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CONTRIBUTION TO THE QUALITY CONTROL OF RAW MILK OF DIFFERENT BREEDS OF DAIRY COWS IN THE TLEMCEN REGION

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Abstract

Our work is geared towards analyzing the quality of the milk we consume daily. This study concerns the analysis of the physico-chemical and microbiological quality of raw milk from 3 different breeds of dairy cows: local, crossed and imported in the region of Tlemcen. The results obtained after physicochemical and microbiological analyzes of the raw milk of the three breeds prove that there are variations in their physico-chemical quality essentially the fat; the milk of the imported breed is the richest followed by the cross breed and lastly the local breed. For microbiological quality it has been found that raw milk of the imported breed contains a significant microbial load, it is above the national and international hygiene standards compared to the raw milk of the local cross breed which are the norm.

Key words: raw milk, imported breed, local breed, crossed breed, physicochemical quality, microbiological quality, Tlemcen

Introduction

Raw milk is a fragile and highly perishable food (Parekh and Subhash, 2008), it can be the source of transmission of pathogens to humans, (Addo et al., 2011). Taking into account the very high water activity and the availability of nutrients, milk serves as an ideal culture medium for the growth and multiplication of various microorganisms. Although, raw milk has germicidal activities or temporary bacteriostatic properties, the growth of microorganisms is unavoidable unless it is treated or stored (Swai and Schoonman, 2011). The main public health problems related to the consumption of raw milk include tuberculosis caused by Mycobacterium bovis and Mycobacterium. tuberculosis, and brucellosis caused by Brucellaspp. (Al-Tahiri, 2005). In some parts of the world, such as developing countries (Shirima et al., 2003), including Algeria (Adjlane-kaouche et al., 2014), milk is still a major source of infections and other food poisoning. These cases of infection still exist in developed countries. Indeed, it has been reported in England and Wales, the existence of an almost annual outbreak of food poisoning related to the consumption of raw milk caused by Salmonella and Campylobacter jejuni for milk not receiving heat treatment . In addition, S.aureus has been isolated from most unheated or slightly heated milk samples (Al-Tahiri, 2005). On the other hand, antimicrobial residues (antibiotics and other anti-bacteria) in milk cause bacterial resistance to common antibiotics (Omore et al., 2005). Our work is therefore part of this analysis of the physiochemical, hygienic and microbiological quality of raw milk locally produced in the region of Tlemcen. The raw milk that will be the subject of this research work comes from a diversified cattle herd composed of dairy cows of local breeds, imported and crossed.

Material and methods

Description of study area and farming systems adopted The wilaya of Tlemcen is made up of a diversified landscape where one meets four distinct physical units from north to south: The northern

zone consists of the mountains of Trara and Sebâa Chioukh which is a massif characterized by erosion quite remarkable and low rainfall. A set of agricultural plains, with the Maghnia Plain to the west and in the center and east a set of plains and inland plateaus called Tlemcen Basin: the low valleys of Tafna, Isser and the plateau of Ouled Riah. South of this complex, the chief town of the Wilaya is established. This complex is characterized by strong agricultural potential, a dense urban fabric, a good road network and an important industrial activity. The Tlemcen Mountains, which form part of the great chain of the Tellian Atlas that crosses Algeria from East to West, are set up as a natural barrier between the high steppe plains and the Tell. The southern zone is constituted by the high steppe plains. Since vegetation cover is the replica of climatic conditions (300 mm), shallow soils that are poor in humus are susceptible to erosion, the aquifer is an economic potential for cellulose for the production of pulp covering an area of 154 000 ha.

Climate

This geological arrangement will serve as a corridor to the sea air that will temper the severity of winters and the heat of summers. The region of Tlemcen is like an island watered in the middle of the semi-arid areas of the Moroccan Moulouya in the West, Sidi Bel Abbes and Mascara in the East and El Aricha in the South. (DSA, 2014)

Beef production systems

Livestock farming in Algeria is not a homogeneous whole (Yakhlef, 1989), so we can distinguish three main systems of cattle production: "Extensive" system: The cattle breeding by this system, is located in the mountainous regions and its feeding is based on pasture (Adamou et al., 2005). This extensive cattle production system occupies an important place in the family and national economy (Yakhlef, 1989), it also ensures 40% of the national milk production (Nedjraoui, 2003). This breeding is based on a traditional transhumance system between highland and lowland areas. It concerns local breeds and cross breeds and corresponds to the majority of the national herd (Feliachi et al., 2003). The extensive system is oriented towards meat production (78% of national production) (Nedjraoui, 2003).

