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Comparison of fatty acid and cholesterol content of Pakistani water buffalo breeds

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Abstract

Present study evaluates the milk fatty acid (FA) composition and cholesterol content of two main Pakistani dairy breeds water buffaloes, i.e. Kundi and Nili-Ravi (n = 25 for each breed). The buffaloes were housed together and received the same diet. The results show a significant variation ($P < 0.05$) in the FA content of the two breeds. The milk fat of Kundi buffalo was found to contain significantly lower ($P < 0.05$) amount of saturated fatty acid content than Nili-Ravi buffaloes (66.96 and 69.09 g/100 g). Determined mean monounsaturated fatty acid (MUFA) contents (27.62 vs. 25.20 g/100g) and total *trans* fatty acids (3.48 vs. 2.48) were significantly elevated ($P < 0.05$) in the milk fat of Kundi buffaloes. Amount of fat and conjugated linoleic acid content was higher ($P = 0.04$) in Kundi buffalo as compared to Nili-Ravi buffaloes (7.00 vs. 7.78 g /100g and 0.80 vs. 0.71g / 100g), while cholesterol content was not different among both breeds ranging from 8.89 – 10.24 mg /dl. Present studies show that in future genetic selection programs along with altered buffalo nutrition may be able to result in optimum levels of various fatty acids in milk.

Key words: *Water buffaloes, Conjugated linoleic acid, Milk fat, Kundi, Nili-Ravi*

Introduction

The structure of the fatty acids (FA's) plays a major role in maintaining health [1]. Increasing public awareness of the health benefits of conjugated linoleic acid (CLA) isomers as anticarcinogenic, antiatherogenic, antiobesity, and antidiabetic [2,3] has stimulated interest in sources of these FA's for human consumption. Ruminant dairy products are the major dietary sources of CLA, and the isomer *cis*- 9, *trans*- 11 is approximately 80 to 90% of the CLA in milk fat [3]. The underlying factor resulting in the variation of FA are predominately related to the ruminants diet, including forage to concentrate ratio [4], level of intake [5] and intake of unsaturated FA's, especially plant oils that are high in linoleic acid [6]. Breed difference in milk FA has been reported by several authors [7-10]. Variation in milk fatty acid composition particularly CLA content among species has been adequately explained [11].

Little attention has been paid to buffaloes milk fatty acids, although among all domestic animals, Asian buffalo holds the greatest promise and potential for milk production [12]. The Food and Agriculture Organization [13] has rightly termed buffalo as an important but 'an asset undervalued'. The world buffalo population, about 130 million has increased by 91% between 1961 and 2000. The buffalo in Far East is called as swamp buffalo and mainly used as a draft animal in paddy crops. This type of buffalo is of small size with compact body and having straight horns. While Mediterranean buffaloes found in Italy, the Balkan states, Turkey and in some part of Russia are of small size giving 1400-1500 liters milk per lactation [14]. South Asia has five groups (Murrah, Gujrati, Uttar Pradesh, Central Indian and South Indian) of buffalo breeds. Among these groups, the Murrah group (Nili-Ravi, Kundi and Murra) is the leading one, both in meat and milk production. Nili-Ravi is the best performing animal of this group, producing more milk than the other breeds of the world (2500 liter per lactation) [15], while milk yield for Kundi breed is 1700-2200 liters with a 6-7% butterfat [16]. The Kundi breed is found in Sindh Province of Pakistan around the River Indus and is jet black in color. Milk yield of this breed varies between 2500-3200 liters per lactation [17].

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Pakistan is fortunate enough in having two best sub-tropical breeds of buffaloes such as Nili-Ravi and Kundi. The best buffalo animals are found in the canal fed areas of the country, where abundant fodder supply and crops by products are available. In Pakistan buffalo (19.7 Million tons) is the major dairy animal contributing maximum in total milk production followed by cattle (9.0 Million tons) and sheep/goat (0.760 Million tons) respectively [18]. Indeed Pakistani buffaloes are the best milch buffaloes in the world. There is considerable genetic variation, which could be judiciously exploited by selective breeding for higher milk production and improved nutritional quality.

Previously we have determined the breed and seasonal differences in ruminant milk from Pakistan [10, 11, 19]. Present study is part of that project and was undertaken to evaluate the difference in fatty acid composition of the two buffalo breeds i.e. Nili-Ravi and Kundi consuming with same diet.

