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Stable Isotope Composition in Moroccan Milk: Feed and Water Relationships

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ABSTRACT

Background: This study investigated the use of stable isotope ratio analysis to trace the geographical origins of Moroccan dairy products, as well as possible applications to other Moroccan agricultural products. Ten agro-ecological zones with different climates were selected, including the Garb, Middle Atlas, Meknes, Lukos, Agadir, Taroudant, Settat and Casablanca regions. Milk samples were collected directly from cows on modern andtraditional farms, as well as the water they consumed.

Methods: Analytical methods such as isotope ratio mass spectrometry (IRMS) and the Piccaro system were used to determine stable isotope ratios in milk andwater samples. Chemometric methods including principal component analysis (PCA) and hierarchical cluster analysis (HCA) showed that stable isotope ratios (H, C, N, O) in milk were highly correlated with environmental factors specific to the region. Three distinct groups emerged, especially for oxygen isotopes, depending on altitude and distance from the ocean. The $\delta^{18}O$ composition was particularly distinct, indicating regional differences: northern, low-altitude coastal areas and high-altitude inl andareas.

Result: The study also showed that the isotopic composition of milk is influenced by the isotopic composition of drinking water. Nitrogen isotopes, especially $\delta^{15}N$, distinguished two groups of milk: one from an area with fertilizer use (low $\delta^{15}N$ values, intensive agriculture) and another from an area with moderate agricultural practices (higher $\delta^{15}N$ values). These results highlight the potential of stable isotope analysis as a tool to verify the geographical origin of dairy products in Morocco.

Key words: Food analysis, Geographical origin, IRMS, Stable isotopes.

INTRODUCTION

Morocco is located in western North Africa and is characterized by a semi-arid climate and a rapidly growing population. Faced with population growth and changes in eating habits, Moroccan authorities launched an ambitious plan in the early 1970s to meet food needs. The revised livestock policy aimed to establish intensive livestock production based on imported dairy cattle breeds and expanded pasture areas. At the same time, the relevant state departments introduced a milk collection policy.

Dairy production plays an important social and economic role in Morocco. In Morocco, approximately 790,000 farms produce milk and calves, of which only about 5% are considered professional dairies (FAO, 2011). Milk and its derivatives have always been considered an important but expensive component of the Moroccan diet, which is mainly based on cereals (bread) and vegetables. Milk has a strong symbolic meaning in local traditions, as it is used together with dates to welcome guests. Milk comes mainly from cattle (2.7 million cows produce more than 96% of annual milk production) (FAO, 2011).

The globalization of the food markets and the facility of transportation of products through and between the countries, generates that the consumers are worried by the origin of the food which they consume.

In recent years, geographical indications and appellation of origin have gained more importance in Morocco, which led to the establishment of the law #25-06 that was published in the Official Bulletin # 5640 on June the 19 (2008). This law regulates the use of distinctive signs of origin as they relate to the quality of food and agricultural products, including labelling of geographical indication. To apply for labelling, geographical indication andorigin appellation, producers associations and food processors have to file a request (cahier des charges) to a national commission created for this purpose (article 17 of the law). Food andagricultural products that have been approved by this commission are registered and published in the Official Bulletin.

To date, there are ten products that have been approved by The Moroccan National Commission for Geographical Indications and Appellation of Origin among them:

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Geographical Indication « Chefchaouan Goat cheese; Minister of Agriculture decision that was published in the Official Bulletin # 5976 on September 8 (2011).

Many techniques proposed in this subject, but the stable isotope ratio and trace elements give a good result concerning the geographic origin of the products food. The $^{18}O/^{16}O$ ratio of milk depends on the water ingested and the proportion of fresh vs. dry fodder. Isotope ratios of precipitation and groundwater depend largely on temperature, latitude, altitude and distance from the sea (Moser and Rauert, 1980; Kern *et al*., 2020).