"Semi intensive" system: This system is located in the east and the center of the country, in the foothills regions. It concerns crossed cattle (local with imported) (Adamou et al., 2005). This system is meat-oriented but provides a significant milk production for self-consumption and sometimes a surplus is released for sale to residents. These animals are judged mediocre in comparison with the imported genetic types and are used alone or in combination with sheep and goats, crop by-products and non-exploited areas. These farms are family-based, with small herds (Feliachi et al., 2003). Most of the animal feed comes from fallow pastures, rangelands and crop residues. As supplements, animals receive hay, straw and concentrate (Adamou et al., 2005,). The use of care and veterinary products is quite rare. (Feliachi et al., 2003). Moreover "Intensive" system: The management of this system clearly shows the mixed tendency of the farms. Indeed, young animals are in most cases kept for up to 2 years and beyond, weaning is late, artificial insemination is not a common practice and production and reproduction performance is low. Herds are generally medium to small (around 20 heads) and maintained by a family workforce. The diet is based on purchased hay and straw. A concentrated complement is regularly brought. Green fodder is rarely available because in most cattle farms, the farm does not have or has very little land (Feliachi et al., 2003). This type of system involves a large consumption of food, a large use of veterinary products as well as equipment for shelter and milking (Adamou et al., 2005).

Description of selected dairy farms

The analyzed samples are raw whole milk of small mixture (about ten females) of three periurban farms of: Beni-Mester, Sebdou and Bensakrane (figure 1). The fist farm is localed in yhe territory of the town of Beni-Mester is located in the center of the wilaya of Tlemcen, about 9 km as the crow flies west of the town of Tlemcen. The second is localed in the territory of the Daira Sebdou is located south of the wilaya of Tlemcen, about 30 km as the crow flies from the city of Tlemcen. While the third is localed in the territory of the municipality of Bensakrane is located north of the wilaya of Tlemcen, about 30 km as the crow flies east of the city of Tlemcen. At each farm there are about 10-20 cows.

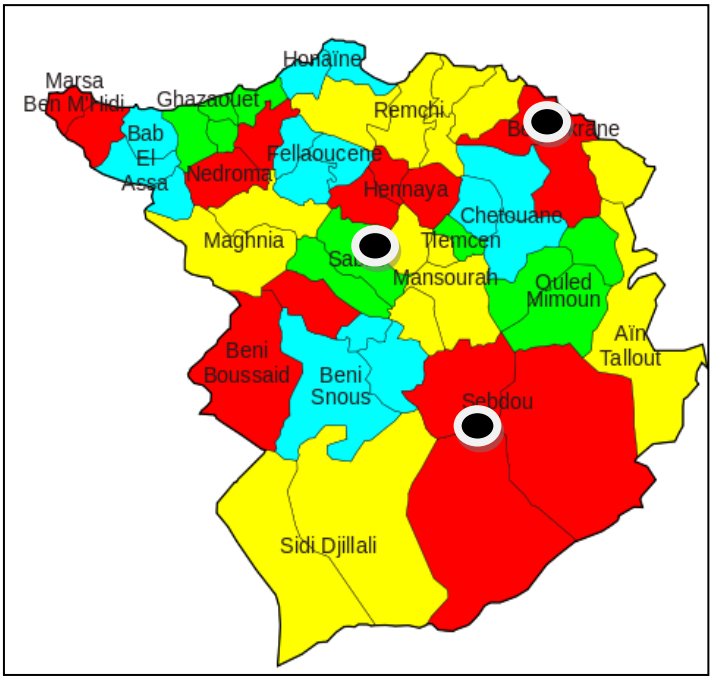

Figure 1. Map showing the sampling point at the Tlemcen wilaya.

