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Aims & Scope

Plant and animal health is closely related to human health. In this century, where the human population is rapidly increasing and technology is developing rapidly, the problem of food supply to the increasing population brings plant and animal health to the fore. Nowadays, when concepts such as artificial meat and capsule feeding are discussed, the process of growing plants and animals has begun to be discussed. For this reason, this conference focused on the changes and innovations in the field of Veterinary, Agriculture and Life Sciences.

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ICVALS 2023: International Conference on Veterinary, Agriculture and Life Sciences

Anti-Inflammatory and Antioxidant Activities of Cow's Milk Supplemented with Aqueous Extract of Malva Sylvestris

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Abstract: Cow's milk is known for its beneficial properties for health, added to this, scientific research is increasingly interested in molecules from medicinal plants. The objective of this study is to evaluate some of the biological activities resulting from the association of cow's milk and the aqueous extract of leaves of malva sylvestris L, a medicinal plant of the traditional pharmacopoeia of Algeria. The combination produced showed appreciable antioxidant activity. Its capacity to trap the DPPH radical, the OH radical and H_2O_2 was respectively estimated by an IC50 of 5.215 ± 0.759 µg/ml, 381.44 ± 37.12 µg/ml and 205.52 ± 12.03 µg/ml. The milk associated with the plant presented an IC50 for its total antioxidant capacity of 362.90 ± 7.04 µg/ml and of 211.02 ± 35.27 µg/ml for its reducing power of the ferric ion. The assessment of the antioxidant capacity of this association was also carried out by the β-carotene bleaching inhibition test where we recorded an inhibition of 83.93 ± 4.82% of the extract at a concentration of 1mg/ml and the ferrous ion chelation test estimated by an IC50 of 506.84 ± 54.50µg/ml. With regard to anti-inflammatory activity, the cow's milk studied showed a stabilizing effect on erythrocyte membranes against osmotic stress (79.71 ± 3.81%), oxidant stress induced by HOCL (80.32 ± 0.92%) and heat (90.1 ± 3.05%). The sum of the results obtained during this study clearly shows that the combination of cow's milk and the aqueous extract of *malva sylvestris* leaves has significant potential for the biological activities investigated. As a result, this association constitutes a potential source of bioactive molecules and thus constitutes a therapeutic alternative to the treatment of a number of pathologies initiated by oxidative stress.

Keywords: Cow milk, Malva sylvestris, Antioxidant , Anti-radical , Anti-inflammatory

Introduction

Throughout the world, plants have always been used as medicines and are a precious heritage for humans, offering a varied and variable source of biomolecules with widely recognized therapeutic potential. The renewed interest in biomolecules from medicinal plants is based on the numerous scientific studies highlighting the numerous merits of medicinal plants (Singh, 2015). Nowadays, the exploration of the antioxidant properties of plant sources has gained a considerable place in therapeutics, thus marking the decline in the use of synthetic antioxidants (Velioglu et al., 1998). This change of direction in scientific research is a logical response given the alarming implications of certain chemical antioxidants. As free radicals inflict often irreversible damage to biomolecules and to protect the integrity of biological systems, scientific research is relying on new antioxidant biomolecules, such as polyphenols, which have shown high antioxidant potential and could help maintain the

oxidative balance of biological systems (Bhattacharyya et al., 2014). Polyphenols, known for their antioxidant properties, owe their biological functionality to their chemical structures, which allows them to be excellent electron donors and efficient hydrogen sensors (Leopoldini et al., 2004). Malva sylvestris, commonly called sylvan mallow, is an annual plant with weakly lobed leaves and purple flowers that bloom in spring. Native to Europe, North Africa and southwest Asia, this hardy plant is found in varied environments, from humid coastal areas to grasslands, ditches and river banks (Razavi et al., 2011). Long known for its use as a vegetable, Malva sylvestris is now renowned as a medicinal plant. numerous scientific studies have confirmed its numerous virtues, particularly as an antioxidant, radical scavenging and antimicrobial agent (DellaGreca et al., 2009; Awwad et al., 2015). Considering the widespread use of aqueous extracts of Malva sylvestris leaves in traditional medicine, coupled with the use as feed for livestock animals (Hassan et al., 2015; KADIOĞLU et al., 2022) associated with this the fact that the cow's diet has a direct impact on the composition of the milk produced, this study aims to evaluate the antioxidant potential of the combination

Materials and Methods

Plant Collection

Malva Sylvestris leaves were collected in October 2022 from M'sila, Algeria. The plant was identified by Doctor Mahmoud Laribi, botanist at Mouloud Mammeri University of Tizi-Ouzou, department of vegetal biology, where a voucher specimen was deposited (FSBSA/MK/oct2722). The sample was dried and then ground to obtain a powder that was stored at room temperature and in the dark until extraction.

Extract Preparation

20g of powder are dissolved in 200ml of distilled water. After 24 hours of maceration at room temperature, the filtrate was lyophilized.

Milk Collection

Collected in October 2022, cow's milk samples are milks of small mixtures, resulting from herds of healthy cows, localized in the area of Tizi-Ouzou.

Skimming

Skimming of milk consists of removing fat from milk by centrifugation at 3500xg for 20 minutes and at 4°C. At the end of the centrifugation we note a phase separation with the formation of a layer on the surface corresponding to the cream of the milk, once this has been removed using spatula, the resulting skimmed milk is filtered through the glass wool. To ensure good skimming, the entire skimming operation is repeated two other times, thus eliminating any residual trace of fat for the rest of the isolation protocol.

Determination of DPPH Radicals Scavenging Activity

The free radical scavenging activity of the extract was measured using the stable free radical DPPH test according to the method described by (Sharma& Bhat, 2009; Santos et al., 2010). 250 µl of 0.8 mM DPPH in ethanol was mixed with 3.65 ml of extract and 100µl of cow milk. The reaction was carried out in triplicate and the absorbance was measured at 517nm after 30 minutes in dark. L-Ascorbic acid was used as reference standard.

Hydroxyl Radical Scavenging Assay

Scavenging activity of hydroxyl radical of the extract was measured according to the method of (Rajamanikandan et al., 2011) Three millilitres of the final reaction solution consisted of aliquots (500 µl) of various concentrations of the extract,100µl of cow milk, 1ml FeSO4 (1.5 mM), 0.7 ml hydrogen peroxide (6

mM) and 0.3 ml sodium salicylate (20 mM). The reaction mixture was incubated for 1 h at 37°C. L-Ascorbic acid was used as the standard. The colour development was measured at 560 nm against a blank.

Hydrogen Peroxide Radical Scavenging Activity

The scavenging ability of water extract of *Malva Sylvestris* on hydrogen peroxide was determined according to the method of (Serteser et al., 2009). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Cow milk (100µl) and extract in distilled water (3.4 ml) was added to a hydrogen peroxide solution (0.6 ml, 40mM). Absorbance of hydrogen peroxide at 230 nm was measured 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide.

Ferrous Ion Chelating Activity

Ferrous ion chelating activity was determined by inhibition of the formation of iron(II)–ferrozine complex, following the method of (Dinis et al., 1994; Nabavi et al., 2012). Briefly, 100 µl of 0.6 mM $FeCl_2$ was added to 500µl of different concentrations of the extract mixed with 100µl of cow milk or EDTA (positive control). The reaction mixture was adjusted to a final volume of 1.5ml with methanol, and then 100µl of 5 mM Ferro zine solution were added. The mixture was shaken vigorously and left to stand at room temperature for 5 min. Absorbance was determined at 562nm.

Ferric Reducing Power Assay

Reducing power was determined by the method described by (Oyaizu, 1986; Hazra et al., 2008). Different concentrations of extract and cow milk were mixed with 1.25 ml of 0.2 M, pH 6.6 sodium phosphate buffer and 1.25 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After incubation, the reaction mixture was acidified with 1.25 ml of trichloroacetic acid (10%) and centrifuged at 3000 rpm for 10 min. Finally, 0.5 ml of freshly prepared FeCl3 (0.1%) was added to this solution, and the absorbance was measured at 700nm. Aascorbic acid at various concentrations was used as standard.

Total Antioxidant Capacity

Total antioxidant capacity was estimated by phosphomolybdenum assay (Prieto et al., 1999; Rao et al., 2010) The tubes containing extract and cow milk and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95°C for 90 min. Then the solution was cooled to room temperature and absorbance was read at 695 nm. Ascorbic acid was used as standard.

Antihemolytic Activity

Red Blood Cell Suspension

Blood was obtained by venipuncture from healthy volunteers collected in heparinized tubes and centrifuged at 2 000 r/min for 10 min at 4 °C. After removing the plasma, red blood cells (RBCs) were washed for three successive times using phosphate buffer saline (PBS) (0.9% NaCl). The study protocol was performed according to the Helsinki declaration and approved by Scientific Committee of the Faculty of Biology (CSFB). Informed written consent was obtained from Hospital Department of Hematology (University Hospital Nedir Mohamed of Tizi-Ouzou).

Hypotonic Solution Induced Hemolysis

Membrane stabilizing activity of extract and cow's milk was assessed using hypotonic solution induced hemolysis, and the method was described by de Freitas et al. (2008). In hypotonic solution, the test sample consisted of washed stock erythrocyte (RBC) suspension (40 mL) with 1 mL of hypotonic solution (0.1%,0.3%, 0.5%, 0.7%, 0.9% NaCl) in sodium PBS (pH 7.4) containing either of the different concentrations of goat's milk.

The mixture was incubated for 30 min at 37 °C under gentle stirring, centrifuged for 10 min at 2 000 r/min and the absorbance of the supernate was measured at 540 nm.

Heat Induced Hemolysis

Different concentrations of the extract mixed with cow's milk (mg/mL) or aspirin dissolved in isotonic PBS (pH 7.4) was mixed with 1 mL of 2% RBCs suspension. The reaction mixture was incubated in a water bath at 56 °C for 30 min. After incubation, the tubes were cooled under running tap water, then centrifuged at 2 000 r/min for 10 min and the absorbance of the supernatants was estimated at 560 nm (Sakat et al., 2010).

Oxidant Induced Hemolysis

One milliliter of RBC suspension (5%) in PBS (pH 7.4) was incubated for 15 mn at 37°C with 1 ml of the extract mixed with cow's milk at different concentrations. After preincubation, the mixture was centrifuged at 2 000 r/min for 10 min at 4°C, the supernatant was removed and packed RBCs were resuspended with 0.5 mmol/L HOCl in PBS. After this, the incubation was performed as previously described. The absorbance was determined at 540 nm (Suwalsky et al., 2007; Chandler et al., 2013).

Inhibition of Albumin Denaturation

A solution of 0.2% (w/v) of egg albumin was prepared in a PBS (pH 6.4). A volume of 50 µL the extract mixed with cow's milk or standard at different concentrations was added to 5 mL of this stock solution.The test tubes were heated at 72 °C for 5 min and then cooled. The absorbance of these solutions was determined at 660 nm (Karthik et al., 2013).

