MILK QUALITY

Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, - Via Appia Nuova, 1411 - Roma

How buffalo milk composition differs from that of cow milk?

Milk yield and composition of buffalo and dairy cow (AIA, 2013)

(Italian Breeders Association)

Breed	Average milk yield (Kg)	Average fat content (%)	Average protein content (%)
Mediterranean buffalo	2,222	8,16	4,66
Holstein Friesian	9,236	3,69	3,30

Average of Fat, Protein, Somatic cells and TBC in bulk milk

Lazio region

	Fat %	Protein %	Somatic cells n/ml x1000	TBC cfu/ml x1000
2008	7.98 ± 0.68	4.46 ± 0.45	241	1659
2009	7.97 ± 0.81	4.43 ± 0.26	207	1069
2010	7.96 ± 0.84	4.56 ± 0.30	244	914
2011	8.06 ± 0.50	4.63 ± 0.28	200	573
2012	8.12 ± 0.50	4.67 ± 0.24	154	705

MILK RECORDING IN THE LAST YEARS IN ITALY

(AIA, 2010)

Year	Average milk yield (kg)	Fat (%)	Protein (%)	Number of recorded heads	Number of herds	Number of heads per herd
1990	1,893	8.1	4.39	14,080	168	84
2000	2,145	8.35	4.74	32,806	284	115
2010	2,180	8.47	4.59	50,240	292	172
2013	2,222	8.16	4.66	56,812	318	179

MILK YIELD AND COMPOSITION DURING THE LAST YEARS IN ITALY

A positive trend of buffalo milk yield and quality registered in Italy is, above all, due to the new feeding criteria, the changed diets, the rearing conditions and the selective breeding

FAT CONTENT

Fat globules: average diameter of 2.8 µm of buffalo milk vs 3.0 - 5.0 µm of dairy cow milk (Martini et al., 2003)

Results of fatty acid composition are not always in agreement because they are affected by genetics, stage of lactation and feed factors

Among the liposoluble compound buffalo milk does not contain the β-carotene, while the vitamin A content is higher than that of cow milk (El Rafey, 1962).

PROTEIN CONTENT

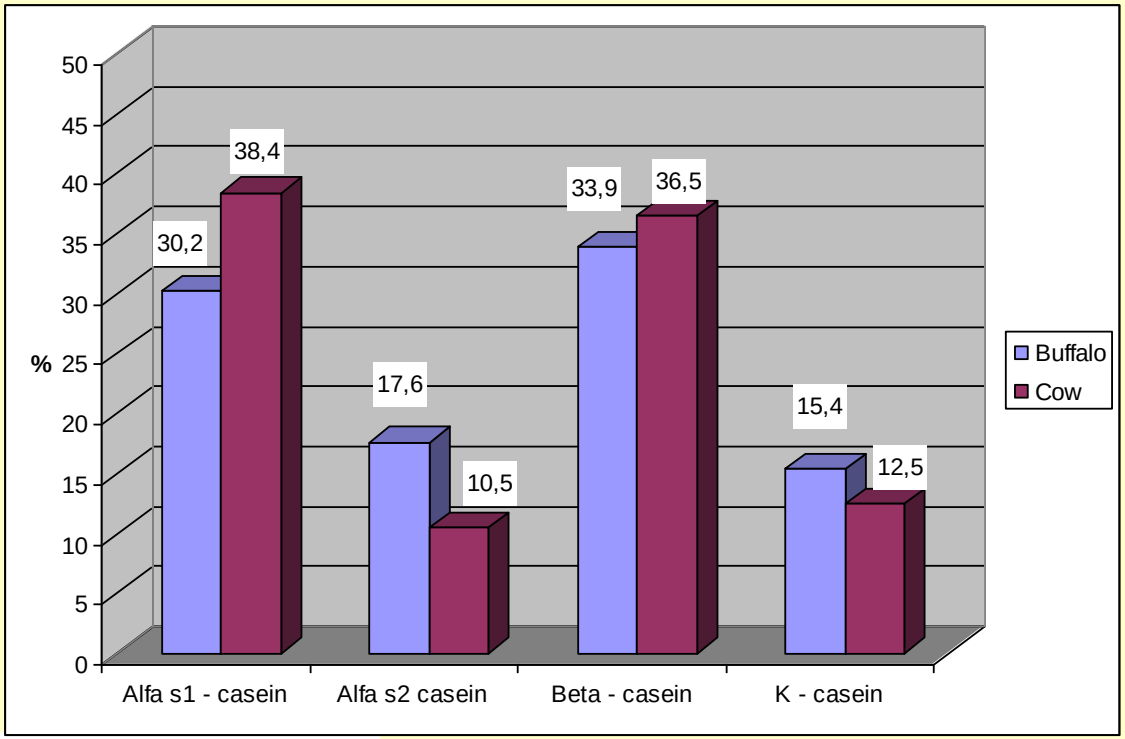
-Casein
-Whey protein
-Non protein nitrogen

Casein content of buffalo milk: 81 to 85% of protein content (Tripaldi et al. 1997) and il was higher than that of cow and goat milk (Alais, 1984).

High correlation was found between protein and casein (r = 0.93) (Giangolini et al., 2005)

PROTEIN CONTENT

The buffalo casein shows a higher content of αs2-casein and k-casein than that of bovine casein and vice versa for αs1-casein and β-casein



PROTEIN CONTENT

Buffalo species is less polymorphic than the bovine one.

Variants for the following proteins were detected (Chianese et al., 2005; Ferranti et al., 1998; Chianerse et al., 2004; Chianerse et al., 2005):

β - casein

αs1 – casein

α – lactalbumin

CASEIN CONTENT

The casein content in milk is very important for cheese production processes and in particular for buffalo milk whose main use is cheese production.

STUDY ABOUT CASEIN CONTENT OF RAW BUFFALO MILK IN LAZIO REGION

Giangolini G., Rosati R., Boselli C., Amatiste S., Filippetti F., Proietti A., Brizioli N.R.

Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana

AIMS

CASEIN AND CASEIN INDEX VALUES IN RAW BULK BUFFALO MILK FROM 5 PROVINCES OF THE LAZIO REGION

MATERIALS AND METHODS

367 bulk milk samples

98 farms

(3 VITERBO, 12 ROMA, 4 RIETI, 15 FROSINONE, 64 LATINA)

Milko-Scan FT6000 (Foss Electric)

Calibration ISO 17997-1:2004

Determination of casein-nitrogen content – Part 1: Indirect method (Reference method)

RESULTS

Averages:

Casein: 3,77% ± 0,40

Protein (total nitrogen): 4,51% ± 0,46

Non casein nitrogen (NCN): 0,83% ± 0,12

Casein index (casein/total nitrogen): 83,9% ± 2,3

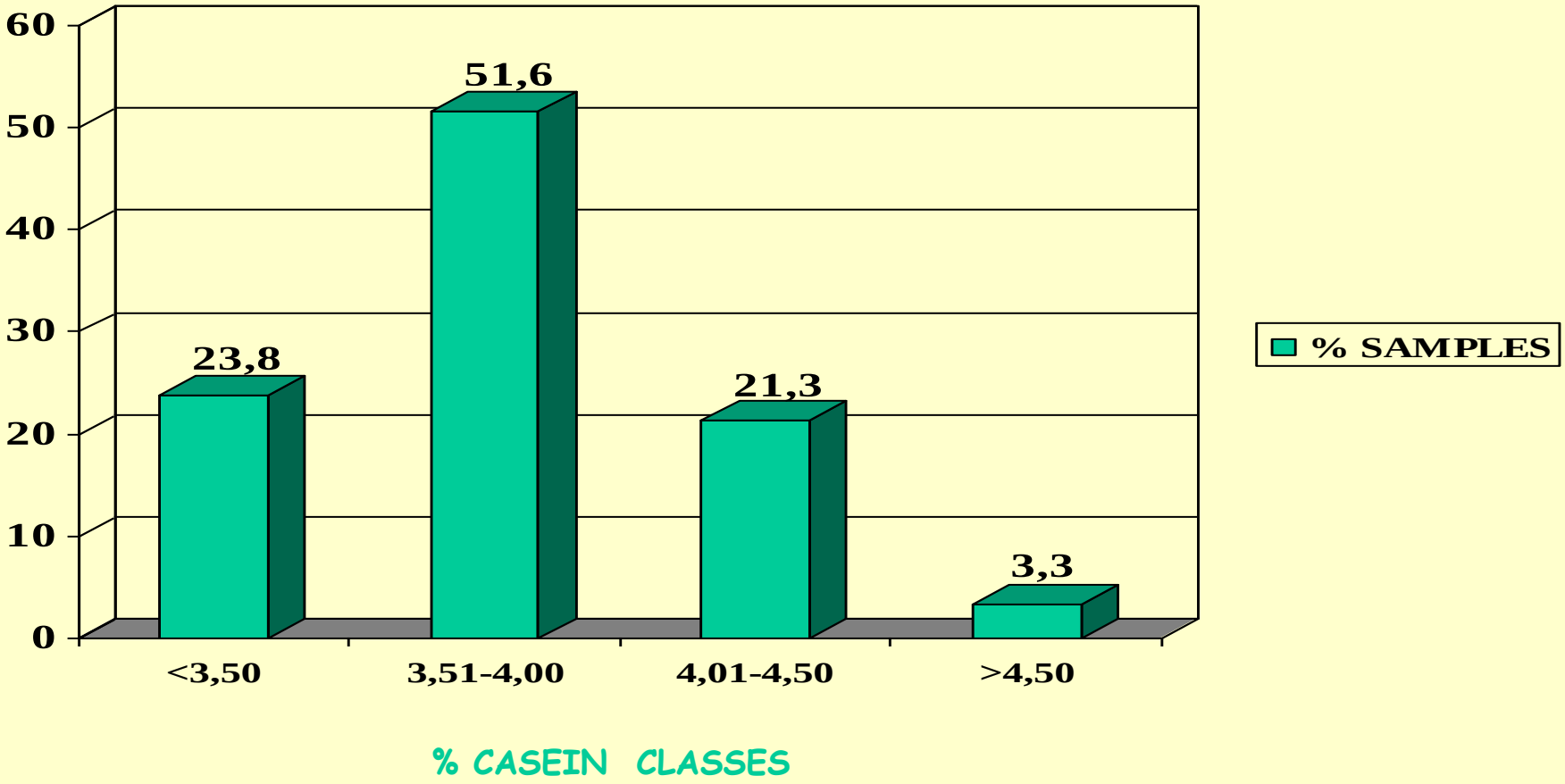
Correlation (r_Pearson):

