Journal of Animal &Plant Sciences, 2018. Vol.36, Issue 1: 5785-5792
Publication date 29/04/2018, **http://www.m.elewa.org/JAPS**; *ISSN 2071-7024*

Effect of somatic cells on the yield, clotting time and organoleptic quality of Wagashi

Vikou R[1,2]., Doko S.Y[3].,Aplogan L.G[4]., Ahanhanzo C[5]., Baba-Moussa L[6]., Gbangboche A.B[1,2].

[1] Laboratory for animal Improvement and Applied Biotechnology (LAABA), Institute of Applied Biomedical Sciences (ISBA), University of Abomey-Calavi 01 BP: 526 Cotonou-Benin

2 School of management and exploitation of animal breeding systems, National University of Agriculture, BP 43 Ketou-Benin.

3. School of food process and agricultural product conservation

4 Laboratory for Veterinary Diagnosis and sero surveillance of animal diseases, Ministry of Agriculture, Livestock and fisheries, Benin.

5 Laboratory of Genetics and Biotechnology (LGB), Department of Genetics and Biotechnology, Faculty of Science and Technology (FAST), University of Abomey-Calavi 01 BP: 526 Cotonou-Benin

[6] Laboratory of biology and molecular typing in microbiology, Department of Biochemistry and Cell Biology, Faculty of Science and Technology, University of Abomey-Calavi 05 BP 1604 Cotonou, Benin.

Correspondence: Ronaldess VIKOU, Email: **vikouronaldess@yahoo.fr**/phone:+22996157243

Key words: yield, cow, production, cheese, somatic cells

1 ABSTRACT

Traditional Wagashi (Peulh cheese) production technology occupies an important place in the artisanal processing of fresh milk in African countries. It faces many quality problems of cheese products due to infectious mastitis. It is in this context that an exploratory study was conducted in the communes of Parakou, Nikki, Tchaourou, Gogonou and Malanvile in Benin, in order to determine in cattle the influence of somatic cells on the yield, the clotting time and the organoleptic quality of Wagashi. The Californian Mastitis Test (CMT) used on 212 bovine milk samples showed positive for mastitis. The threshold for CMT is 300 × 103 cells/ml, with the distribution of samples by proportion (%) somatic cell (CS) as follows: 900 × 103 CS/ml at 2700 × 103 cells/ml (61%), 8100 × 103 CS/ml (11%), 300 × 103 cs (26%), 100 × 103 CS/ml (2%). The time of coagulation and cheese yield varied significantly ($p < 0.001$): For the CCS + line, it is 30 minutes with a yield cheese 1.72 kg/100L versus 20 minutes for the CCS line-and a yield cheese 1.93 kg/100L. The triangular test used for degustation of cheeses showed a significant difference (P value = 0.01%) between Issu CCS + and CCS-cheeses. 58.53% indicate a bitter taste and a friable mechanical aspect for the CCS + cheese compared to 41.46% a sweet taste and a mechanical aspect of firmness for sac-derived cheese.

2 INTRODUCTION

The somatic cells of milk are mainly leukocytes, which include macrophages, lymphocytes and neutrophils (Harmon and Reneau, 1993) which have the role of digesting microorganisms invading the mammary gland. Among the methods for assessing the overall health status of the Udder, Somatic cell count (CCS) is a reference (Sadak *et al.,* 2016). They are considered as indicators of the resistance and susceptibility of cows to mammary inflammations and are used as a monitor of the level of the sub-clinical state of udder inflammation of herds or Individual cows (Bala *et al.,* 1999; Chaiyotwittayakun *et al.,* 2008; Sharma *et al.,* 2011). Sharma *et al.* (2007)