Sampling plan for raw milk

The physicochemical and microbiological analyzes concern a total number of 15 samples with a volume of 300 ml to 500 ml, (Table 1). The samples are taken in the morning, at different times (with a gap of about 5 minutes) for better representativeness of the samples.

Table 1: Presentation of samples from the three farms.

Béni Mester farm (B.L.L)	Bensakrane farm (B.L.C)	Sebdou farm (B.L.I)
Ech 1	Ech 6	Ech 11
Ech 2	Ech 7	Ech 12
Ech 3	Ech 8	Ech 13
Ech 4	Ech 9	Ech 14
Ech 5	Ech 10	Ech 15

B.L.L: local dairy cattle; B.L.C: crossbred dairy cattle; B.L.I: imported dairy cattle; Ech: sample.

Each of the samples is a representative unit of a quantity of mixing milk (approximately 1000 to 2000 liters) intended for dairies.

The sample intended for the analyzes requires the use of a sterilized ladle which is immersed inside the tank by its upper opening. The milk samples are placed in a cooler with ice accumulators and are sent to the laboratories for analysis. The study was conducted during the period from February to March 2017, at the level of the quality control laboratory of the dairy "GIPLAIT" located at Tlemcen (head office). This dairy is contracted with 22 collectors and 350 dairy farmers. It receives about 21,000 liters of raw milk per month. It has a varied range of products based on cow's milk.

Determination of physicochemical parameters

The density is measured using a thermo-lactodensimeter calibrated so as to give (by simply reading the line corresponding to the point of touch) the density of the milk sample to be analyzed. It is reduced to 20 ° C by the following formula: Corrected density = density read + 0.2 (temperature of the milk - 20 ° C) (Mathieu, 1998). The acidity is determined by the lactic acid assay using 0.11 mol / l sodium hydroxide. The presence of phenolphthalein, as a colored indicator, indicates the limit of neutralization by color change (pale pink). This acidity is expressed in degrees Dornic (° D) where: 1 °D represents

0.1 g of lactic acid in one liter of milk (Mathieu, 1998). The principle of this method is based on the dissolution of the fat to be assayed by sulfuric acid. Under the influence of a centrifugal force and thanks to the addition of a small amount of isoamyl alcohol, the fat separates into a clear layer whose graduations of the butyrometer reveal the rate (standard AFNOR, 1980). A sample is dried on a bath of boiling water and the remaining water and subsequently evaporated in an oven at a temperature of 120 ° C (Journal Officiel, 2013).

Determination of microbiological quality

Total sprouts: These are the microorganisms able to give rise to visible colonies after 3 days of incubation at 37 ° C. on nutrient agar. The search for total germs informs us of the degree of safety of the milk. Seeding is carried out in the mass of P.C.A medium (Plat Count Agar, MERCK) at a rate of 1 ml per dish. The petri dishes thus sown are incubated in an oven set at 37 ° C. for 72 hours. All visible yellow colonies on the agar are counted. The result obtained is multiplied by the inverse of the dilution and compared with the norms.

Total coliforms: The coliform bacteria belong to the family Enterobacteriaceae. The estimation of coliforms makes it possible to assess the importance of contaminations as well as the risk of presence of pathogenic germs. Seeding is carried out in bulk on D.C.L.S. (Desoxycholate Citrate Lactose Sucrose, MERCK). The incubation is 24 h at 37 ° C for total coliforms and 44 ° C for faecal coliforms. Colonies of coliform bacteria occur as dark red colonies 0.5 mm in diameter.

Fecal Streptococci? : Streptococci are part of the family of treptococcaceae, which includes Genres very common in the food industry as contaminants and especially as lactic fermenting agents. Streptococcaceae are gram-positive, sporulated shells that are generally grouped in pairs or, especially, in variable length chains, usually immobile. They are catalase negative, some pediococci have a pseudo catalase and can appear catalase positive. The differentiation between genera is based on the arrangement of the cells and the type of lactic fermentation (homo or hetero lactic). (Guiraud and Galzy, 1980).