Protein - Casein 0,93

Protein - NCN 0,62

Casein - NCN 0,39

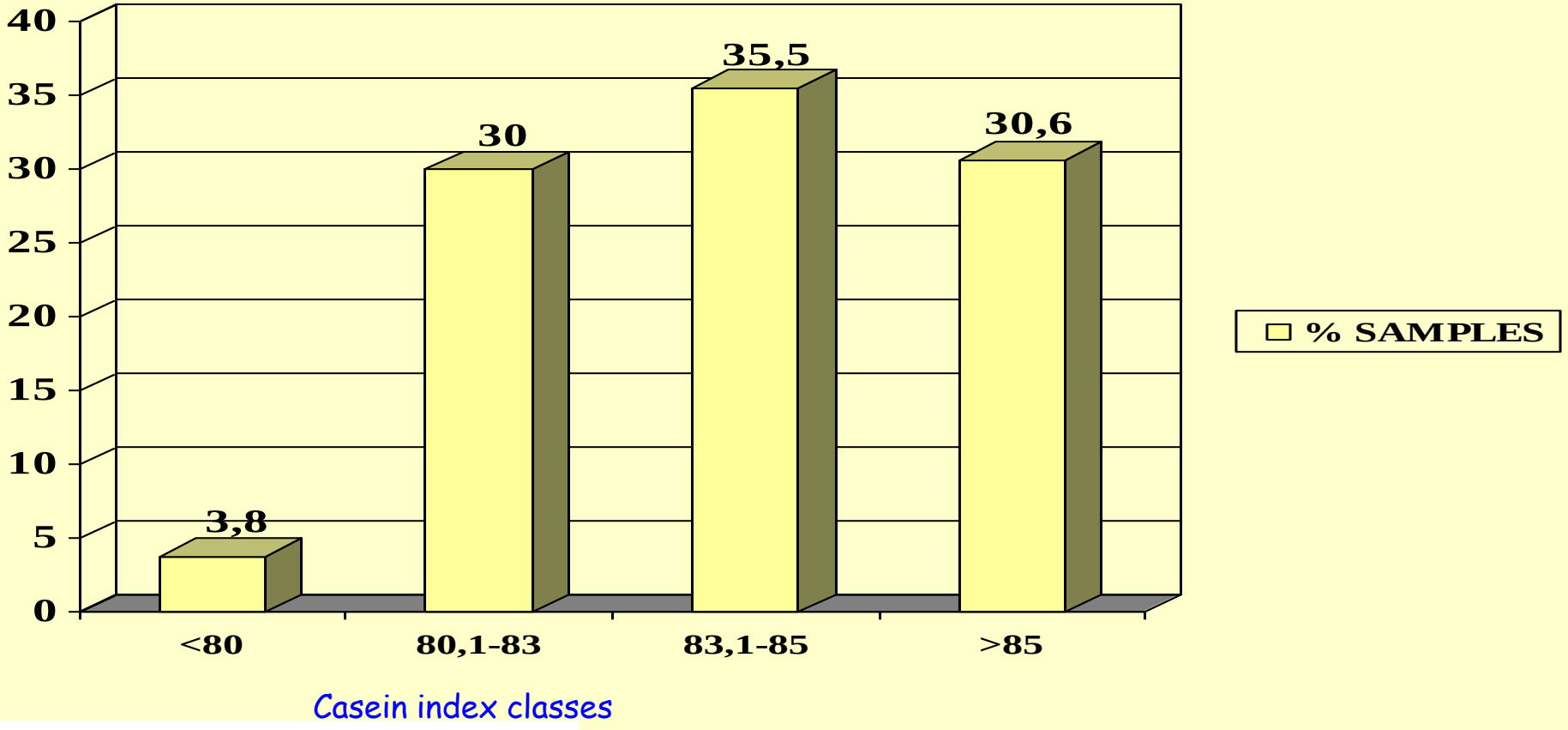
% OF MILK SAMPLES IN THE CASEIN CLASSES



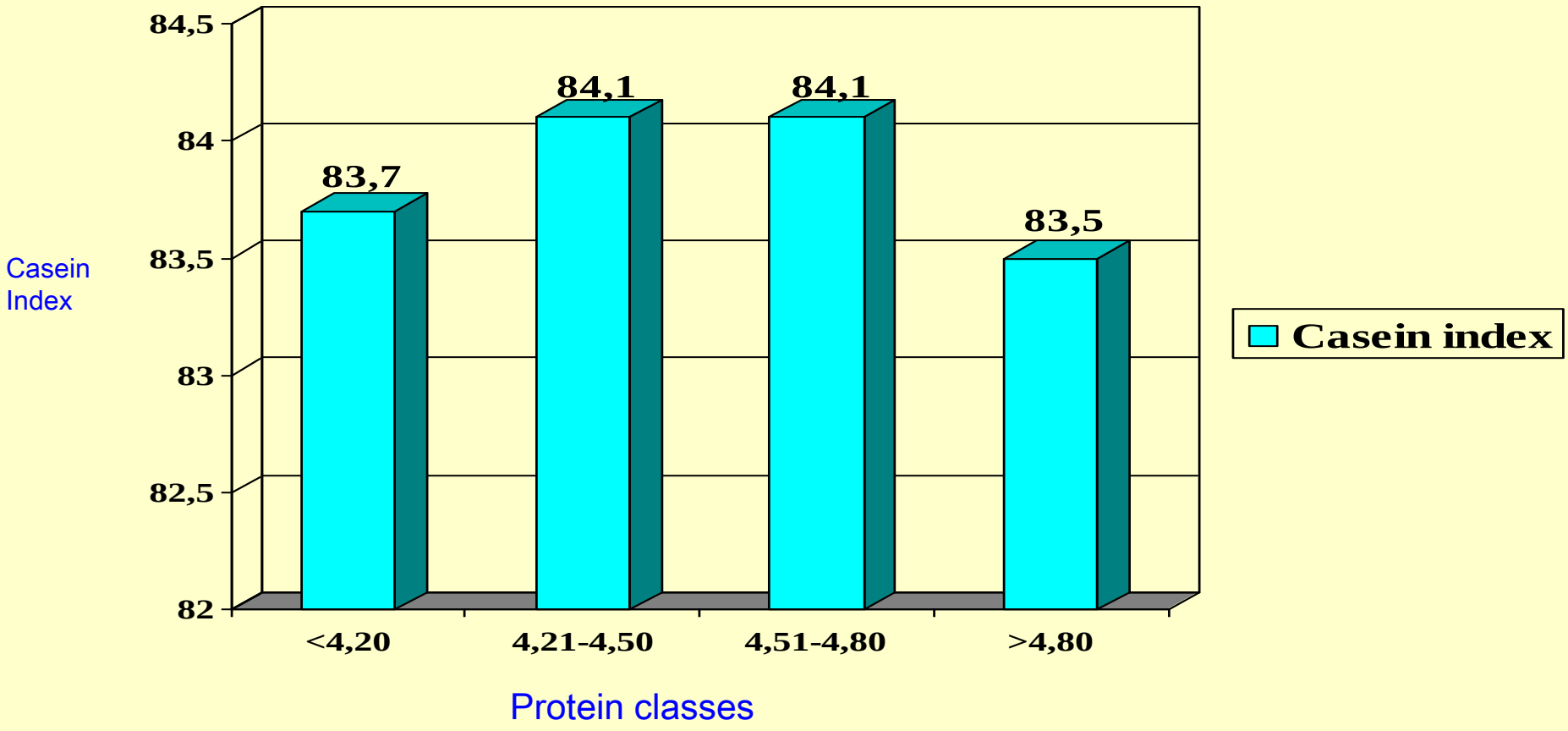
CASEIN INDEX (casein:protein ratio)

% OF MILK SAMPLES IN THE CASEIN INDEX CLASSES

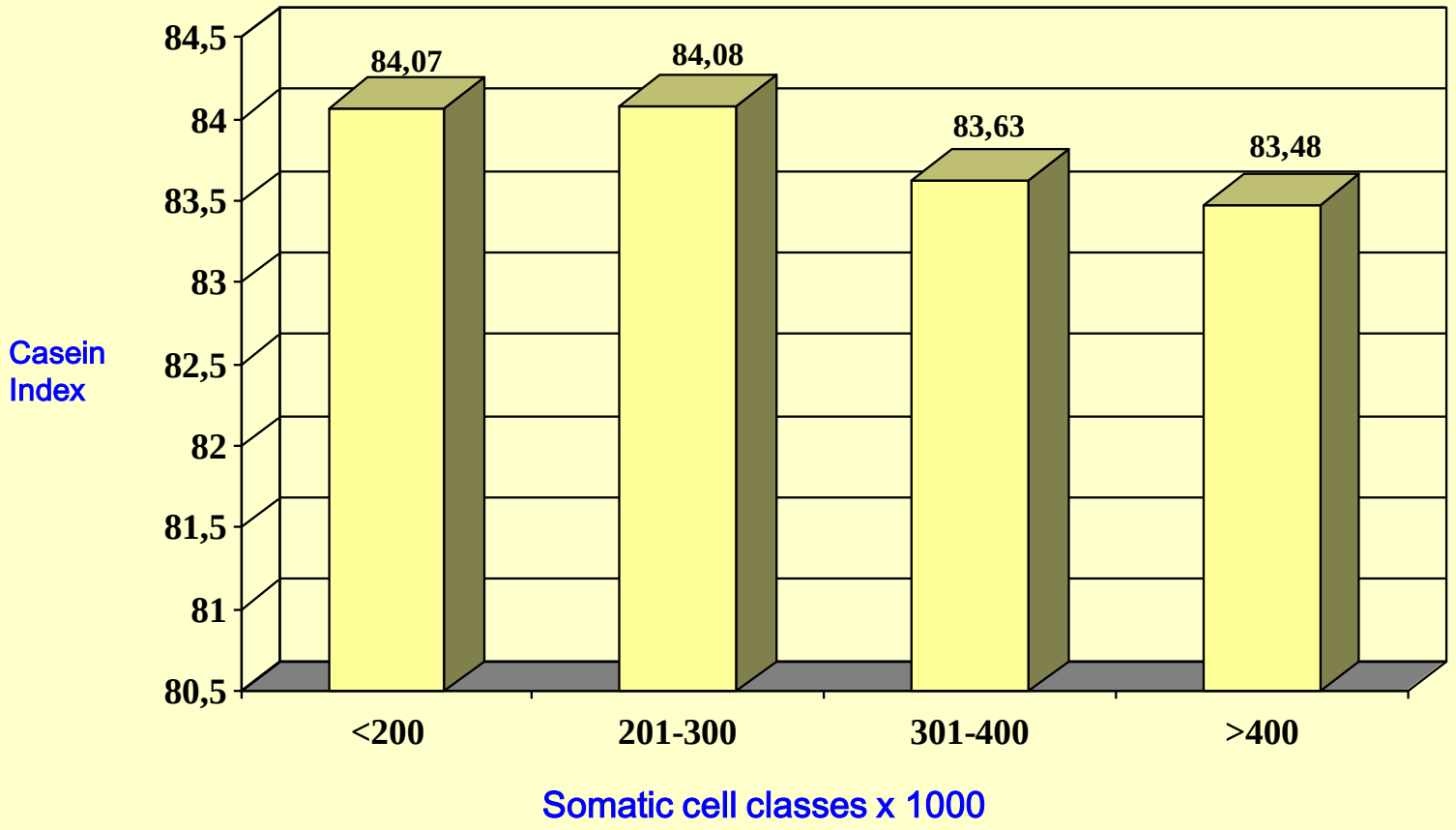
Average Casein index: 83,9% ± 2,3
(Min.71,2%; Max 89,8%)