The$^{13}C/^{12}C$ ratio for both milk fat and cheese protein gives information on the type of forage fed to the cows. The carbon isotope value of plants depends on their photosynthetic cycles for CO_2 fixation (Smith and Epstein, 1971). C_4 plants such as maize show higher $\delta^{13}C$ values than C_3 plants, which constitute the major part of a cow's fodder (Potoènik *et al*., 2020). Differences in the $^{15}N/^{14}N$ ratio also result essentially from forage. The nitrogen isotopic compositions of animal manures, composts and other fertilizers permitted in organic production systems are significantly enriched compared to synthetic nitrogen fertilizers (Sáez *et al*., 2020; Bateman and Kelly, 2007). The mean $\delta^{15}N$ values of organic fertilizers cluster around +8‰ and some fertilizers (*e.g.*, animal manures) can have values higher than +35%, due to the preferential volatilization of ^{15}N-depleted ammonia in the field or during storage (Philippe *et al*., 2022; Bateman and Kelly, 2007; Mie *et al*., 2022; Bhargav *et al*., 2025).

The 87Sr/86Sr ratio can also be useful for origin assignments as it is dependent only on the types of rocks and soils and not on human activity, climate or season of production (Rossmann *et al*., 2000).

In this study we decided to conduct a study on the variability of $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, D/H isotope ration and trace elements in milk in the order to determine the geographic origin of dairy products in Morocco. For that the sampling strategy will be based on nature of the rock and soil, climate of the region and irrigated areas or rainfall agriculture practices.

MATERIALS AND METHODS

Sampling

For sampling, we selected agro-ecological zones (Ducrotoy *et al*. 2015). The ten geographical units described by the soils can be considered important agro-ecological zones because they have a certain homogeneity in terms of geomorphology/subsoil, precipitation and growing season (bioclimate and its thermal zoning).

1- The Middle Atlas.
2- The Rif.
3- The Loukkos area.
4- The Rharb area.
5- The Sais plateau.
6- The Mamora and central plateau.
7- The Chaouia plain and casablanca.
8- The plains and plateau north of the Atlas.
9- The Argan zone.
10- The High Atlas.

The samples (cow milk and farm water) have been collected from various farms during the spring season for all agro-ecological zones, as climatic factors influence the isotopic composition of the samples (milk and water). Samples were collected early in the morning. In total, 49 samples of cow milk from various geographical regions were collected. We also collected the farm water that the cows drink.

Table 1 show the geographical information (longitude and latitude and some farms characteristics) of each farm where the samples have been taken.

Analysis of samples

In the laboratory the all samples will be kept frozen until the following analyses were performed. The ratios of $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, will be determined by spectrometer coupled to element analyzer for the stable isotope. Also the farm water will be collected for the isotope analysis.

Stable isotope data are expressed as δ-values according to (Brand *et al*., 2014; Huang *et al*., 2013) :

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right)$$

Where,

R = Ratio of the heavy to light isotope of the element (*e.g.*, H^2/H^1.

In the laboratory, fresh milk samples were subjected to vacuum distillation to isolate the water for the analysis of isotopes ^{18}O and ^{2}H (Boito *et al*., 2021). Additionally, raw milk samples were directly freeze-dried to produce milk powder for the analysis of ^{13}C and ^{15}N Ataro *et al*. (2008). Statistical data analyses were carried out on the data obtained from the various environmental and chemical and isotopic data. The data will be processed using the statistical software package Xstat using principal component analysis and Analytic hierarchy process (OECD, 2006).

RESULTS AND DISCUSSION

In this section the results obtained from isotopic ratio mass spectrometer will be discussed.

Stable isotope of water farm

The first parameter examined in this study concerns the stable isotopes in the drinking water of dairy cows, focusing on the stable isotopes of oxygen ($\delta^{18}O$) and hydrogen ($\delta^{2}H$) (Fig 1). The $\delta^{18}O$ and $\delta^{2}H$ values of the drinking water source samples are below the global atmospheric water line (GMWL) proposed by Craig (1961) and are consistent with the local atmospheric water line (LMWL) proposed by Raibi *et al*. (2004) findings. This scenario suggests that the water undergoes some form of evaporation, resulting in isotopic depletion, as the lighter isotopes of oxygen and hydrogen are preferentially lost in this process. This observation

Table 1: Background information of sampling sites.