Rapid detection of antibiotics in milk

A quick test kit of ANTIBIO is used. This kit contains a number of ampoules containing spores of a standardized bacterium, immobilized in a solid agar culture medium, loaded with a colored indicator (Bromocresol purple). The kit of Delvotest contains all the elements to realize in a simple and fast way these tests. Milk can be tested either cold or at room temperature.

Methods of statistical analysis

SPSS software and R software were used for the statistical processing of the data of our study. The data is organized and entered in a large Excel spreadsheet, organized by communes, the farms on lines and the variables on the columns for their data processing, different analyzes are carried out (descriptive analysis, ACP, CAH). The variables selected for the analysis of milk quality are physicochemical and microbiological characteristics (Table 2).

Table 2. List and description of analyzed parameters.

Abbreviations	Description
EST	Total dry
MG	matterFat
DEN	density
AC	Acidity
CT	Total coliform
GF	Total sprouts
Strfec	Fecal streptococci
antibio	Antibiotic test

Results and discussion

Physicochemical quality

Raw milk from local cows "Beni Mester Farm" (Table 3).

Table 3. Physico-chemical analysis of local cow's milk: Beni Mester farm

	N samples	Minimum	Maximum	Mean	Standard Deviation
AC (°D)	5	16	17	16,60	0,548
MG (g/l)	5	22	23	22,60	0,548
DEN	5	1029	1029	1029,00	0
EST %	5	11,4	11,5	11,480	0,0447

The samples of raw milk from local cows at the Beni Mester farm contain on average 11.48 ± 0.0447 mg / ml total dry extract (TSE); 22.60 ± 0.548 of fat (MG) with a density (DEN) of 1029 ± 0. The acidity (AC) varies between 16 and 17 ° D (Table 3). For the density, the standards of the interministerial decree of 18/08/1993 requires 1034, which is above the values recorded for this sample. The results we obtained can be explained by adding water from the breeder. Regarding fat, national and international standards require 34g / l. Out of the five samples analyzed, the fat measurement revealed the poor nutritional quality of the milk, given that the recorded rate was 23 g / l. This drop in fat in the milk of local cows is mainly due to the quality and quantity of the feed given to the animal (extensive farming method). The stage of lactation can also influence the fat content. The lowest rates are during the second and third months of lactation and higher at the beginning but especially at the end of lactation. For titratable acidity the regulation followed requires a value between 16 and 18D°. The values obtained in the 5 samples vary between 16 and 17 and comply with the standard. This can be explained by the fact that the collection of milk is done under conditions of proper hygiene. The total dry matter measurements for the 5 raw milk samples from local cows are below the standard of 13% (FAO, 1995). These values vary between 11.4 and 11.5% this can be explained by the decrease in fat which is probably due to a poor diet.

Raw milk from crossed cows "Bensakrane Farm", (Table 4).

Table 4: Physicochemical analysis of milk from crossed cows: Bensekrane farm

	N samples	Minimum	Maximum	Mean	Standard Deviation
AC (°D)	5	17	18	17,40	0,548
MG (g/l)	5	27	28	27,80	0,447
DEN	5	1032	1032	1032,00	0
EST %	5	11,9	11,9	11,900	0

The raw milk samples from the crossed cows of the Bensakrane farm contain on average 11.9 mg / ml total dry extract (TSE); 27.80 g / l of fat (MG) with a density (DEN) of 1032. The acidity (AC) varies between 17 and 18 ° (Table 4). The fat and the density are the standards set by the interministerial decree of 18/08/1993. The value of total dry matter in crossbred milk is close to norms (13%) (FAO, 1990).

c. Raw milk of cows imported "Sebdou's farm", (Table 5).

Table 5: physicochemical analysis of milk from imported cows: Sebdou farm

	N samples	Minimum	Maximum	Mean	Standard Deviation
AC (°D)	5	17	18	17,40	0,548
MG (g/l)	5	34	34	34,00	0
DEN	5	1032	1032	1032,00	0
EST %	5	12,3 0	12,3	12,300	0

Samples of raw milk from cows imported at Sebdou farm contain an average of 12.3 mg / ml total dry extract (TSE); 27.80 fat (MG) with a density (DEN) of 1032. The acidity (AC) varies between 17 and

18 ° (Table 5). The milk of this type of farm shows a good quality, these values can be explained by the fact that these cows are subjected to a rich diet (intensive farming mode).

