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CONTRIBUTION TO THE STUDY OF TWO LOCAL BOVINE BREEDS IN WILAYA OF TLEMCEN: MORPHOMETRIC CHARACTERIZATION AND DNA BIOBANK.

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Abstract

This work is carried out a study of the typology of local cattle breeding in the Wilaya of Tlemcen, a phenotypic characterization of two local breeds Guelmoise and Tlemcenienne with a preparation of biobank of DNA concerning the latter and relating to 40 blood samples from unrelated animals. The results of the typology (supported by a questionnaire) showed that there is an leaving of the breeding of local breeds in favor of imported breeds are that the local breed is raised exclusively in e extensive system and conditions breeding are really precarious. Quantitative measures (age, chest circumference, height at withers, sacral height, diagonal length, length of head, length of ears, length of horns, length of tail, milk yield) and qualitative (color of the dress, regions) submitted to statistical analyzes show that the two breeds are almost identical, and that there are introductions of exogenous breeds especially in the Tlemcenienne breed, this was confirmed when calculating the diversity index, which showed that the Tlemcenienne breed is more diversified (H '= 0.31) given the exchanges practiced by breeders and the anarchic crossbreeding of animals, while that of Guelmoise equal to H' = 0.28. This very important preliminary work deserves to be pursued by a molecular characterization of more populations and a wider geographical area.

Keywords: local cattle, Tlemcenienne breed, Guelmoise breed, DNA biobank, morphometric measurements, typology of the breeding.

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Introduction

Livestock represents all the operations that allow the reproduction and the life of the animals for the needs of the man. Providing a shelter, administering care, meeting food needs are all obligations for breeders vis-a-vis their animals. (INRA, 2013). The birth of livestock is contemporaneous with an important turning point in human history: the transition from hunter- gatherer status to that of farmer-breeder. This period, which today we call "the Neolithic Revolution" has been spread over millennia (from 14 000 BC. BC to 7000 BC. J.-C.) (INRA, 2013)

At that time, the man has stopped living from hunting and gathering by moving towards the establishment of domestication and breeding. He intuitively influenced natural selection by choosing the best performing animals. Subsequently, domesticated animals followed the man in his great

migrations; with as a first consequence a relative genetic distance between not only the domestic species and the wild species, but also between the different domestic species (Moula, 2012) as well than between breeds of the same domestic species. Demand for livestock products in sub-Saharan Africa is growing rapidly. The current upward trend in demand is not accompanied by a similar increase in local production. Several African governments, as well as regional organizations, are currently working on how to better ensure that their farmers can contribute to better availability of high quality livestock products, thus reducing the need to depend on increased imports. . At the same time, governments are increasingly aware that if increased production of livestock products is not followed closely, there will be negative consequences, including increased pressure. Natural resources (including water and land), greenhouse gas emissions, and zoonotic disease risks (Herrero, 2014).

Crossbreeding with exotic animals is also widely practiced to increase production levels. If, as is often the case, these marks are indiscriminate, they may represent a major threat to local breeds. (FAO, 2006). A breed that disappears is an irreversible phenomenon. It is a heritage that is at once genetic, cultural and economic lost forever (Coutard, 2002).

Algeria has the resources needed to face the challenge of sustainable agricultural development. However, controlling this challenge requires sensible, efficient and coordinated management of these resources. In the livestock sector, as in other agricultural sectors, the concern for sustainability and efficiency leads to the prioritization of local resources and capacities as a basis for rural development.nThe knowledge and preservation of local breeds of production animals is therefore crucial in this respect, as only the latter have the qualities of adaptation and appropriation necessary for successful breeding projects. Often poorly known and poorly described in the literature, these local breeds are today largely threatened by changing patterns of production. Their disappearance would be a disaster, by the loss of characters ignored today and potentially useful tomorrow. (Moula, 2012).

The domestication of the Aurochs, the ancestor of most domestic cattle, including the bull, began around 9000 years ago in the Near East and Pakistan. (INRA, 2013). He is considered to be at the origin of the brown race of the atlas spread throughout the Maghreb (Encyclopaedia Universalis, 1990).

In Algeria, the composition of the herd has changed considerably with the introduction, since 1970, of the Pie-Noire, Pie-Rouge and Tarentaise breeds. The crossbreeding, often anarchic, as well as artificial insemination based on imported semen have greatly reduced the blood of local breeds "brown atlas" which only survive in pure breeds in marginal areas (mountains, cattle breeding in extensive) (Abdelguerfi and Bedrani, 1997).

The aim of this work is to describe the characteristics of local cattle breeding in the Tlemcen wilaya and to identify within breed phenotypic differences as well as differences with the local Guelmoise breed. This step is very important to carry out before any procedure of management and genetic improvement.