Results and Discussion

DPPH Scavenging Activity

Figure 1 shows the percentage inhibition of DPPH radical scavenging activity by the samples tested. Corresponding to the concentration of antioxidant required to trap 50% of DPPH radicals, the IC50 was used as a comparative value for the different samples studied. The IC50 value of ascorbic acid was 2.359 ± 0.091 µg/ml, which was relatively lower than the IC50 (7.81 ± 0.402 µg/ml) of the aqueous extract. Despite this disparity between these two values, it remains significantly closer to that recorded for the mixture of plant extract and cow's milk IC50 (5.215 ± 0.759 µg/ml).

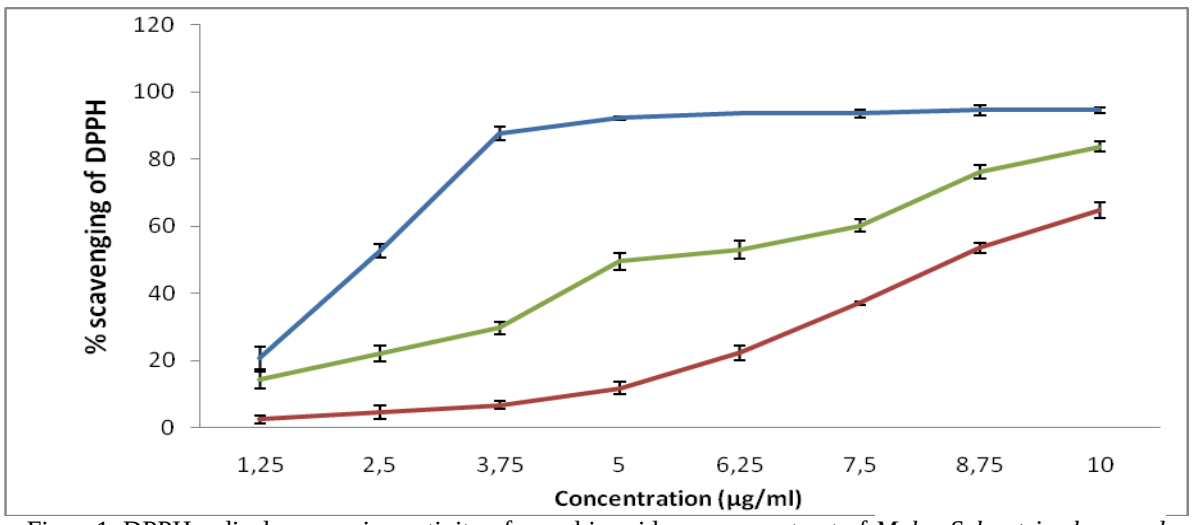

Figure1. DPPH radical scavenging activity of ascorbic acid, aqueous extract of *Malva Sylvestris alone and combined with cow milk.*

Hydroxyl Radical Scavenging

The ability of *Malva Sylvestris* aqueous extract alone and combined with cow milk to compete with salicylic acid for hydroxyl radicals is the principle applied to the evaluation of its ability to scavenging these radicals.As shown in Figure 2, Hydroxyl radical scavenging increased with increase in concentration. The ascorbic acid (IC_{50} = 758.83 ± 7.40 µg/ml) showed more effective scavenging ability when compared to that of aqueous extract (IC_{50} = 971.28 ± 27.12 µg/ml) and combined with cow milk (IC_{50} = 381.44 ± 37.12 µg/ml).

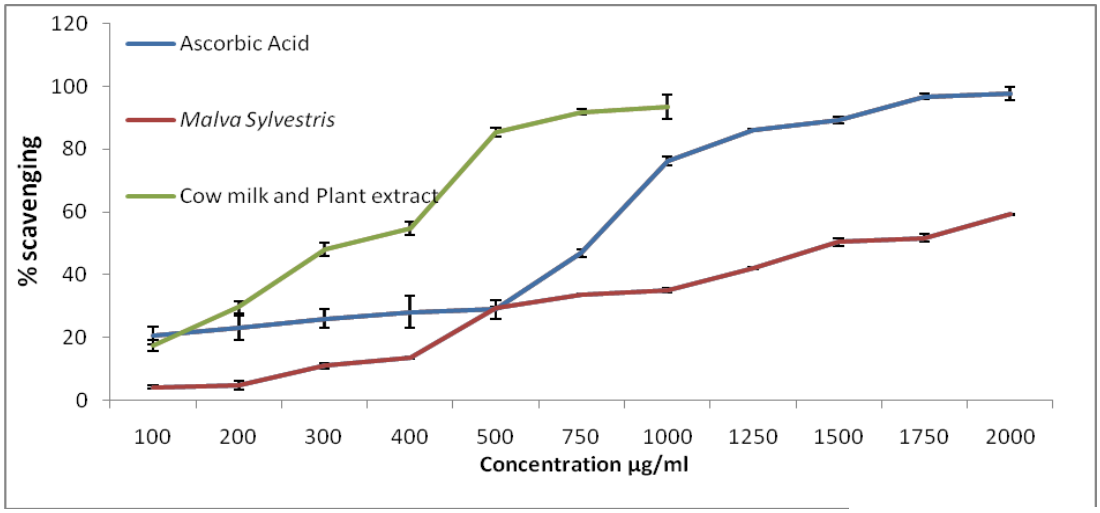

Figure 2. Hydroxyl radical scavenging activity of ascorbic acid, aqueous extract of *Malva Sylvestris alone and combined with cow milk.*

Hydrogen Peroxide Radical Scavenging Activity

Scavenging activity of hydrogen peroxide for extract alone, combined and ascorbic acid as reference compound in terms of effective concentration was remarkably different and shown to be 69.12%, 95.46%, 96.4% and respectively (Figure 3).

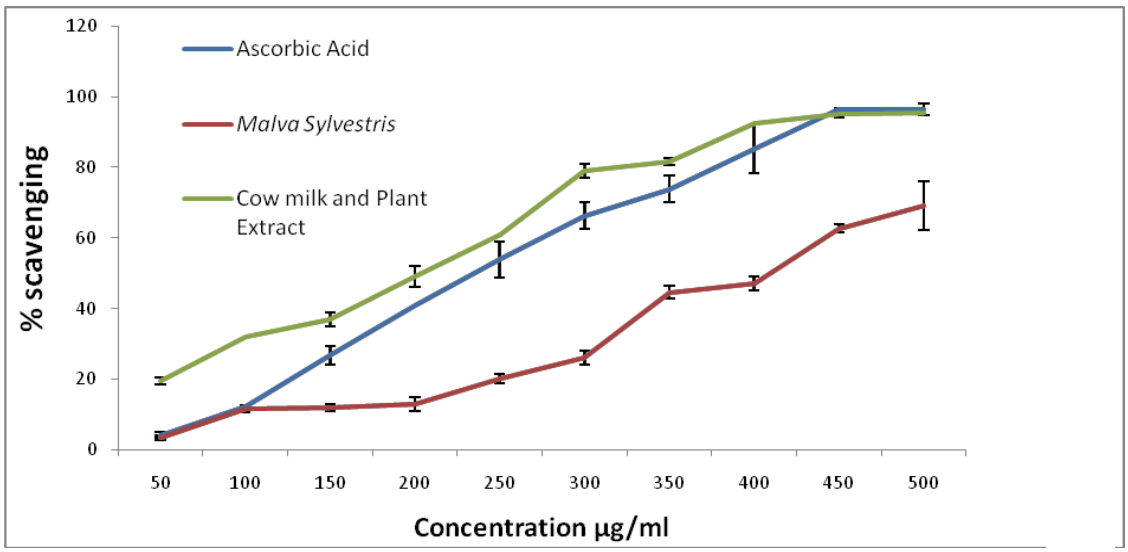

Figure 3. Hydrogen peroxide radical scavenging activity of ascorbic acid and aqueous extract of *Malva Sylvestris alone and combined with cow milk.*

According to the results, *Malva Sylvestris* showed an activity dependent on the concentration and the H_2O_2 scavenging IC_{50} was 431.06 ± 11.72 µg/ml, which indicates a distant effective scavenging potential referring to ascorbic acid IC_{50} (259.95 ± 9.33 µg/ml). the data collected shows a rapprochement between this last value and that recorded for the combination of plant extract, cow's milk IC_{50} (205.52 ± 12.03).

Ferrous Ion Chelating Activity

Because of a possible secondary antioxidant activity, we have been interested in one of the most important mechanisms and that is the chelating of pro-oxidant metals such as Iron. The test put in place is based on the formation of a complex between Ferrozine and Fe2+ with a characteristic red color. In the presence of chelating agent, the complex formation is disrupted and the red color is decreased. Measurement of color reduction, therefore, allows the estimation of the chelating activity of the plant extract. The metal chelating effect of investigated extract and EDTA were dependent on concentration (Figure 4). EDTA (IC_{50} = 57.21± 0.44 µg/ml) in this assay demonstrated relatively high activity in comparison to extract (IC_{50} = 74.631 ± 1.19µg/ml). However, this difference is accentuated when milk is added to the aqueous extract studied (IC_{50} = 506.84 ± 54.50 µg/ml)

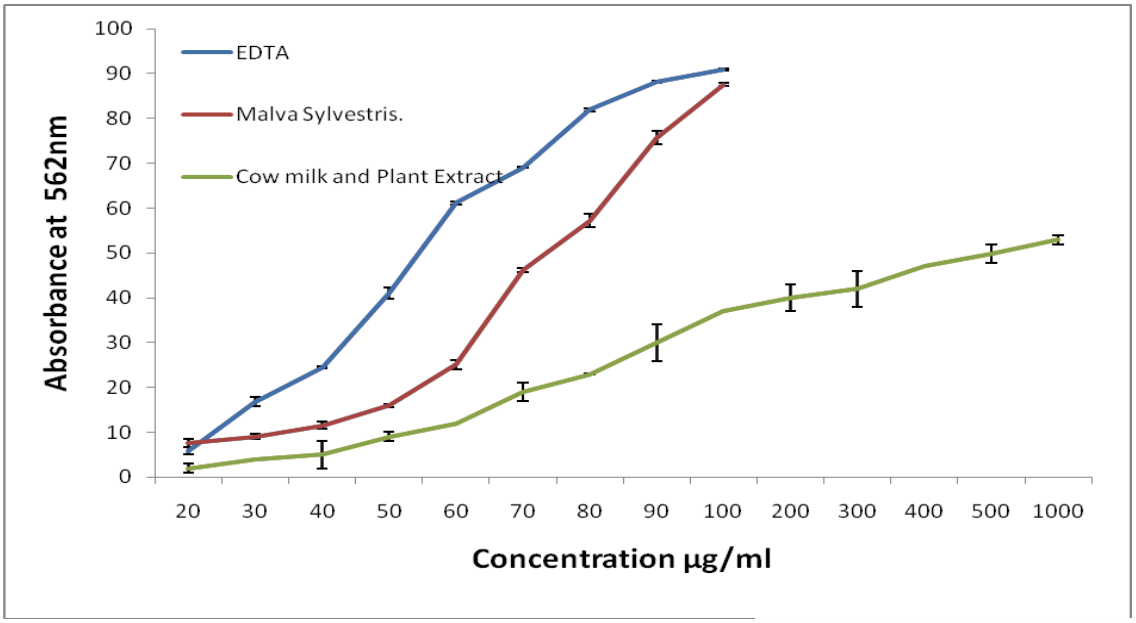

Figure 4. Ferrous ion chelating activity of EDTA and aqueous extract of *Malva Sylvestris alone and combined with cow milk.*

Ferric Reducing Power

Extract showed concentration-dependent reducing power. However, its reducing power (IC_{50}=46.7 ± 0.85 µg/ml) was lower than that of ascorbic acid (IC_{50}=88.17±1.39 µg/ml) (IC_{50}=211.02 ± 35.27 µg/ml).

