



Actilait

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EXPERTISE ET CONTROLE DES ANALYSES
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EVALUATION OF THE BENTLEY SOMACOUNT FCM™ SOMATIC CELLS COUNTER ON EWE AND GOAT MILK

The Somacount FCM, manufactured and distributed by Bentley Instruments, is an apparatus which permits the enumeration of somatic cells in milk. It works using the fluoro-opto-electronic method with the following principle: in a first time, the test portion is mixed to a dye (detergent buffered solution of ethidium bromure) in order to disperse fat globules and to dye somatic cell nuclei. An aliquot is then injected into a laminar flow carrier fluid. The cells stained are separated by the flow and are exposed to the light beam of a laser and consequently emit a fluorescent light. The luminous impulses emitted by the somatic cells are then detected and amplified thanks a photomultiplier tube. Only impulses, whose the intensities are beyond to a threshold, are counted and converted in term of cellular concentration using a calibration equation.

The instrument is composed of two separate counting units which operate alternately. The apparatus is connected to a computer that ensures the signal treatment.

The tests:

The evaluation tests were performed in Actilait-Cecalait's physico-chemistry laboratory (reference and instrumental analyses) from December 2009 to February 2010. The repeatability and the accuracy were evaluated.

The appreciation criteria of the estimated parameters were taken from ISO 13366-2/IDF 148: 2007, or from the CNIEL/IE handbook concerning the use of somatic cells counters within the context of milk payment and milk control in France.

The following instrumental parameters were used:

- Rate: 500 samples / hour;
- No correction of contamination;
- Use in combined mode with the Bentley FTS infrared analyser (draining assistance).

A- EWE MILK

A1- Samples

The tests were performed using 100 samples of milk from the Roquefort region (12). Bronopol was added to the samples to give a final concentration of 0.02%.

A2- Repeatability

A2.1- Procedure

The repeatability of the instrument was evaluated using all the milk samples. The quantitative determination were performed in automatic analysis mode, in duplicate for each set of 10 samples according to the following sequence: (Set 1 rep 1 – Set 1 rep 2 – Set 2 rep 1 – Set 2 rep 2...Set n rep 1 – Set n rep 2). A control milk was analysed every 30 samples to verify the stability of the analyser.

A part of the samples was also analysed by the Bentley Somacount 150 (SCC 150) somatic cells counter.

A2.2- Results

The table below presents the results obtained:

	n	min	Max	M	Sx	Sr	Sr (%)	r
BLUE CANAL	100	103	1788	540	314	15.2	2.8	42
RED CANAL	100	105	1807	555	323	13.4	2.4	37
SCC 150	79	123	1808	554	330	9.5	1.7	26

Table 1: FCM repeatability criteria on ewe milk samples

n: number of results; *min* and *max*: minimum and maximum value; *M* and *Sx*: mean and standard deviation of the results; *Sr* and *Sr%*: absolute and relative standard deviation of repeatability; *r*: maximum deviation of repeatability in 95% of cases

A2.3- Conclusion

With no regulation for milk payment to quality for this type of milk, it can be noted that the relative standard deviations of repeatability obtained are in accordance with the recommendations of the method ISO 13366-2/IDF 148: 2007, which fixes a maximum limit to 4% (mean level 450.10^{E03}/ml) and with the recommendations of the manufacturer (< 5%).

A3- Evaluation of the accuracy

A3.1- Procedure

The accuracy of the analyser was evaluated using a part of the samples. The quantitative analyses were performed in accordance with the evaluation of the repeatability (cf B2.1). The evaluation concerns the values obtained after calibration of the instrument with commercial SRMs (cow milk) produced by Actilait-Cecalait.

The reference method ISO 13366-1/IDF 148: 2007 was used for the enumeration of the somatic cells by microscope counting.