Experimental

Animals and feeding

Milk samples were obtained from 25 lactating buffaloes from each of the two breeds, Kundi and Niili Ravi, from a farm situated in Hyderabad region.

Table 1 Ingredient and composition of experimental diet given to the buffaloes

Composition	Content
Ingredients (% of DM)	
Corn silage	65.00
Rapeseed meal	13.13
Soybean meal	13.13
Wheat feed (middling)	5.47
Blended cane molasses and urea	1.10
Limestone	0.80
Mineral and vitamin supplement	2.27
Chemical composition (% of DM)	
CP	17.2
NDF	40.5
ADF	17.7
EE	3.35
Ca	1.22
P	0.46
NE (Mcal /kg of DM)	1.87

CP= Crude fiber, NDF= Neutral detergent fiber, ADF= Acid detergent fiber, EE= Ether extract, NE= Net Energy

All buffaloes were multiparous (parity, 2.31 ± 0.30, body weight, 400 ± 50 kg; milk yield, 16.00 ± 3.12 kg/d; days in milk, 85 ± 33 (mean ± SD), free from mastitis or any other inflammatory diseases, and were used in a 12-week trail. All buffaloes were offered 7.00 kg/d (DM basis) of an identical TMR (Table 1), formulated to meet the nutrition requirement [20].

Buffaloes were housed together and milked at 0500 and 1600 h on the last 2days of experimental period. Milk samples were composed for each buffalo and divided into three sub samples in order to obtain triplicate analysis. The milk samples were then frozen at -20 ^{0}C until subjected to fat analysis.

Fat extraction

Lipids in milk (1 mL) were extracted using a modified Folch et al.[21] procedure for total lipids in human milk as described by Jensen et al. [22] except: 1mL of an internal standard (1 mg/mL of methyl 10-heptadecenoate [C17:1] (Nu-Chek-Prep, Inc., Elysian, MN, USA) dissolved in chloroform/methanol, 2:1, vol/vol) was added to each sample before extraction. The extracted lipids were evaporated under nitrogen and then dissolved in 1mL of iso-octane.

Preparation of methyl esters

The lipids were esterified in capped screw top tubes (Teflon liners) with 6mL of 0.5N sodium methoxide heated at 50 ^{0}C for 10 min [23]. The fatty acid methyl esters (FAME) were then cooled to room temperature, and 2mL of iso-octane and 3mL of 10% acetic acid were added. The tubes were recapped to prevent evaporation of the short chain FAs. The FAME were centrifuged (2000g) for 10 min, and a portion of the top layer removed and placed in sealed gas chromatography vials and kept at -20 ^{0}C until analyzed.

Analysis by gas chromatography

The FAME were analyzed in a gas chromatograph (Hewlett Packard Co., model 5890) fitted with SP-2560 fused silica capillary column (100m × 0.25mm i.d. × 0.2 mm film thickness: Supelco, Inc., Bellefonte, PA, USA) using manual injection. Hydrogen was the carrier gas, which was set at a 40 psi. The injection volume was 2 mL with a split/splitless ratio (80/20). The column parameters were as follows: initial column temperature was held at 70 ^{0}C for 2 min; increased 15 ^{0}C/ min to 155 ^{0}C (held for 25 min), then increased at 3 ^{0}C/min to 215 ^{0}C (held for 8 min). The total run time was 61 min.

Data were collected automatically and FAs were identified by comparison to a general standard containing 32 FA methyl esters (GLC reference standard-461, Nu- Chek-Prep, Inc.), using the computer program: Chrom- Perfect for Windows (Justice Innovations, Mountain View, CA, USA). Retention times of CLA isomers and C18:1 trans-11 was checked by co-elution of samples with commercial preparations (Matreya, Inc., Pleasant Gap, PA, USA) of these FAs. Peak area and percent of individual FAs were quantified by comparing the peak areas of the samples corrected for losses by internal standard to the area of external standards (GLC reference standards, Nu-Chek-Prep, Inc., Elysian, MN, USA).