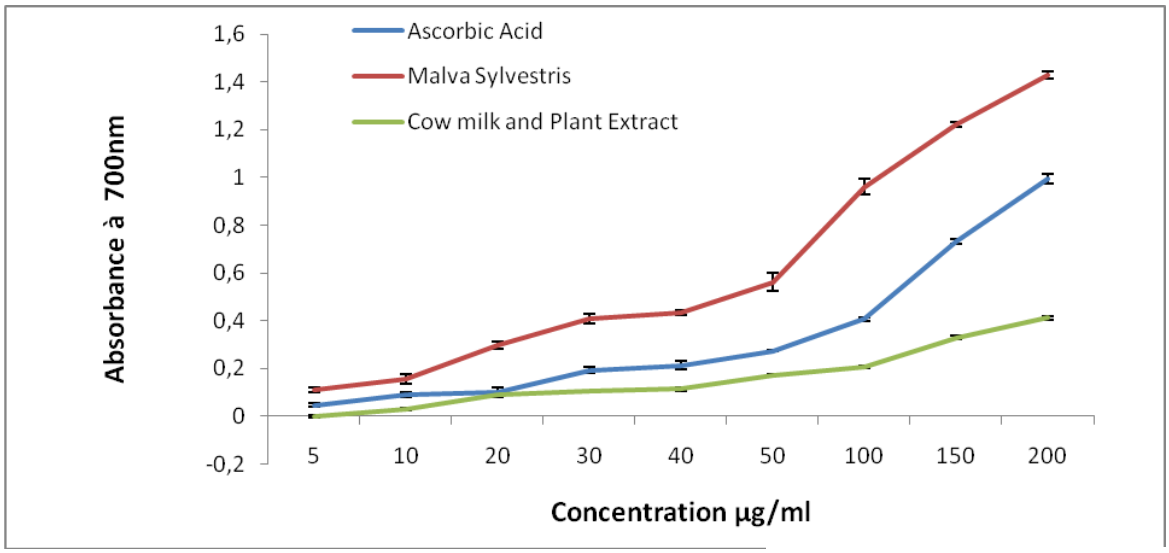

Figure 5. Ferric reducing powerof ascorbic acid and aqueous extract of *Malva Sylvestris alone and combined with cow milk.*

Total Antioxidant Capacity

The basic principle of this test is based on the formation of a green phosphate / Mo (V) complex resulting from the reduction of Mo (VI) to Mo (V) by the acidic pH extract. Results showed antioxidant activity of extract and ascorbic acid in dose dependent manner at concentration 100 to 500 µg/ml. The IC_{50} value of antioxidant capacity for the ascorbic acid (292 ± 7.54 µg/ml) was greater than extract IC_{50} (348.357 ± 6.03 µg/ml).

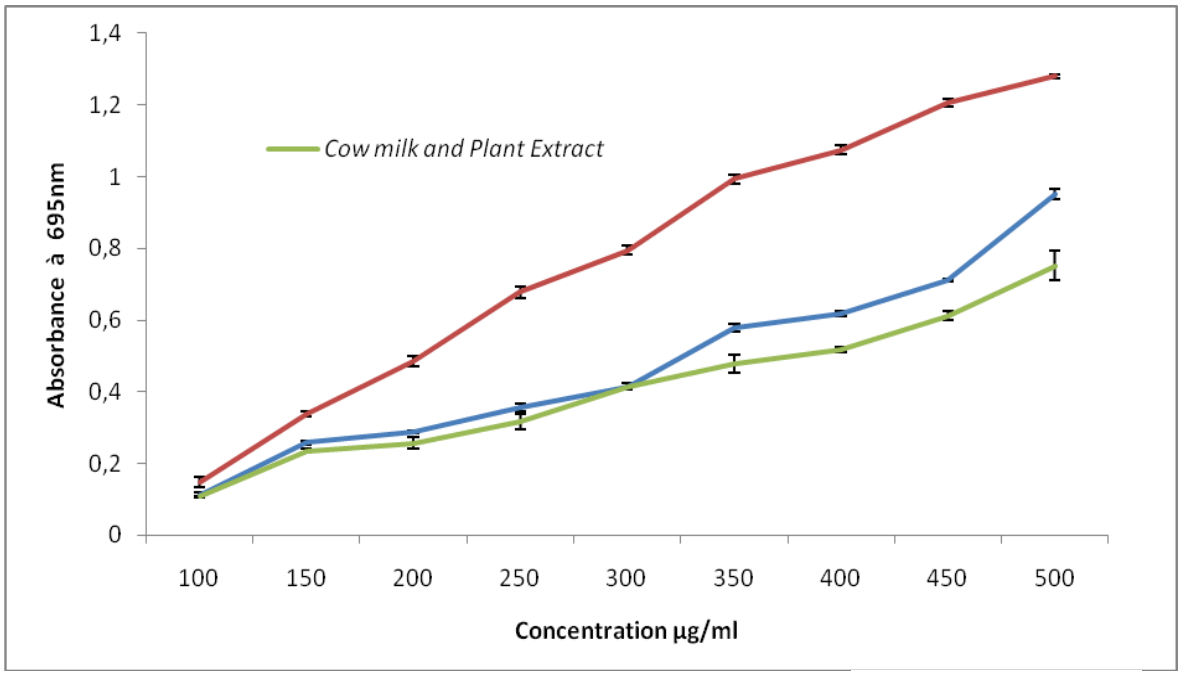

Figure 6. Total antioxidant capacity of ascorbic acid and aqueous extract of *Malva Sylvestris alone and combined with cow milk.*

Heat Induced Hemolysis

As shown in Figures 7, the extract prevented the erythrocyte membrane against lysis induced by heat compared with aspirin. The maximum protection recorded at 1200 µg/ml is 62.97 ± 2.1% for aspirne followed by the two samples studied for which no significant difference in their percentage of protection was found and around 90% for both

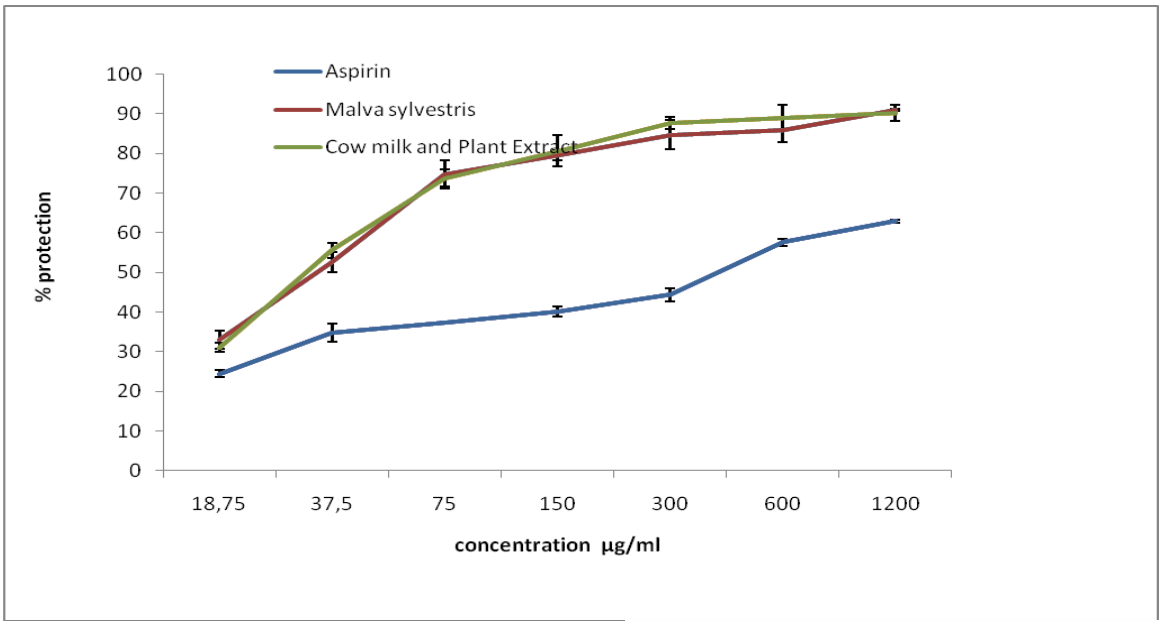

Figure 7. Effect of ascorbic acid and aqueous extract of *Malva Sylvestris alone and combined with cow milk.* on heat-induced hemolysis.

Hypotonic Solution Induced Hemolysis

In favor of the results indicated in Figure 8, it clearly appears that there are few differences between the two samples studied, namely the native extract of malva sylvestris and that with added cow's milk, taking into account their protective power of red blood cells against -with respect to osmotic stress. Thus we respectively record a maximum of protection at the concentration 0.3% Na Cl of the order of 79.57% and 80.48% and at the concentration of 0.7% NaCl 60.3% 66.12%.

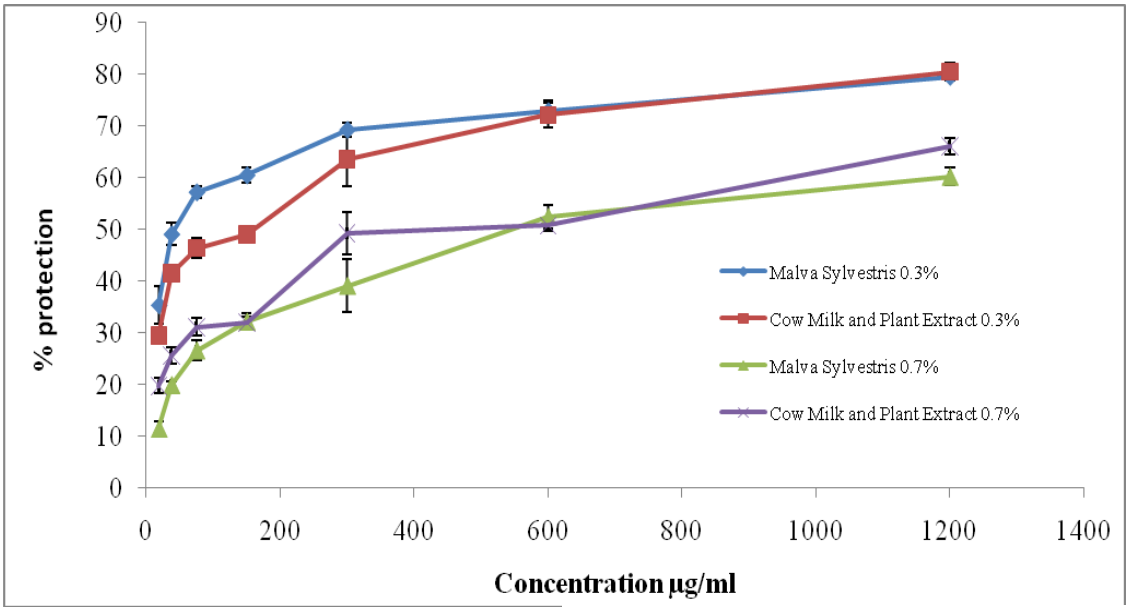

Figure 8. Effect of ascorbic acid and aqueous extract of *Malva Sylvestris alone and combined with cow milk.* on hypotonicity-induced hemolysis.