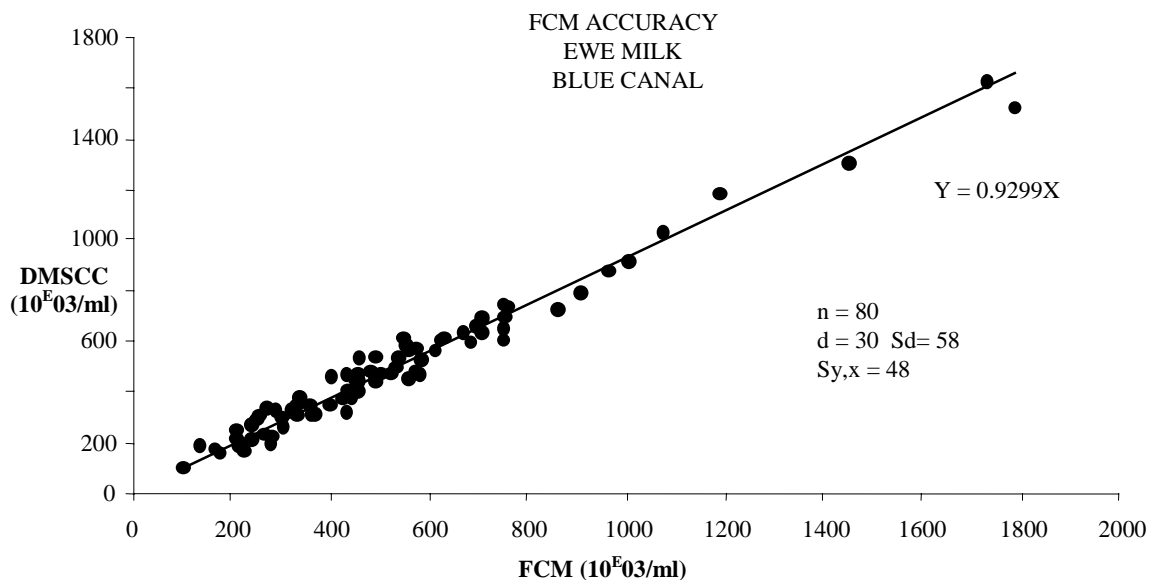
A3.2- Results

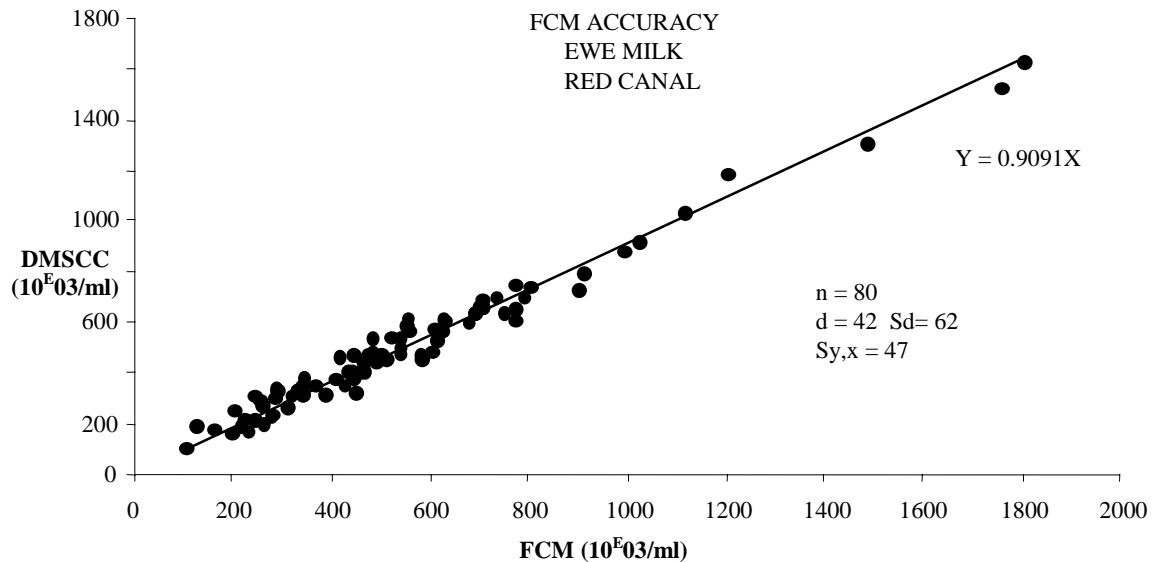
The following tables and figures present the results obtained:

	BLUE CANAL	RED CANAL	SCC 150	COMPARISON BLUE CANAL / SCC 150
n	80		60	60
min	103		172	123
max	1625		1625	1808
Y (10^{E03}/ml)	507		532	559
X (10^{E03}/ml)	537	550	559	555
Sy (10^{E03}/ml)	284		292	338
d (10^{E03}/ml)	30	42	28	-4
Sd (10^{E03}/ml)	58	62	65	19
Sy,x	46	46	43	19
Sy,x (%)	9.1	9.0	8.2	3.4
b	0.887	0.868	0.856	1.014
a	31	30	53	-4
Sy,x	48	47	49	19
Sy,x' (%)	9.4	9.3	9.2	3.4
b'	0.930	0.909	0.926	1.009

Table 2: FCM accuracy criteria on ewe milk samples

n, min, max: number of results, minimum and maximum value; Y,X: mean of the results using the reference and instrumental methods; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x (Sy,x%): absolute and relative residual standard deviation; b, a: slope and intercept of the linear regression; Sy,x' (Sy,x'%): absolute and relative standard deviation forced by zero; b': slope of the linear regression forced by zero





Figures 1 and 2: Relation between FCM and reference results on ewe milk samples (regression forced by zero)