and Seegers *et al.* (2003) and Caillat *et al.* (2012) reported that a selection on somatic cell concentration with a level greater than 305000/ml modifies the suitability of milks for coagulation, the organoleptic properties of cheeses, and affects cheese yield. Le Roux and Laurent (1999) reported that the degradation of the health status of Udder, the main factor in the endogenous proteolysis of milk, induces a change in the biochemical and technological composition of milk that can cause loss of Yield and taste defects. Thus, among the factors affecting milk production and its quality, mastitis occupies a good place and presents three forms (Bonnefont *et al.,* 2011): Clinical form (easy to detect); The Sub-clinical form (difficult to detect) and the chronic form. In addition, Mastitis has an evolutionary character and due to the severity of infection and the number of sick animals in the herd, its incidence is not noticeable in the breeding and is characterized by physical, chemical and bacteriological changes in milk plus, pathological changes in the glandular tissues of the udders (Sharma *et al.,* 2007; Dragana *et al.,* 2012). In dairy cattle, mastitis is ranked at the forefront of diseases in terms of their economic consequences (Davies et al., 2009), in relation to the decrease in milk production, the devaluation of the selling price, and the costs of High treatment. In Benin, cow milk contributes more than 50% to the annual incomes of PEULH households (Ogodja, 1991). It is unanimously regarded as a very perishable commodity, but of great economic value, as food, and of nutritional importance. Also, among the products derived from the processing of cow milk in Benin (curd, butter, Wagashi), the Wagashi remains the most widespread and the most consumed in both rural and urban settings. It is also the best form of milk conservation (Dossou *et al.,* 2006). Therefore, the objective of this study is to evalute the influence of somatic cells on the yield, clotting time and organoleptic quality of Wagashi.

3 MATERIALS AND METHODS

The studies focused on 212 samples of fresh milk previously diagnosed positive for the Californian Mastitis (CMT) test. The samples come from the Borgou and Alibori departments (Figure 1, table 1): including 51 samples (Parakou), 48 (Malanville), 45 (Nikki), 42 (Tchaourou) and 26 (Gogounou).

3.1 Diagnosis of Mastitis: The Californian Mastitis test (CMT): For the diagnosis of mastitis, the Californian Mastitis (CMT) test (Lot 10 LEUST35 manufactured by COOPHAVET) was used to digitize somatic cells, to specify the status of cell lines (CCS + or CCS-) and the level of inflammation of the gland Breast (M'Sadak *et al.,* 2016). This test is based on the use of a surfactant detergent that is the solution of 10% Teepol and a coloured indicator (purple of Bromocresol ®) on the milk. This active surfactant works by causing the cells present in the milk to lysis. The destruction of cell walls releases the cellular DNA that forms an imprisoning network of fatty and other particles. The effect of this reaction is to increase the viscosity of the milk, or even to induce a flocculate whose importance and consistency depend on the cell content of the milk sample. The colourful indicator accelerates the turn of the green colour that evolves towards the purple. The test is easy but cleanliness is necessary. In practice, at the beginning of the milking process, after elimination of the first draft, a little milk from each quarter is taken from each of the four identified cups of the tray. Then the tray is inclined to remove excess milk to the line that indicates the amount of milk necessary for the reaction (about 2 ml). After adding 2 ml of leukocytest reagent to each cup, a circular motion is printed on the tray for a few seconds to mix the milk with the reagent. Finally, the presence and appearance of the flocculate, an indicator of mastitis, whose reading and interpretation of CMT is made in reference to the Table1, is also transparent.

Journal of Animal &Plant Sciences, 2018. Vol.36, Issue 1: 5785-5792
Publication date 29/04/2018, **http://www.m.elewa.org/JAPS**; *ISSN 2071-7024*