Oxidant Induced Hemolysis

The results that we recorded taking into account oxidative stress indicate in Figure 9 that the extract added with cow's milk displays a protection percentage of 80.32%, which remains relatively higher than the native extract where we find a protection rate of 63.10%.

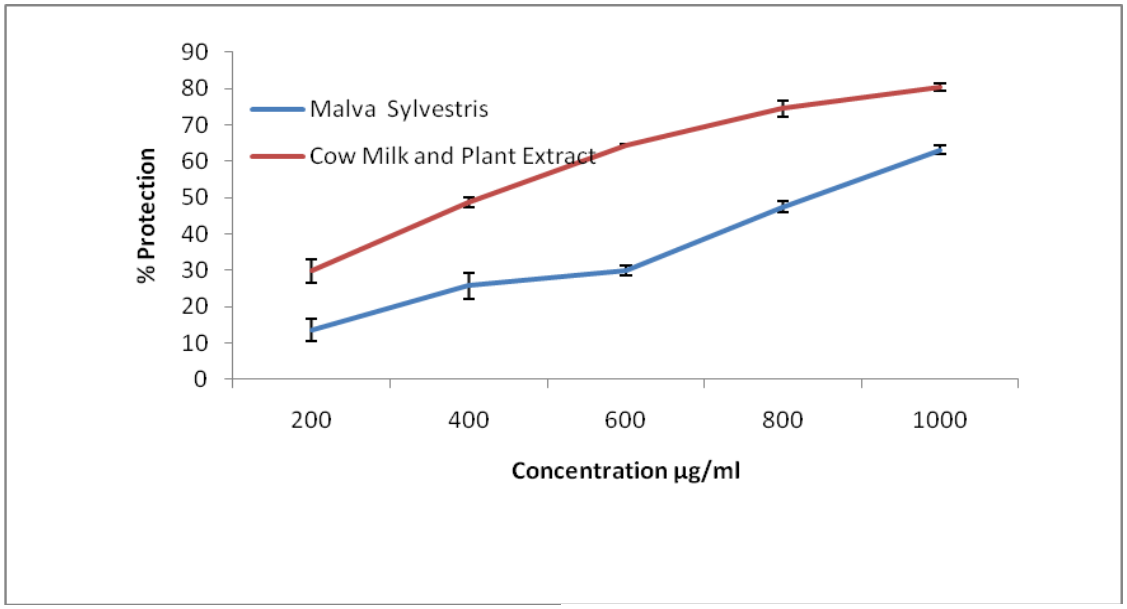

Figure 9. Effect of ascorbic acid and aqueous extract of *Malva Sylvestris alone and combined with cow milk.* on HOCl induced hemolysis.

As little data is available in the literature regarding the anti-inflammatory and antioxidant potential of the combination of the aqueous extract of Malva Sylvestris and cow's milk, it is difficult to compare our results. Compared to the data we collected on the aqueous extract of Malva sylvestris leaves in the Tizi-Ouzou region, the sample from the M'sila region presents relatively superior antioxidant and anti-inflammatory properties (Moualek et al., 2020). As for the potential of the activities expressed by the combination of the aqueous extract of Malva sylvestris leaves with cow's milk, the results recorded mark three levels of interactions. Thus, we note the existence of a synergy between cow's milk and the plant extract for radical inhibition ($DPPH^{\bullet}$ and $OH^{\bullet}$) as well as the protection of biological membranes against an oxidant (HOCl). Considered as the most reactive free radical, hydroxyl radical is most often implicated in the pathology of free radical because of its ability to interact with intracellular targets such as DNA, thus causing significant damage. In a second level of interaction and for the chelation tests, reduction of iron and molybdate, we find a negative effect of the association which gives rise to a loss of the original potential of the plant extract. Knowing that the chelation potential of a biomolecule indicated a significant protective activity of the extract against oxidative damage by sequestering iron (II) ions that may turn into catalyst for Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions (Adefegha & Oboh, 2011). The absence of repercussions of the association between the plant extract and cow's milk was recorded for the trapping of H2O2 as well as the protection of erythrocyte cells against osmotic and thermal stress. Knowing that Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it can give rise to hydroxyl radical in the cells (Halliwell, 1991; Kumar et al., 2012). Thus, the removal of H2O2 is very important for antioxidant defense in cell systems or food (Turkoglu et al., 2010). Knowing that the erythrocyte membrane resembles lysosomal membrane and as such, the effect of extracts on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomalmembrane (Omale & Okafor, 2008). The anti-inflammatory activity can also be explained by the inhibition of release of lysosomal content at the site of inflammatory (Govindappa et al., 2011). This study demonstrated in vitro antioxidant and anti-inflammatory activities of, through scavenging, chelating and reducing activities indicated in the performed tests, showed a good antioxidant activity. Furthermore, the protection of RBCs indicated a membrane stabilizing effect of the extract. Conclusion It emerges from this preliminary study that the mixture studied presents a significant anti-radical potential as well as strong protection of erythrocyte membranes with respect to HOCl. The sum of the data collected indicates a disparity of results taking into account the uniformity of the impact of the combination of plant extract and cow's milk on the antioxidant and anti-inflammatory potential of the mixture. As we are faced with two matrices (plant and animal) rich in compounds of diverse and varied natures, it would be interesting to deepen the investigations and to highlight the type of interactions existing between the constituents of the mixture. It would also be interesting to verify the impact of Malva Sylvestris supplementation on the composition of cow's milk as well as on its antioxidant and anti-inflammatory potential, in vitro and in vivo.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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Determining Nutritive Value of Dry Ash Leaves (*Fraxinus Angustifolia*) Harvested in Fall via the Regression Method for Growing Rabbits

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Abstract: Shortage acute and high price of animal proteins can be mitigated by sustainable farming of small, very prolific animal species with a short production cycle and valuing non-competitive foods on the resources and space reserved for human food. The rabbit (*Oryctolagus cuniculus*) combines all these assets. Ash leaves are little or not known nutritionally, nutritive value information relating them is very fragmentary and relates specifically to small ruminant herbivores.Therefore, the present work aim is to determine the nutritional value of oxyphylle dry ash leaves(*Fraxinus angustifolia*), distributed with increasing rates incorporation in pelleted food for growing rabbits. The nutritive value of Ash leaves collected in fall, dried under shade conditions and distributed for growing rabbits. We compared diets containing an increasing incorporation of Ash (*Fraxinus angustifolia*) leaves (0 to 40%) in substitution to a basal mixture. The crude protein (CP) concentration of Ash leaves was 10.9 % dry matter (DM), while neutral detergent fibre (NDF) and acid detergent fibre were 30.5 and 19.9%, respectively. A basal diet was formulated (32.51% NDF and 18.2% CP, on DM basis) and pelleted. Two others diets were obtained through substitution of 20 and 40% of basal diet by Ash leaves. Faecal digestibility was measured between 45 and 49 d of age on 12 young rabbits per diet, fed ad libitum since weaning (35 d, 802±197 g). The substitution of 40% of basal diet by Ash leaves didn't reduce the digestibility of organic matter, however digestibility of crude proteins, energy and NDF were reduced from 76 to 70%, 71 to 67% and 34 to 32, ($P < 0.01$), respectively. The digestible energy obtained by regression for shade-dried Ash leaves was 8.67±0.47MJ/kg DM, and the digestible protein content of Ash leaves was 71.55±7.3 g/kg DM.

Keywords: Dry ash leaves (*Fraxinus angustifolia*), Diet, Nutritive value, Growth rabbit, Fall

Introduction

Overall, acute shortage and animal proteins high price can be alleviated by small breeding sustainable, very prolific animal species with a short production cycle and valorizing non-competitive feeds on resources and space reserved for human food (Pothin et al., 2017). Concretely, the rabbit (Oryctolagus cuniculus) brings together all these assets. However, feeds lack affects negatively rabbit productivity and stimulates nutritionist's curiosity to seek and find new alternative and unconventional sources that are cheap and locally available.
Fodder trees and shrubs are important sources t fed small ruminants (Papachristou and Platis (1999); El hasan et al., 2000; Khanal & Subba (2001); Dini-Papanastasi et al., 2005; Pereira et al., 2008 ; Ahmed et al., 2015 and Kholif et al., 2015 & 2016), however the use available shrubs and trees remains a relatively little explored

subject in rabbit feeding locally. Tree leaves available locally and at low cost are in contrast to commercial feeds sold at high prices and often lacking essential raw materials due to fluctuations in their prices on global market. Algeria, like developing countries, has a weakness in animal proteins due livestock poor performance of, resulting from often irreversible increases prices of conventional raw materials and imported inputs. Thus, new "low-cost" feeds alternatives are being sought to replace conventional commercial concentrate raw materials. In rabbit nutrition there are some 170 plant foods and or by-products, recognized for their nutritional qualities in developing countries, while only 10% of them are introduced into the formulation of granulated rabbit diet (Finzi, 2008). Currently, new feed solutions have even become a *sine qua* non condition for meeting this constantly growing animal proteins demand. And this, can be accomplished by fully exploiting alternative feed resources advantage, such as volunteer herbaceous plants trees and shrubs leaves in rabbit diet (Raharjo et al., 1986; Deshmukh et al., 1993a,b; El-Gendy et al., 1999; Tedonkeng Pamo et al., 2007; Samkol & Lukefahr 2008; Kadi et al., 2011; Akoutey et al., 2012; Zeweil et al., 2013; Abu Hafsa et al., 2014).

Rabbit farming, for its multiple advantages, is becoming increasingly sought after as an alternative source to the deficit and animal proteins diversification in meat market local (Djellal et al., 2006; Kadi et al., 2013). Concretely, rehabilitating the use certain local and unconventional feeds sources can partly improve this food situation. Certain local plants, such as sulla (Abdelguerfi-Berrekia et al., 1991; Kadi et al., 2011) and certain fodder shrubs, were once used as a ruminant basic ration and monogastric herbivores. Such an alternative can improve and promote sustainable crop and livestock production diets.

Ash (Fraxinus ssp.) is fodder tree per excellence in the North Country. Mainly in Kabylia, it is subject of rigorous and regular exploitation. The presence of ash trees in the Kabyle landscape attests to strong link that exists between this tree and agriculture. Indeed, due to energy and nitrogen balance (Jayanegara et al., 2011), ash branches and leaves are widely used as a valuable dietary supplement for ruminants consuming poor basic diets (Pereira *et al.,* 2008). In addition to fodder reserves it constitutes, this tree plays a very important ecological and productive role in protecting land against erosion, produces firewood and cabinetry wood and has great capacity for bioabsorption of heavy metals and aqueous acid solutions (Abbasi et al., 2017).

Little or no ash leaves nutritional information is available, since information relating to their nutritional value is very fragmentary and concerns specifically small ruminant herbivores (Pereira et al., 2008). However, in rabbit feeding, ash leaves (F. angustifolia) are imperfectly known nutritionally, while characteristics knowledge relating to nutritional value (digestibility, nitrogen and energy value, etc.) and ingestibility determine their rational use. Therefore, the present work aim is to determine ash leaves nutritional value harvested in autumn and shade dried, distributed with increasing incorporation rates in pelleted fattening rabbits feed : this is the so-called range substitution method or regression method.