Materials and methods :

Two cattle breeds the Tlemcenienne and the Guelmoise breed are being studied to make a morphological comparison. The equipment used for this experiment is a tape measure for taking measurements and many parameters are studied such as : Age, Length of the ears (LO); Chest size (TP); Diagonal length (LD) ; Height at withers (HG); Sacral height (HS); Length of the head (LT); Length of the tail (LQ); Horn length (LC); Milk production (PL). (**Figure 1**)

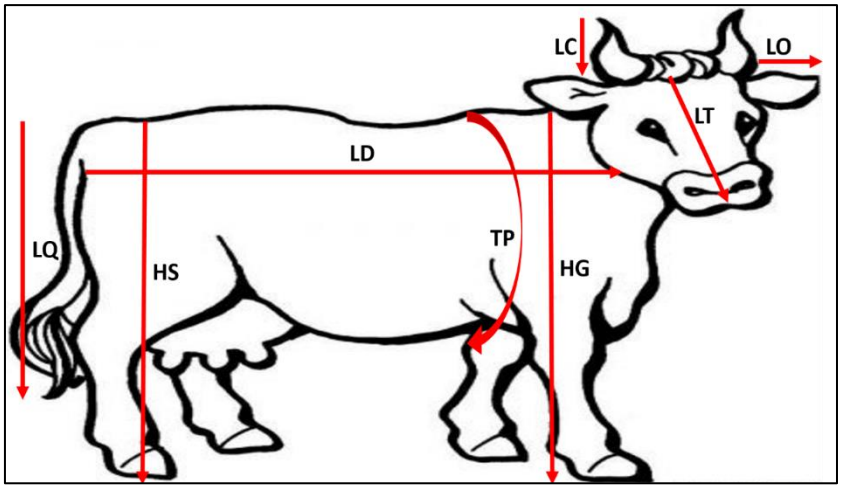

Figure 1. Schematic representation of the different measures taken in animals.

Statistical analysis:

The data collected were the subject of statistical analyzes (comparison of means, principal component analysis " ACP », Hierarchy tree "CAH"), Using a statistical program that is R. thus Software as calculating the diversity index of Shannon-Weaver via the Excel program.

Result and interpretation :

Comparison of averages :

Table 1. presents the mean and standard deviation of each quantitative variable measured with the results of the comparison of means with Student's t-test for a level of significance less than 0.05.

We note that the p-value is greater than 0.05, it cannot be said that there is a statistically significant difference HG characters, PL, PT, between Tlemcenienne and Guelmoise breed. This is probably because both breed belong to the same ancestor (brown of the atlas). For the LD character, we notice that there is a highly significant difference p <0.001 between the two breeds, this difference is probably because the Tlemcenienne breed undergoes a lot of anarchic crossing with exogenous breeds.

Table 1. Mean comparison results for body measurements: mean and standard s- kinds of local cattle in the province of Tlemcen.

	Tlemcenienne breed	Guelmoise breed	p-value
	Mean±SD	Mean±SD	
Age	5.46 ± 3.01	6.44 ± 2.09	> 0.0707
HG	119.90 ± 11.73	115.92 ± 7.93	> 0.0561
LD	138.66 ± 20.69	117.94 ± 8.19	<6.396e-09
PL	6.44 ± 2.40	6.84 ± 2.16	> 0.4077
TP	154.46 ± 14.52	152.78 ± 8.96	> 0.4981

Principal Component Analysis (PCA) :

a. Guelmoise breed

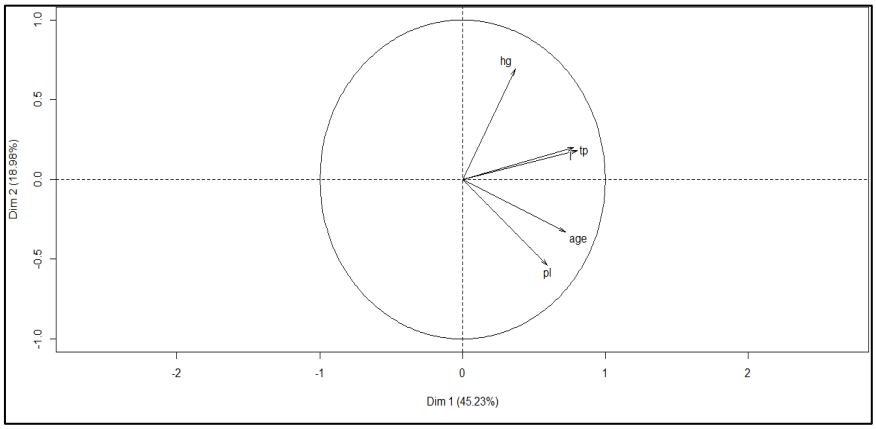

Figure 2: graphical representation of the PCA of the variables of the Guelmoise breed.