Statistical analysis

The data obtained were statistically analyzed by one-way ANOVA using SAS (SAS Inst. Inc., Cary, NC, USA) for significant F-statistics. If the overall F-test was significant ($P < 0.05$) a Fachers T-test was performed to discern differences between the breeds.

Result and Discussion

Fatty acid composition of Kundi and Nili-Ravi breed buffaloes milk is depicted in Table 2. Average parity and DIM were similar for the two breeds. There were several significant differences ($P < 0.05$) in the concentration of fatty acids between the Kundi and Nili-Ravi buffaloes. Of total fatty acids (weight basis), short and medium chain length fatty acids (< 16 carbons) represented 23.53 g/100g for Kundi and 25.95 g/100g for Nili-Ravi breed. Similar values for 16 carbon fatty acids were 32.15 g/100g and 32.13 g/100g for the two breeds and longer chain fatty acids (> 16 carbons) represented 46.28 g/100g and 41.79 g/100g of total fatty acids for Kundi and Nili-Ravi buffaloes, respectively. As far as author knowledge is concern, there is no comprehensive data available earlier, regarding milk fatty acid composition of different breed buffaloes. However, similar differences in milk fatty acids composition have been previously observed by Kelsey et al. [24] and Pesek et al. [25] for Holstein and Brown Swiss cows.

Table 2. Amount of fatty acids (g / 100 g) in milk fat of Kundi and Nili -Ravi buffalo breeds determined by Capillary GC

Fatty acids	Milk of Kundi (g /100g)				Milk of Nili -Ravi (g/100g)				Signif:
	Min	*Max*	*Mean*	*SD*	*Min*	*Max*	*Mean*	*SD*	*P-Value*
C-4:0	3.12	4.12	3.72	0.22	3.58	4.56	4.20	0.62	***
C-6:0	1.85	2.58	2.08	0.18	2.00	3.20	2.45	0.50	***
C-8:0	1.30	2.30	1.91	0.51	1.78	2.36	2.10	0.42	**
C-10:0	1.20	1.82	1.45	0.60	1.65	2.00	1.80	0.63	**
C-12:0	1.36	2.66	1.98	0.24	2.00	3.56	2.59	0.74	**
C-14:0	10.21	12.01	11.35	0.57	10.84	12.20	11.82	0.91	**
C-16:0	31.23	33.45	32.15	1.04	30.26	33.64	32.13	1.23	NS
C-17:0	0.79	1.05	0.92	0.17	0.82	0.99	0.90	0.62	NS
C-18:0	11.58	13.03	12.40	0.46	10.78	11.88	11.10	1.01	**
∑ SFA	64.55	68.21	66.96	2.28	65.23	70.58	69.09	3.21	***
C-14:1	0.89	1.21	1.04	0.27	0.82	1.25	0.99	0.23	NS
C-16:1	1.23	1.78	1.53	0.09	0.87	1.30	1.06	0.36	***
C-18:1	23.56	25.80	25.05	0.25	21.69	24.04	23.51	0.65	**
∑ MUFA	26.12	28.45	27.62	1.68	22.89	26.67	25.20	0.92	***
C-16:1 *trans*	0.11	0.34	0.28	0.05	0.08	0.22	0.15	1.21	***
C-18:1 t 11 *trans*	1.92	3.00	2.72	0.07	1.68	2.43	2.02	0.43	**
C-18:2 t9, t12 *trans*	0.32	0.51	0.43	0.06	0.23	0.42	0.31	0.08	***
∑ TFA	3.02	4.02	3.43	0.13	1.85	3.12	2.48	0.41	**
C-18:2 *cis*	1.00	1.36	1.22	0.10	1.08	1.39	1.21	0.34	NS
C-18:2 c9, t11 (CLA)	0.50	1.00	0.80	0.15	0.40	0.94	0.71	0.18	**
C-18:3 n-3	0.60	0.75	0.69	0.05	0.63	0.79	0.71	0.24	NS
C-20:4 n-6	0.07	1.00	0.09	0.06	0.05	1.01	0.08	0.04	NS
C-20:5 n-3	0.06	1.01	0.09	0.08	0.06	0.08	0.07	0.03	NS
C-22:6 n-6	0.05	0.09	0.06	0.06	0.04	0.07	0.05	0.05	NS
∑ PUFA	1.98	3.00	2.77	1.02	1.95	3.14	2.76	1.12	NS

n = 75 (25 samples triplicate) for each breed cows; SD = standard deviation;
NS = non-significant at $P > 0.05$, Significant at **$P < 0.05$; ***$P < 0.01$