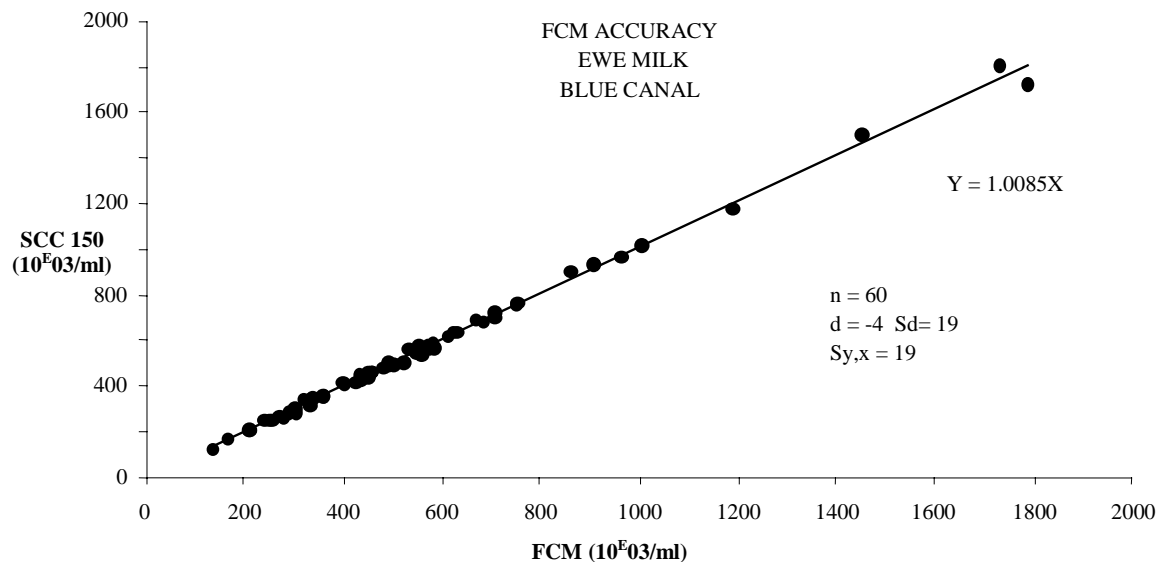


Figure 3: Relation between FCM and SCC 150 results on ewe milk samples (regressions forced by zero)

It can be noted that the means and standard deviations of deviations, which are about $-35.10^E03/ml$ and $60.10^E03/ml$ respectively, are equivalent between the both units in relation to the reference method. The regression lines are significantly different from 1.00 ($P = 1\%$). The residual standard deviations (about $46.10^E03/ml$, relative 9.3%) are equivalent. The FCM results are very near to the values obtained with the SCC 150 analyser with a slope not significantly different from 1.00, a deviations mean of $4.10^E03/ml$ and a standard deviation of deviations of $19.10^E03/ml$.

A.3.3- Conclusion

With no regulation for milk payment and standard limits for this type of milk, it can be noted that the regression line obtained is significantly different from 1.00. The origin of this deviation can be probably due to the nature of the ewe milk somatic cells (the apparatus are calibrated with cow milk SRMs). It can be also noted that the accuracy results obtained (regression line, residual standard deviation and mean bias) are very near of the European FAIR programme results (1997 to 1999) on this type of milk and of the Somacount 150 results.

B- GOAT MILK

B1- Samples

The tests were performed on 90 samples of milk from Poitou-Charentes region (17).

B2- Repeatability

B2.1- Procedure

The repeatability of the instrument was evaluated using all the milk samples. The quantitative analyses were performed in automatic analysis mode, in duplicate for each set of 10 samples according to the following sequence: (Set 1 rep 1 – Set 1 rep 2 – Set 2 rep 1 – Set 2 rep 2 ...Set n rep 1 – Set n rep 2). A control milk was analysed every 30 samples to verify the stability of the analyser. As the both units of the instrument operate alternately, each unit was evaluated on the half of the samples.

The samples were also analysed by the Bentley Somacount 150 (SCC 150) somatic cells counter.

B2.2- Results

The following table presents the results obtained:

	n	min	max	M	Sx	Sr	Sr (%)	r
GLOBAL	89	415	2713	1479	499	43.4	2.9	120
BLUE CANAL	45	668	2601	1476	507	48.8	3.3	135
RED CANAL	44	415	2713	1482	497	37.0	2.5	102
SCC 150	89	415	2668	1434	461	16.2	1.1	45

Table 3: FCM repeatability criteria on goat milk samples

n: number of results; min and max: minimum and maximum value; M and Sx: mean and standard deviation of the results; Sr and Sr%: absolute and relative standard deviation; r: maximum deviation of repeatability in 95% of cases

B2.3- Conclusion

With no regulation for payment of milk for this type of milk, it can be noted that the global relative standard deviation of repeatability is in conformity with the recommendations of the standardised method ISO 13366-2/IDF 148: 2007, which fixes a maximum limit of 3% (mean level $1500 \cdot 10^3/ml$).