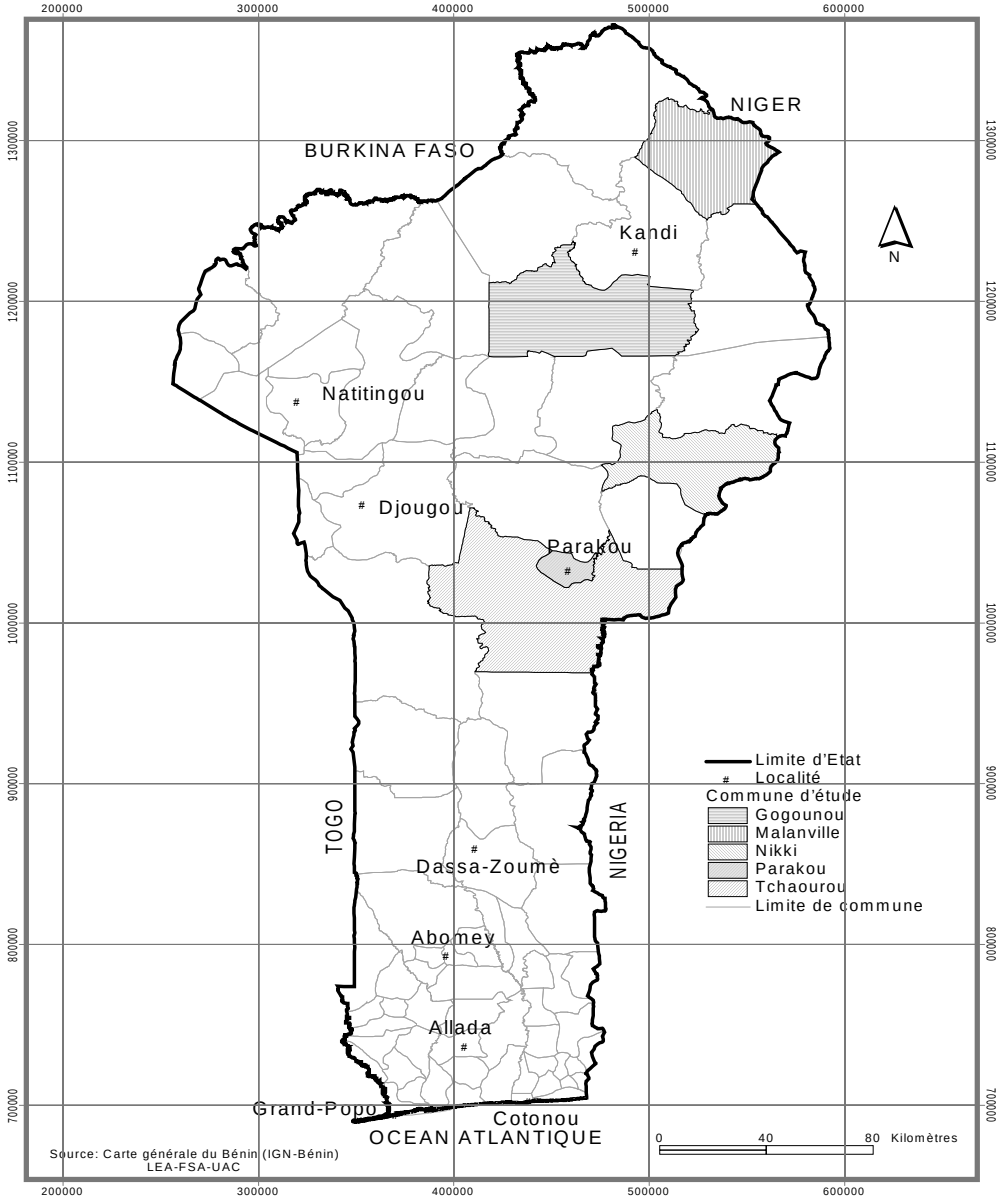

Figure 1: Area of study of infectious mastitis in Benin

Journal of Animal &Plant Sciences, 2018. Vol.36, Issue 1: 5785-5792
Publication date 29/04/2018, **http://www.m.elewa.org/JAPS**; *ISSN 2071-7024*

Table1: Reference for the reading of CMT results and distribution of infections by localities and nature of infections