Agro-ecological zones	Coordonnée X	Coordonnée Y	Élévation (m)	Distance from sea (km)	Water sources	Main product	Dominant breed	Stock lifestyle
Rharb area	33.79419	-6.11152	474.00	67.00	Ground Water	Milk	straw and local herb	Pasture
Rharb area	33.76108	-6.117877	453.00	73.00	Ground Water	Milk	straw and local herb	Pasture
Sais plateau	33.78849	-5.50289	668.00	120.00	Ground Water	Milk	straw and local herb	Mixte
Middle Atlas	33.61827	-5.43091	973.00	135.00	Surface water	Milk	straw and local herb	Pasture
Middle Atlas	33.3638	-5.553877	824.00	142.00	Ground Water	Milk	straw and local herb	Pasture
Middle Atlas	33.110262	-5.58392	1155.00	155.00	Ground Water	Milk	straw and local herb	Pasture
Middle Atlas	33.02809	-5.61646	908.00	163.00	Ground Water	Milk	straw and local herb	Pasture
Middle Atlas	32.866758	-5.626805	862.00	172.00	Ground Water	Milk	straw and local herb	Pasture
Middle Atlas	32.77009	-5.66733	870.00	178.00	Ground Water	Milk	straw and local herb	Pasture
Middle Atlas	32.74449	-5.68693	853.00	179.00	Ground Water	Milk	straw and local herb	Pasture
Middle Atlas	32.6301	-5.95201	713.00	170.00	Ground Water	Milk	straw and local herb	Pasture
Plains and plateau north of Atlas	32.2561	-6.56871	438.00	180.00	Ground Water	Milk	straw and local herb	Without pasture
Plains and plateau north of Atlas	32.253567	-6.589554	443.00	181.00	Tap water	Milk	straw and local herb	Without pasture
Plains and plateau north of Atlas	32.208757	-6.824253	433.00	175.00	Tap water	Milk	Straw and composite	without pasture
High Atlas	31.729758	-7.643462	535.00	160.00	Ground water	Milk	Straw and composite	Without pasture
High Atlas	31.729758	-7.643462	535.00	162.00	Ground water	Milk	Straw and composite	Without pasture
High Atlas	31.694982	-7.703827	512.00	158.00	Tap water	Milk	Straw and composite	Mixte
Argan zone	31.574807	-9.270985	425.00	38.00	Ground water	Milk	Straw and composite	without pasture
Argan zone	31.537647	-9.54612	220.00	15.00	Ground water	Milk	Straw and composite	Pasture
Argan zone	31.537647	-9.54612	220.00	15.00	Ground water	Milk	Straw and composite	Pasture
Loukkos area	35.18184	-6.128093	3.00	3.00	Ground water	Milk	local herb	Pasture
Loukkos area	35.201349	-6.085113	3.00	5.00	Surface water	Milk	local herb	Pasture
Loukkos area	35.201042	-6.068602	3.00	7.00	Surface water	Milk	local herb	Pasture
Loukkos area	35.264232	-5.617586	196.00	44.00	Ground water	Milk	local herb	Pasture
Loukkos area	35.305539	-5.631101	196.00	42.00	Surface water	Milk	local herb	Pasture
Loukkos area	35.309041	-5.644547	196.00	41.00	Surface water	Milk	local herb	Pasture
Loukkos area	35.37188	-5.778763	196.00	26.00	Ground water	Milk	local herb	Pasture
Argan zone	30.481339	-9.254076	132.00	45.00	Ground water	Milk	composite and local herb	Without pasture
Argan zone	30.492297	-9.223522	134.00	39.00	Ground water	Milk	composite and local herb	Without pasture
Argan zone	30.468816	-9.153006	133.00	43.00	Tap water	Milk	composite and local herb	Without pasture
Argan zone	30.270904	-9.504519	54.00	12.00	Ground water	Milk	composite and local herb	Without pasture
Argan zone	30.228748	-9.562233	50.00	6.00	Ground water	Milk	composite and local herb	Without pasture
Chaouia plain and casablanca	30.170873	-9.511819	83.00	12.00	Ground water	Milk	composite and local herb	Without pasture

Table 1: Continue..