B3- Evaluation of the accuracy

B3.1- Procedure

The accuracy of the analyser was evaluated using 71 samples (9 on the 80 samples initially selected did not be read by the reference method). The quantitative analyses were performed in accordance with the evaluation of the repeatability (cf C2.1). The evaluation concerned the values obtained after calibration of the instrument with commercial SRMs (cow milk) produced by Actilait-Cecalait.

The reference method ISO 13366-1/IDF 148: 2007 was used for the enumeration of the somatic cells by microscope counting.

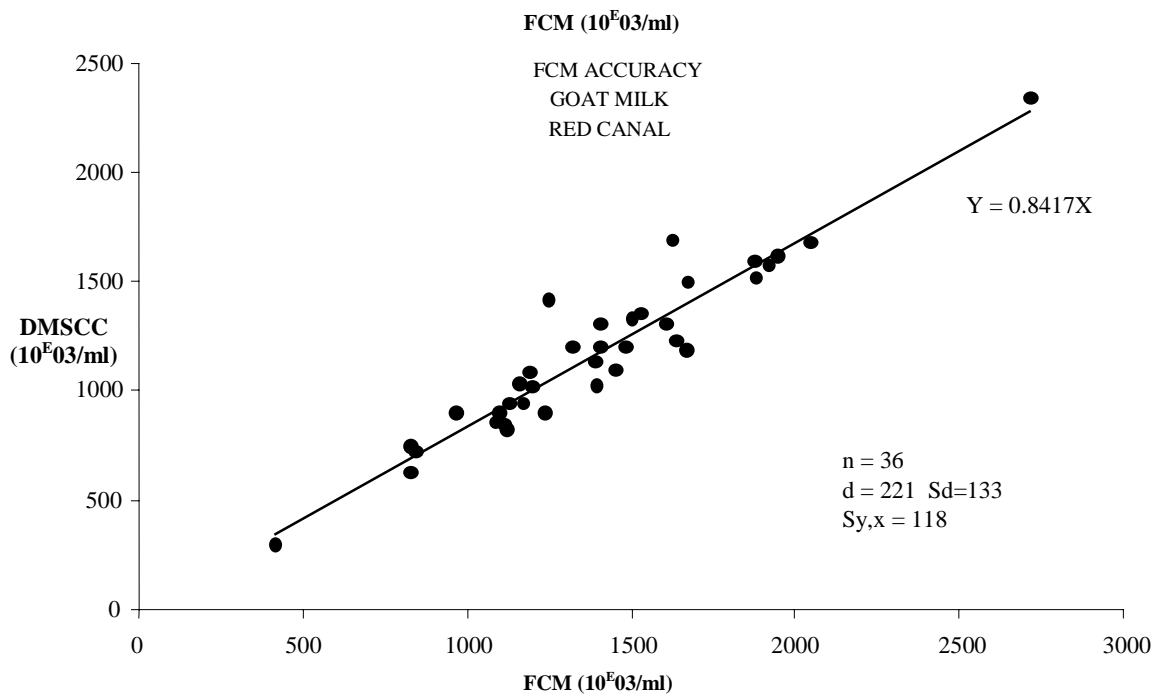
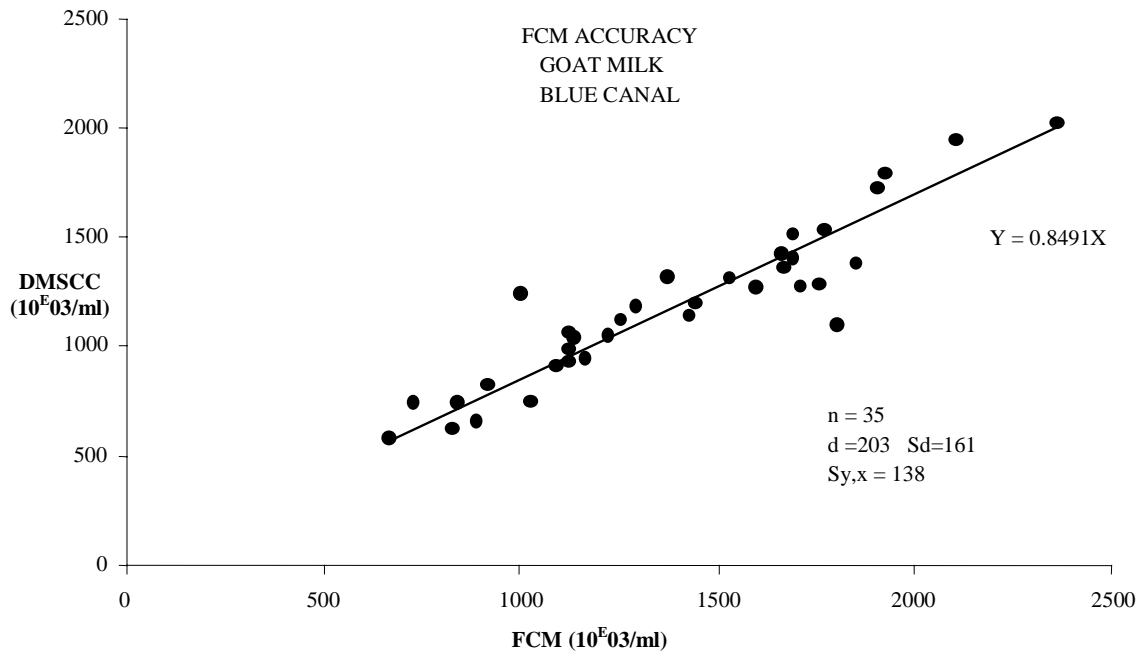
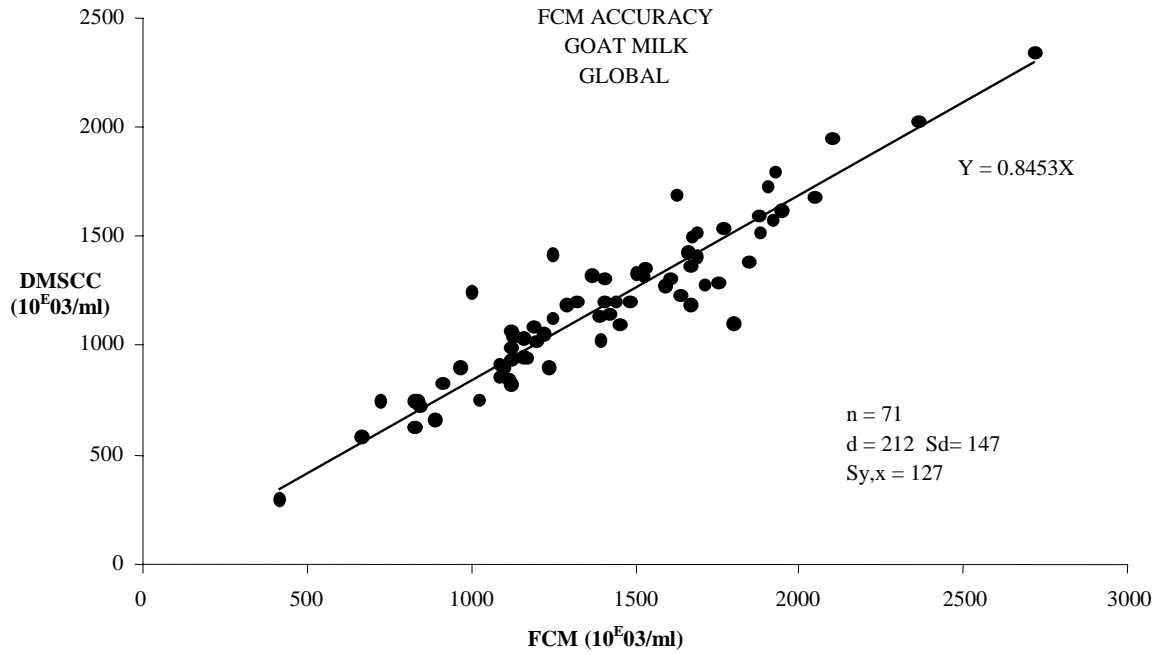
B3.2- Results