RELATIONSHIP BETWEEN % OF PROTEINS AND CASEIN INDEX



RELATIONSHIP BETWEEN THE AVERAGE OF CASEIN INDEX AND SOMATIC CELL



CONCLUSIONS

•Examining the medium value of the casein index, relative to the various classes of frequency for the content in proteins, the casein index is significant low in the classes <4,20 e >4,80 so high and low values of proteins correspond excesses of not casein nitrogenous

•Since the determination of this parameter is fast and reliable, the casein content could be inserted as a parameter for the appraisal of quality of buffalo milk

•96% of the samples have a casein index > 80%

Mineral content
Comparison with the cow's milk

	Calcium	Magnesium	Phosphorus
Buffalo (1)	0,18-0,24	0,013-0,027	0,12-0,14
Buffalo (2)	0,21	0,018	0,13
Cow (3)	0,11-0,15	0,010-0,015	0,09-0,10

(1) Alborico et al. (1988)
(2) De Maria e Tripaldi, dati non pubblicati
(3) Walstra e Jenness (1984)

UREA

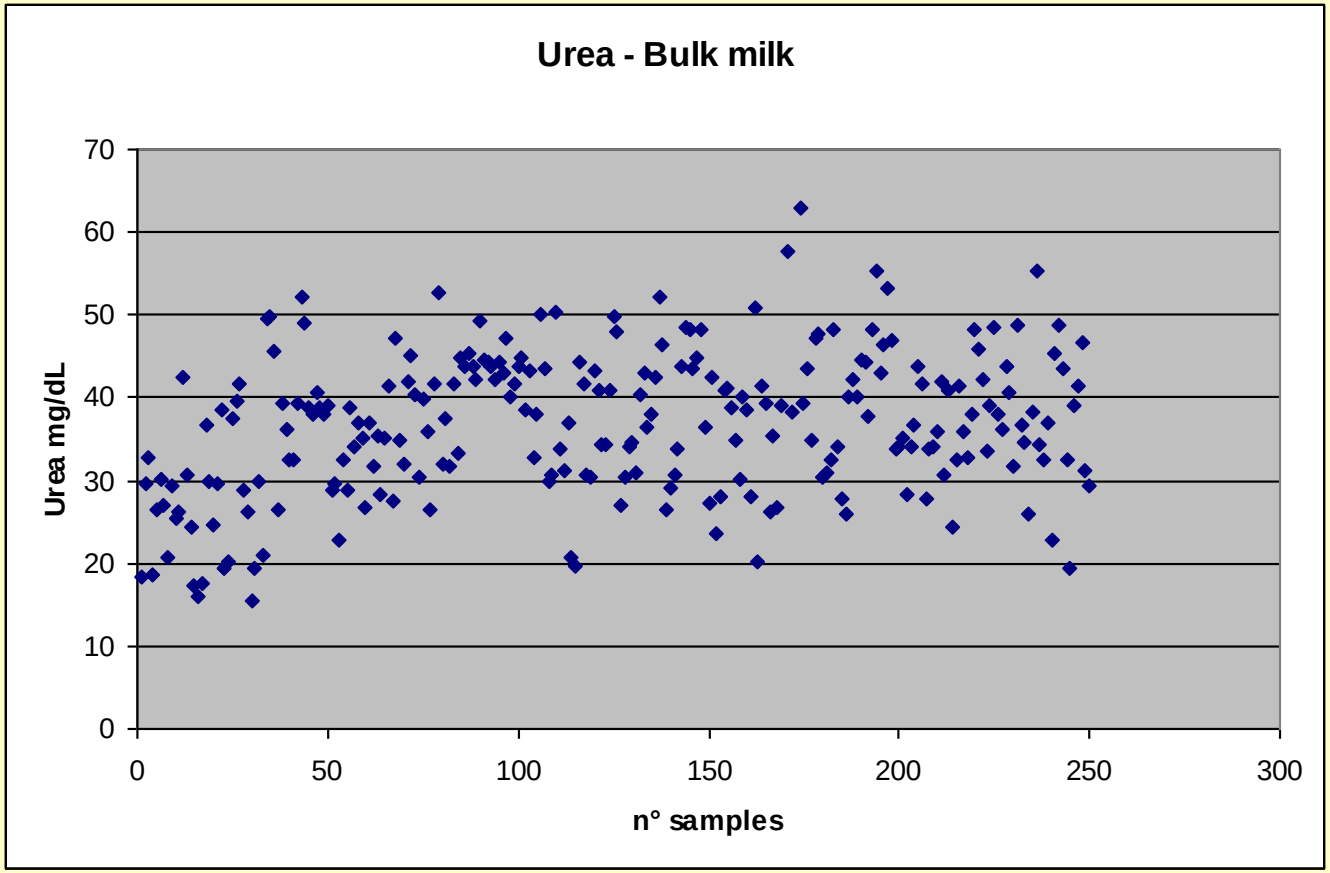
The values recorded in in buffalo milk are higher than that of dairy cows (Campanile *et al.*, 1998; Sarubbi *et al.*, 2000; Di Francia *et al.* 2003; Rov *et al.*, 2003)

Dairy cows: range 25 – 33 mg/dL

UREA IN BULK MILK

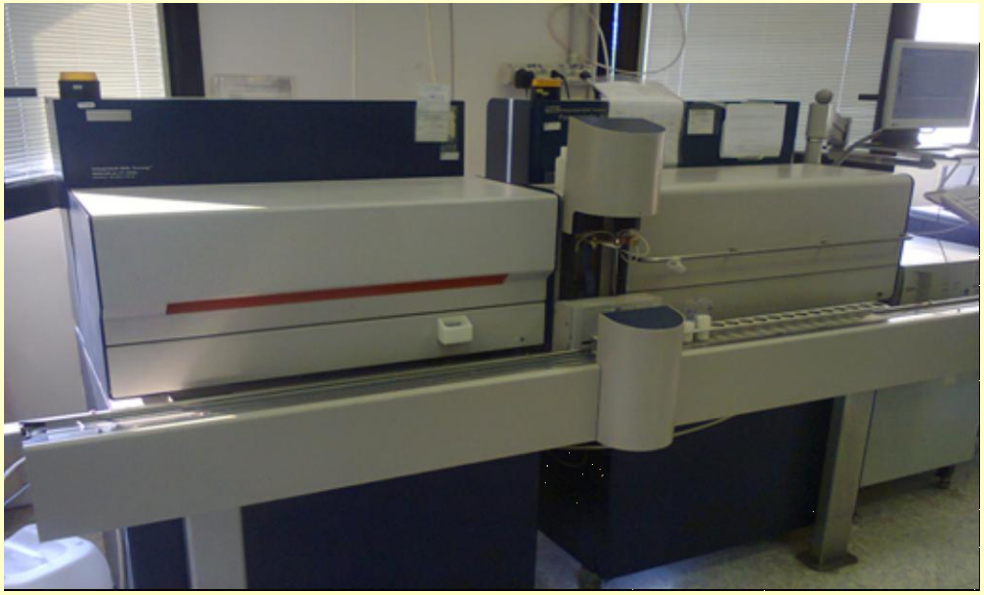
April, May and June 2014 – Latina province

