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Original paper

Influence of varying ranges milk urea nitrogen on chemical, hygienic and physical quality traits of cow milk

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Abstract The aim of our research was to investigate theinfluence of predetermined value ranges incow milk components on milk quality regarding chemical, physical and hygienic aspects. It also aims to identify optimal values o be used in dairy cow farm management, with potential economic efficiency impact, through a morerational use of ration protein. The predetermined value ranges of milk urea were structured between <22 mg/dl and >40 mg/dl, milk urea nitrogen (MUN) within values <10 mg N/dl and >19 mg N/dl, protein from <3% to >3.5% and somatic cell count (SCC) with values between <100 SCC/ml x 10^3 and >400 SCC/ml x 10^3 and investigated urinary excretion nitrogen losses. We analyzed 1591 samples of milk obtained during spring period, which were processed and interpreted statistically. Milk samples were obtained from 1591 head Friesian – Black Spotted Breed raised in Transylvania, Romania with a body weight of 645-680 kg, milk production of 9.981 kg / year with a UNIFEED feeding system. The results of this research show that the optimal value range for milk urea isbetween 23-31 mg/dl, with milk urea nitrogen (MUN) between 10-15 mg N/dl and urinary excretion losses (UEN) between 197-270 g N/day/head.

Keywords Dairy cattle, urea, MUN, UEN, SCC, protein, fat, milk yield.

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Introduction

Urea is a small organic molecule composed of carbon, nitrogen, oxygen and hydrogen which is a common constituent of blood and other body fluids. Milk urea nitrogen (MUN), which is a normal constituent of milk, represents a portion of milk nonprotein nitrogen and a variable fraction of milk total nitrogen. Milk urea nitrogen is synthetized as urea in blood serum, so it can pass through the secretory cells of the mammary gland, which would be an indication of the amount of degradable protein in the rumen (Gustafsson and Palmquist, 1993 [8]).

Urea, a major end product of nitrogen metabolism in dairy cows, is mostly synthesized in the liver and transported to the kidneys for excretion via urine. The concentration of urea in the blood rapidly equilibrates with other body fluids, including milk (Gustafsson and Palmquist, 1993 [8]). The conversion of ammonia to urea, primarily in the liver, prevents ammonia toxicity. Animals convert excess ammonia to urea because ammonia is toxic while urea is non-toxic and can be at very high levels without causing any problems.

Milk urea nitrogen and lactose concentrations in milk may vary from herd to herd, between cows of the same herd, and along the course of lactation. It is important to determine how both metabolites fluctuate as well as their influence on other milk fractions because concentrate feed is an important component of the cost structure of dairy farms and excessive dietary nitrogen can affect the reproductive efficiency of cows and cause negative environmental impacts (Rajala-Schultz and Saville, 2003 [14]). Milk urea nitrogen (MUN) is one possible tool to assess the protein and energy balance status of a group of dairy cows and can be used for minimizing feed costs while maximizing production and how efficiently the cow is utilizing the protein or nitrogen to produce milk. The mean MUN could be used to signal potential problems with our feeding program.

Most ruminants are fed low-quality roughages, agricultural crop-residues and industrial byproducts, however, roughages are low in nutritive value, protein level, high content of ligno-cellulose and low digestibility, thus resulting in low voluntary feed intake (Wanapat & al, 2013 [16]). The MUN values on farms can vary significantly due to a variety of non-dietary and dietary factors. Non-dietary factors such as breed, parity, days in milk, milk production and feeding to milking interval can all affect MUN values. Diet or ration has the greatest effect on MUN values, however, with high MUN values indicating excess protein in the diet or a deficiency in energy. The improvement of low quality roughages can be fulfilled by supplementation of true protein sources and non-protein nitrogen (NPN) like urea (McAllen, 1991; Huntingto and Archibeque, 1999 [2]). In addition, the efficiency of protein utilization should always consider economical as well as environmental aspects. Urea in rumen is converted to ammonia by urease and the ammonia released from urea has the capacity to weaken the lignified outer walls, allowing better penetration by rumen microorganisms to produce more effective fermentation and liberation of nutrients (Chenost, 1995 17]). However, the addition of urea to animal diet should be done under limitations to avoid the risk of hyper ammonia. The hydrolysis of urea to NH3 in the rumen by microbial enzymes is rapid and occurs at a faster rate than NH3 utilization by the rumen bacteria (Highstreet & al, 2010 [1]).