Microbiological quality

Raw milk from local cows "Beni Mester Farm", (Table 6).

Table 6: Microbiological analysis of local cow's milk: Beni Mester farm

	N samples	Minimum	Maximum	Mean	Standard Deviation
GT	5	2000	4000	2800,00	836,660
Col	5	7	8	7,20	0,447
Strfec	5	0	0	0	0
antibio	5	0	0	0	0

The number of total sprouts obtained for the 5 samples of raw milk from local cows varies between 2000 and 4000 for an average of 2800 ± 836.66 CFU / ml. This number is below the threshold set in the official journal, which is of the order of 105 CFU / ml, (JORA, 1998). They are also lower than the maximum tolerated loads by the two French and American regulations which are respectively 5.105 CFU / ml and 3.105 CFU / ml (Alais, 1984). The total coliform analyzes of the raw milk of local cows is also below the threshold set in the official journal, which is of the order of 2,106 CFU / ml; this varies between 7 and 8 for an average of 7.20 ± 0.447 CFU / ml. All samples have a lower than standard load (JORA, 1998). These germs are indicators of faecal contaminations, and an index of unhygienic manipulations. The absence of faecal streptococci in all samples of raw milk from local cows. According to the Interministerial Order No. 35 of 27 May 1998, fixing the microbiological criteria of certain foodstuffs, faecal streptococci in raw milk must be absent. This is indicative of proper hygienic handling during milking. No antibiotics (ANTIBIO) were detected in the milk of local cows, which complies with the standard (JORA, 1998). The correct use of ANTIBIO by farmers and veterinarians as well as the respect of waiting times after the treatment of animals lead to the absence of residues of ANTIBIO in milk and other animal products (Aning, 2007).

Raw milk from crossed cows "Bensakrane Farm", (Table 7).

Table 7: Microbiological analyzes of milk from crossed cows: Bensakrane Farm

	N samples	Minimum	Maximum	Mean	Standard Deviation
GT	5	20000	22000	20600,00	894,427
Col	5	30	40	36,00	5,477
Strfec	5	0	0	0	0
antibio	5	0	0	0	0

The number of total sprouts obtained for the 5 samples of raw milk from the crossed cows varies between 20,000 and 22,000 for an average of 20600 ± 894.427 CFU / ml. This number is lower than the threshold set in the official journal, of the order of 105 CFU / ml. The total coliform analyzes of the raw milk of the crossed cows are below the threshold set in the official journal, which is 2.106 CFU / ml; this varies between 30 and 40 for an average of 36 ± 477 CFU / ml. This total coliform concentration is greater than that of local cow's milk. According to Larpent (1990), the presence of coliforms is not necessarily a direct indication of faecal contamination. Some coliforms are in fact present in the wet residues encountered in dairy equipment. Fecal streptococci are absent in all samples of raw milk from crossbred cows. A complete absence of antibiotics in crossbred milk has also been noted, which is consistent with the standard (JORA 1998).

Raw milk from imported cows "Sebdou's farm", (Table 8).

Table 8: Microbiological analyzes of milk from imported cows: Sebdou farm.

	N samples	Minimum	Maximum	Mean	Standard Deviation
GT	5	270000	290000	282000,00	8366,600
Col	5	2300	2500	2420,00	83,666
Strfec	5	0	0	0	0
antibio	5	0	0	0	0