Palmquist & Beaulie [26] have also reported that Holstein cows produced 8 to 42% more short chain and medium chain fatty acids (C6:0 to C14:0) compared with Jersey cows when fed similar diets. In present study a significant effect of breed ($P \leq 0.01$) was found in the case of butyric acid (C 4:0) with higher concentration in Nili-Ravi breed. Previously Reklewska et al. [27] has reported differences in butyric acid content among Black and White, Polish Red and Simmental breed cows during winter (indoor) and summer (grazing periods). Recently butyric acid has identified as one of the functional milk components due to its potent antimicrobial and anticarcinogenic affects [26]. Our data shows that Kundi buffaloes produced 7% higher stearic (C18:0) and 12% higher oleic acid (C18:1) content as compared to Nili-Ravi buffaloes milk fat. Palmiquist and Beaulie [26] also reported that Jersey cows produced 13% more stearic acid and 15% lower Oleic acid concentrations. Further studies may offer explanations of breed differences for fatty acid production. Preliminary work by Medrano et al. [28] shows differences between breeds in the activity of the mammary enzyme Stearoyl coenzyme A desaturase. Stearoyl coenzyme A desaturase oxidizes palmitic (C16:0) and stearic (C18:0) acids to Palmitoleic (C16:1) and (C18:1) oleic acids is involved in CLA production. Results of *cis*- monoenes showed no difference in the levels of C14:1 but did show that Nili-Ravi produced 44% lower C16:1. Mammary desaturase activity in vitro was reported to use both C16:0 and C18:0, but not C14:0, as substrate [29].

In contrast, recent in vivo studies implicate mammary desaturase activity as the major source of C14:1 found in milk [30]. Few comparisons between cow breeds for C14:1 content in milk fat is available; two studies reported no difference between breeds [6], whereas three other studies did not report C14:1content [8, 31, 32]. Drackley et al. [32] and Morales et al. [6] reported a lower content of C16:1 in milk fat from Jersey as compared to Holstein cows, but Bitman et al. [31] and DePeters et al.[32] found no statistical difference between breeds. Lower contents of C14:1 and C18:1 in milk fat of different cow breeds with changed and unchanged C18:0 suggests less desaturase activity in mammary tissues [8].

The proportion of C18:2 and C18:3 were not significantly different ($P > 0.05$) between Kundi and Nili-Ravi buffalo's milk fat. Other reported breed comparisons have also not detected differences in milk C18:2 and C18:3 between Holstein, Jersey and Czech Paed cows [7, 8, 25]. Breed differences on fat content, cholesterol, *cis*-9, *trans*-11 CLA concentration in milk fat and CLA desaturase index is summarized in Figure 1. Kundi buffalo contain higher ($P = 0.04$) amount of fat (7.00 vs. 7.78 g /100g) as compare to Nili-Ravi buffaloes, while cholesterol content was not different among both breeds ranging from 8.89 – 10.24 mg /dl. Comparable values for fat (7.58-8.30 g/100g) and cholesterol concentrations (9.18-12.75 mg /dl) has been reported for Italian mediterranean buffaloes [34] and lactating buffaloes from Nepal [35]. CLA content was significantly higher in Kundi (0.80 vs. 0.71g / 100g of total fatty acids) than Nili-Ravi buffaloes and CLA index was also different. We are not aware of any previous investigations regarding the effect of buffalo breeds on desaturase index.

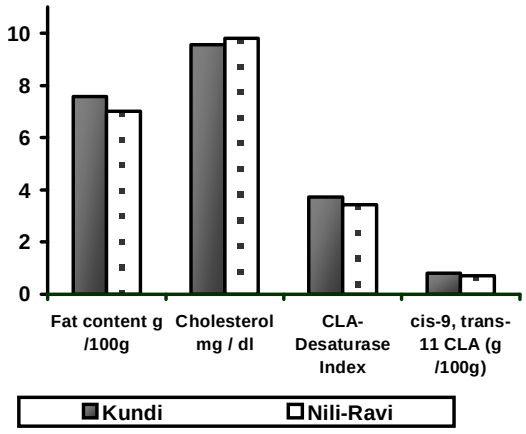

Figure 1 Total fat, cholesterol, conjugated linoleic acid and CLA-desaturase index* for Kundi and Nili-Ravi buffaloes

