improve the activity of Δ9-desaturase enzyme (SCD), taking part in the conversion of stearic to oleic acid and TVA to CLA, especially increasing n-3 and n-6 fatty acids at a par with rumen biohydrogenation, as well as α-linoleic acid (Roy et al., 2002; Cabiddu et al., 2010). Such limited data about bioactive compounds on lactic fatty acids were revealed in our previous, still unpublished review, which found no reports on buffalo milk.

The results of our experiment on Bulgarian buffaloes (Ilieva et al., 2022) indicate that the content of MCFA, such as lauric and capric acids, decreased while the concentrations of LCFA (11-hexadecanoic and stearic), increased in the group of buffaloes supplemented AyuFertin, containing bioactive compounds such as carotenoids, flavonoids, tocopherols, and fatty acids. In another experiment of our team (Penchev et al., 2022) was concluded that, in comparison to the control group, in the milk from the buffaloes consuming curcumin total and individual SFA have not changed, which applies also to the produced yoghurt. There was increased concentration of the valuable TVA and total *trans*-isomers in the raw milk product of supplementation, which is obliterated after processing to yoghurt. The dominating MUFA, oleic acid, was found constant.

Studies also demonstrate that for the significant individual variation in unsaturation of the milk fats also genetics plays a role, Kelsey et al. (2003) and Soyeurt et al. (2006) having even established that in cows the within-breed variation is greater than between breeds. Similarly, in national cattle Mihaylova and Peeva (2007) has found greater variation among regions than among breeds, supported by Dimitrov (2007) for the Bulgarian Black and White breed. In the Bulgaian Murrah buffalo the author (Mihaylova and Peeva, 2007) observed general uniformity among herds, with greater variability of 14- to 18-atom FAs (especially of the most highly presented palmitic acid) in our herd as compared to three other herds.

In the processing from milk to dairy products – namely white brine cheese, Bulgarian yoghurt and curd – in our study (Ivanova S. et al., 2021) in Bulgarian Murrah buffaloes myristic acid was found to change to little extent. As compared to milk, in the yoghurt from two studies of Naydenova (Naydenova, 2005; Naydenova et al., 2013) were observed similar changes in the 4- to 14-carbon FAs – especially the increase of capric, lauric and myristic acids. But the increased content of the palmitic acid and the decreased stearic and oleic acids are in disagreement with the unchangeable values found by Ivanova S. et al. (2021). In agreement between the three studies, total dienes were established to decrease in yoghurt, while Naydenova (2005) and Naydenova et al. (2013) observed an increase in the trienes and decrease in total MUFA which is a difference. In the same time, using technological methods to elongation of shelf life in yoghurt from buffalo milk, Ivanova S. et al. (2021) have found unchangeable SFA.

Ivanova S. et al. (2021) also established no specific changes in MUFA (TVA in particular) and *trans* isomers in the studied processing to the three dairy products of buffalo milk.

As for the saturation of the cheese, the lauric, caprylic and capric acids that showed some increase in the cheese making process, except for its adverse atherogenic effect, have also some benefits in association with antivirus and antibacterial effects (Sun et al., 2002; Thormar and Hilmarsson, 2007), the latter two together with butyric acid forming the specific flavor of cheese (Naydenova, 2005; Güler et al., 2005).

More special is the increase of C4:0 as it is found only in fats of ruminant origin and which, together with the fat-soluble vitamins (A, D, E) and CLA, has protective function against various diseases (German, 1999; Parodi, 2004). In disagreement, in buffalo un-ripened cheese, Van Nieuwenhove et al. (2007) established increased rumenic, total CLA, stearic acid, and ∑C18:1*cis*. Similarly, Tyagi et al. (2007) found in the production of three types of cheese that the improved total CLA, rumenic acid, PUFAn-3 (respectively n-6/n-3 ratio) after feeding the high-protein berseem to buffaloes were preserved in the end dairy products.

Similar significant increase in the concentration of butyric acid in the dairy processing to the fermented buffalo milk Dahi was found by Yadav et al. (2007), when using germination wih *L. acidophilus* and *L. casei*. Moreover, the levels of CLA were twice higher and the storage at 4°C preserved all CLA isomers.