Reference for the reading of CMT results				**Distribution of infections by localities**					**Distribution according to Values/Crosses**	
Value/Cross	Aspect	Infection	Relationship to average cell count	Tchaourou	Parakou	Nikki	Malanville	Gogounou	Total	% by location
0 (0)	Natural consistency, Grey	Absent	(x 1000/ml)	1	3	1	0	0	5	2.36
1 (+/-)	Light gel disappearing after agitation. Purplish grey colour	Risk of infection by minor pathogen	300	14	8	14	10	9	46	21.70
2 (+)	Slight persistent gel. Lumpy filaments. Colour Grey Violet	subclinical mastitis	900	26	12	15	21	10	94	44.34
3 (+ +)	Immediate thickening. Viscous cluster at the bottom of the cup	subclinical mastitis	2700	1	16	11	12	6	46	21.70
4 (+ + +)	Thick Gel, egg white consistency. Dark purple Colour	subclinical mastitis at the limit of clinical expression	8100	0	12	4	5	1	22	10.38
Total				42	51	45	48	26	212	100
% by location				19.81	24.05	21.22	22.64	12.26	100	

3.2 Manufacture of Wagashi: Five (05) liters of fresh milk from cell line (CCS +) and (CCS-), obtained immediately after milking, were used in the manufacture of a single wagashi. Ten (10) tests were performed by lineage. The milk is filtered to remove the impurities. A first slice (3.75 liters) is put on low heat in a large pot. The second slice (1.25 liters) is mixed with the grinding of the leaf and stem of *Calotropis procera* (a plant coagulant) and then filtered. The filtrate thus obtained is added to the first slice on the low fire until the curd forms and goes back to the surface. With the help of a ladle, the curd is then transferred into braided plastic sieves. The cheeses are then coloured with sorghum panicules that are dipped in the water of a pot that is already on the fire. The cheeses are plunged in turn in the water for about ten minutes and come out colourful. They are then drained again and dried at room temperature. The weight of each cheese was recorded using an electronic weighing scale (METTLER PJ600 ®). The yield of cheese (r) [r = (weight of Wagashi (kg) x 100)/(5 liters)] was calculated and the coagulation time for each wagashi from cell lines (CC +) and (CCS-). The duration of coagulation (minute) is the time that elapses between cooking and the formation of the stalled.

3.3 Tasting of Wagashi: The tasting of Wagashi made from the two types of cell lines (CCS +) and (CCS-) was conducted in a population who of 41 people actually consume wagashi. The triangular tests according to a scoring grid drawn from the procedure by Waitt *et al.*(1991) was used to assess the organoleptic quality of wagashi, i.e. taste and mechanical appearance. The tastes of cheeses or the tasting description is defined by sweet, sour, salty and bitter while the mechanical aspect or texture by elasticity, firmness, friability and adhesion of cheeses.

3.4 Statistical analysis: The weight of the cheeses, the yield and the coagulation time of the milk, were calculated with their standard deviation. The ANOVA procedure was used to estimate the 5% threshold, the effect of milk quality (CCS-and CCS +) on yield and coagulation time. For the effect of milk quality (CCS-and CCS +), the binomial test has a tail with a probability of correct judgments equal to or greater than X with n tests (p = 1/3) as described by Waitt *et al.*(1991), has been used for the assessment of the organoleptic qualities of cheeses (taste or tasting description and mechanical appearance or texture). This difference is significant at the 1% threshold.

4 RESULTS AND DISCUSSION

The values of the average cell counts are presented in the Table1. It is inferred that the milk of the sac + cows used for wagashi manufacture is an average of 2.7 million CS/ml with a ph of 6.5 versus 300,000 CS/ml with a ph of 6 for the CCS-lineage. More than half of the lines are between 900 × 103 CS/ml at 2700 × 103 CS/ml, 11% of the somatic cells are 8100 × 103 cs/ml, 26% of 300 × 103 cells and 2% of 100 × 103 cells/ml. As shown in table 1, sub-clinical mastitis accounted for 66% of infections and this rate would be higher than that reported in France of 47% and 53% respectively by Fabre *et al.* (1997) and Faye *et al.* (1994). In California, it varies between 22 and 34% Fox *et al.* (1995), Denmark between 37 and 55% (Aaerstrup and Jensen in 1997), Quebec 17 and 33% between 42 and 74% (Nickerson *et al.,* 1995). This high rate in the farms from which the milk samples originated in northern Benin could be explained by the mechanical technique of milking, as illustrated by the work of Luís *et al.*(2001) suggesting that if the number of somatic cells exceeds 500,000 cells/ml, the milking procedure should be implicated, including the lack of hygienic conditions (no use of dry towels, disinfectants for Cleaning of teats before milking) as recognized by Rasmussen (2000) and Ryszard Skrzypek (2006) who report that cleaning with a dry towel or towel soaked with a disinfectant are the best methods to reduce The CCS in the milk.