The accumulated share of information returned on the two axes is 64.21%, this will provide a good representation of the relationship between all data. The projections on the one component (x-axis) show that variable Age, PL, PT and LD positively correlated. The projections on component 2 show that HG correlates positively with the axis.

b. Tlemcenienne breed

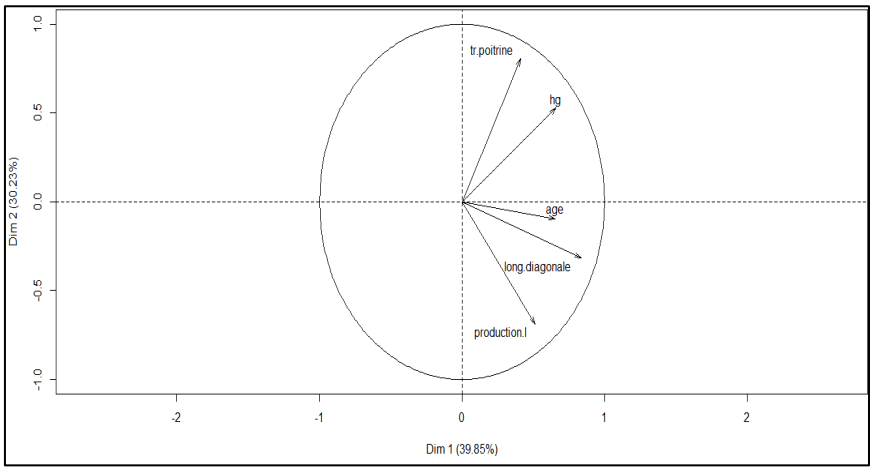

Figure 3: graphical representation of the PCA of the variables of the Tlemcenienne breed.

The accumulated share of information restored on the two axes is 70.08%, this allows to have a good representation of the relationship between all the data. The projections on the component 1 (x-axis) show that the variables: age, LD, and TP correlate positively. Projections on component 2 show that TP correlates positively with the axis and PL correlates negatively with the axis. The variable age and girth variable as an orthogonal angle, which leads us to say that the two variables are not correlated, this is due to the fact that the measured animals arrived at adulthood.

Figure 2 and 3 show that the two ACPs do not share the same correlations between variables, which means that the characters are either genetically or environmentally influenced, and since the local breed generally lives in the same environment (mountainous regions, system of breeding in an extensive environment, food based mainly on fodder) so the difference between the two breeds is probably due to genetics.

c. ***Both Guelmoise and Tlemcenienne breeds:***

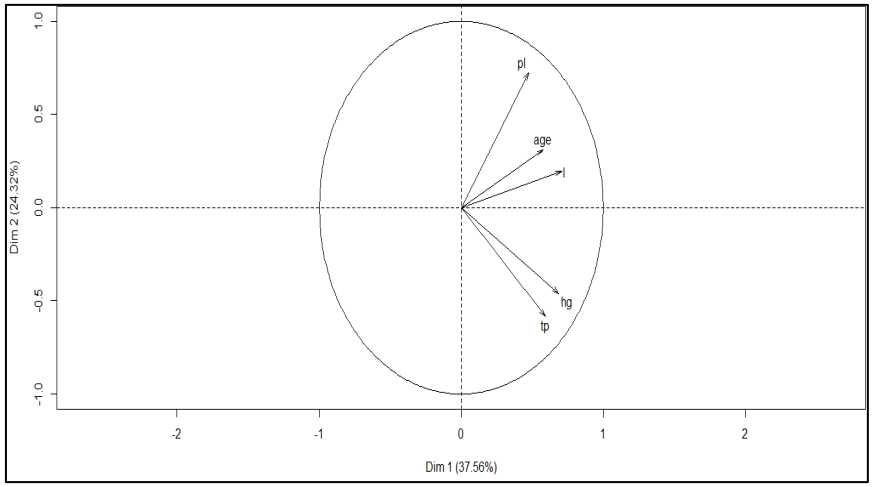

Figure 4: graphical representation of the PCA of the variables (circle of correlation) of the two breeds Tlemcenienne and Guelmoise .

The cumulative share of information retrieved on both axes is 61.88%, this will provide a good representation of the relationship between all data. Projections on component 1 (x-axis) show that the variables Age, HG, TP and LD correlate positively and its length increase, ditto for the bust. Projections on component 2 show that PL correlates positively with the axis.