The aerobic mesophilic flora always informs us about the hygienic quality of raw milk, it is considered as the factor determining the shelf life of fresh milk (Guinot-Thomas et al, 1995). It is the most sought-after flora in microbiological analyzes. The enumeration of this flora for the 5 samples of raw milk has shown that there is a significant contamination of the raw milk. Indeed, we noted that all the analyzed samples have a total flora which is between 270000 and 290000 CFU / ml, an average of 282000 ± 8366.6 CFU / ml. These values exceed the critical threshold of alteration of milk fixed according to some authors to 105 CFU / ml. According to Ameur et al., (2011) in Algeria, raw milk collected has a very high microbial contamination rate (between 105 and 107 CFU / ml), which is detrimental both to the transformation in the dairy industry and to health. public. Our samples are of poor quality in view of the Algerian standards that set the threshold of contamination at 105 CFU / ml, despite relatively low temperatures during the study period. They reveal a lack of respect for good production practices and the storage of evening milking milk which will then be mixed with milking milk the next morning, as well as at the level of multitudes of transfers (Amhouri, 1998). The results show a high count of total coliforms of 2420 ± 83.666 CFU / ml. This high load is observed for all samples with a count of between 2300 and 2500 CFU / ml. According to Larpent (1990), the presence of coliforms is not necessarily a direct indication of faecal contamination. Some coliforms are in fact present in the wet residues encountered in dairy equipment. According to Magnusson et al. (2007), heavily soiled litters contain more coliforms and the prevalence of mastitis, in this case, increases, suggesting teat and milk contamination. Other sources of contamination are also to be considered such as poor transport conditions and lack of hygiene during milking. The analysis of milk samples from imported cows shows a total absence of faecal streptococci which is in line with the standards of the official newspaper of the Algerian Republic. In the analyzes, there was a total absence of antibiotics in imported cow's milk, which is in line with the standard (JORA 1998).

Principal Component Analysis (PCA)

Physicochemical parameters

The PCA carried out on 15 milk samples from 3 farms of 3 cattle breeds (local, crossed and imported) represents 95.10% of the total variability (Figure 2). The first axis, which explains 80.10% of the total variation, generally represents milks possessing a density, fat and total dry matter which approaches the standards (with exceptions for some milk samples belonging especially to the local breed). The second axis, which accounts for 15.56% of the total variation, represents milks with acidities complying with the standards (but with maximum values). It is noted at the PCA that the density, fat, and total solids parameters represent a first group (have a strong positive correlation between them), which suggests that they are probably controlled by the same genes. The acidity parameter represents another group that has a low positive correlation with the first group.

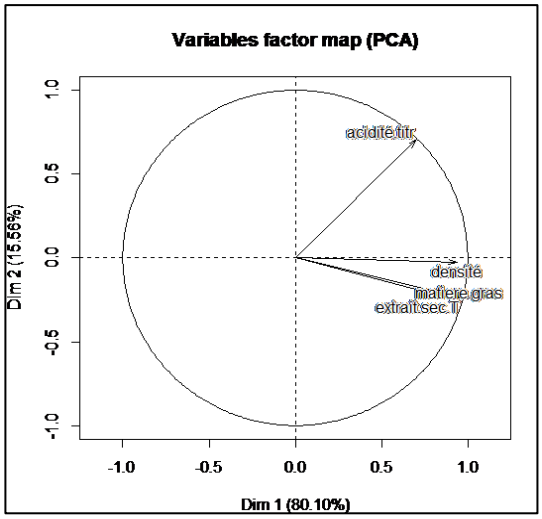

Figure 2: Correlation circle: Representing the active physicochemical variables in the foreground of the PCA.

From Figure 3 it is clear that the milk sampled at the farm level, which practices extensive rearing on the local breed, is very specific to that of the other two types of farms which breed semi-breeders. extensive and intensive on cross-breed and imported breeds.