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Chaouia plain and casablanca	32.960727	-7.601045	495.00	63.00	Ground Water	Milk	Straw and composite	Pasture
Chaouia plain and casablanca	32.898679	-7.573739	495.00	71.00	Ground water	Milk	Straw and local herb	Pasture
Chaouia plain and casablanca	33.080422	-7.625909	255.00	52.00	Ground Water	Milk	Straw and local herb	Pasture
Chaouia plain and casablanca	33.163701	-7.618816	231.00	44.00	Ground water	Milk	Straw and local herb	Pasture
Chaouia plain and casablanca	33.295244	-7.577252	212.00	32.00	Ground water	Milk	Straw and local herb	Pasture
Chaouia plain and casablanca	33.421881	-7.520807	177.00	23.00	Ground water	Milk	Straw and local herb	Pasture
Mamora and central plateau	34.478842	-6.385339	17.00	11.00	Ground water	Milk	Straw and local herb	Pasture
Mamora and central Plateau	34.525358	-6.31043	10.00	16.00	Ground water	Milk	Straw and local herb	Pasture
Mamora and central plateau	34.603329	-6.170604	18.00	20.00	Ground water	Milk	Straw, maize, sugarcane and local herb	Pasture

is consistent with previous work by Bailey *et al*. (2018) and recent work by Gallart *et al*. (2024), who highlighted that evaporation enriches the remaining water with heavier isotopes, causing its isotopic distribution to change along the $\delta^{18}O$ and $\delta^{2}H$ axes. Detailed inspection of the graphs reveals clear differences in isotopic signatures from different regions, reflecting local environmental and climatic factors that influence the isotopic composition of meteoric water. The Local Meteoric Water Line (LMWL) relationships help distinguish water from different regions and provide valuable insights into the local water cycle and potential sources of evaporation and contamination.

Oxygen and deuterium isotopes of fresh milk

On the other hand, when we compared the diagram on the Dairies waters with the diagram of the water coming from milk, we can observe enrichment on the milk (Fig 2). The $\delta^{2}H$ and $\delta^{18}O$ values of water extracted from dairy milk samples were found to be isotopically enriched relative to cow drinking water (Chesson *et al*. (2010); Gregorèiè *et al*. (2020). This enrichment is attributed to the fractionation processes that occur during the metabolism and physiological processes in the cow's body, leading to the enrichment of the milk with heavier isotopes of hydrogen and oxygen compared to the drinking water Boito *et al.* (2021). Therefore, the stable isotope analysis of hydrogen and oxygen in dairy milk can be used to authenticate the geographical origin of the milk and to detect any potential adulteration or fraudulent practices in the dairy industry Emad *et al*. (2015). All milk samples exhibit enrichment with respect to water samples presenting a slope that reminds waters that have undergone evaporation process.

There was a significant linear relationship between between $\delta^{18}O$ of farm water and $\delta^{18}O$ milk water shows that isotopic composition if milk is influenced by drinking water (r^2 = 0.5 $p<0.05$) (Fig 3) . The oxygen isotope ratios in milk samples were found to have a clear relationship with the water from the farm, which aligned with the expected effects

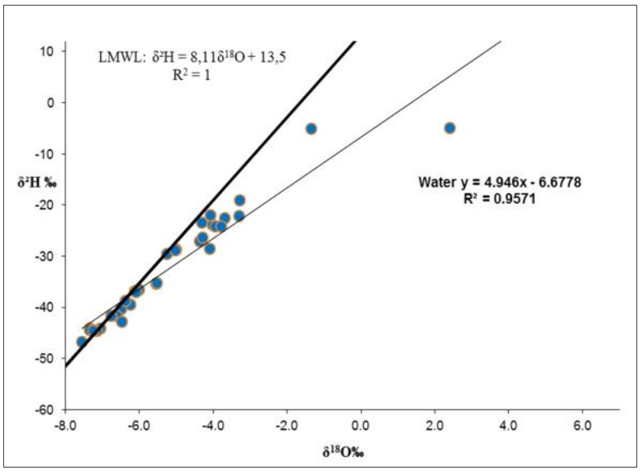

Fig 1: The plot of $\delta^{2}H$ vs. $\delta^{18}O$ values of watering water from farms.