ACP of individual's observations:

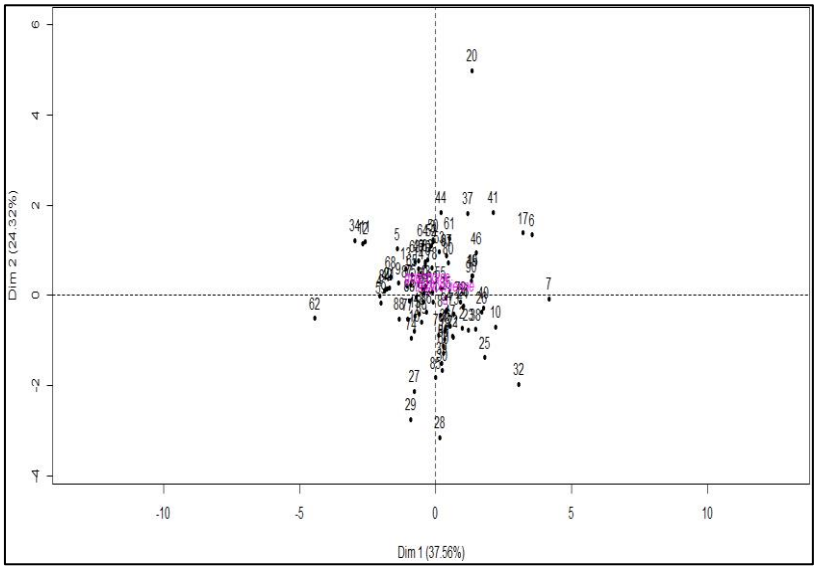

Figure 5. graphical representation of the ACP of the individuals of the two breeds Tlemcenienne and Guelmoise

Individuals form a cloud of points that are closer to the center, making PCA difficult to interpret, this can be explained by the fact that individuals are phenotypically similar, but there are some exceptions for individuals such as the individual. (Belongs to the Tlemcenienne breed) and the individual 62 (belongs to the Guelmoise breed) which are very far phenotypically, with respect to the three variables HG, TP and LD (according to the correlation circle). Nevertheless, The individual 20 is different from the others probably by the milk production (according to the previous correlation circle), that is to say that despite its significant age which is 12 years, the animal can still produce up to 14L / D, this difference is likely to genetics factors.

a. According to the color of the dress "Tlemcenienne breed":

i. (ACP variables):

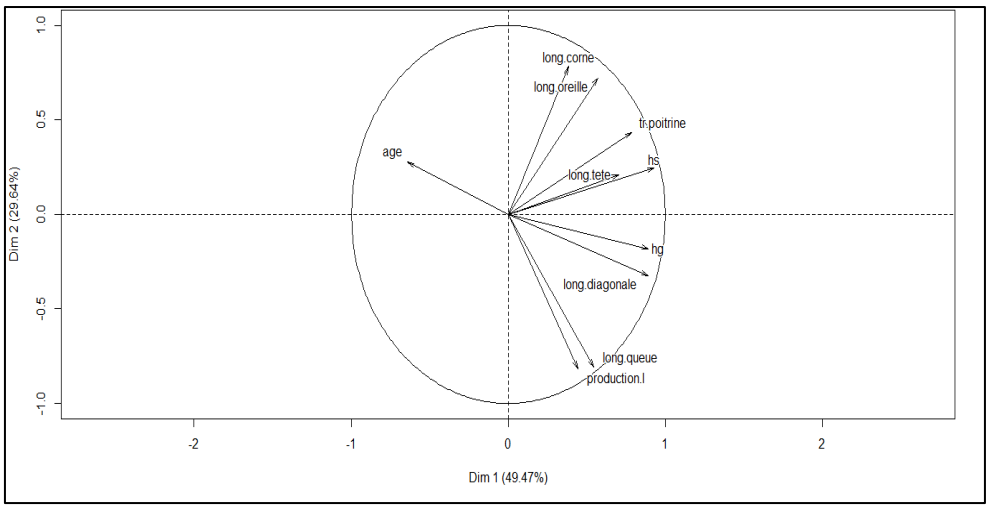

Figure 6: graphical representation of the PCA of the variables of the Tlemcenienne breed according to its color of the dress.

The x-axis represents 49.47% of the information and the ordinate 29.64%, so the graphs represent 79.11%.