The amount of non-protein N, such as in urea, that can be used in diets is limited due to their rapid hydrolysis to ammonia in the rumen by microbial enzymes. Rumen ammonia and plasma urea levels generally peak 1 to 4 hours post-feeding in meal-fed animals and decline thereafter (Gustafson and Palmquist, 1993 [8]). MUN values can be used in conjunction with evaluating milk production records, feeding management practices and dry matter intake, energy intake, degradable and undegradable protein, nonstructural carbohydrates, water intakes, liver function and urinary output. The very rapid degradation of most nonprotein N forms to ammonia is often faster than the ammonia can be utilized by rumen microbes, resulting in ammonia being absorbed through the rumen wall as the ammonium ion (Satter and Roffler, 1975 [20]), which is converted to urea and subsequently excreted in urine. Slowly ruminally released urea compounds, as a replacement for urea in ruminant rations, have a long history in ruminant feeding. Biuret (Fonnesbeck & al, 1975 [6]) is likely the most widely researched slow release N compound historically, although others (e.g., Huntington & al, 2006 [9]) have been examined in recent years.

It is generally known that mean urea concentration and the range in urea values for a group of cows should fall into specific ranges. Thus, urea concentration may serve as a monitoring tool for assessing protein nutritional status. Urea concentration in milk may provide an opportunity to look at problems with the feeding program and system within the respective farm;on the other hand it is necessary to optimize the feeding of dairy cows with effects in limiting protein losses so as to improve theeconomic efficiency of the process.

Materials and Methods

Producers of raw milk from EU countries area must comply with European regulations on hygiene and food safetyand quality requirements according to regulations quality for raw cow's milk obtained on farms in accordance with Regulation 852/2004 EC and EC Regulation 853/2004. During the pre-accession period and after accession to the EU Romania has implemented agricultural policies to improve the quality of milk with a transition period from 2005-2010, in order to improve the quality of raw milk and implicitly the quality of collection system and hygiene in the dairy farms. The extension provided to farmers, especially to the small size exploitation must be constructive in order to apply measures and implement programs designed to improve the quality and safety of raw cow milk, the collection system and its modernization as well as development of farms according to European requirements and regulations on animals' welfare, good practices and safety (MACIUC & al, 2003 [21]; MACIUC & al, 2008 [22]).

The present research aims to identify optimal values to be used in dairy cow farm management with economic efficiency influences through the rational use of protein from fodder ration and checking the influence of some predetermined value ranges of milk components on milk quality in terms of chemical, physical and hygienic aspects. We analyzed 1591 samples of milk obtained during the spring (March) period, which were processed and interpreted statistically. Milk samples were obtained from 1591 head Friesian – Black Spotted Breed raised in Transylvania, Romania with a body weight of 645-680 kg, average daily milk production was 33.3 L/day and milk production of 9.981 kg/year. The influence of some milk urea ranges between <22 mg/dl and >40 mg/dl, milk urea nitrogen (MUN) at values <10 mg N/dl and >19 mg N/dl, protein from <3% to > 3.5% and somatic cell count (SCC) with values between <100 SCC/ml x 10^3 and >400 SCC/ml x 10^3 and urinary excretion nitrogen losses. Analyzes of samples of milk were carried out with the apparatus certificated for each method of analysis as folows: urea (mg/dl) Milkoscan, protein (g/100ml) ISO 9622|IDF 141 2013; fat (g/100 ml) ISO 9622|IDF 141 2013; lactose (g/100 ml) ISO 9622|IDF 141 2013; conductivity (S/m) Milkoscan, SU (g/100 ml) Milkoscan; number of somatic cells, SCC/ml x 1000 Fossomatic ISO 13.366-2 IDF 148-2:2006. Milk urea nitrogen (MUN) was determined by calculation, because MUN contains 47% Nand can be influenced by conditional factors and shows signs of protein level or energy protein imbalance infodder ration. Urinary excretion of nitrogen (g N/day UEN) was calculated using an equation to predict the loss of nitrogen based on body weight and MUN values (M. HUTJENS, L.E. CHASE, 2012 [18]).