of climate on $^{18}O/^{16}O$ fractionation Boito *et al*. (2021); Huang *et al*. (2017). The $\delta^{18}O$ water values in milk water show seasonal variability and are enriched in ^{18}O compared to cow milk, reflecting the isotopic composition of the drinking water source and the effect of differences in the water uptake by the animals (Gregorčič *et al*., 2020; Crittenden *et al*., 2007). However, clear correlations between geography and isotopic fractionation of the other elements examined were difficult to discern, due to limited available knowledge of the main factors influencing isotope abundance for these elements at the various locations.

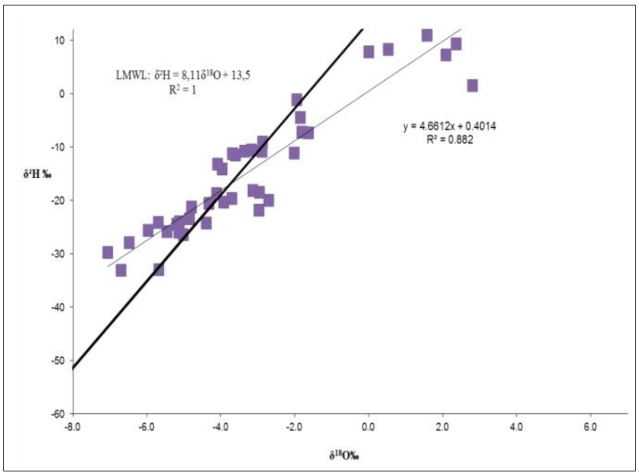

Fig 2: The plot of δ^2H vs. $\delta^{18}O$ values of liquid Milk.

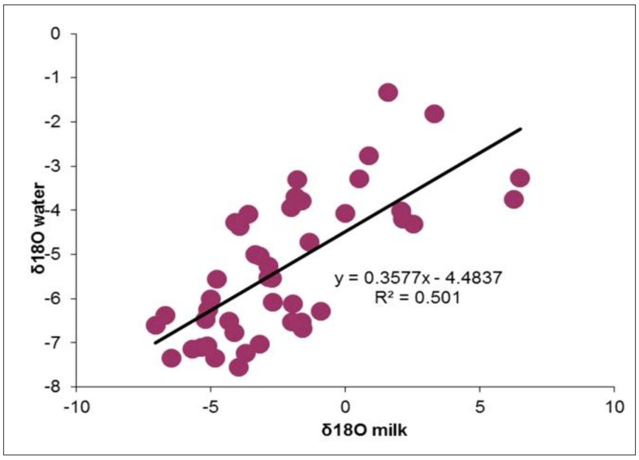

Fig 3: Relation between $\delta^{18}O$ fresh milk and $\delta^{18}O$ of farm water.

The data presented shows a significant negative correlation between the isotopic value of fresh milk and altitude (Fig 4), as well as between the isotopic value of fresh milk and distance from the Atlantic Ocean. The correlation can be attributed to progressive rainout or the 'continental effect' (Gremaud *et al*., 2004). Other studies have also found negative correlations between milk isotopic values and latitude, δO Rain and δH Rain (Shima *et al*., 2020). Additionally, elevation has been found to be positively correlated with one component of milk fatty acid composition and negatively correlated with two other components (Shima *et al*., 2020). Environmental factors such as solar radiation, temperature, relative humidity and precipitation have also been found to have varying degrees of correlation with milk quality parameters such as fat content, total solids and mesophilic count (Vélez-Terranova *et al*., 2023; Enrique *et al*., 2020).

The composition of stable isotope milk (Fig 5, Fig 6) can be distinguished into three groups based on the influence of different geographical and environmental factors.

Milk from the north influenced by the Mediterranean Sea

This group of milk has a specific isotopic composition due to the influence of the Mediterranean Sea, which affects the water isotope composition in the region.