Method

Feeds, Animals and Experimental Setup

Ash leaves (*Fraxinus angustifolia*) were harvested fresh in autumn and immediately dried by displaying them in the shade for a week, in Ait Hague village located in Tizi-Ouzou (Kabylia). Then, they were crushed (sieved with a diameter of 3 mm) and transported to SARL "local production" livestock feed manufacturing unit in Algiers, to incorporate them into two experimental granulated feeds. A ash leaves sample was taken at the factory to determine its chemical composition. Ash leaves nutritional value was studied by measuring fecal digestibility of three granulated diets corresponding to a control feed; also called basic diet (FFA0) and two other diets (FFA20 and FFA 40) with an increasing ash leaves incorporation rate (Table 1).

Table 1. Feeds Ingredients experimental (%)

	Experimental diets		
Raw materials	FFA0	FFA20	FFA40
Basic diet[1]	97	77	57
Crushed Ash leaves	00	20	40
CMV	03	03	03
Total	100	100	100

[1] Basic diet composition (%) : barley (INRA 84) 12.06, olive cake 12.13, soybean meal 46 ("48"->INRA 190) 10.7 wheat bran (INRA 104) 62, Salt (NaCl) 0.8, CL25 premix rabbit vit+mineraux 0.5 et Calcium carbonate 1.8. Basic diet was formulated to meet nutritional recommendations for growing rabbits (De Blas and Mateos,

2010). This contains barley, olive cake, soybean meal and wheat bran (Table 2). Feeds containing ash leaves were prepared by substituting the basal diet (mineral-free and premix) with 20 or 40% ash leaves. Minerals and premixes were added to three diets a fixed rate of 3%. The ingredients and chemical composition of diets are listed in Table 1. The mixtures were pelleted to 4mm in diameter and 9mm in length using moist heat.

Table 2. Chemical composition and estimated nutritional value basal diet

Nutriment	Feed intake
Chemical composition (%)	
Dry matter	89,71
Crude ash	6,96
Crude Protein	15,85
NDF	37,31
ADF	14,96
ADL	06,20
Lysine	00,72
Méthionine	00,24
Total sulfur amino acids	00,53
Nutritive value	
Digestible Proteins (%)	11,82
Digestible Energy (MJ/kg)	09,64

36 rabbits from a local white population (Zerrouki et al., 2008) weaned at age 35 days with (Live weight: 802±197 g) were used to determine nutritional value of ash leaves in a private hutch (temperature: 10 to 25° C and lighting routine from 7:00 a.m. to 7:00 p.m.), located in the Isser region in Boumerdes. The animals were housed in cages 76 × 46 × 30 centimeters (length, width and height) and arranged in a Flat-Deck. They have all been equipped with a system designed to recover all droppings. Three groups of 12 rabbits were formed and assigned to three pelleted diets. The rabbits had free access to feeds and water. After an adaptation period of 12 days, feces (droppings) were collected from 45 to 49 days of age, according to harmonized and standardized European procedure of the EGRAN group (Perez et al., 1995).

Chemical Analyzes

Chemical analyzes of diets and ash leaves were carried out at INRA in Toulouse GenPhySE (Genetics, Physiology and Livestock Systems), according to ISO methods, respecting the recommendations proposed by the EGRAN group. (EGRAN, 2001): dry matter (MS; ISO 6496:1999), crude ash (ISO 5984:2002), crude protein (PCF; N×6.25, Dumas method, ISO 16634-2:2009), gross energy (ISO 9831:1998) and also NDF, ADF and ADL via the sequential Van Soest method (ISO 16472:2007 and ISO 13906:2008).

Table 3. Chemical composition of shade-dried ash leaves of and experimental diets (g/kg DM).

	Ash leaves (*F.angustifolia)*	Expérimental diets		
		FFA0	FFA20	FFA40
Humidy	09,32	12,88	05,06	05,20
Organic matter	89,68	91.33	91,00	92,40
Crude ash	10,19	08,67	09,00	07,60
Crude Protéins (N*6.25)	10,26	18,21	18,00	16,00
Neutral detergent fibre (NDF)	30,50	32,56	30,00	28,00
Acid detergent fiber (ADF)	19,94	12,53	13,00	13,90
Acid detergent lignin 5ADL)	08,56	03,72	04,00	04,70
Gross Energy (Kcal/kg DM)	4212	4635	4301	4304

Statistical Analyzes

The data were analyzed using a completely random system according to the GLM procedure of the SPSS software (Version 26) and as main of source variation the diets type. Comparison of means was carried out using the Tukey test. In addition, the effect of incorporating ash leaves was treated by regression using SAS software. The nutritional value of ash leaves was calculated according to regression method described by Villamide et al. (2001).

Results and Discussion

Research work on use dry or moist ash leaves in feeding rabbit is not available either in the ingredient tables (INRA, 2004), not in those updated by Lebas (2004), although chemical composition and nutritional value are available in some small ruminants (Perioro et al., 2008). Shade-dried ash leaves have a moderate concentration of crude proteins, i.e. 119 g/kg DM. This content is close to that reported by Jayanegara et al. (2011) about common ash leaves (Fraxinus excelsior) and variant from 14.1 for the first year of harvest to 12.1 g/kg DM just for the following harvest. It is close to that determined by Cazzato et al. (1994), 109 g/kg DM. However, it is lower than the crude protein concentration of plant Pueraria phaseoloides flowers, which is a legume naturally occurring everywhere in tropical and humid regions, ranging from 176 to 230 g/kg DM (Hiep & Man, 2008; Djago et al., 2010; Akoutey et al., 2012). While the average concentrations of NDF and ADF ash leaves in autumn are 305 and 199 g/kg DM, respectively. This NDF content is close to that ash common leaves (Fraxinus exscelsior) reported by Cazzato et al. (1994) and which is equal to 290 g/kg DM. On the other hand, ADF concentration of ashleaves emerging in autumn is much lower than that flowering Pueraria phaseoloides plant reported by Akoutey et al. (2012): 344 versus 199 g/kg DM.

The energy digestibility coefficient only decreased by approximately 4 points compared to the control diet (Table 4). As a result, the digestible energy (DE) content of the experimental diets decreased with the ash leaves incorporation from 13.71 to 11.99 MJ/kg DM (Table 5). When we extrapolated to 100% (using linear backtracking), the predicted digestible energy content of shade-dried ash leaves is 10.64 MJ ED/kg DM for a 0-40% range incorporation In comparison with "Alfalfa Meal 12" (7.5 MJ/kg DM; Maertens et al., 2002), the DE content of ash leaves is 41% higher. The apparent crude protein (CP) digestibility coefficient decreases linearly and abruptly from 76 (FFA0) to 69% (FFA40) with an incorporation rate of 40% ash leaves (Table 5). As a result, the experimental diets digestible protein (DP) decreased from 138 to 111 g PD/kg DM with ash leaves incorporation. When we extrapolated to 100% (using linear backtracking), the DP content was estimated to be 65.9 g/kg DM in 0-40% incorporation range of ash leaves. On the other hand, this content is higher in 0-20% incorporation rate, predicted at 112.7 g/kg DM. This value is higher than that Alfalfa "12" and which is 78 g/kg DM according to Maertens et al. (2002). Overall, nutritive value of ash leaves, shade-dried and pelleted, is acceptable for growing rabbits, compared to other leguminous plants such as alfalfa, sulla (*Hedysarum fluxiosum*). This is partially explained by the average growth achieved by rabbits during trial period with two rate incorporation into the experimental diets FFA20 and FFA40 (Table 4).

Table 4**.** Effect of ash leaves dietary level on feed consumption and rabbit growth during the two periods of the trial.

	Experimentals diets			ES	p
	FA0	FA20	FA40		
n	12	12	12		-
Weight live at 35 j (g)	837.14	803.12	759.16	43	0.794
Weight live at 45^2 j (g)	1117.14	1195.62	1119.16	50	0.772
Weight live at 49 j (g)	1253.57	1333.75	1239.16	51.7	0.734
Consumption 35-45j (g)	76.78	68.00	77.50	0.4	0.611
Consumption 45-49j (g)	85.71	97.03	99.58	1.1	0.417
Consumption 35-49j (g)	79.34	76.30	83.83	1.4	0.758
Gain de poids 35-45 d (g/j)	36.16	39.25	36.00	1.6	0.586
Weight Gain 45-49 d (g/j)	34.10	34.53	30.00	1.6	0.472
Weight Gain 35-49 d (g/j)	36.06	37.90	34.26	2.6	0.487

ES: standard error, 45^2 d: start digestibility test (Collection period).

It appears that these ash leaves are well balanced in essential amino acids since their PD content is moderate. What is already reported by Gidenne et al. (2015) for certain raw materials used in feeding growing rabbits achieving weight increases. Therefore, it would be convenient to obtain ash leaves with a higher protein content, taking care to carry out pruning in smart or planters depending on the shape and age of the ash and Preferably use leaves from young branches a few years old. Presumably, tree leaves nutrient content is related to age of the tissues that form leaves and stems of plants that have never been used before (Tsiouvaras & Nastis, 1990; Cazzato et al., 1995; Jayanegara et al., 2011).

Hiep & Homme (2008) showed that *Pueraria phaseoloides* whole plant can be introduced in a rate of 20 to 40% as a source of fiber in rations of growing rabbits. Also, Nieves et al. (2004) recommends a incorporation rate of *Leucaena leucocephala* green leaves into fattening rabbit diets ranging from 24 to 40%. However, no rate of

incorporation of ash leaves under all its forms have not been previously decided for growing rabbits. Precisely, the present study demonstrates that ash leaves, harvested in autumn, can be incorporated into diets for growing rabbits at a level of 20 to 40% as a fiber and energy source.

Table 5. Effect of ash leaves level incorporation (Fraxinus angustifolia) on apparent digestibility (%) and nutritive value of experimental diets in growing rabbits diet.

	FFA0	FFA20	FFA20	*SEM*	*P value*
n.	12	12	12		
Digestibility Coefficient de (%)					
Dry matte	70,5	69,6	71,0	0,74	> 0.05
Organic matter	70,8	69,5	71,1	0,75	>0.05
Crudes proteins	76,1	74,9	69,7	1,16	>0.05
Gross Energy	70,7	66,6	66,9	0,91	>0.05
Neutral detergent fibre	33,7	30,6	31,7	1,72	>0.05
Acid detergent fibre	21,1	22,5	32,5	2,10	>0.05
Nutritive Value					
Digestible enrrgy (Mj/kg)	13,71	11,99	12,05		
Digestible proteins (g/kg)	138,6	134,8	111,5		

Conclusion

Ash leaves, harvested in autumn, can be considered a good source of energy (8.67 ± 0.47 MJ/Kg DM) and fiber, but a moderate source of digestible protein (71.55 ± 7.3 g PD/kg DM). However, additional trials are necessary to verify growth and health performance using a large number of rabbits, fed with a complete and balanced diet including a high proportion of ash leaves and harvested in autumn.