The feeding technology was in the UNIFEED system with unique feed mixture (UFM) and the feed ration was made up of corn silo, alfalfasilo, beer draff, maize meal, sunflower groats, hill hay, soy groats, wheat, straw, premix, calcium carbonate, sodium bicarbonate, salt administered in the form of a single feed mixture with a composition adapted to the lactation stage, milk production and body weight (Table 1).

Table 1. Fodder ratios for a dairy cow at different lactation stages with body weight, 650-680 kg

Lactation Interval (day)	Milk L/day	UFM kg	DM kg	UNL	PDIN (g)	PDIE (g)	Ca (g)	P (g)
30-100	39-42	57.2	25.8	23.2	2949.6	2668.1	194.0	109.9
100-200	32-37	52.9	22.5	23.7	2516.0	2429.0	185.5	119.9
>200	15-20	42.4	20.1	18.2	1883.8	1827.2	130.5	79.5

Results and Discussion

The results shown in Table 2 show that 75.42% of dairy cows fall between 25.1-40 mg/dl urea in milk, with mean values between 31.05±0.1-37.36±0.06 mg/dl, MUN between 14.59-17.56 mg N/dl and UEN between 268.4-327.7 g N/day. Intervals with the lowest average values of the milk urea and the urinary excretion nitrogen losses (UEN) with an efficient use of the protein in the ration range between 20.36±0.3-23.6±0.1 mg/dl, MUN 9.57-11.09 mg N/dl and UEN of 169.4-197.9 g N/day. Also in range between 20.36-23.6 mg/dl were maintained good milk protein values at 3.34%±0.08, fat at 3.78% ± 0.1, lactose at 4.49%±0.01, SCC at 255.8±33.49/ml x 10^3, higher by 25% than the average, and conductivity of 899.78±19.28 S/m which was higherthan the average by 6.2% (Table 2).

Table 2. The influence of some milk urea value ranges on the composition of cow's milk

Specification		Range:					
Urea mg/dl		<22	22-25	25-35	35-40	>40	Average
MUN mg N/dl MUN Average		<10 9.57	10-12 11.09	12-16.5 14.59	16.6-19 17.56	>19 20.21	16.5
UEN g N/day		169.4	197.9	268.4	327.7	360.6	302.5
n		25	49	676	524	317	1591
Urea, mg/dl	*X*	20.36	23.6	31.05	37.36	43.01	35.11
	±*sx*	0.3	0.1	0.1	0.06	0.14	0.14
	S	1.61	0.82	2.67	1.41	2.64	5.81
	V%	7.90	3.48	8.6	3.78	6.14	16.55
Protein, g/100ml	*X*	3.34	3.29	3.33	3.45	3.59	3.42
	±*sx*	0.08	0.04	0.01	0.02	0.02	0.009
	S	0.43	0.28	0.32	0.36	0.41	0.37
	V%	12.8	8.65	9.55	10.43	11.55	10.75
Fat, g /100ml	*X*	3.78	3.96	3,77	4,00	4,35	3,97
	±*sx*	0.1	0.12	0.03	0.03	0.04	0.02
	S	0.80	0.86	0,77	0,73	0,74	0,78
	V%	21.23	21.69	20,34	18,34	17,1	19,75
SCC/ ml x1000	*X*	255.8	256.16	222,52	195,67	166,09	203,99
	±*sx*	33.4	16	5.99	6.29	6.8	3.7
	S	167.49	158.47	155,85	143,94	121,37	147,71
	V%	65.48	61.86	70,04	73,56	73,08	72,41
Lactose, g/100ml	*X*	4.36	4.42	4,49	4,49	4,49	4,49
	±*sx*	0.05	0.038	0.01	0.01	0.012	0.05
	S	0.27	0.27	0,2	0,2	0,21	0,21
	V%	6.29	6.05	4,49	4,37	4,61	4,57
Conduct. S/m	*X*	899.7	869.7	854,24	844,16	829,4	847,1
	±*sx*	19.2	10.8	2.63	2.72	3.57	1.68
	S	96.41	76.08	68,4	62,3	63,6	67,3
	V%	10.72	8.75	8,01	7,38	7,67	7,94
DM, g/100ml	*X*	12.52	12.71	12,6	12,95	13,43	12,88
	±*sx*	0.21	0.14	0.04	0.04	0.05	0.02
	S	1.06	1.01	0,95	0,93	1,0	1,0
	V%	8.48	7.97	7,53	7,17	7,45	7,8

Research on the level of urea in milk showed a connection with the feeding of dairy cows. Thus, the amount of urea found in milk, and which is highly correlated (0.88 to 0.98) with blood urea nitrogen (BISWAJIT ROY & al, 2011 [5]), can be one of the useful tools (PETERSON & al, 2004 [13]) to detect when major inadequacies in protein or energy nutrition are occurring at the rumen level.