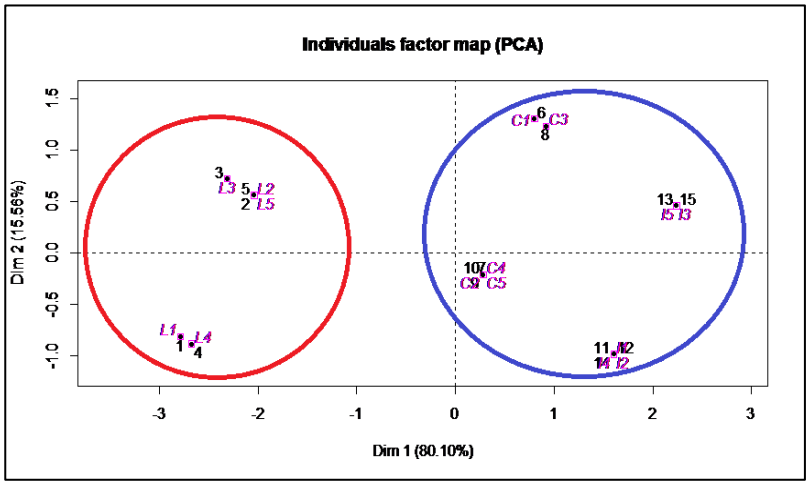

Figure 3: Presentation of the distribution by PCA in the studied bovine population Physicochemical parameters.

Analysis of microbiological parameters

The PCA carried out on the 15 Samples of milks coming from the 3 farms of 3 cattle breeds (local, crossed and imported), makes it possible to release two main axes of variation which forms the foreground by relating almost 100% of the total variability (figure 4). Since the two parameters, Strfec and antibio have no variability they are therefore unusable at the level of a PCA. From Figure 4 there is a significant positive correlation between GT and Col.

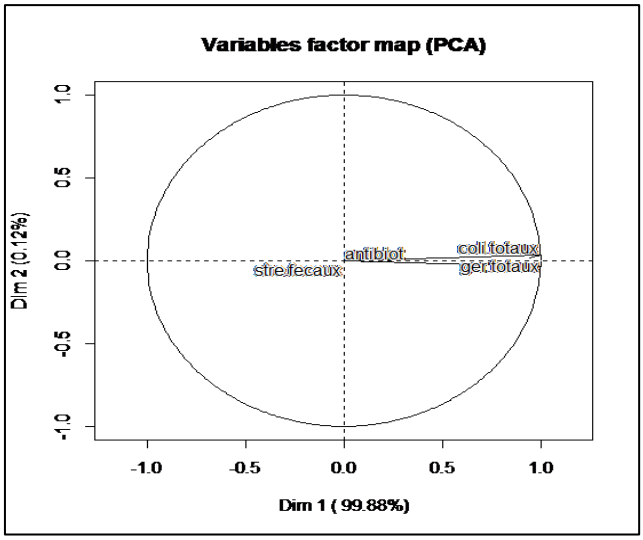

Figure 4. Correlation circle: Representing the active Microbiological variables on the foreground of the PCA.

With regard to microbiological parameters, it can be seen from Figure 5 that milk samples sampled from farms that practice extensive and semi-intensive rearing respectively on local and cross breeds are grouped together and therefore show very similar and different profiles about milk samples from the farm which practice intensively farmed.

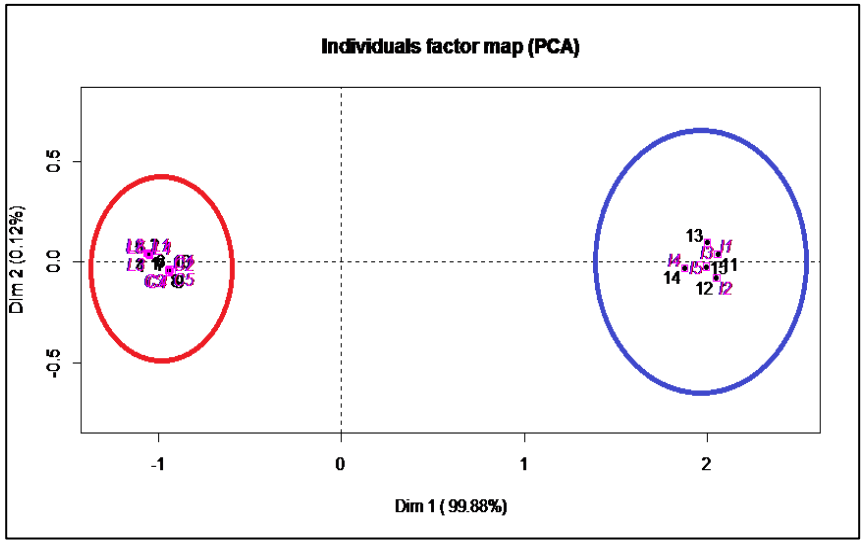

Figure 5. Presentation of the distribution by PCR in the bovine population studied for microbiological parameters.