Recommendations

Ash leaves, harvested in autumn, can be incorporated into meat rabbit diets. They are an excellent source of energy and fiber, and moderate source protein digestible.

Scientific Ethics Declaration

Ash leaves, harvested in autumn, can be incorporated into meat rabbit rations. They are an excellent source of energy and fiber, and a moderate source of digestible protein.

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Prevalance of Hereditary Disorders in Imported Brown Swiss Bull Sperms throughout Ten-Year Period

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Abstract: The purpose of this study was to determine hereditary defects of frozen Brown Swiss bull sperms imported to Turkiye during the period between 2013 and 2022. The data were obtained from website of General Directorate of Livestock of Ministry of Agriculture and Forestry. The hereditary status of each imported bull was examined on various website producing bull sperms or including their databases. Totally, 37 of 375 bulls whose frozen sperms imported to Turkiye carry at least one genetic defect. A total of 30 bulls carried Braunvieh Haplotype 2 which was the most prevalent disorders among examined ones. The carried bulls were reported to be originated from USA, Germany and Italy respectively. The study was first to examine occurrence of hereditary disorders of imported Brown Swiss bull sperms in Turkiye. The result of this study would be a beneficial for authorities to make more precautions during importing frozen bull sperms against genetic pollution.

Keywords: Brown swiss, Frozen sperms, Hereditary disorders, Genetic defects, Genetic pollution,

Introduction

The artificial insemination (AI) implementation has been popular for decades in Türkiye. Almost all heifers and cows are getting pregnant via AI implementation. For this purpose, a considerable amount of frozen sperm has been imported annually. The procedures and principles regarding the import of sperm, ovum and embryo were issued by General Directorate of Livestock of Ministry of Agriculture and Forestry.

Although considerable number of Brown Swiss Bulls bred for the purpose of producing frozen sperms carried some hereditary disorders, there is no regulation against importing frozen sperm straws of carriers. Hereditary disorders cause genetic pollution throughout worldwide. So, the frozen sperms should be tested for common hereditary disorders and the results should be specified in pedigrees. The aim of this study was to determine hereditary disorders of frozen Brown Swiss Bull sperms imported to Turkey between 2013 and 2022.

Method

The excel worksheets including the name and ID of the bulls, the related companies and the number of straws were downloaded in the website of General Directorate of Livestock of Ministry of Agriculture and Forestry. In this study, hereditary status of imported Brown Swiss bulls throughout the last ten-year period were examined and separated into years and origins. Some of imported bulls were the same bulls which were imported in different years. So, they combined for general examination which reduced to 375 bulls (1777220 straws). Table 1 showed websites of various companies that produce sperms, bull catalogues and databases of Brown Swiss bulls. Effect of number of carriers on different year was analyzed using Chi-Square analysis in SPSS (ver. 25.0). %5 confidence interval was accepted for the significance level of the tests.

Table 1. Websites of various companies investigated in the research

Company	Websites
Braunviehzuchtverband	www.braunvieh.it
Zuchtwert Austria	www.zuchtwert.at
ST Genetics	www.stgen.com
Accelerated Genetics	www.accelgen.com
ABS Global	www.absbullsearch.absglobal.com
World Wide Sires	www.ct.wwsires.com
Genex	www.catalog.genex.coop
Aberekin	www.aberekin.com
New Generation Genetics	www.brownswiss.com
Superbrown	www.superbrown.it
Club Brown Swiss du Quebec	www.brownswissquebec.com
Synetics	www.evolution-xy.international.com
Swiss Genetics	www.swissgenetics.com
Brown Swiss Association	www.brownswissusa.com
Brune Genetique Services	www.brune-genetique.com
DataGene	www.datagene.com.au

Results and Discussion

Brown Swiss cattle has been breeding in Turkey for years and well accepted by farmers in terms of its beef and adaptation capabilities. The study was the first to examine all Brown Swiss frozen sperms imported to Turkiye throughout ten-year period. Table 2 shows hereditary defects of Brown Swiss frozen sperms imported throughout the last ten-year period. The results showed that 37 of 375 imported bulls carried at least one genetic defect. BH2, characterized by high newborn mortality (Schwarzenbacher et al, 2016), was the most commonly seen hereditary disorder to compare with other defects.

Table 2. Hereditary defects of the Brown Swiss frozen sperms imported between 2013 and 2022.

Hereditary defects	Carriers (n)	Straw (n)
BH2	30	184388
SM	1	18605
W	2	11100
BHD	2	8775
A	1	1950
BH14	1	5000

Frequency of hereditary defects in different years was presented in Table 3. Significant differences were observed among carriers imported in different years. No carriers were determined in the bulls imported after 2018.

Table 3. Effect of year on frequency of hereditary disorders of the Brown Swiss frozen sperms

Year	n	Carriers rate	Straw Number of Carriers	Total Straw Number
2013	38	21,05^{ab}	23332	173677
2014	50	30,00^{a}	55797	214682
2015	64	14,06^{b}	55543	361153
2016	44	9,09^{bc}	14127	191161
2017	36	2,78^{c}	3010	178228
2018	26	30,77^{a}	66409	157762
2019	15	0	0	54365
2020	43	0	0	121982
2021	74	0	0	191553
2022	52	0	0	132657

^{a,b,c}: Different superscripts in the same column show significant differences.

Frequency of carriers from different countries were shown in Table 4. The results showed that majority of Brown Swiss bull frozen sperms were imported from Italy, Germany, Switzerland and USA respectively. More than 10% of imported Brown Swiss bulls from USA, Germany and Italy carried at least one genetic defect.

Table 3. Frequency of carriers from different origins.

Year	n	Carriers rate (n)	Straw Number of Carriers	Total Straw Number
Austria	13	0	0	61425
Switzerland	54	3,70 (2)	14092	284011
Germany	77	11,69 (9)	33642	349117
Spain	4	0	0	41476
France	19	5,26 (1)	18605	76577
Italy	159	10,06 (16)	128801	817444
Netherlands	1	0	0	20192
USA	48	16,67 (8)	26078	126978

Conclusion

The study showed that the highest frequency among investigated hereditary disorders was Braunvieh Haplotype 2. The highest frequency of carriers was originated from the bulls imported from USA, Germany and Italy respectively.

Recommendations

The result of this study encourages authorities to make more precautions during importing frozen bull sperms against genetic pollution.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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Growth Performance of Ouled Djellal Male Lambs at Semi-Arid Region of Setif/Algeria

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Abstract: Herd sheep is the leading supplier of red meat in Algeria, which places fattening lambs the animal speculation of choice. Despite the extent of this practice and its importance in the national economy, few studies have been devoted to characterizing it. Precisely, the aim of the present work is to study the growth performance Ouled Djellal lambs male in the fattening phase at semi-arid region of Serif. A set of 43 weaned lambs aged 7 months and over are monitored and weighed regularly every ten days for two months. The animals are fed with a commercial concentrate, wheat straw and water *ad-libitum*. The results obtained on the male lambs growth remain acceptable. Thus, the initial average live weight is 33±1.48 kg and the final average live weight is 43±1.44 kg, which gives us an average daily gain (ADG) of 165±45 g/d. This reveals the growth potential of Ouled Djellal lambs and its preferred choice by breeders-fatteners. While remembering that the majority of lambs were grass-fed before fattening period. There is therefore poor exploitation growth potential of these lambs, food deficits and poor management breeding are probably the cause.

Keywords: Male lambs, Ouled Djellal, Growth, Fattening, Meat

Introduction

A polygastric herbivore ruminant, sheep is not a direct competitor to man. Formerly domesticated by humans, it is among the most efficient livestock species. It enhances grass it grazes on, even when it concerns plants rich in parietal carbohydrates; it transforms poor quality fodder into high biological value proteins. Sheep meat comes from a wide farming systems variety using extensive outdoor or intensive indoor rearing methods with different ages at slaughter animals. There are strong country-specific preferences meat quality sheep, linked to production system characteristics such as pasture-based systems (Prache et al., 2022).

Algeria occupies a strategic geographic position in Africa. The heterogeneity of its bioclimatic levels gives it a natural capital of animal biodiversity and plant genetic resources. Sheep farming is practiced almost throughout the national territory. However, its concentration is greater in North Country, and even with a greater concentration in steppe and semi-arid cereal-producing high plains. It represents 25 to 30% in animal production and 10 to 15% in agricultural production and provides more than 50% red meat production national (MADR, 2020). Ouled Djellal breed is the first and main sheep breed in Algeria in number and distribution terms. It is characterized by its hardiness and its most extreme environmental conditions tolerance (Chellig, 1992).

One objectives observatory sheep is, through long-term monitoring, to highlight income factors main and their evolution. These factors can be external (economic conditions, support linked to agricultural policies) or internal, linked to routes and technical performances. These vary greatly and have a strong impact on technical and economic results (Benoit & Laignel, 2006). Considerations for improvement must focus on herd rational exploitation in addition to increasing numbers, as well as an evaluation of performance and their genetic improvement.

Supplying animal proteins local market with a population that continues to evolve requires need to increase livestock systems productivity and diversification their products. This requirement to supply sufficient quantities from meat local production, milk and eggs is the justification intensive systems development, the only ones capable guaranteeing productivity gains desired (Chniter, 2013). However, for them to be profitable, sheep must be well managed. This requires application of certain well-reasoned management practices to ensure herd overall well-being (Boujenane, 2005).

Little research work is devoted studying growth Ouled Djellal breed lambs performance finished at the trough. In this regard, we have started this study whose main objective is to characterize lambs growth performance at fattening phase (live weight and ADG) of lambs kept extensively. Moreover, this contribute to resolve an economic problem, which is animal productivity improvement. In other words; know where to intervene? To improve said trough finishing phase growth performance.

Method

Animals and Experimental Conditions

Our study focused on 43 males aged 7 months and over, sold after fattening. All animals belonging to Ouled Djellal breed, given that it is the dominant breed in numbers terms not only on a national scale, but also in the east country, which constitutes breed cradle (Figure 01). The lambs tracked were identified by an ear tag bearing a number for each lamb tracked.

Figure 1. Lambs fattening in a sheepfold.

Figure 1. Lambs fattening in sheepfold

Method Breeding

With same origin, all lambs have in common particularity of having been raised generally in the same way: extensive breeding since lambs were raised only on range from their second month of age and without their mothers separation (that is to say without weaning), they then underwent a fattening period of approximately 2 months before their sale.

The Breeding Method

The fattening diet consists concentrate commercial and wheat straw, introduced gradually at fattening start, then distributed at will until removal day. Antiparasitic treatment was started. Like all domestic herbivores, lambs need receive a transitional ration before switching new diet to preserve rumen health. The adaptation period allows the microbial flora to new diets adapt. Water was available ad libitum.

Collection of Data

The measurements were carried out for all lambs retained at pilot farm MAKHLOUFI Aïssa ; located in El Eulma (Sétif) during two months.