Analysis of maximum protein and milk fat values showed that values of >40 mg/dl urea with a mean of 43.01±0.148 correlate with a protein of 3.59±0.02 and a fat content of 4.35±0.04, MUN of 20.21 mg N/dl, a high level for urinary excretion nitrogen losses of 360.3 g N/day and a relatively constant value of lactose. Investigating the value range of > 40 mg/dl milk urea was found very good SCC/ml values of 166.09±6.81 x 10^3 and the lowest conductivity of 829.46±3.57 S/m, which attests good health of the mammary gland. The differences between the highest and smallest values in the predetermined urea ranges (<22 and >40 mg/dl) determine reduced effects on the protein of + 0.25% and to a fat content of + 0.57% but with significant effects on milk urea, of +22.65 mg/dl, milk urea nitrogen (MUN) of +10 mg N/dl and significant urinary excretion nitrogen losses of + 191 g N/day. In order to establish the optimum values of protein and milk fat, the value ranges for milk protein were set as well: <3% with 177 cows (11.12%), between 3.01-3.3% with 481 cows (30.21%), between 3.31-3.5% with 336 cows (21.12% and >3.5% with 597 cows (37.52%), (Table 3).

Table 3. Effect of protein value intervals on the composition of cow's milk

Specification		**Range:**				
Protein g/100ml		< 3%	3,0-3,3%	3,3-3,5%	>3.5%	Average
MUN mg N/dl		15.80	15.95	16.14	17.33	16.50
n		177	481	336	597	1591
Urea, mg/dl	*X*	33.63	33.94	34.34	36.87	35.11
	±*sx*	0.41	0.27	0.29	0.23	0.15
	S	5.4	6.0	5.47	5.5	5.8
	V%	16.17	17.83	15.95	14.93	16.55
Protein g/100 ml	*X*	2.87	3.15	3.4	3.8	3.42
	±*sx*	0.007	0.004	0.003	0.01	0.009
	S	0.1	0.09	0.06	0.24	0.37
	V%	3.43	2.7	1.66	6.39	10.75
Fat g/100ml	*X*	3.6	3.65	3.8	4.4	3.97
	±*sx*	0.06	0.03	0.03	0.03	0.02
	S	0.82	0.73	0.62	0.69	0.78
	V%	22.63	19.86	16.29	15.78	19.75
SCC/ml x 1000	*X*	191.83	193.8	194.71	222.88	203.99
	±*sx*	11.0	6.96	7.78	5.95	3.70
	S	147.63	152.68	142.7	145.3	147.71
	V%	76.96	78.78	73.28	65.19	72.41
Lactose g/100 ml	*X*	4.53	4.54	4.52	4.41	4.49
	±*sx*	0.013	0.008	0.011	0.009	0.005
	S	0.17	0.17	0.19	0.22	0.21
	V%	3.8	3.85	4.28	5.05	4.57
Conduct S/m	*X*	855.51	849.85	847.44	842.94	847.18
	±*sx*	5.18	2.91	3.54	2.89	1.69
	S	68.92	63.82	64.82	70.54	67.29
	V%	8.06	7.50	7.65	8.37	7.94
DM g/100 ml	*X*	12.02	12.35	12.71	13.65	12.88
	±*sx*	0.06	0.03	0.03	0.03	0.02
	S	0.87	0.78	0.66	0.82	1.00
	V%	7.22	6.33	5.18	6.03	7.8

Protein intervals between 3.3-3.5% with MUN of 16.14 mg N/dl and urea of 34.34 ± 0.29 mg/dl provide the best values of milk protein of 3.4±0.003, fat of 3.8±0.03, lactose 4.52±0.01 and SCC at 194.71±7.78 /ml x 10^3. Research by various authors have previously shown that there is a strong correlation between milk quality parameters and nutrition (especially protein and energy nutrition). Therefore, it is very important to limit protein losses from the ration of dairy cows by establishing optimal levels for milk urea and implicitly MUN and UEN but maintaining milk parameters within optimum values.