The projections on axis 1 show that the variables HG, LD, HS are strongly correlated, that the variables TP, LT are also positively correlated. The projections on axis 2 show that the variables LQ, PL are strongly correlated, and that the LD, LO are also positively correlated. The factor Age negatively correlates with the axis 1, and according to the figure (6) it correlates negatively with the diagonal length, theoretically, this is false, and this is probably due to the fact that when grouping individuals by the color of the dress, the averaging of the age of each color cannot be interpreted theoretically.

ii. ACP of individuals :

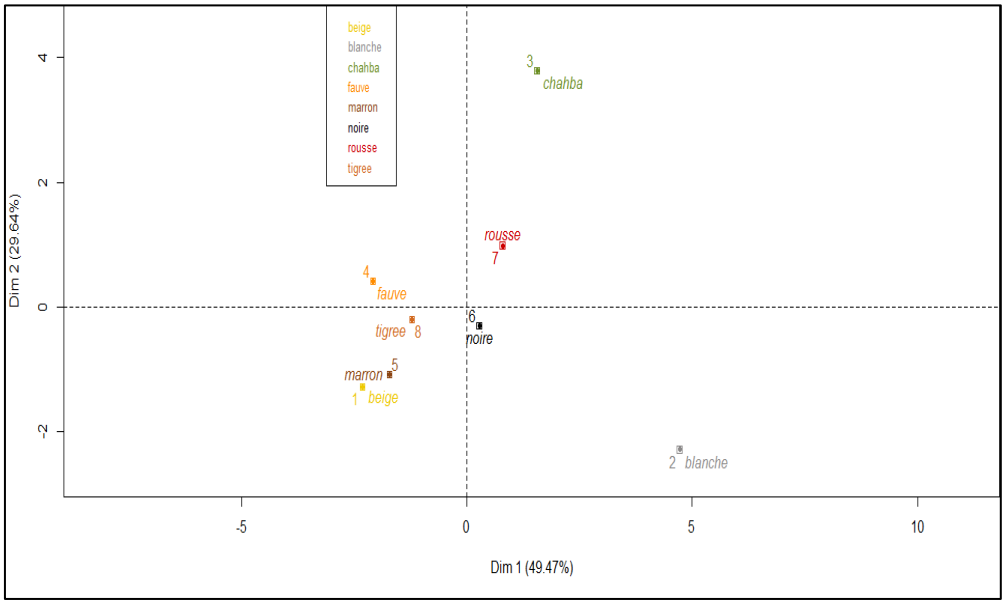

Figure 7. Graphical representation of the ACP of individuals of the Tlemcenienne breed according to its color of the dress.

Individuals beige, tan, brown, black, red, tiger get too close to the center which makes interpretation difficult, but it pushes us just say that this is probably û of the fact that individuals are close on the phenotypic plane (so they respond in the same way to the effect of the environment). For the white color, it correlates positively with the axis 1 and it differs with the other individuals by the characters diagonal length, height at the withers and sacral height.

This difference is probably due to the fact that individuals with a white dress color and chahba have few repetitions.

a. According to the sampling region "Tlemcenienne breed":

i. ACP variables

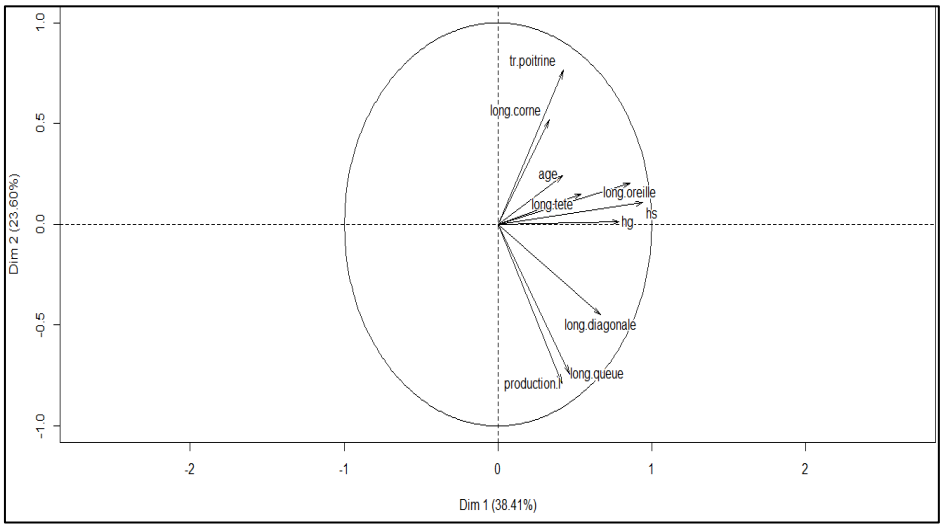

Figure 8. graphical representation of the PCA of the variables of the Tlemcenienne breed in relation to the regions.