Santos-Junior et al. (2012) found rumenic acid to undergo dramatic drop due to pasteurization and to partially regain its content during fermentation. The author’ conclusion that the drastic decrease in CLA obliterates the efforts to increase it via diet supplementation explains the findings of our study (Ivanova S. et al., 2021) but is not commensurate with the following review.

Gutiérrez (2016) finds the literature on the effect of fermentation during dairy processing rather controversial and concludes that the content of CLA in dairy foodstuffs depends more on feeding and management than on the specifications of the milk processing. According to Dave et al. (2002), the concentrations of CLA, TVA and PUFAn-3 remain unchanged in yoghurt produced via germination with *L. d. bulgaricus* and *S. thermophilus*, although the diet manipulation of fatty-acid profile resulted in substantial increase of beneficial FAs in milk. But still, there are evidences that these two probiotic strains efficiently biosynthesize CLA from linoleic acid (Lin et al., 1999; Yang et al., 2014).

In the study of Ivanova S. et al. (2021) the thermal regime of the cheese making is not very high, for which our practical observations in laboratory conditions have shown that it does not affect fatty acids, so the alterations in dairy processing are to be associated with the further processes – to lesser extents with fermentation and to greater with ripening and aging. The different behavior of CLA can be explained with the difference in pasteurization specifications. Martínez-Monteagudo and Saldaña (2014) established that both CLA and TVA are oxidized to a great extent when the temperature increased from 90 to 120°C.

A process of oxidation was suggested to happen to the *trans*-vaccenic acid in the yoghurt production (Ivanova S. et al., 2021), meaning that a further increase must have also occurred presumably due to biosynthesis. This is for compensation of the otherwise serious reduction of total CLA, as TVA is reported to be the only known precursor of rumenic acid and a definite portion of it from the human diet is converted into it (Turpeinen et al., 2002; Field et al., 2009).

Except thermal specifications of the processing of milk, according to Khan, I.T. et al. (2020) cooling after coagulation also plays effect which is expressed in decreased concentrations of MUFA (C18:1) and PUFA (C18:2) in yoghurt cooled down to 25 and 18ºC but when it is cooled from 43ºC way down to 5ºC they retain their levels.

Like in the yoghurt, in the curd produced via acidification of Ivanova S et al. (2021) was established dramatic decrease in total CLA and in all individual CLAs, excluding rumenic acid, as it had been in a similarly produced but germinated ricotta cheese during 6-month ripening (Bergamaschi and Bittante, 2017).

CONCLUSIONS

As indicated by their reaction to the change in roughage in our earlier study and the metabolic variations mostly during the transition phase, the buffalo's body response to the complex synthesis of fatty acids should be distinguished from that of dairy cattle. One specific feature of this species is its seasonality (at least with regard to reproduction), so respective dynamics of the valuable FAs can be expected, as some studies have shown, but diet changes should be carefully weighed to avoid masking of the *per se* factor. The most mitigative effect is the animal factor lactation stage, expressed in improvement of beneficial FAs with the advance of lactation. But the highest effect is associated with managemental practices.

Though, as a ruminant product, buffalo milk acquires only limited number of FAs directly from the diet, farming system involving feeding plays the major impact on the composition of fatty acids in it. It can also include fatty-acid manipulation via different feeding strategies (rumen-inert fats, seeds, bioactive compounds, etc.) to affect rumen biohydrogenation but the inconsistent success in this implies that the doze should be studied very well.

The best manipulation, as many studies show, is natural grazing, not only because it provides soluble sugars, vitamins, polyphenols and proteins, or even some FAs that enter unchanged the bloodstream, but mostly because of the stimulation of the microbial synthesis, and the activity Δ9-desaturase and acetyl-CoA carboxylase responsible for the *de novo* synthesis, in these pathways improving omega ratio, conjugated linolenic (CLA) and *trans*-vaccenic acid (TVA). The results about the transformations in the health-related fatty acids in the yoghurt production are controversial, while those about the cheese confirm to some extent the general conception that such technological processes alter the individual isomers but not the groups of beneficial fatty acids (CLA) as a whole.

The resemblance in the changes between yoghurt and curd (with no germination) presumptively denies the existence of a notable effect of fermentation and emphasizes the negative effect of pasteurization. In cheese making, to these effects should be added the impact of ripening processes and that of pasteurization only if the temperature applied is high, but not the effect of rennet, which has no lipolytic activity.

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