The normal value of blood urea nitrogen in cows is 15 mg/dl (ROSELER & al, 1993 [15]) and milk urea nitrogen concentration for individual cows ranges from 8 to 25 mg/dl, while the optimum concentration for a herd ranges from 12 to 17 mg/dl (ROSELER & al, 1993; BAKER et al, 1995 [3]; HWANG & al, 2000 [10]; BASET & al, 2010 [4]).

In order to analyse the somatic cell count (SCC) with values between <100 SCC/ml x 10^3 and> 400 SCC/ml x 10^3 all 1,591 samples obtained from official control of milk production during March controls were grouped into five value ranges, as follows: under 100 cell/ml x 10^3, between 101 and 200 cell/ml x 10^3, between 201 and 300 cell/ml x 10^3, between 301 and 400 cell/ml x 10^3 and over 400 cell/ml x 10^3 with the specification that cows have been raised under the same technological conditions (Table 4).

Table 4. Effect of somatic cell count (SCC) value ranges on cow's milk components

Specification		Range:					
SCC/ml x 1000		< 100	100-200	200-300	300-400	>400	Average
MUN mg N/dl		16.83	16.82	16.70	16.38	15.45	16.50
n		559	394	198	120	320	1591
Urea, mg/dl	*X*	35.82	35.79	35.54	34.85	32.88	35.11
	±sx	0.23	0.29	0.39	0.57	0.32	0.14
	S	5.48	5.79	5.56	6.25	5.86	5.81
	V%	15.30	16.18	15.63	17.94	17.81	16.55
Protein g/100ml	*X*	3.37	3.41	3.51	3.49	3.42	3.42
	±sx	0.01	0.02	0.02	0.04	0.02	0.009
	S	0.35	0.37	0.35	0.44	0.36	0.37
	V%	10.39	10.91	9.83	12.63	10.58	10.75
Fat g/100ml	*X*	3.89	4.01	4.04	4.07	3.97	3.97
	±sx	0.03	0.04	0.05	0.07	0.04	0.02
	S	0.75	0.82	0.76	0.82	0.79	0.78
	V%	19.28	20.41	18.82	20.20	19.88	19.75
SCC /ml x 1000	*X*	62.74	144.88	245.96	340.93	446.22	203.99
	±sx	0.80	1.3	2.04	2.46	0.06	3.70
	S	18.83	27.60	28.78	27.02	10.85	147.71
	V%	30.01	19.05	11.7	7.92	2.43	72.41
Lactose g/100ml	*X*	4.57	4.51	4.45	4.41	4.35	4.49
	±sx	0.01	0.01	0.013	0.02	0.01	0.005
	S	0.17	0.16	0.18	0.22	0.23	0.21
	V%	3.62	3.65	3.98	4.94	5.39	4.57
Conduc S/m	*X*	822.19	837.17	853.48	872.97	889.58	847.18
	±sx	2.4	2.9	4.27	6.11	4.17	1.68
	S	56.71	58.12	60.10	67.01	74.71	67.29
	V%	6.90	6.94	7.04	7.68	8.4	7.94
D.M., g/100ml	*X*	12.83	12.93	13.01	12.99	12.79	12.88
	±sx	0.04	0.05	0.07	0.09	0.05	0.02
	S	0.98	1.03	0.98	1.08	0.99	1.0
	V%	7.65	7.98	7.55	8.33	7.70	7.80