The cumulative share of information is 62.01%; this will provide a good representation of the relationship between the data set. The projections on axis 1 show that the length of the ears, the height at the withers, and the sacral height are highly correlated are they are also correlated with the diagonal length and the length of the head. This is obvious since the length of the animal increases, the height at the withers and the sacral height increase as well as the length of the head. Axis 2 projections show that chest circumference and horn length correlate positively, and that milk production and tail length are strongly correlated as well.

ii. ***ACP of individuals***

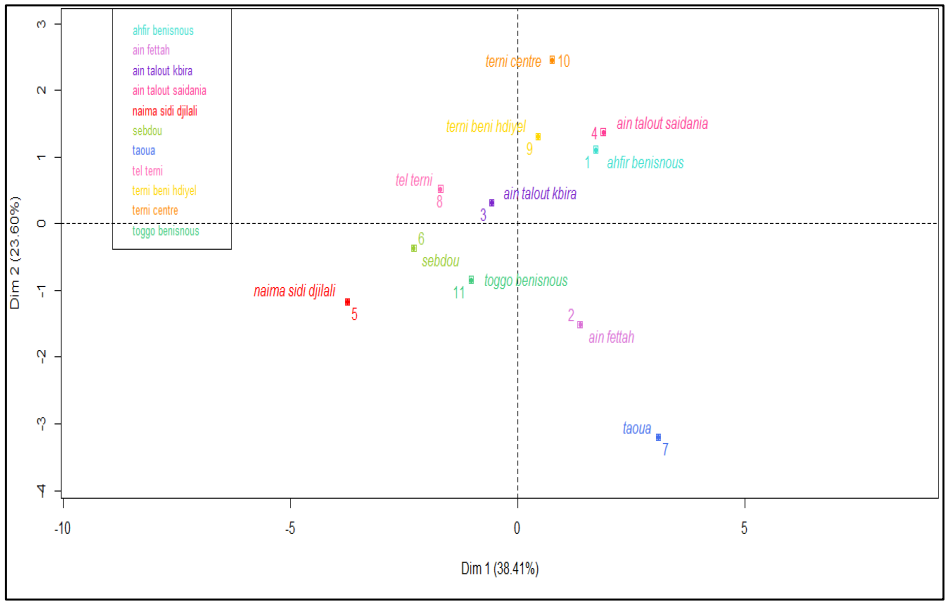

Figure 9. Graphical representation of the PCA of individuals of the Tlemcenienne breed in relation to the regions.

Individuals belonging to ahfir regions benisnous , ain fettah, ain Talout kbira , ain Talout saidania, sebas, tel terni, terni beni hdiyel are too close to the center which makes interpretation difficult, this is probably due to the fact that the individuals in these regions are similar because the breeders of the different regions exchange animals with each other. The projections on the axis 1 show that individuals of Naima Sidi Djilali region correlates positively with the shaft and those of the latter differs other individuals by Height at the withers, their sacral height and length of ears as well as their diagonal length and head length. The projections on axis 2 show that individuals in the Taoua region differ from other individuals in their milk production.

The hierarchy tree (CAH)

i. both breeds:

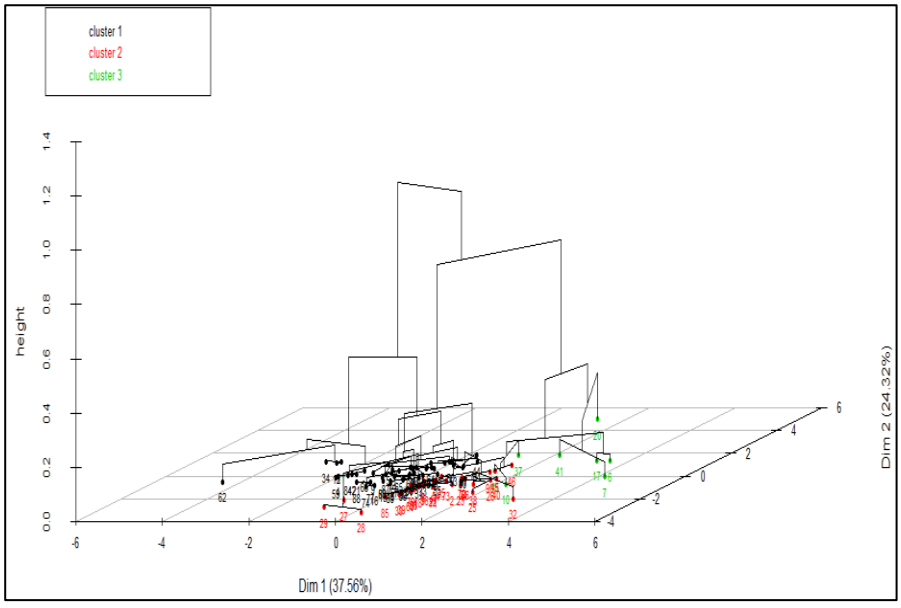

Figure 10. Graphical representation of the tree of hierarchy (CAH) of the individuals of the two breeds Tlemcenienne and Guelmoise.