The following table and figures present the results obtained:

	GLOBAL	BLUE CANAL	RED CANAL	SCC 150	COMPARISON GLOBAL APPARATUS / SCC 150
n	71	35	36	71	89
min (10^{E03}/ml)	293	588	293	293	415
max (10^{E03}/ml)	2339	2024	2339	2339	2668
Y (10^{E03}/ml)	1178	1186	1171	1178	1434
X (10^{E03}/ml)	1391	1389	1392	1344	1479
Sy (10^{E03}/ml)	366	359	379	366	461
d (10^{E03}/ml)	212	203	221	165	45
Sd (10^{E03}/ml)	147	161	133	124	111
Sy,x (10^{E03}/ml)	127	138	118	113	100
Sy,x (%)	10.8	11.6	10.1	9.6	7.0
b	0.822	0.792	0.850	0.870	0.902
a	35	86	-13	9	99
Sy,x '(10^{E03}/ml)	127	138	117	113	104
Sy,x' (%)	10.8	11.6	10.0	9.6	7.2
b'	0.845	0.849	0.842	0.876	0.963

Table 4: FCM accuracy criteria on goat milk samples

n, min, max: number of results, minimum and maximum value; Y,X: mean of the results using the reference and instrumental method; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x (Sy,x%): absolute and relative residual standard deviation; b, a: slope and intercept of the linear regression; Sy,x' (Sy,x' %): absolute and relative residual standard deviation of the regression forced by zero; b': slope of the linear regression forced by zero