4.1 Effect of milk quality on the weight of cheese, cheese yield and milk coagulation time: Table 2 shows that the loss of milk yield

varies considerably according to the SCC level, and highlights the significant variation ($p < 0.001$) in weight, cheese yield, and coagulation time as a function of milk quality. For this purpose, the weight, the cheese yield and the time of coagulation are + 11, respectively; + 2.17 and + 9.60 in favour of CCS milk-in relation to CCS + milk (table 2). The same observation is made by Philipsson *et al.* (1995) which considers that a cow in clinical mastitis with a rate of 200 000 CCS/ml would generate an individual milk yield loss of 1.29 kg/day for first lactating cows versus 2.04 kg/day for older cows (Koldeweij *et al.,* 1999). Juozaitiene *et al.* (2005), indicate a negative correlation ($R = -0.35$, $P < 0.01$) between a CCS level greater than 200000/ml and milk yield and that the increase in CCS from 100 000/ml to 800 000/ml would increase the number of inseminations per design in the first three Lactations.

Table 2: Variation of weight of wagashi, the yield and clotting time according to milk quality

Parameters	**Quality of milk**		**p-Value**
	CCS-	CCS+	
Weight	0,96± 0,001	0,85± 0,001	<0,001
Yield	19,36±0,14	17,19±0,02	<0,001
clotting time	20,10±1,19	29,70±1,33	<0,001

4.2 Organoleptic Quality of cheeses: The tasting tests show that 58.53% reported the bitter taste and a friable mechanical aspect for the cheese from CCS + and 41.46% a sweet taste and a mechanical aspect of firmness for the cheese from CCS-. This difference is significant with a P-value of 0.01%. These results confirm the work of Sharma *et al.*(2007), Seegers *et al.* (2003) and Caillat *et al.*(2012), which indicated that a selection on the concentration of somatic cells with a level greater than 305000/ml altered the organoleptic properties of the cheeses. Roux and Laurent (1999) reported that the degradation of the state of Health of the udder induced a change in the biochemical and technological composition of milk, which could cause cheese taste defects. The observed difference in the taste of cheeses could be due on the one hand to the feeding of dairy cows shortly before milking or during milking (Kuzdzal-Savoie and Suraj, 1960) and on the other hand to the lactation rank of dairy cows. Lucey *et al.*(1992) observed that in areas where milk production is very seasonal, cheeses produced when animals are at the end of lactation are frequently described as wetter, with protein degradation and have a Less firm and less elastic texture with pronounced taste defects. In their study, the effects attributed to the lactation stage were confused with those of the season, diet or cell count of milks often higher at the end of lactation.

5 CONCLUSION

This study is carried out in some cattle farms in the departments of Borgou and Alibori and whose objective is to show the effect of somatic cells (CCS) on the yield, coagulation time and organoleptic quality of Wagashi. In fact, somatic cells have the role of digesting microorganisms that invade the mammary gland. As a result, their presence in milk informs the state of the udder and is considered as indicators of the resistance and susceptibility of cows to breast inflammation. Given CCS rates in the 212 milk samples analyzed, the results showed that sub-clinical mastitis accounted for 66% of infections. It is also noted that more than half of the lines are between 900 × 103 CS/ml at 2700 × 103 CS/ml, 11% of somatic cells are 8100 × 103 cs/ml, 26% of 300 × 103 cells and 2% of 100 × 103 cells/ml. The study also highlighted the loss of the weight of the cheese, the cheese yield and the lengthening of the coagulation time of the milk to CCS + than CCS-. The organoleptic quality of the resulting cheese is also affected showing a

bitter taste, a friable mechanical aspect for CCS + cheese, a sweet taste and a mechanical aspect of firmness for CCS-.