Average: 36.8 mg/dL



The routine methods of milk composition analysis

The routine methods to determine fat, protein, casein, urea and lactose content are based on interferometry FTIR (Fourier Transformed InfraRed analysis)



Milkoscan FT 6000 and Fossomatic 5000 apparatus for analysis of chemical characteristics and somatic cell count

Fat %

Protein %

Lactose %

Total Solids %

Urea mg/dL

Citric Acid %

Feezing Point (Screening)

Casein %

With this technology, we can determine the following parameters

(200 samples per hour)

Parameters that can be determined with the new calibrations

Free Fatty Acids (mmol/10 kg)

C:16_0

C:18_0

C:18_1

Total saturated fatty acids

Total unsaturated fatty acids

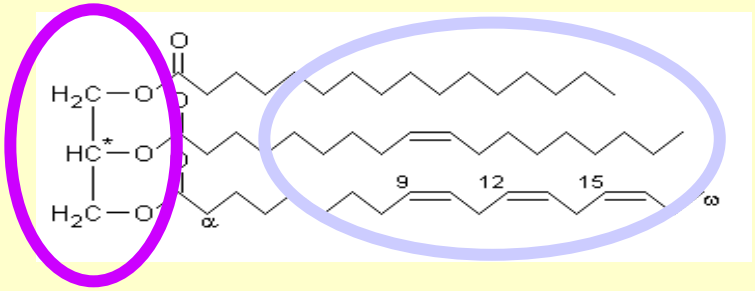
Mono unsaturated fatty acids

Poly unsaturated fatty acids

Free Fatty Acids

Raw milk has lipoprotein lipase, an enzyme that will rapidly hydrolyse milk fat to free fatty acids (FFAs).

Bacterial lipase causes serious degradation of milk fat.



RENNET ABILITY

In Italy buffalo milk is assigned to cheese making and it is important to achieve milk able to obtain high yields and good quality cheese.

To evaluate the renneting ability, the lactodynamographic analysis is utilized

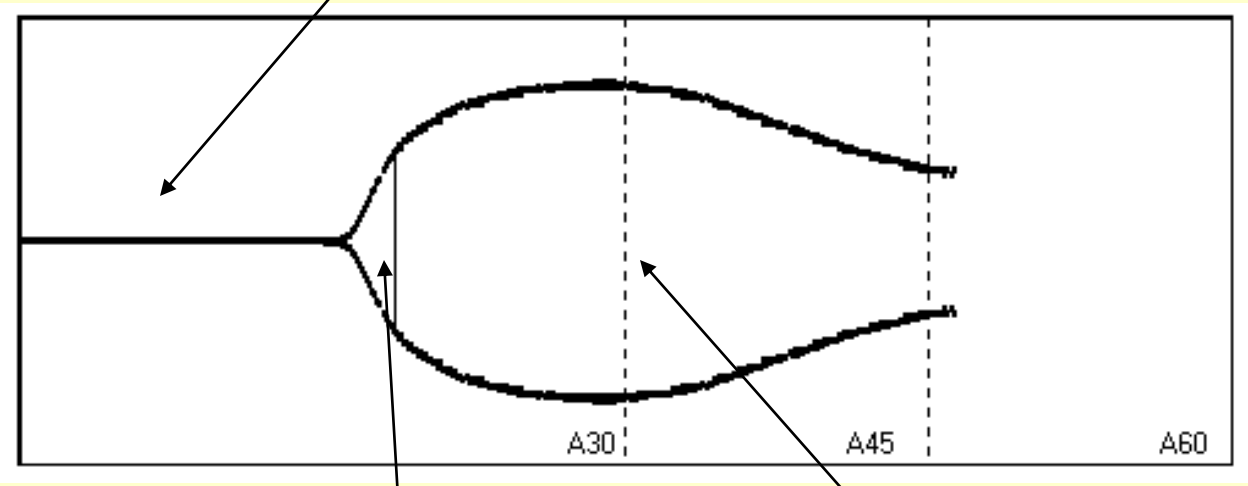
Formagraph instrument for lactodynamographic analysis

RENNET ABILITY

The examined parameters are: clotting time (r), curd firming time (k20), curd firmness at 30 minutes from rennet addition (a30) and curd firmness at 2r (A2r).

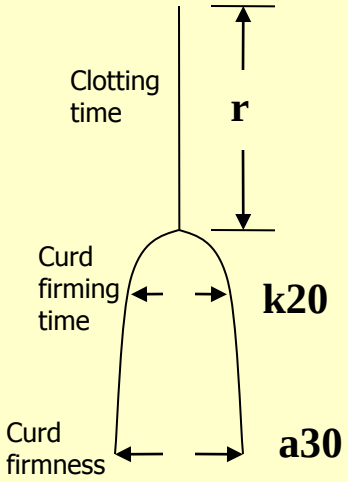
It is to make allowance that milk with better renneting ability is characterized by a brief clotting and curd firming time and by an elevated curd firmness.

Lactodynamographic parameters



Rennet ability - results from 20 farm located in Lazio (Tripaldi *et al*., 2002)

	Good rennet ability	Fairly good rennet ability	Poor rennet ability	Very poor rennet ability
Samples (%)	71.92	18.64	3.76	5.68
r (min)	13.61C	18.71B	25.11A	
k20 (min)	2.81C	4.46B	5.92A	
a30 (mm)	52.24A	36.27B	17.44C	



RENNET ABILITY

If we compared buffalo with cow milk (Fossa *et al*., 1994), we can observe that cow milk has a higher clotting and curd firming time (19.0-19.6 min and 9.8-12.7 min) and a lower curd firmness (23.8-18.1 mm).

The elevated curd firmness of buffalo milk is probably due to the high casein content of this milk

National Association of Buffalo Breeders (ANASB)

take into account the **PKM index**, that is based on the evalutation of the kg of mozzarella production per lactation.

This index considers simultaneusly milk quantity (Kg per lactation) and milk chemical composition, which allows to **calculate cheese yield** by the formula of Altiero (Altiero *et al.*, 1989).

Cheese Yield = 1.23 x fat% + 3.50 x protein% - 0.88

$$PKM = \text{milk yield} \times \frac{3.5 \times N(\text{protein}\%) + 1.23(\text{Fat}\%) - 0.88}{100}$$

SOMATIC CELL COUNT

Somatic cell count (SCC) are recognized worldwide as a measure of udder inflammation (Smith, 2002).

The increase in leucocytes in milk and in the mammary gland, as a response to the invading pathogens or to their metabolites or toxins, leads to an increase in SCC

SOMATIC CELL COUNT

Different measures of mammary inflammation in buffalo as polymorphonuclear leukocytes (PMN), N-Acetyl-β-glucosaminidase (NAG) and chloride content were compared to SCC (Piccinini *et al*., 2006; Tripaldi *et al*., 2010).