**Desaturase index was calculated as cis-9 trans-11 CLA/cis-9 trans-11 CLA + trans-11 18:1*

However, similar values (0.77 g/100g) of *cis*-9, *trans*-11 is reported for Murrah buffloes [36] fed on green fodder, while Nieuwenhove et al. [37] has described lower CLA proportions (0.483 g/100g) in river buffaloes from Argentina. The CLA content in different breeds of cows is well documented. Lawless et al. [38] compared four breeds of cows, Irish Holstein/ Friesian, Dutch Holstein/ Friesian, Montbeliaardes, and Normandes that were grazing pasture. They reported that breed had a small effect with Montbeliardes, averaging about 13 % greater CLA content in milk fat than the other three breeds. White et al. [39] compared Holstein and Jersey cows that were either fed a TMR confinement or grazing pasture ; they found that Holstein cows had slightly higher milk fat concentrations of CLA (~ 18% greater overall). Several other authors had also established breed effect on CLA concentration while studying different breeds of cows, fed on identical diets [24, 40].

The mean proportions of PUFA especially long chain fatty acids EPA (C20:5) and DHA (C22:6) were not differ significantly ($P > 0.05$), and are comparable with literature data for different breeds cows [27]. Although EPA and DHA are present in milk fat in negligible amounts, they can not be ignored because they are important components of the cell membrane associated process. However it should be noted that an uncontrolled, increased intake of these PUFA, may potentially result elevated risk of exposure to PUFA auto oxidation toxic products [41].

Conclusions

Present study reveals the difference in milk fatty acid composition between Kundi and Nili-Ravi water buffaloes, comparable with earlier reported data for various cattle breeds. Breed differences in fatty acid production could have implication on milk and dairy product consumption. Most of the buffalo milk in Sindh is produced from Kundi, while in Punjab province it is produced from Nili-Ravi buffaloes, which are mostly proceeds for the dairy products. In the future, genetic selection programs along with altered buffalo nutrition may be able to result in optimum levels of various fatty acids in milk.

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References

1. M. W. Christine. *Ann. Zootech.* 49 (2000) 165.
2. M. A. McGuire and M. K. McGuire. Proc. Am. Soc. Anim. Sci. Annu. Mtg. 1999. (2002). Online. Available: http://www.asas.org/jas/symposia/proceedings/0938.pdf
3. M. A. Belury. *Annu. Rev. Nutr.* 22 (2002) 505.
4. P. W. Parodi . *J. Dairy Sci.* 82, (1999) 1339.
5. C. R. Stockdale, G. P. Walker, W. J. Wales, D. E. Dalley, A. Birkett, Z. Shen, P. T. Doyle, *J. Dairy Res*. 70 (2003) 267.
6. A. T. Ward, K. M. Wittenberg, H. M. Froebe, R. Przybylski, L. Malcolmson. *J. Dairy Sci.* 86 (2003) 1742.
7. M. S. Morales, D. L. Palmquist, W. P. Weiss . *J. Dairy Sci.* 83 (2000) 2112.
8. A. D. Beaulieu, D. L. Palmquist. *J. Dairy Sci.* 78 (1995) 1336.
9. M.J. Auldist, K. A. Johnston, N.J. White, W. P. Fitzsimons, M. J. Boland. *J. Dairy Res*, 71 (2004) 51.
10. F. N. Talpur, M. I. Bhanger, M. Y. Khuhawar. *J. Food Compos Anal.* 19 (2006) 698.
11. F. N. Talpur and M. I. Bhanger. *Pak. J. Anal. Enviorn. Chem.* 6 (2005) 22.
12. W. R. Cockrill. Present and future of buffalo production in the world. Proceedings of the Fifth World Buffalo Congress, 27-30 June (1994) Sao Paulo, Brazil.
13. FAO . Water Buffalo: an Asset Undervalued, (2000) pp.1-6. FAO Regional Office for Asia and the Pacific, Bangkok, Thailand. http://www.aphca.org/publications/files/w_buffalo.pdf.
14. Buffalo Information Society. (2006).Available online at: http://huntingsociety.org/BuffaloInfo.html.
15. M. Q. Bilal and A Ahmad. Dairy hygiene and disease prevention. (2004). Usman and Bilal printing linkers, Faisalabad, Pakistan.
16. S. I. Shah. A textbook of animal husbandry .(1994) . National Book Foundation, Islamabad, Pakistan.
17. B. B. Khan, M. Younas, S. H. Hanjra. Breeds and types of livestock in Pakistan. Department of Livestock Management, University of Agriculture, Faislabad, Pakistan (2005).
18. Agricultural statistics of Pakistan 2004 – 2005. Government of Pakistan, Ministry of food, agriculture & livestock (Economic wing) Shaheed-e-millat ecretariat, 8th floor, Islamabad, Pakistan. (2006) 190.
19. F. N. Talpur and M. I. Bhanger..Current topics in Dairy Production, Volume 11, Published by: University Printing Service Sydney, Australia. (2006) 132.
20. S. K. Ranjhan, S. K. Chemical composition of Indian feeds and feeding of farm animals. ICAR, New Delhi (1991).
21. J. Folch, M. Lees, G. H. Sloane-Stanley. *J. Biol. Chem.* 226 (1957) 497.
22. R. G. Jensen, A. M. Ferris, C. J. Lammi-Keefe. *J. Dairy Sci.* 74, (1991) 3228.
23. J.K.G. Kramer,V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba, M. P. Yurawecz. *Lipids*, 32 (1997) 1219.
24. J. A. Kelsey, B. A. Corl, R. J. Collier, D. E. Bauman. *Dairy Sci*, 86 (2003) 2588.
25. M. Pesek, J. Spicka, E. Samkova. *Czech J. Anim. Sci.* 50 (2005) 122.
26. D. L. Palmquist and A. D Beaulieu. *J. Dairy Sci.* 75 (1992) 292.