In the lowest range, of <100 SCC/ml x 10^3, entailed 559 cows (35.15%), these revealed the highest urea content in milk (35.82±0.23 mg/dl, with 16.83 mg N/dl (MUN)). Also, the animals found in this range of SCC values showed good protein and milk fat values and excellent values. The 394 cows (24.76%) between 100-200 x 10^3 SCC show good values of protein and fat with MUN values between 16.70 and 16.82 mg N/dl. A total of 320 heads cows (20.11%) displayed SCC values over 400 SCC/ml x 10^3; they also displayed 32.88±0.327 mg/dl urea and MUN 15.45 mg/dl; while all these dairy cows were suspected of subclinical and other by clinical mastitis, they retained good values for protein (3.42±0.02) and fat (3.97±0.04). The analyzed ranges of SCC high lights a relatively constant of MUN between 15.45-16.83 mg N/ml with an average of 16.50 mg N/dl and the interval between 100-300 SCC/ml x 10^3 displays the highest protein values of 3.41-3.51% and fat of 4.01-4.04%. Somatic cells are an important component of milk used in the assessment of qualityaspects, hygiene and mastitis control, which can be used in order to monitor the level oroccurrence of subclinical mastitis in herds or individual cows (SHARMA N. & al, 2011 [19]). According to Sharma 2011 [19], high cell-count milk is associated with a decrease in lactose, α-lactalbumin, fat in milk and affects the activity of yogurt fermentation and cheese production.

Other research on the quality of milk for Friesian – Black Spotted Breed raised in Transylvania area was carried out by other authorson a number of 1,391 milk samples obtained and highlighted the fact that the somatic cell count in milk obtained from cattle included in this study recorded an average value of 390.54±11.16 cells/ml x 10^3 with the variability between controls of 314.94±28.93 and 482.36 ± 91.12 cells/ml x 10^3. Regarding the fat content, the average values were 4.19%, 3.63% for protein content, a pH value of 6.56 and the values of urea were 20.09 mg/dl an variable seasonal values: spring 17.65±0.32 mg/dl, summer, 26.36±0.49 mg/dl, autumn, 20.43±0.32 mg/dl,winter, 12.01±0.52 mg/dl. (ONACIU & al, 2016 [11]).

Also, data on milk quality and Agri-Environmental impact werereportedon breeds raised in the Transylvania area on main productivity traits. (ONACIU & al, 2016 [12]). In terms of milk qualityobtained from Friesian – Black Spotted Breed raised in Moldova area, the seasonal effect must be also specified which significantly affected the BC and the SCC, higher values being observed in summer ($p < 0.001$). Also, the accommodation system of the cows influenced the same safety traits of the raw milk (BC and SCC). Thus, the straw yard system proved to supply cleaner milk than the cubicle system ($p<0.001$) (MACIUC & al, 2017 [23].

Conclusion

Intervals with the lowest average values of the milk urea and the urinary excretion nitrogen losses (UEN) with an efficient use of the protein in the ration, range between 20.36±0.3 and 31.05±0.1 mg/dl, MUN 9.57 and 14.59 mg N/dl and UEN of 169.4 and 268.4 g N/day. Regarding the value range between 20.36-31.05 mg/dl maintains milk protein values at 3.29-3.34%, fat at 3.77-3.96%, lactose at 4.36-4.49%, SCC at 222.52-255.8/ml x10^3 and conductivity of 854.24-899.78 S/m.

The results of this research recommend a value range for milk urea from 23-31 mg/dl with milk urea nitrogen (MUN) between 10-15 mg N/dl. Interval values above 35 mg/dl bring a high value of over 17 mg N/dl and urinary excretion losses (UEN) more than 327 g N/day/head, values that show that it is necessary to optimize the feeding system for dairy cows. Protein intervals between 3.3-3.5% with MUN of 16.14 mg N/dl and urea of 34.34±0.29 mg/dl provide the best values of milk protein of 3.4%, fat of 3.8%, lactose 4.52% and SCC at 194.71±7.78 / ml x 10^3.

The analyzed ranges of SCC did not significantly change the values of MUN, which remained relatively constant from 15.45-16.83 mg N/ml with an average of 16.50 mg N/dl MUN and the interval between 100-300/ml x 10^3 is with the highest protein values of 3.41-3.51% and fat of 4.01-4.04%.

With the increase in milk production the milk urea content drops, so in productions over 25 L/day the milk urea was 30.58 mg/dl and 14.37 mg N/dl MUN compared to 35.94 mg/dl and 16.89 mg N/dl in the case of productions between 10-15 L/day.

Considering the variability of the data regarding the influence of milk urea on the quality of raw cow's milk, we consider that quality standards may not be unique. This is because it is possible to develop different standards of milk urea according to the production level and feeding technology with standardized optimal levelsto hopefully be included in European regulations on hygiene and food safety for raw cow's milk.

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