Figures 4, 5 and 6: Relation between FCM and reference results on goat milk samples (regressions forced by zero)

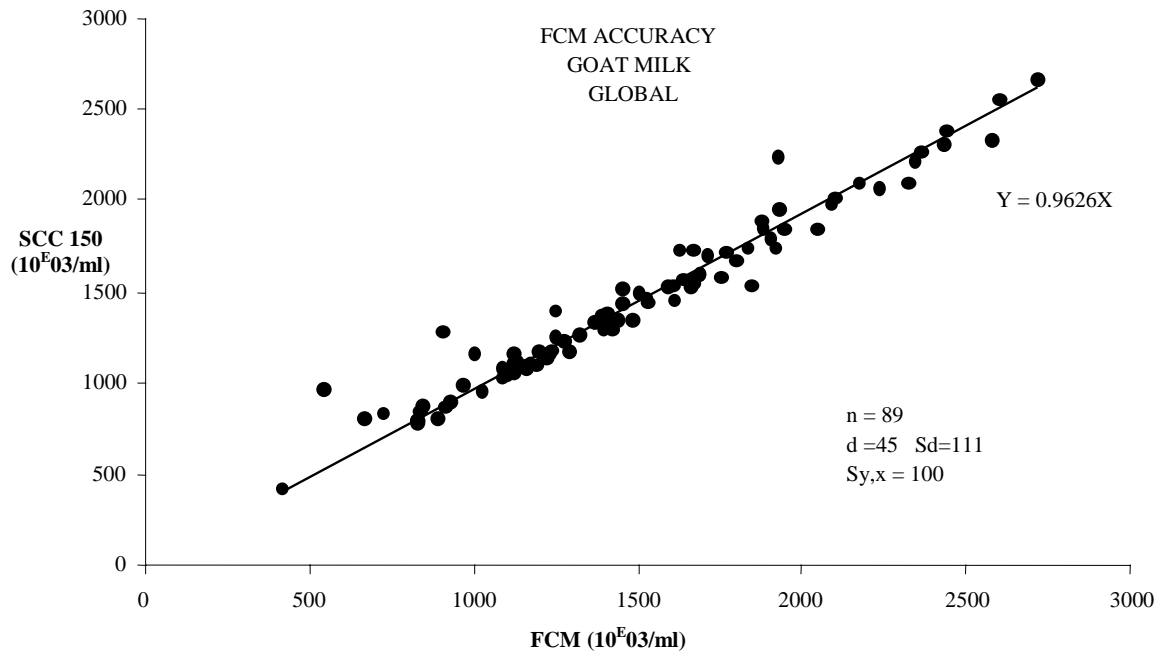


Figure 7: Relation between FCM and SCC 150 results on goat milk samples (regressions forced by zero)

It can be noted that the slope equal to 0.845 is significantly different from 1.00 ($P = 1\%$). The residual standard deviation of regression is equal to 127 (relative 10,8%). These values are equivalent to the values obtained with the SCC 150 analyser.

As the samples analysed by the both units concern two different under-populations, their characteristics (mean and standard deviation next) are equivalent. The results and performances obtained by the both canals are then comparable.

B.3.3- Conclusion

With no regulation for milk payment and standard for this type of milk, it can be noted that the regression line obtained is significantly different from 1.00. The origin of this deviation can be probably due to the nature of the goat milk somatic cells (the apparatus are calibrated with cow milk SRMs). It can be also noted that the accuracy results obtained (regression line, residual standard deviation and mean bias) are very near of the European FAIR programme results (1997 to 1999) on this type of milk.

According to the evaluation report of the Bentley FCM™ somatic cells counter on ewe and goat milk - X. QUERVEL and Ph. TROSSAT – Actilait / Cecalait – April 2010

STANDARDS - REGULATIONS

STANDARDS, DRAFT STANDARDS

Classification in alphabetic order by theme

ISO standards under development

CHEESE AND PROCESSED CHEESE		
NITROGENOUS FRACTIONS	ISO/DIS 27871 June 2010	CHEESE AND PROCESSED CHEESE Determination of the nitrogenous fractions
LACTOSE		
WATER	ISO/DIS 12779 September 2010	LACTOSE Determination of water content – Karl Fischer method
MICROBIOLOGY OF FOOD AND ANIMAL FEEDING		
ALTERNATIVE METHODS	ISO/DIS 16140/A1 March 2010	MICROBIOLOGY OF FOOD AND ANIMAL FEEDING Protocol for the validation of alternative method – Amendment 1: Interlaboratory study on quantitative methods
MILK AND MILK PRODUCTS		
CHYMOSIN / PEPSIN	ISO/DIS 15163 October 2010	MILK AND MILK PRODUCTS Calf rennet and adult bovine rennet – Determination by chromatography of chymosin and bovine pepsin
SENSORY ANALYSIS		
SENSITIVITY OF TASTE	ISO/DIS 3972 July 2010	SENSORY ANALYSIS Methodology – Method of investigating sensitivity of taste