Hierarchical ascending classification (HCA): The HAC in Figure 6 confirms what was reported by the PCA in Figure 3.

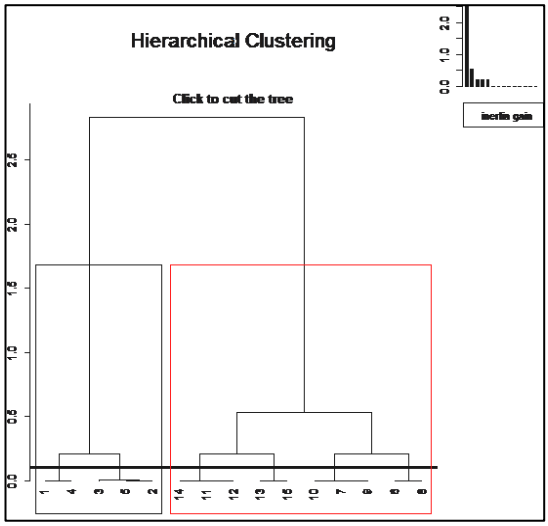

Figure 6. Ascending classification hierarchical physico-chemical parameters (HCA)

Table 9 reports the physicochemical characteristics of the milk of the two classes identified by the statistical tests.

Table 9. Characterization of the two classes of milk (physico-chemical parameters)

Class	1 (local)	2 (crosed +imported)
Samples	5	10
AC	16.5	17.5
MG	22.5	30.5
DE	1029	1032
EST	11.45	12.1

The HCA in Figure 7 confirms what was reported by the PCA in Figure 5. The Table 10 reports the microbiological characteristics of the milk of the two classes identified by the statistical tests.

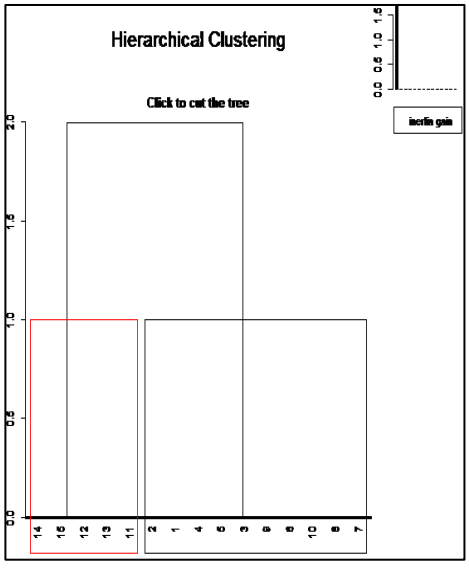

Figure 7: Hierarchical ascending classification for microbiological values (HCA)

Table 10: Characterization of the two classes of milk (microbiological parameters)

Class	1 (imported)	2 (crossed +local)
Samples	5	10
Total sprouts	280000	12000
Coliform Totals	2400	23.5
Strept Feces	0	0
Antibiotic	0	0

Conclusion

The validity of consumption of raw milk is based essentially on its bacteriological and physicochemical quality, to consume this product under acceptable conditions, it is important to ensure a good technical delivery from milking to the stage of the finished product. Indeed, we analyzed the physicochemical and microbiological parameters of milk produced by cows belonging to 3 types of cattle known in Algeria; local dairy cattle (BLL) and imported dairy cattle (BLL) and crossbred dairy cattle (BLC). Our study on raw milk has allowed us to draw some remarks. Regarding density, fat content, titratable acidity and dry extract. It was noted that the majority of samples taken from different breeds met the standards. With regard to total germs, total coliforms, faecal streptococci and antibiotics, it has been observed that the milk of imported cows contains a high microbial load above national and international standards. This may be due to the fact that the rearing conditions are non-compliant or that these animals are resistant to the local microbial agent.

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