Class 3 individuals all belong to the Tlemcenienne breed, and to the Taoua and Ain Fettah regions, Class 1 and 2 individuals belong to the Tlemcenienne breed and the Guelmoise breed, with a low distance this confirms the fact that the individuals have a similarity between them (they are not phenotypically distant and therefore are not also genotypically), unlike with class 3 which move away from the two groups by a greater distance, this is probably due to the fact that the individuals are phenotypically and therefore genetically distant, this can be explained by the anarchic crossings practiced in the study areas (the individuals probably belong to cross breeds and not local)

i. Tlemcenienne breed according to the color of the dress

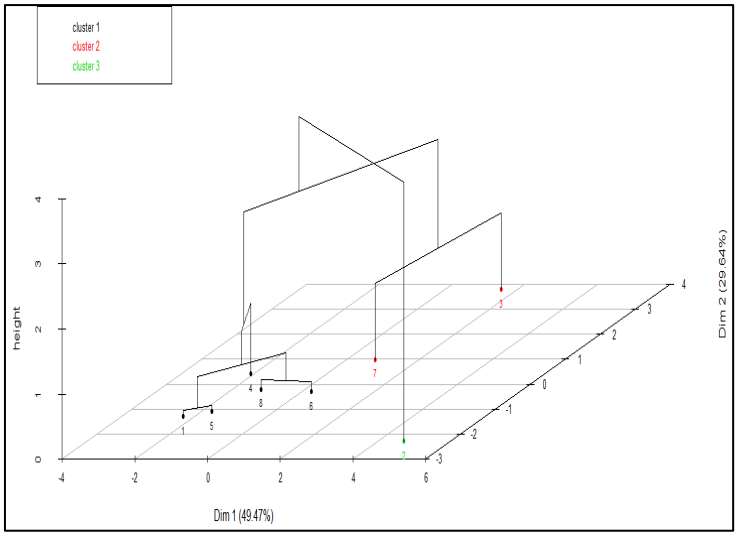

Figure 11*:* graphical representation of the tree of hierarchy (CAH) of the individuals of the Tlemcenienne breed according to the color of the dress.

Figure (11) shows the distance between class 3 and the other two classes (1 and 2), class 2 containing only white individuals confirms the result found in the PCA of figure (7).

ii. Tlemcenienne breed according to the region of sampling

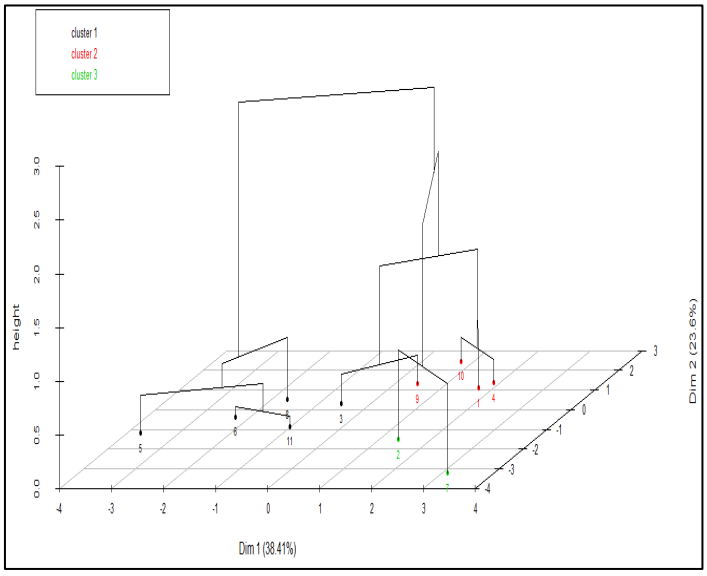

Figure 12. Graphical representation of the tree of hierarchy (CAH) of the individuals of the breed Tlemcenienne according to the regions.

Figure (12) shows that Class 3 individuals are distant from individuals in the other classes (1 and 2), belong to the Taoua and Ain fettah regions, this result confirms that individuals from these regions are probably cross breeds. For class 2, individuals belong to nearby regions, this explains the closeness of individuals, this rapprochement is probably due to the fact that the breeders exchange their herd between them. Same for class 3.

The Shannon-Weaver Diversity Index:

Table (4) represents the fairness of the Shannon-Weaver diversity index (this is the ratio between the calculated diversity of each character H 'and the maximum diversity (Hmax), it varies between 0 and 1.

The fairness varies from 0.24 to 0.36 in the breed Tlemcenienne, and from 0.22 to 0.34 in the Guelmoise breed, from "milk production" character trait "chest" This is probably explained that the genes that control milk production are responsible for the important physiological characters eg heart function, unlike the chest character is probably controlled by genes that do not show significant physiological function for the body.

In Tlemcenienne breed, the characters have a greater diversity (H '= 0.31) than those of Guelmoise breed (H = 0.28), this is probably due to the fact that Tlemceniennes breeds undergo uncontrolled intersections and the important exchange of animals between breeders.