27. B. Reklewska, E. Bernatowicz, Z. Reklewski, B. Kuczynska, K. Zdziarski, T. Sakowski,K. Sloniewski. *Elec. Pol. Agric. Uni.* 8 (2005) 1.
28. J. F. Medrano, A. Johnson, E. J. DePeters, A. Islas. *J. Dairy Sc.* 82 (1999) 71.
29. J. H. Moore and W. W. Christie. Prog. Lipid Res. 17 (1979) 347.
30. J. M. Griinari, B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. V. Nurmela, D. E. Bauman, D. E. *J. Nutr.* 130 (2000) 2285.
31. J. Bitman, D. I. Wood, R. H. Miller, H. F. Tyrell, C.K. Reynolds, H. D. Baxter. *J. Dairy Sci.* 79, (1996) 1595.
32. E. J. DePeters, J. F. Medrano, B. A. Reed. *Canad. J. Anim. Sci.* 75 (1995) 267.
33. J. K. Drackley, A. D. Beaulie, J. P. Elliott. *J. Dairy Sci.* 84 (2001) 1231.
34. L. Zicarelli. Vet. Res. Commun. 28 (2004) 127.
35. Y. Shah, S. Shah, S. K., Kumagai, H. *Livest. Res. Rural Dev*. 17 (2005) 10.
36. A. K. Tugia, N. Kewalramania, T. R. Dhiman, H. Kaura, K. K. Singhala, S. K. Kanwajiaa. *Anim. Feed Sci. Technol.* 133 (2007) 351.
37. C. Nieuwenhove, S. P. Gonzalez, A. P. De Ruiz Holgado. *Milchwissenschaft* 59 (2004) 506.
38. F. Lawless, C. Stanton, P. L'Escop, R. Devery , P. Dillon, J. J. Murphy. *Livest. Prod. Sci.* 62 (1999) 43.
39. S. L.White, J. A. Bertrand, M. R. Wade, S. P. Washburn, J. T. Green, T. C. Jenkins. *J. Dairy Sci.* 84 (2001) 2295.
40. V. A. Capps, J. E. Depeters, J. S. Taylor, H. Perez-Monti, M. W. Rosenberg. *J. Dairy Sci.* 82 (1999) 45.
41. G. Valivety. *Trends Biotechnol*. 15 (1997) 401.