From practical point of view SCC resulted the best compromise between accuracy and suitability

SOMATIC CELL COUNT

MEAN VALUE

Mean value of SCC (cell/ml) Individual milk samples	Mean value of SCC (cell/ml) Bulk milk samples	Authors
	169,000	Esposito et al. (1997)
	309,000	Tantillo et al. (1997)
	255,147	(Giangolini et al., unpubl. data)
181,000		Moioli et al (2003)
221,000		Tripaldi et al. (2003)
259,000		Tripaldi et al. (2010)

European legislation on somatic cell count

There is no limit observe for somatic cell count of buffalo milk

A limit to abserve for cow raw milk is 400,000 scc/ml
(Bulk milk)

(Geometric mean)

Threshold of somatic cell count in milk of buffalo reared in Italy (Piccinini *et al*., 2006; Tripaldi *et al*., 2010)

As it was already demonstrated in dairy cows, the threshold of 200.000 cells/ml could be used to identify and early sign of subclinical mastitis

Significant changes in milk components can be observed when SCC are higher than 400.000 cells/ml

Effect of SCC content on milk production

Inflammation of the mammary epithelium has the following effects on milk production: reduced milk yield, reduced synthesis of milk components synthesized by tissue cells (fat, casein and lactose), increased passage of blood component to milk (albumin and immunoglobulin) (Anderson and Andrews, 1977; Kitchen, 1981; Verdi et al., 1987).

Effect of SCC content on milk production

Estimated mean values for yield, components and coagulation properties of buffalo milk according to the three SCC classes (Tripaldi et al., 2010).

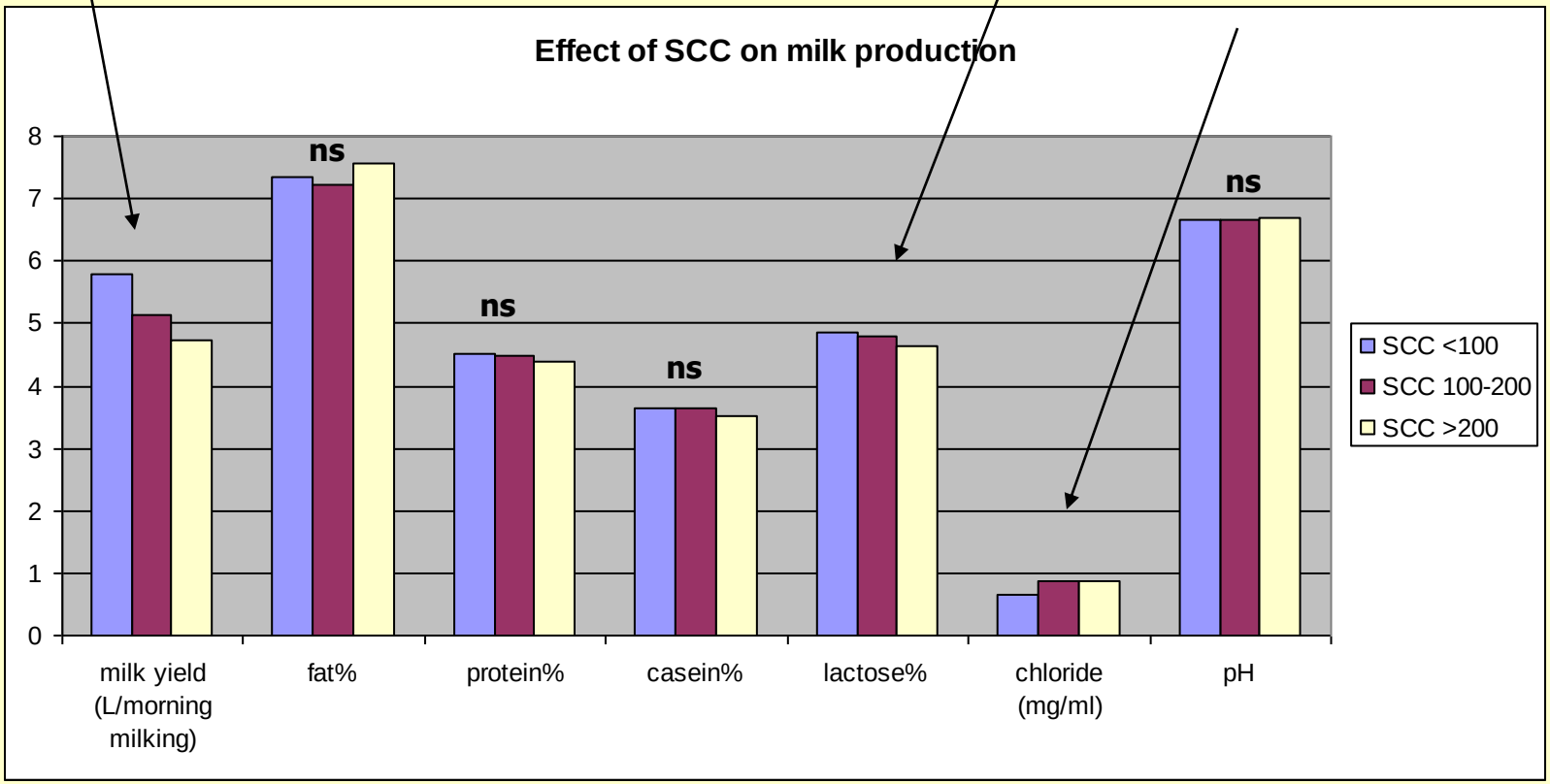
SCC (×103/ml)	<100			100 - 200			> 200		
milk yield (l/morning milking)	5.79	±	0.21a	5.14	±	0.45ab	4.73	±	0.33b
fat (%)	7.35	±	0.15 ns	7.23	±	0.31	7.55	±	0.23
protein (%)	4.52	±	0.05 ns	4.47	±	0.10	4.38	±	0.08
casein (%)	3.65	±	0.06 ns	3.63	±	0.12	3.53	±	0.09
lactose (%)	4.87	±	0.03a	4.80	±	0.07b	4.64	±	0.05b
chloride (mg/ml)	0.650	±	0.039b	0.862	±	0.0862ab	0.882	±	0.068a
pH	6.66	±	0.01 ns	6.67	±	0.02	6.69	±	0.02
r (min)	19.82	±	0.75 ns	20.75	±	1.50	22.39	±	1.16
k20 (min)	2.05	±	0.16b	2.21	±	0.31ab	2.72	±	0.24a
a30 (mm)	43.57	±	1.32 ns	40.67	±	2.67	32.23	±	2.06
a2r (mm)	50.40	±	0.77 ns	50.13	±	1.56	48.42	±	1.21