ISO published standards

CASEINS AND CASEINATES		
pH	ISO 5546 (IDF 115) June 2010	CASEINS AND CASEINATES Determination of pH (reference method)
CHEESE AND PROCESSED CHEESE PRODUCTS		
PHOSPHORUS CONTENT	ISO 2962 (IDF 33) June 2010	CHEESE AND PROCESSED CHEESE PRODUCTS Determination of total phosphorus content – Molecular absorption spectrometric method
DRIED MILK		
TITRATABLE ACIDITY	ISO 6091 (IDF 86) June 2010	DRIED MILK Determination of titratable acidity (reference method)
MILK		
FAT CONTENT	ISO 1211 (IDF 1) June 2010	MILK Determination of fat content – Gravimetric method (reference method)
CALCIUM CONTENT	ISO 12081 (IDF 36) June 2010	MILK Determination of calcium content – Titrimetric method
MILK AND MILK PRODUCTS		
IRON CONTENT	ISO 6732 (IDF 103) June 2010	MILK AND MILK PRODUCTS Determination of iron content – Spectrometric method (reference method)

STANDARDS - REGULATIONS

ZINC CONTENT	ISO 11813 (IDF 156) June 2010	MILK AND MILK PRODUCTS Determination of zinc content – Flame atomic absorption spectrometric method
QUALITY		
CUSTOMER SATISFACTION	ISO/TS 10004 April 2010	QUALITY MANAGEMENT Customer satisfaction – Guidelines for monitoring and measuring

NEW EU REGULATIONS

Classification is established in alphabetical order of the first keyword

ENTEROBACTERIACEAE

O.J.E.U. L 107, 29th April 2010 – Commission Regulation (EU) n° 365/2010 of 28 April 2010 amending Regulation (EC) n° 2073/2005 on microbiological criteria for foodstuffs as regards *Enterobacteriaceae* in pasteurised milk and other pasteurised liquid dairy products and *Listeria monocytogenes* in food grade salt
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:107:0009:0011:EN:PDF>

HEALTH CLAIMS

O.J.E.U. L 111, 4th May 2010 – Commission Regulation (EU) n° 375/2010 of 3 May 2010 refusing to authorise a health claim made on foods, other than those referring to the reduction of disease risk and to children's development and health
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:111:0001:0002:EN:PDF>

O.J.E.U. L 111, 4th May 2010 – Commission Regulation (EU) n° 376/2010 of 3 May 2010 amending Regulation (EC) n° 983/2009 on the authorisation of certain health claims made on food and referring to the reduction of disease risk and to children's development and health
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:111:0003:0004:EN:PDF>

O.J.E.U. L 113, 6th May 2010 – Commission Regulation (EU) n° 382/2010 of 5 May 2010 refusing to authorise certain health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:113:0001:0003:EN:PDF>

O.J.E.U. L 113, 6th May 2010 – Commission Regulation (EU) n° 383/2010 of 5 May 2010 refusing to authorise a health claim made on foods, other than those referring to the reduction of disease risk and to children's development and health
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:113:0004:0005:EN:PDF>

O.J.E.U. L 113, 6th May 2010 – Commission Regulation (EU) n° 384/2010 of 5 May 2010 on the authorisation and refusal of autorisation of certain health claims made on foods and referring to the reduction of disease risk and to children's development and health
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:113:0006:0010:EN:PDF>

LABELLING

O.J.E.U. L 102, 23rd April 2010 – Commission Decision (2010/229/UE) of 22 April 2010 concerning the draft Decree from Italy setting out standards governing the labelling of shelf-stable milk, UHT milk, micro-filtered pasteurised milk and high-temperature pasteurised milk, as well as milk products
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:102:0052:0053:EN:PDF>

PESTICIDES

O.J.E.U. L 94, 15th April 2010 – Commission Regulation (EU) n° 304/2010 of 9 April 2010 amending Annex II to Regulation (EC) n° 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 2-phenyl phenol in or on certain products
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:094:0001:0014:EN:PDF>

O.J.E.U. L 129, 28th May 2010 – Commission Regulation (EU) n° 459/2010 of 27 May 2010 amending Annexes II, III and IV to Regulation (EC) n° 396/2005 of the European Parliament and of the Council as regards maximum residue levels for certain pesticides in or on certain products
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:129:0003:0049:EN:PDF>

PROTECTED DESIGNATION OF ORIGIN

O.J.E.U. L 94, 15th April 2010 – Commission Regulation (EU) n° 306/2010 of 14 April 2010 approving non-minor amendments to the specification for a name entered in the register of protected designations of origin and protected geographical indication [Pecorino Toscano (PDO) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:094:0019:0020:EN:PDF>