Farm Level Measures

The characteristics and measurements retained for animal assessment:

Live Weight (PV)

Lambs were weighed individually and regularly every 10 days, until end fattening period (Figure (08) i.e.: two months. Arithmetically, fattening phase is divided into six typical periods (Period 01, 02, 03, 04, 05 and 06). Lambs are weighed using an electronic scale (±10g). The seven weighing were carried out before ration distribution. The average daily gains for each lamb (ADG) were calculated between two successive standardized weighings and according following formula:

$$ADG = Initial\ live\ weight - Final\ live\ weight\ /10$$

Quantities of Feed Distributed

Several samples feed distributed (wheat straw and barley) were taken at each visit. And this, to characterize and quantify them with precision. The adaptation period new diet was respected. For this, the new fattening ration was introduced gradually at fattening period start, in order to avoid any possible metabolic deviation. The feed quantities distributed to animals monitored are given in table (01) below:

Table 1. Quantity and feed distributed to animals during the fattening phase

Feeds / Périodes	Period (01)	Period (02)	Period (03)	Period (04)	Period (05)	Period (06)
Pasture (h)	00	00	00	00	00	00
wheat straw (Kg)	03.00	03.00	02.50	01.50	01.50	01.50
Commerce concentrate (Kg)	00.30	00.50	00.750	01.00	01.00	01.00
Water	*Ad-libitum*	*Ad-libitum*	*Ad-libitum*	*Ad-libitum*	*Ad-libitum*	*Ad-libitum*

Centesimal composition of commercial concentrate manufactured by the National cattle Feed Office (ONAB) is recorded in following Table (02):

Table 2. Percentage composition of ONAB commercial concentrate distributedto lambs in the fattening phase

	Corn	Wheat bran	Limestone	CMV	Salt	Non-conventional phosphate
Content (%)	50.00	46.08	02.10	01.00	00.50	00.32

Statistical Processing

The raw data obtained from carried out weighings are entered into a workbook Excel. They are then subjected to a descriptive statistical analysis (means, standard deviations, standard errors, etc.) using the XLSTAT software

version 2017. In addition, an analysis of variance is carried out to check the effect of the lambs live weight at the start of fattening on the live weight at the fattening phase end.

Results and Discussion

Evolution of Live Weights

The overall averages obtained for male lambs live weight gains in our study are represented in table (03) below:

Table 3. Overall average live weight during the different fattening periods

	BW(01)	BW (02)	BW (03)	BW (04)	BW (05)	BW (06)	BW (07)
BW mean (kg)	33	35	37	39	41	42	43
Standard Deviation	08	09	09	09	09	10	10
standard Error	1.21	1.29	1.33	1.32	1.37	1.40	1.44
BW Gain (kg)	01.48	02.11	02.65	01.44	01.26	01.26	00.93
	Typical periods of the fattening phase						
BW Gain (kg)	P1	P2	P3	P4	P5	P6	P. Globale
	1.48	2.11	2.65	1.44	1.26	0.93	9.88

BW : Body weight

The overall averages live weight gain obtained in our study are different from one period to another. It displays a maximum (02.65 ± 01.33 kg) in period (03) and a minimum (00.90 ± 01.44 kg) in period (07) during the fattening period. This difference can be explained by compensatory power of breed following a sufficient feed supply. This observation is already reported by Djellal et al. (2021) for male lambs of the Ouled Djellal breed growing post-weaning and managed extensively at Bordj-Bou-Arreridj region. Contrary to our results, Adaouri (2019) records a better live weight gain of crossbred Ouled Djellal breed lambs (Ouled Djellal × D'man), and D'man breed with respective weights of 49.92; 49.13 and 47.95 Kg at the end fattening trial. This can be explained a priori by diet. Indeed, the scientific literature reports that the ration composition is even more influential since some authors (Droguaul et al., 2004) managed to measure the differences in body composition generated in lambs by using different nature of cereals in fattening diet. In other words: wheat promotes fixation a higher proportion of fatty tissue than barley which itself gives fattier carcasses than corn. These results confirm that fattening method and diets are behind the variations in fattening state of lambs since they constitute the most influential factors on the composition and quality of both carcass and meat in sheep. Concretely Adaouri (2019) confirms this, expressed in g of DM/kg Metabolic weight, The quantities of average dry matter voluntarily ingested during the entire test period stand at 89.55±8.57 g of DM/kg P0 .75; 80.05±9.12 g of DM/kg P0.75 and 62.01±11.18 g of DM/kg P0.75 respectively for crossbred lot lambs, the Ouled Djellal lot and the D'man lot . This quantity ingested is statistically different between three genotypes ($p<0.001$).The overall weight gain trend line is bearish as shown in Figure (02) below:

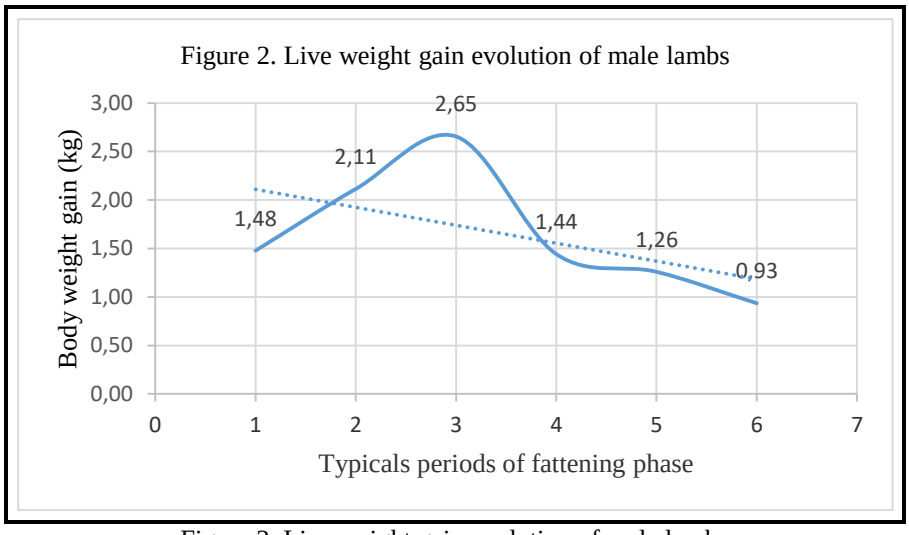

Figure 2. Live weight gain evolution of male lambs

It shows accelerated growth at start of following fattening phase followed by another growth decline (1.48, 2.11 2.65, 1.44, 1.26 and 0.93 kg). This downward trend in live weight gain is widely known in ruminants. After compensatory growth and with animals advancing age we observe consumption index increase. This has a negative impact on sheep live weight gain and growth rate.

Lamb Growth

The overall male lamb average daily gains (ADG) during different periods of fattening phase are recorded in table (04) below:

Table 4. Overall averages of average daily gains of male lambs during the different typical periods of fattening

Statistical parameters	Typical periods of the fattening phase						
	P1	P2	P3	P4	P5	P6	P. Globale
Average daily gain (g)	148	211	266	144	126	94	165
Standard Deviation	134	179	106	74	65	52	45
Standard error	20	26	15	11	9	8	7

It should be remembered that from birth to 110 age days, lambs had a weight that was higher the heavier they were at birth. During the milky phase, the average daily gain (ADG) is positively linked to birth weight, despite greater relative growth at the same lambs age (Djellal et al., 2016). After weaning and during the fattening phase, the estimated consumption at same age or the same weight is birth weight independent. The same is true for feed efficiency calculated overall fattening period (Ziani, 2016).

In our study, the highest average daily gains (ADG) are recorded during P3 and P2 and lowest at the end fattening phase; i.e. period P6. The growth speed increase at start of our study can also be explained by the diet adopted. Several studies show existence a positive relationship between diet protein level and carcass muscle tissue development (Lebret and Mourot, 1998). And also, between energy level and fat deposition (Atti et al., 2003). However, research intended to quantify protein level effect of diet on quality and carcass composition still remains limited. Concretely, fattening lambs with barley is a very widespread practice in sheep farming. Many studies have shown effect of diets based on cereals, such as barley, on the rapid growth of sheep and cattle (McDonald et al., 1996).

With an overall ADG equal to 165±45, the sheep weight evolution monitored is considered acceptable. Our results are higher than ADG (110-200 g/d) observed by technical institute of cattle and sheep breeding ITEBO (1995) in Ouled-djellal lambsbreed which reached a live weight varying between 38-40 Kg. Still significantly higher than that provided by Arbouche et al. (2014), reporting one of the most reliable ADG recorded in Ouled Djellal breed lambs in fattening phase (84 g/d), i.e. at 90 days of age.

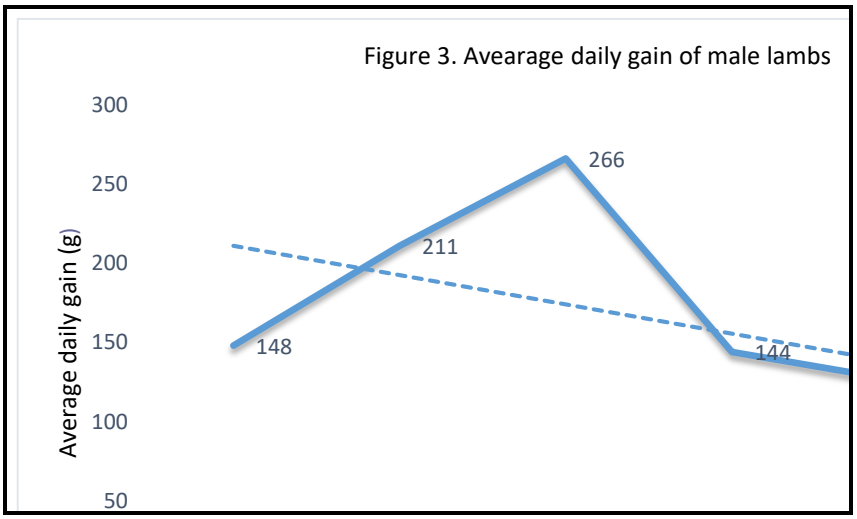

Figure3. Avearage daily gain of male lambs

However, daily growth rate of our animals is lower than those reported by Ziani (2016) in Hamra breed lambs fed with two different diets (EC vs CC) and which is on average 230.26±15.49 and 213.16± 17.69g/d;

respectively without any significant difference, with feed efficiency (6.09 vs 5.63). These weight and growth performances demonstrate the satisfactory growth potential of Ouled Djellal lambs. This value is significantly lower than those obtained with Hmra breed lambs (199 g to 227 g per day) by Boujenane (2005) ; El-Fadili, (2009) and El-Fadili and Lakhssassi, (2010), and other values obtained in sheep breeds Moroccan by Chikhi and Boujenane (2005) ; El-Fadili (2009) (Sardi: 282 g/d; Boujaâd: 278 g/d and Beni Guil (195 g per day). While Ibnelbachyr et al. (2014) report a lower value (127 g/d) in D'Man breed at Tafilalet oases and averages recorded by Saïdi et al. (2011) in Tunisia Queue Fine de l'Ouest breed lambs (QFO: 101 g/d) and QFO x D' crossbred sheep Man (152 g per day). Finally, Atti and Mahouachi (2011) report that fat-tailed Barbarine (FTB) lambs generally achieve moderate growth with an average daily gain between 100 and 350 g.

One causes could explain this weight gain fluctuation is the increase in body weight and the age of the lamb. This tends to increase nutritional requirements in relation to the increase in lamb weight; maintenance needs in particular. Indeed, diet may be one of the determining causes of this downward trend.

Effect of Live Weight at Entry into Fattening on Lambs Absolute Growth

The live weight at fattening phase start does not significantly affect (P= 0.9) the live weight lambs at the fattening phase end (table 5).