Table 2. Results of the Shannon-Weaver diversity index equitability calculation of the different body measurements of the local Tlemcenienne and Guelmoise cattle.

	Tlemcenienne breed	Guelmoise breed
PL	0,24	0,22
HG	0,33	0,32
TP	0,36	0,34
LD	0,30	0,26
Moyenne	0,31	0,28

2. DNA extraction protocol

DNA extraction was performed for subsequent genetic characterization.

i. Sampling strategy:

The samples were made from the tail vein of 40 females with different ages, avoiding the consanguineous ones as much as possible.

ii. Blood sampling

The local cattle is difficult to control, it is necessary to ensure a restraint of the animal, putting his fingers in his nostrils. Despite this, the blood test requires the help of breeders to maintain the animal. We used sterile vacuum tubes with a pierceable stopper containing ethylene-Di-Amine-Tetraacetic acid (EDTA), which is an anticoagulant for blood. During collection, the needle is inserted into the tail vein of the animal, the blood is stored on site in a cooler, then at -4 ° C in the freezer.

The extraction of nucleic acids from a biological material requires cell lysis, inactivation of cellular nucleases, and separation of the desired nucleic acid from cellular debris. The ideal lysis procedure is often a compromise of techniques and must be sufficiently rigorous to break up the complex starting material (e.g., tissue), but soft enough to preserve the target nucleic acid. Common lysis procedures are as follows:

- mechanical failure (eg grinding or hypotonic lysis),
- chemical treatment (eg detergent lysis, chaotropic agents, reduction of thiols)
- and enzymatic digestion (eg proteinase K).

Membrane disruption and inactivation of intracellular nucleases can be combined. For example, a simple solution may contain detergents for solubilizing cell membranes and powerful chaotropic salts to inactivate intracellular enzymes. After cell lysis and inactivation of nuclease, cell debris can be easily removed by filtration or precipitation. (Somma, 2004). The extraction was carried out at the research laboratory of physiology, physiopathology and biochemistry of nutrition (PpBioNut) of the University of Tlemcen Abou bekr Belkaid

iii. NACL technique for DNA extraction

After thawing the samples at room temperature, the blood from each tube is transferred into conical tubes, the TE10 / 10 buffer is added to each tube to a final volume of 30 ml. After homogenization, the tubes are put in ice for 30 min, which will cause thermal shock and bursting of the cells, followed by centrifugation at 2500 rpm for 15 min, the supernatant is removed and the TE10 / 10 is added. pellet obtained up to 30ml volume, for maximum elimination of red blood cells.

A second centrifugation is performed, followed by removal of the supernatant. The whitish pellet obtained corresponds to white blood cells. For the bursting of the white blood cells, 1500 µl of a lysis solution are added; the SLB which consists of the tris / HCl of EDTA and the SDS to the obtained pellet. The SDS (Sodium Dodecyl Sulfate) contained in this solution has the role of solubilizing the lipids of plasma membranes in order to structure the latter, inhibit nucleases and denature proteins.

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Subsequently, vigorous stirring and centrifugation at 4000 rpm for 15 minutes, the supernatant thus formed containing the DNA is then recovered by transferring it to another tube, avoiding peeling off the pellet. Two volumes of absolute ethanol cold from that of the supernatant are added to the tube.

It is noted that after the addition of ethanol, the solution becomes whitish and the DNA begins to precipitate. The DNA rushes in the form of filaments visible to the naked eye that compact to form what is called a "jellyfish". A second wash with 70% and 100% cold ethanol will then be carried out. The mass of DNA extracted will then be recovered in a sterile Eppendorf tube to dry. For the dissolution of the jellyfish, 200 to 500 µl of 10/1 TE buffer are added according to its size, then the tubes will be transferred to an agitator at room temperature for a period of 24 hours.

Conclusion:

Field surveys have shown that the breeding of the local cattle breed in the wilaya of Tlemcen is practiced only by men because of the aggressiveness of the animal. Farmers do not practice any rational feeding system (except for a few exceptions in the case of pregnant females) with little veterinary care since the cattle are reared in an extensive system, to meet individual profit. A phenotypic characterization of 42 females with 40 blood samples was carried out, showing the similarity between the individuals and their similarities with the Guelmois breed since they belong to the same branch except for some animals estimated to be crossed with other exogenous breeds. The genetic diversity between the two breeds is rather visible, this diversity is more distinct in the Tlemcenienne breed, and this is due to the exchanges practiced by the breeders and to the anarchic crossings of the animals. Finally, it is important for genetic progress to have its mark in the development of indigenous breeds for heritage conservation and local development taking into account their diversity as well as safeguarding programs for extinct breeds.

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