Milk from continental regions with low altitude and far from the Atlantic Ocean

This group of milk has a different isotopic composition compared to the first group, as it is not directly influenced by the Mediterranean sea. The isotopic composition of milk in these regions is more related to the continental climate and the altitude of the area.

Milk from regions near the Atlantic sea and low altitude

This group of milk has a distinct isotopic composition due to the proximity to the Atlantic Ocean and the low altitude of the region. The isotopic composition in these areas is influenced by both the ocean and the continental climate. These differences in isotopic composition can be used to trace the origin of milk and verify its provenance, as well as

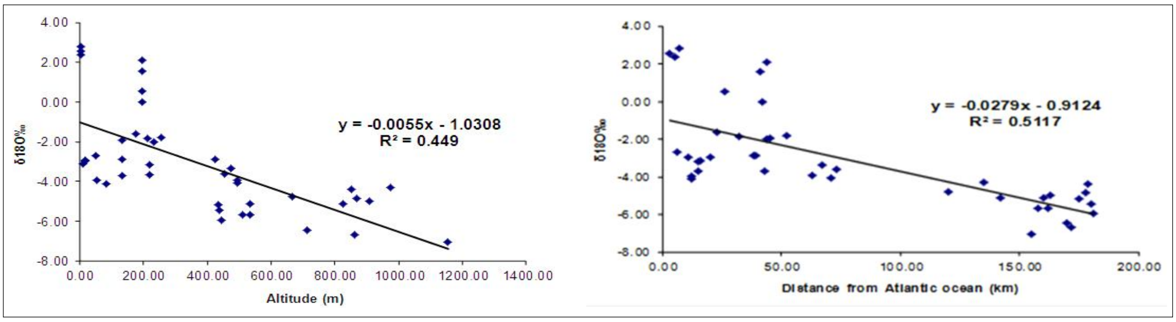

Fig 4: Relation between $\delta^{18}O$ of fresh milk, distance from the sea and Altitude for the studied farms.

to study the effects of different environments on the isotopic composition of milk (Gregorčič *et al*., 2020).

Carbon and nitrogen isotopes of powder milk

The dominant geographical phenomena influencing isotopic fractionation vary for each element. In the case of carbon, the differing photosynthetic CO_2 fixation pathways used by plants (C_3 or C_4) leads to characteristic $\delta^{13}C$ in the plant tissues, which is then rapidly reflected in the milk of cattle eating those plants Roffet-Salque *et al.* (2017); Wijenayake *et al*. (2020); Boutton *et al*. (1988). Carbon isotope tissue ratios vary from 16% to 7% in C_4 plants and from 35% to 20% in C_3 plants Kirkels *et al*. (2022); Pate and Noble (2000); Nandini *et al.* (2018).

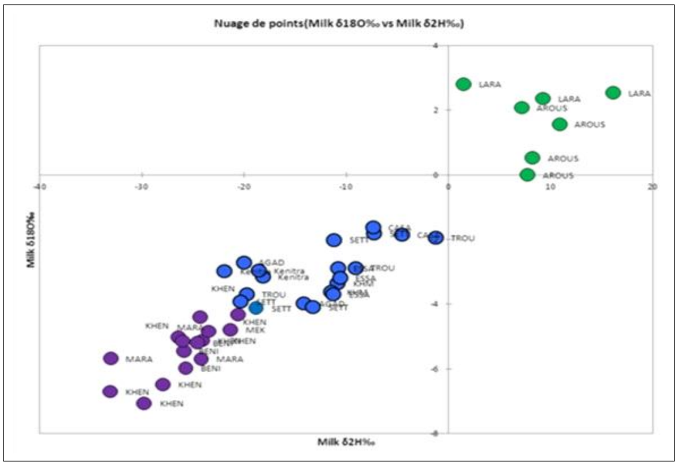

Fig 5: $\delta^{18}O$ versus $\delta^{2}H$ in liquid milk from different regions.