6 ACKNOWLEDGEMENTS

Authors are grateful to Ministry of higher education and scientific research for financial support.

7 REFERENCES

Anoper: 2014. La situation actuelle de l'élevage et des éleveurs de ruminants au Bénin.9-14p.

Barnouin J, Geromegnace N, Chassagne M, Dorr N. and Sabatier P : 1999. Facteurs structurels de variation des niveaux de comptage cellulaire du lait et de fréquence des mammites cliniques dans 560 élevages bovins répartis dans 21 départements français. inra prod. Anim., 12: 39-48.

Bonnefont C, Toufeer M, Caubet C, Foulon E, Tasca C, Aurel MR, Bergonier D, Boullier S, Robert-Granie C, Foucras G. and Rupp R : 2011.Transcriptomic analysis of milk somatic cells in mastitis resistant and susceptible sheep upon challenge with *Staphylococcus epidermidis* and *Staphylococcus aureus*. BMC Genomics12: 208.

Caillat H, Augerat D, Petrier M, Bouvier F, Leroux V, Delacroix-Buchet A. and Rupp R : 2012. Consequences of divergent selection based on somatic cell counts in dairy goats on the transformation of lactic type of milk. Renc. Rech. Ruminants, 19.

Chaiyotwittayakun A, Aiumlamai S, Chanlun A. and Sri S: 2008. Alternative Method for Determination of Milk Somatic Cell Count in Dairy Cow. Proceedings, The 15th Congress of FAVA, 27-30 October à Bangkok, Thailand.

Davies G, Genini S, Bishop SC. and Giuffra E: 2009. An Assessment Of Opportunities To Dissect Host Genetic Variation In Resistance To Infectious Diseases In Livestock. Animal 3 (03): 415-436.

Dehoux JP. et Hounsou-Ve G : 1992. Productivité de la race bovine Borgou en milieu traditionnel au nord-est du Bénin. Rapport d'étude n° 1. Projet Développement de l'élevage dans le Borgou-Est, Ministère du développement rural, Bénin.

Dossou J, Adote S. and Soulé H : 2006. Fiche technique de production et transformation du lait frais en fromage peulh au Bénin. Guide de Bonnes Pratiques 33 pp.

Dragana PM, Milan PP and Raycheva E: 2012. Effect of variations in somatic cell count on cheese yield on the stara planina in Serbia. IJRRAS 12 (1). July. www.arpapress.com/Volumes/Vol12Issue1/IJRRAS_12_1_09.pdf

Egounléty M : 1981. Fabrication du "Woagashi" (Fromage pâte molle) en République du Bénin. Enquête technologique FSA/UNB 50p.

Fabre JM, Morvan H, Lebreux B, Houffschmitt P. et Berthelot X : 1997. Estimation de la fréquence des différents germes responsables de mammites en France - Partie 1: mammites cliniques. 17-23.

Faye B, Dorr N, Lescourret F, Barnouin J. et Chassagne M : 1994. Les infections intramammaires chez la vache laitière dans l'enquête éco-pathologique Bretagne. *INRA Prod. Anim.*, sect.7.

Fox LK, Chester ST, Hallberg JW, Nickerson SC, Pankey JW. and Weaver LD: 1995. Survey of intramammary infections in dairy heifers at breeding age and first parturition. J. Dairy Sci. 78: 1619-1628.

Harmon RJ: 1994. Physiology of mastitis and factors affecting somatic cell counts. *J Dairy Sci* 77: 2103 - 2112.