O.J.E.U. C 101, 20th April 2010 – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) n° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Ricotta Romana (PDO) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:101:0020:0025:EN:PDF>

O.J.E.U. C 123, 12th May 2010 – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) n° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Fontina (PDO) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:123:0018:0024:EN:PDF>

O.J.E.U. L 119, 13rd May 2010 – Commission Regulation (EU) n° 414/2010 of 12 May 2010 entering a name in the register of protected designations of origin and protected geographical indications [Nieheimer Käse (PGI) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:119:0003:0004:EN:PDF>

O.J.E.U. L 126, 22nd May 2010 – Commission Regulation (EU) n° 441/2010 of 21 May 2010 entering a name in the register of protected designations of origin and protected geographical indications [Canestrato di Moliterno (PGI) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:126:0006:0007:EN:PDF>

O.J.E.U. L 126, 22nd May 2010 – Commission Regulation (EU) n° 443/2010 of 21 May 2010 entering a name in the register of protected designations of origin and protected geographical indications [Piave (PDO) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:126:0010:0011:EN:PDF>

O.J.E.U. L 135, 2nd June 2010 – Commission Regulation (EU) n° 480/2010 of 1 June 2010 approving non-minor amendments to the specification for a name entered in the register of protected designations of origin and protected geographical indications [Spessa della Giudicarie (PDO) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:135:0036:0037:EN:PDF>

O.J.E.U. C 162, 22nd June 2010 – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) n° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Pecorino Sardo (PDO) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:162:0007:0010:EN:PDF>

O.J.E.U. C 164, 24th June 2010 – Publication of an application pursuant to Article 6 (2) of Council Regulation (EC) n° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Piacentinu Ennese (PDO) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:164:0026:0029:EN:PDF>

O.J.E.U. C 168, 26th June 2010 – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) n° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Robiola du Roccaverano (PDO) (cheese)]

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:168:0010:0015:EN:PDF>

SWEETENERS

O.J.E.U. L 152, 18th June 2010 – Commission Directive 2010/37/EU of 17 June 2010 amending Directive 2008/60/EC laying down specific purity criteria on sweeteners

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:152:0012:0013:EN:PDF>

BOOKSHOP: LATEST PUBLICATIONS

The classification in alphabetic order of the first keyword allows you to consult the references according to your interests. The web site allows you to know more, or to order the book.

QUALITY

GRIFFITHS M. – **Improving the safety and quality of milk: volume one; milk production and processing** – CRCPress Edition – June 2010 – ISBN 9781439802182 – 700 pages

<http://www.crcpress.com>



The first section of this book introduces milk biochemistry and raw milk microbiology. Part two then reviews major milk contaminants, such as bacterial pathogens, pesticides and veterinary residues. The significance of milk production for product quality and safety is the focus of part 3. The 4th part then treats of the improvement of the quality of pasteurised and UHT milk.

IN THE PRESS – ON THE WEB

Classification in alphabetical order of keywords

MELAMINE

EFSA reduces tolerable intake level for melamine

<http://www.efsa.europa.eu/en/press/news/contam100413.htm>

► The European Food Safety Authority (EFSA) has lowered the tolerable intake level for melamine to 0.2 milligrams per kilogram of bodyweight, which is in conformity with the tolerable intake level set by the World Health Organisation in 2008

STANDARDISATION

Report of the 42th session of the Codex Committee on food additives – Beijing, China, 15-19 March 2010

Report of the 31st session of the Codex Committee on methods of analysis and sampling - Budapest, Hungary, 8-12 March 2010

Report of the 42nd session of the Codex Committee on pesticide residues - Xian, China, 19-24 April 2010

Report of the 5th session of the Codex Committee on contaminants in foods - Izmir, Turkey, 26-30 April 2010

Report of the 38th session of the Codex Committee on food labelling – Quebec City, Canada, 3-7 May 2010

<http://www.codexalimentarius.net/web/archives.jsp?lang=en>

► These reports present the conclusions which will be considered / adopted by the 33rd session of the Codex Alimentarius Commission, which will be held at Geneva, Switzerland, from 5 to 9 July 2010.

VETERINARY DRUG

EFSA publishes technical report on veterinary drug residues in animals and food

<http://www.efsa.europa.eu/en/press/news/datex100419.htm>

► Following a request for assistance from the European Commission, EFSA has published a technical report on the occurrence of residues of veterinary drugs in live animals and animal products. The report, which also covers other substances such as contaminants, compares reported levels with limits set out in EU legislation in order to protect consumers.

FORTHCOMING EVENTS

Classified in chronological order

FOOD

22-26 August 2010
Cape Town, South Africa

15th World congress of food science
and technology

www.iufost2010.org.za

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