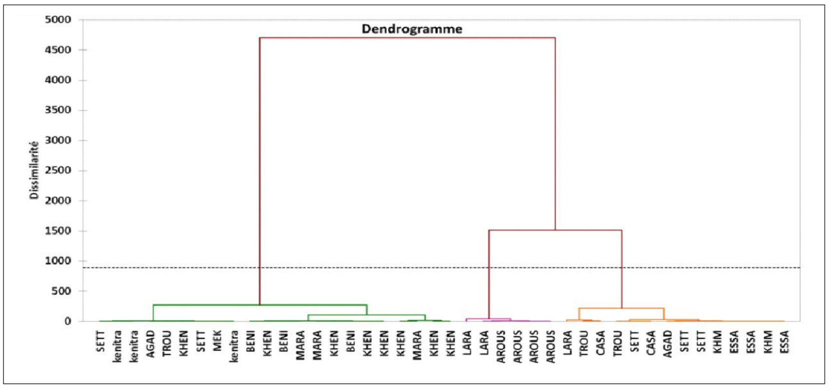

Fig 6: Dendrogram for the origin of fresh milk.

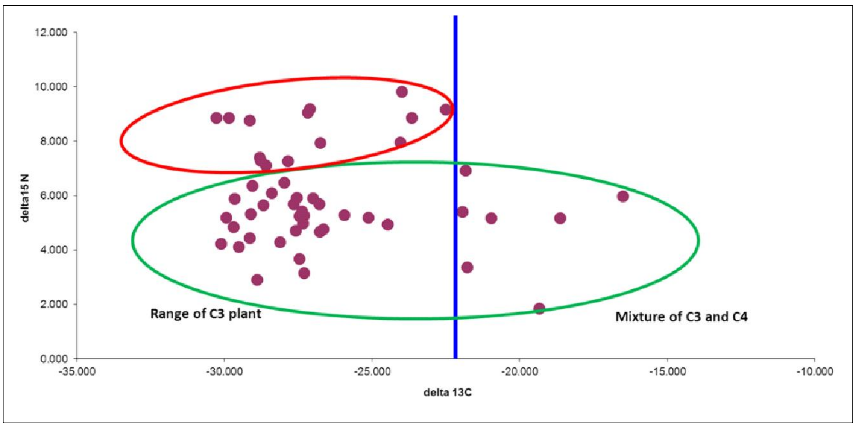

Fig 7: ^{13}C versus ^{15}N in powder Milk.

According to ^{13}C (Fig 7), we have two groups the first one ranged from -23,98 to -30,27% corresponding to C_3 plant and second one from -16,5 to -21,9% corresponding to mixture of C_3 and C_4 plant respectively (Kirkels *et al*., 2022).

However, there is an understanding of at least some of the parameters that influence the fractionation of ^{15}N in soils and in plants. The intensity of agricultural practices, fertilization regimes, crop types, tillage practices and diet can all impact the ^{15}N levels of tissues in animals and plants (Chanda *et al*., 2024). These factors can influence the nitrogen cycle and affect the $\delta^{15}N$ values in soil, plants and animal products (Caio *et al*., 2020); Kornexl *et al*., 1997). According to ^{15}N signature (Fig 7), we have two group the first one ranger from 7.2 to 9.8% corresponding to region with moderate agriculture, second one ranger from 1.8 to 6.4% corresponding to intensive agriculture.

CONCLUSION

The results show that the H, C, N, O stable isotope ratios in milk are related to the region, especially the vegetation type and environment. ^{18}O and H isotopes distinguish three groups according to altitude and distance from the Atlantic Ocean. The correlation between ^{18}O in milk and dairy water confirms the influence of drinking water. ^{13}C isotopes distinguish milk from C_3-only equipment from milk from equipment with a mixture of C_3 and C_4. ^{15}N isotopes distinguish milk from intensive agriculture (low ^{15}N) and moderate agriculture (high ^{15}N).

These results emphasize that geographical, climatic and agricultural factors affect the stable isotope ratios incorporated into animal tissues through diet, water and environmental exchanges. Therefore, stable isotope analysis of H, C, N and O offers great potential for determining the geographical origin of milk.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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