a, b: $P \leq 0.05$

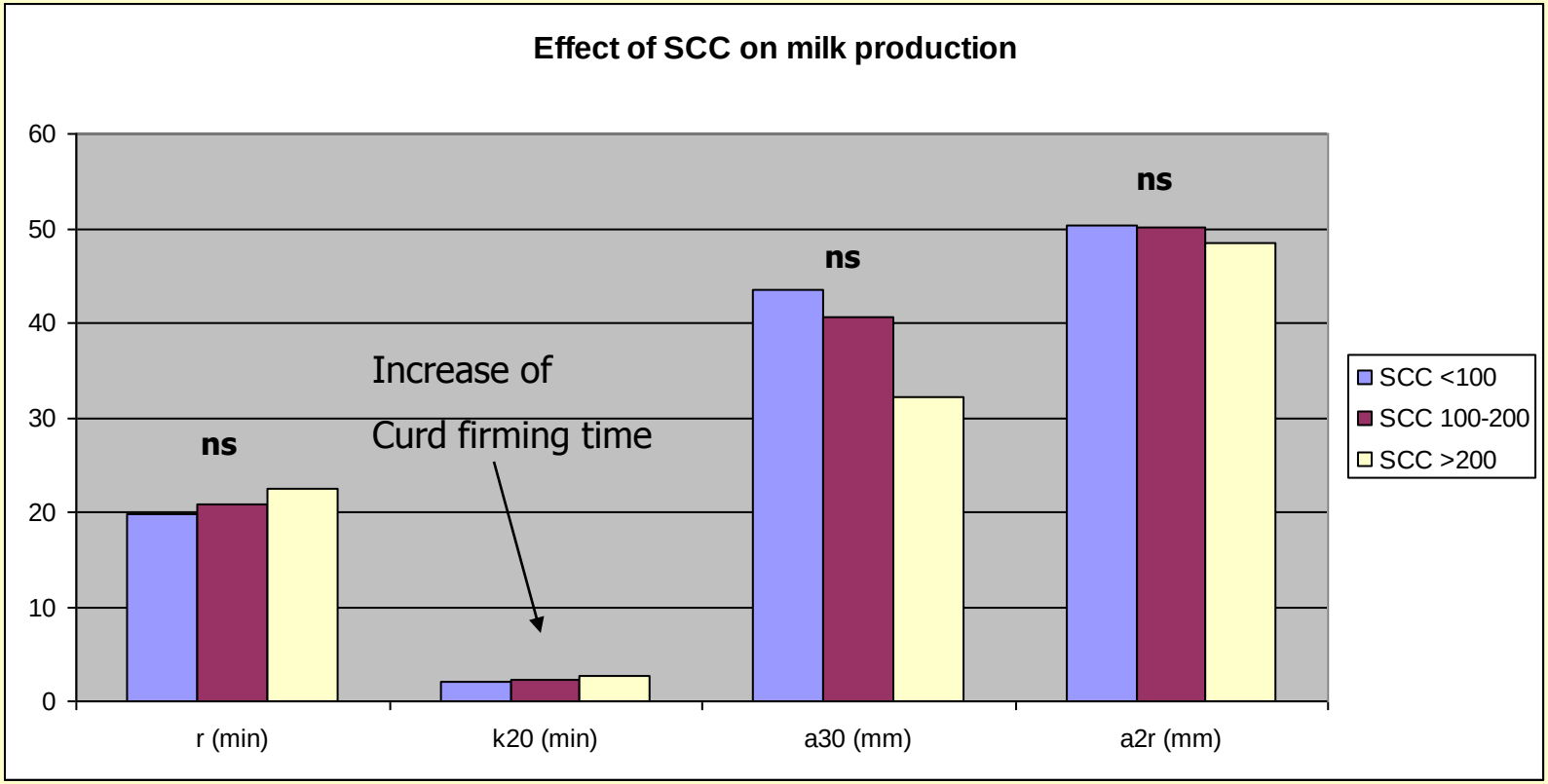
Reduced milk yield

Decrease in lactose

Increased chloride



Effects on rheological parameters (Lactodynamographic parameters)



Controlling buffalo's milk somatic cells content could help to produce high quality milk with hygenic parameters more suitable for cheese manufacturing.*

Somatic cell content affected the distribution of casein and whey proteins in buffalo's milk.*

A low number of Somatic cell count implies a higher quantity of casein, which could increase cheese yield during milk transformation.*

*M. Pasquini et al.,2003

European legislation on Total bacterial count (TBC)

The limit observed in European Countries (Reg CE 853/04) concerning the bacterial count is 1,500,000 CFU/ml;

for milk destined to manufacture of products from raw milk the limit is 500,000 CFU/ml.

The routine methods of TBC analysis

The routine methods to determine bacterial count are based on flowcytometry

Bactoscan FC apparatus for TBC analysis

The routine methods of TBC analysis

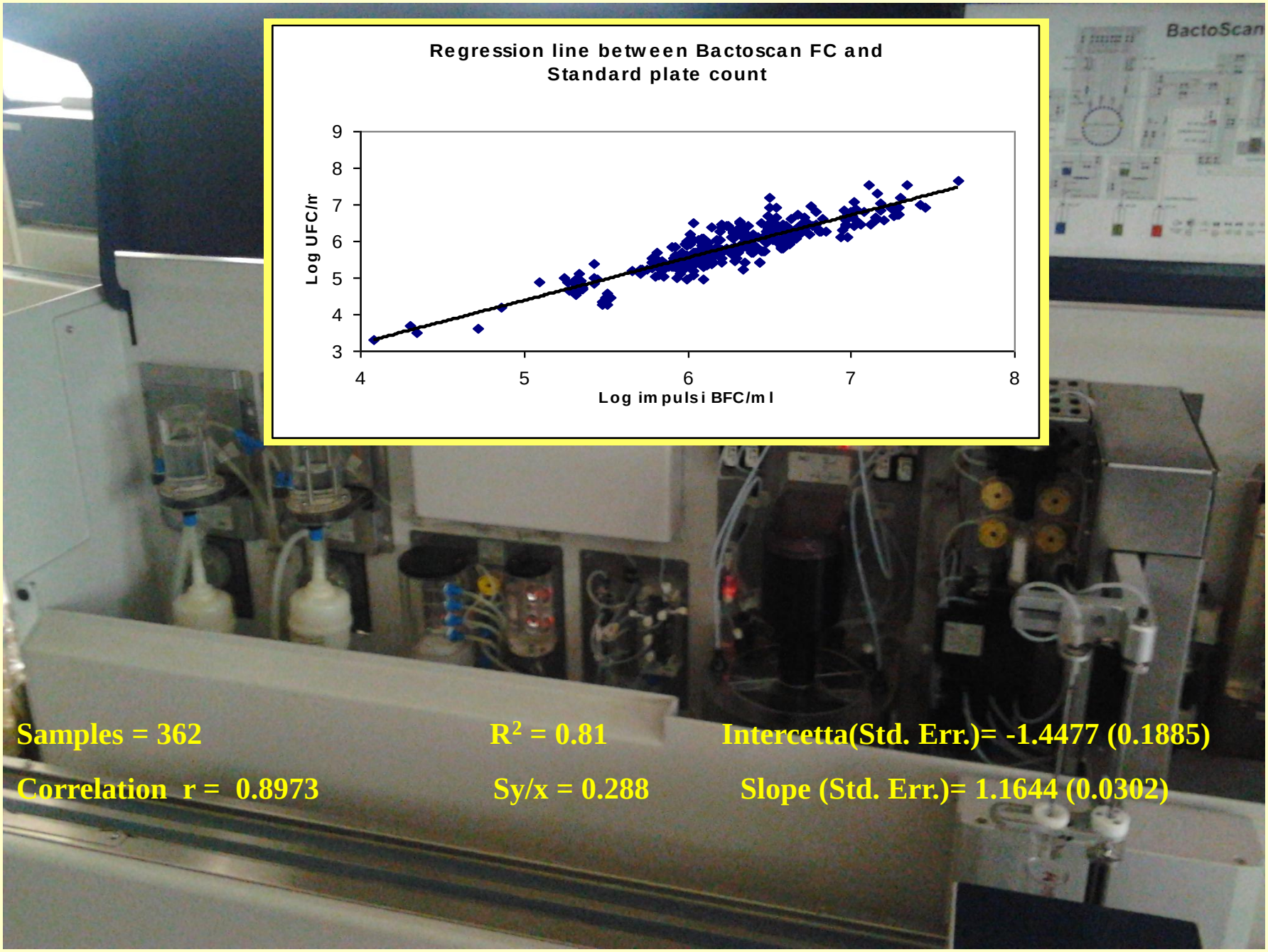
The Bactoscan FC is an instrument for the determination of total bacteria count in milk.