Harmon RJ. and Reneau JK: 1993 Factors affecting somatic cell count in milk. *Proc. Natl. Mast. Counc.,* v. 33, p. 33.

Koldeweij E, Emanuelson U. and Janson L : 1999. Relation of milk production loss to milk somatic cell count. Acta Vet. Scand. 40, 47-56.

Kuzdzal-Savoie S. and Mocquot G : 1960. Observations sur les qualités organoleptiques du lait. Le lait, INRA Editions, 40 (399 400), pp.603-620.

Laevens H, Deluyker H, Schukken YH, De Meulemeester L, Vandermeersch R, De Muêlenaere E. and De Kruif A: 1997. « Influence of parity and stage of lactation on the somatic cell count in bacteriologically negative dairy cows ». *J. Dairy Sci*80 (12):3. 219-3226.

Le Roux Y. and Laurent F : 1999. Relations between somatic cell count of bulk milk and composition of milk .Renc. Rech. Ruminants,6.

Lucey JA, Kindstedt PS. and Fox PF: 1992. Seasonality: its impact on the production of good quality Mozzarella cheese. In: 3rd Cheese Symposium, National Dairy Products Research Centre, Moorepark, 28-29 Octobre 1992. 4149.

Luís CV, Ítavo GT, Vagner de Alencar A, Camila CB, Ítavo F. and Newton PR: 2001. Milk quality and subclinical mastitis detection through somatic cells counting. Maringá, v. 23, n. 4, p. 1065-1068.

M'Sadak Y, Haj Mbarek R. and Mighri L: 2016. Description and variation factors of individual cell counts of milk in of units bovins aboveground (Tunisian Sahel). J. Fundam. Appl. Sci. (Algérie) 8: 61-72.

Nickerson SC, Owens WE. and Boddie RL: 1995. Mastitis in dairy heifers: initial studies in prevalence and control. J. Dairy Sci. 78: 1607-1618.

Ogodja JO, Hounsou-Vè G. and Dehoux JP: 1991. Part I: Rôle et activité de la femme Peulh dans son ménage dans le sud Borgou au Bénin. Part II : Commercialisation du lait et des produits laitiers dans le sud Borgou en République du Bénin. Projet de Développement Pastoral intégré dans le Borgou. Parakou Bénin. 20p.

Paape MJ. and Capuco AV: 1997. Cellular defense mechanisms in the udder and lactation of goats. J. Anim. Sci. 75: 556–565.

Philipsson J, Berglund B. and Ral G: 1995. Somatic cell count as a selection criterion for mastitis resistance in dairy cattle. Livest. Prod. Sci. 41, p. 195-200.

Seegers H, Fourichon C. and Beaudeau F: 2003. Production effects related to mastitis and mastitis in dairy cattle herds economics. Vet. Res. Commun. 34: 475-491.

Rasmussen MD: 2000. A review of milking preparation: the science. 2000, in: Proceedings of the 39th National mastitis Council, Atlanta-Madison.pp. 104-110.

Ryszard S: 2006. Factors affecting somatic cell count and total microorganisms count in cow's milk. Pol.J.Food Nutr.Sci.Vol.15/56, SI 1, pp.209-2013.

Sharma N, Singh NK. and Bhadwal MS: 2011. Relationship of Somatic Cell Count and Mastitis: An Overview Asian-Aust. J. Anim. Sci. 24(3):429-438.

Sharma N, Singh NK. and Bhadwal MS: 2011. Relationship Of Somatic Cell Count And Mastitis: An Overview Asian-Aust. J. Anim. Sci. 24., 3:429-438.

Sharma N, Maiti SK. and Sharma KK: 2007. Prevalence, etiology and antibiogram of microorganisms associated with sub-clinical mastitis in buffaloes in Durg, Chhattisgarh State (India).Int. J. Dairy Sci. 2 (2): 145-151.

Waitt BM, Ylimaki LG, Jefferey LE. and Elias LG: 1991. Méthodes de bases pour l'évaluation sensorielle des aliments. CRDI Ottawa (Canada), 123p.