We have to calculate the relationship between Standard Plate Counts (CFU/ml) (ISO 4833-1:2013) and Impulse BFC/ml in buffalo raw milk. (Giangolini et al., 2003)

$$\text{Log}_{10}\ \text{CFU/ml} = 1{,}1644 \times \text{Log}_{10}\ \text{BFC/ml} - 1{,}4477$$

Accuracy: 0.288 log/ml and correlation coefficient r = 0.90;

$R^2 = 0.81$.



Samples = 362 R^2 = 0.81 Intercetta(Std. Err.)= -1.4477 (0.1885)

Correlation r = 0.8973 Sy/x = 0.288 Slope (Std. Err.)= 1.1644 (0.0302)

Milk can deteriorate since milk contains high nutrient contents such as fat, protein and lactose which required by bacteria to grow.

Raw milk price

In Italy the price of raw buffalo milk is not in agreement with fat and protein content and somatic cell and bacterial count, that is generally applied for cow milk

POSSIBLE DETERMINANTS OF RAW MILK PRICE AND REJECTION LIMITS

Increased price factors	Reduced price factors	Rejection criteria
High Fat & Protein	Low Fat & Protein	Added Water
Low Bacteria Content	Moderate Bacteria Content	Too High Bacteria Content
Low Somatic Cell Count	Moderate Somatic Cell Count	Too High Somatic Cell Count
Good Taste	Acceptable Taste	Bad Taste
Fresh	Stale	Inhibitory Substance/Antibiotics
Cool	Too high temperature	Harmful Contaminants

Milk quality and cheese yield

Cheese manufacturers are interested in the protein, casein and fat contents, because these components determine the cheese yield

Casein: fat ratio is one of primary factor related to cheese yield (Barbano and Sherbon, 1984)

Milk quality and cheese yield

The casein percentage of the protein is reduced when the milk contains a large number of somatic cells. This makes somatic cell count a very interesting parameter for cheese manufacturers. (Lawrence, 1991)

Milk quality and cheese yield

Estimated equation proposed by Altiero*et al*. (1989) to evaluate buffalo mozzarella cheese yield

Cheese yield (kg per 100 litres of milk)
= (3,5 * % proteina) + (1,23 * % grasso) - 0,88

Price of buffalo and cow milk and cheese yield

	Milk price (€/litre) VAT not included	Cheese yield (%)
buffalo	1,00-1,25	24
cow	0,44	13

The price of buffalo milk varies according to the demand of Mozzarella cheese and it was higher during the hot season, when the consume of this cheese is particularly elevated

AFLATOXIN

Aflatoxin are a group of mycotoxins produced by mycetes of the *Aspergillus* genus.

Aflatoxin B1 is considered one of the most potent known natural hepatic-carcinogen for mammals. The major metabolite of AFB1 is **aflatoxin M1**, which is detectable in the urine, blood, **milk**.

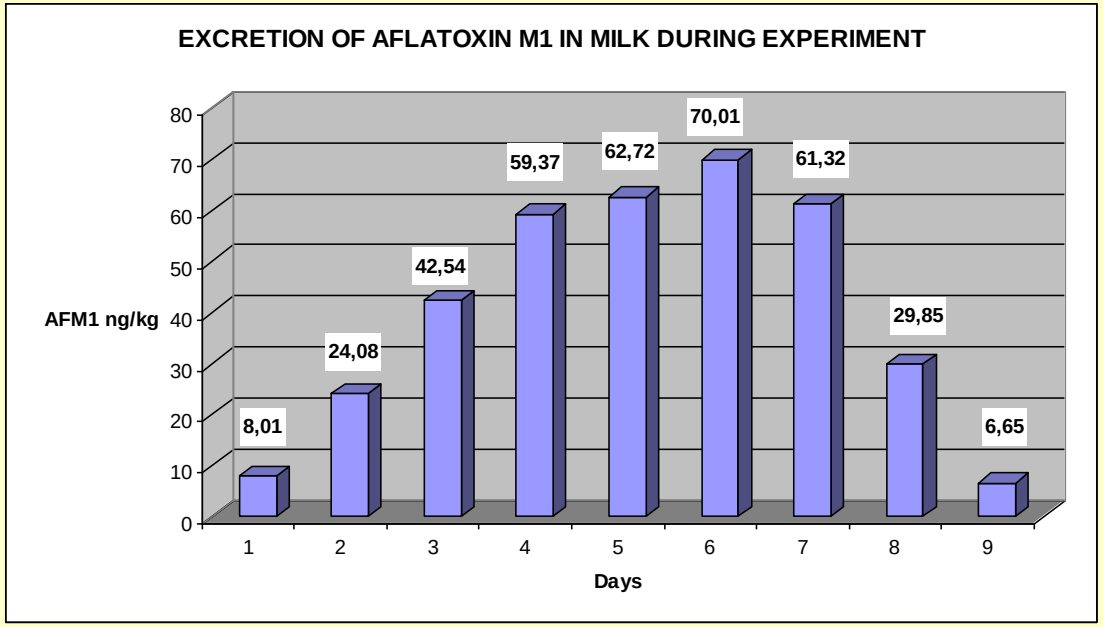
The concentration of AFM1 in animal milk is related to the dose of AFB1 ingested with the diet and is affected by milk yield, stage of lactation, the duration of the period of ingestion.

The European law limit for AFM1 in milk is 50 ng/Kg.

Excretion of Aflatoxin M1 in milk of Mediterranean Italian buffalo cow fed diet naturally contaminated of Aflatoxin B1*

Eight Mediterranean Italian buffalo cow (parity of 3 ± 1, in mid-lactation), were fed for 6 days with naturally contaminated corn meal (217 µg AFB_1/buffalo/day) and monitored for 3 days with a diet free of AFB_1.

Excretion of AFM_1 increased rapidly, reaching the maximum value at the sixth day of trial (70.01 ± 16.19 ng/kg), then decreases linearly until it reaches 6.65 ± 2.39 ng/kg in the last day of study.



*Giangolini G. *et al.* (Buffalo Bulletin Vol.32 – Special Issue 2 – 2013)

The rate of transfer of AFB_1 from feeds to milk AFM_1 (AFM_1/AFB_1 ratio) during the experimental period was 0.240%.

The CO of AFM_1, determined during the steady-state occurred from the third to sixth day, was 0.190% (range: 0.123% - 0.280%).

The individual daily milk production did not show significant variation during the experimental period, the average was 6.88 ± 1.39 kg / buffalo / day.

The low CO observed in this study may be due to several factors: the low level of milk yield, similarly as observed in dairy cows where AFM_1 excretion depends on the level of production (Britzi *et al.*, 2013) and the capacity of the ruminal microbial ecosystem to degrade or convert AFB1 into other metabolites (Fedele *et al.,* 2010).

In our experiment the contamination of milk with AFM_1 leads to clearance times 72 hours after the last administration of AFB_1.

THANK YOU FOR YOUR ATTENTION