Table 5. Effect of initial live weight at entry into fattening at fattening performance

Live weight at the beginningof fattening (kg)	live weight at the endof fattening (kg)	*P value (P)*
33±1.21*	43±1*	0.9

*: mean ± standard Error

This Result Is Contrary To the growth results displayed by Ouled djellal lambs in the post-weaning period. This result is contrary to growth results displayed by ouled djellal lambs in post-weaning period. According to Djellal *et al.* (2021), whatever considered period (between 120-150 d, 150-180 d, 180-210 d and 120-210 d), the growth levels are characterized by significant individual variations, overall they are more pronounced in skinny animals. It is also reported that lambs sex and ewes parity do not influence growth performance from birth to weaning (Baa *et al.*, 2020). Other studies have shown that food consumption does not increase after food restriction period (Kabbali et al 1992, Turgeon et al 1986). In any case, it has been shown that cattle having compensated had a heavier digestive tract (Wright and Russell 1991), while Kamalzadehab et al. (1998) report that only the weight of the small intestine was higher in cattle compensatory animals. This is also indicated by Pérez-Clariget (1998), stating that at the end of the experiment, the weight of small intestine was higher in the group of sheep that underwent food quality restriction.

Conclusion

The results obtained on male lambs Ouled Djellal breed growth remain appreciable. Thus, the average weights are 43±10 at end of fattening. Male lambs showed an average daily gain of 165±45 g/d. The live weight at fattening phase start does not significantly affect (P= 0.9) the live weight lambs at the fattening phase end This shows the acceptable growth potential Ouled Djellal breed lambs and its preferred choice by breeders-fatteners. However, there is poor exploitation growth potential of these animals, feed deficits and poor breeding management are probably the cause, especially when we know that majority lambs were grass-fed before the fattening period. And often, this phase still fails to fill this deficit. Consequently, these results make it possible to consider the exploitation and optimization performance of this breed in breeding systems oriented towards meat production in the Algerian context.

Recommendations

The particular interest that we must have in technical aspects of sheep breeding must not make us forget the importance of economic aspects, since the final objective remains the best possible valorization the product, whether meat, wool or milk. At the end of this research applied to Ouled Djellal breed in the semi-arid context of Sétif, it appears that breeding activities of this breed observed and experienced suggest significant margins of progress by applying action plans appropriate technique in relation with breeders who adopt this breed thanks to

better optimization of its production potential on the one hand and technical support services closer to these breeders on the other hand..

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

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A Comparison of Two Estrus Synchronization Protocols Utilizing Progesterone Supplement 24 Hours before CIDR-G Removal in Sheep

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Abstract: Two experiments investigated the effect of progesterone administration 24 h prior to intravaginal CIDR-G withdrawal using two protocols in two geographical locations and different breeds of sheep. Ewes in both experiments were administered with intravaginal CIDR-G either for 8 days (Expt. 1, n=36 thin tail mixed-breed ewes) in early April or for 12 days (Expt. 2, n=28 fat tail Awassi ewes) in late May. Half of the ewes in each experiment were assigned randomly one day before CIDR-G removal and given 25 mg P4 (P4-treated) and the second half were given saline solution and served as controls. Fertile rams were allowed with the ewes of both experiments immediately following CIDR-G removal (day0, 0h) and estrus was monitored at 6-h intervals for 4 days. Blood samples were collected for analysis P4 and LH levels. Estrus responses differed between the two experiments. P4 profiles during the period of CIDR-G insertion behaved the same way among ewes of both experiments, but levels differed ($P < 0.05$) on day 0 in response to P4 supplement. The preovulatory LH surge was greater among groups treated with P4 supplement than those that did not. In both experiments, the percentage of ewes exhibiting estrus was greater ($P < 0.05$) and intervals to estrus were longer ($P < 0.05$) in P4-treated than control ewes. In the 12-day protocol in Expt. 2, ewes treated with P4 supplement produced greater ($P < 0.05$) incidence of estrus, longer ($P < 0.05$) intervals to onset of estrus and LH surge and had higher ($P < 0.01$) magnitude of LH surge and pregnancy rate than controls and those of the 8-day protocol. In conclusion, P4 supplement 24 h prior to CIDR-G removal in a 12-day protocol can be used successfully to improve reproductive performance of ewes.

Keywords: Progesterone supplement, Sheep, LH surge

Introduction

Reproductive performance is one of the major factors having an impact on sheep industry that will ensure future success and long-term sustainability. In sheep, estrus synchronization protocols implemented for 12 to 14 days increase reproductive efficiency through hormone administration (Kuru et al., 2022). Incorporation of additional injection of P4 in an estrus synchronization protocol produced superior reproductive performance in Awassi ewes (Husein and Ababneh, 2008). Such a protocol was based on an injection of 25 mg P4 administered 24 h before intravaginal inserts removal in a 12-day CIDR-G scheme (Husein & Ababneh, 2008). These authors reported 100% pregnancy and lambing rates in non-prolific Awassi ewes treated with P4 supplementation out-of-season.

There have been concerns that results reported by Husein and Ababneh (2008) are repeatable and may produce similar outcomes despite changes in latitude, season, nutrition, breed and duration of the synchronization protocol. The intention, therefore, was to compare the effect additional P4 supplement in two different estrus synchronization protocols using prolific breeds of sheep raised in non-tropical areas and non-prolific sheep raised under subtropical environment. Effectiveness of P4 supplement may be altered by any of these factors.

The aims of this study were to compare the effectiveness of an 8-day CIDR-G-P4 with a 12-day CIDR-G-P4 supplement protocols in two different geographical locations and to investigate whether or not results obtained are repeatable. Other aims were to clarify changes in hormonal profiles of P4 and LH in plasma samples of ewes and to identify related factors that are conducive to pregnancy).

Method

General

Different breeds of multiparous ewes were used in two experiments to investigate the effect of progesterone (P4) administration 24 h prior to intravaginal CIDR-G withdrawal using two protocols conducted in two geographical locations. Ewes were handled humanely in the two experiments and all procedures pertaining to the use of animals complied with the rules approved by the Animal Care committee of each location. Ewes in both experiments had previously lambed during the past lambing season and had their last lambs been weaned about 8-10 weeks before the start of each experiment. During the experimental period, ewes in each experiment were housed together in a single pen and were fed 0.5 kg concentrate mixture (13% CP) per ewe per day, and had *ad libitum* access to alfalfa hay or wheat straw, water, shade and mineral salt blocks.

In experiment 1, 36 thin tail multiparous mixed-breed ewes (Montadale, Southdown, Tunis and Colombia) were assigned randomly to two treatment groups (P4-treated, n= 18 and control n= 18). All ewes were administered with intravaginal CIDR-G Pharmacia and Upjohn Ltd. Co., Mt Wellington, Auckland, New Zealand) devices containing 300 mg P4 for 8 days. Devices were inserted on April 6 (day −8) and were removed 8 days later on April 14 at 08:00 (Day 0 and hour 0). This study was conducted in a farm located in Goodhue County in Minnesota (latitude 44°23'N, longitude 92°26'W) at an altitude of 340 m. Ewes ranged in age from 3 to 6 years and weighed 65.4 ± 1.4 kg (range= 52 to 81) and had a body condition score of 2.5 to 3 (scale= 0 lowest to 5 highest).

In experiment 2, 28 Awassi ewes were assigned at randomly to two treatment groups (P4-treated and control) of 14 ewes each. Ewes were induced to estrus using intravaginal CIDR-G (Pharmacia and Upjohn Ltd. Co., Mt Wellington, Auckland, New Zealand) devices containing 300 mg P4 for 12 days. Devices were inserted on May 27 (day −12) and were removed 12 days later on June 8 at 08:00 (Day 0 and hour 0). This study was conducted in a farm located at the Agricultural Center for Research and Production at Jordan University of Science and Technology (latitude 32º33'N, longitude 35º51'E). Ewes ranged in age from 3 to 7 years old and weighing 51 ± 1.2 kg with a body condition score of 2.5 to 3 (scale= 0 lowest to 5).

One day before CIDR-G removal in both experiments (day −7 in Expt. 1 and day −11 in Expt. 2), half of the ewes (P4-treated) were given an exogenous injection of 25 mg P4 (Intervet UK Ltd., Science Park, Milton Road, Cambridge, UK) supplement each and the second half were given saline solution and served as controls. Fertile rams were allowed with the ewes of both experiments immediately following CIDR-G removal (Day 0 and hour 0) and estrus was monitored at 6-h intervals for 4 days. Blood samples were collected for analysis P4 and LH levels. Jugular venous blood samples were collected every other day starting immediately before CIDR-G insertion until day −2 and once daily from day−1 until day 3 to compare P4 concentrations. Blood samples also were collected at 6-h intervals starting immediately after CIDR-G removal for 72 h for LH analysis and then on alternate days thereafter until day 19 for pregnancy diagnosis.

Plasma P4 concentrations in Expt. 1 were measured using a solid-phase RIA. Sensitivity was 0.1 ng/mL and intraassay coefficient of variation was 2.9%. Plasma P_4 concentrations in Expt. 2 were measured by a solid-phase RIA using a commercial kit [Coat-A-Count procedure, Diagnostic Products Corporation (DPC), Los Angeles, CA]. Sensitivity was 0.1 ng/ml and intraassay coefficient of variation was 5.3%.

Plasma LH concentrations in Expt. 1 were measured in duplicate of 100 µl plasma aliquots using a double-antibody RIA (Husein et al., 1996). Assay components were NIH-LH-S19 for reference, NIADDK-oLH-I-4 (AFP-8614B) for radioiodination, and NIADDK-anti-oLH-1 (AFP-192279) serum. Sensitivity was 0.5 ng/ml and the intraassay CV was 7.1%. Plasma LH levels in Expt. 2 were determined using ovine LH ELISA commercial kit (Endocrine Technologies Inc., Newark, CA). Sensitivity was 0.1 ng/ mL and intraassay coefficient of variation was 7.9%. Pregnancy was diagnosed on day 30 using transrectal ultrasound transducer (Aloka 500V scanner and a 7.5-MHz human prostate transducer; Corometrics Medical Systems, Inc., Wallingford, CT).

Statistical Analysis

Data were analyzed by using SAS/STAT ANOVA procedures (SAS Inst. Inc., Cary, NC., 2006). Data in text, tables and figures are presented as means ± SEM. Onset of estrus was considered to have occurred 6 h before observation of a breeding mark among ewes of both experiments. The effects of P_4 supplementation on incidence of estrus, preovulatory LH surge and pregnancy were analyzed using the *Chi-square* test. The effects of P_4 supplementation on the amplitude of LH surge and various intervals to onset of estrus and LH surge were analyzed using least-square means of the GLM procedures. Plasma P_4 and LH concentrations were analyzed for the effect of treatments and time using the repeated-measures procedure of GLM.

Results and Discussion

Experiment 1

Progesterone Concentration during the 8-Day Period in Which CIDR-G were in Place:

Ewes maintained CIDR-G devices during the period of insertion until they were pulled out. Progesterone concentrations in plasma samples of ewes during the period from day –8 to day 3 are illustrated in Figure 1. Initial P4 concentrations in plasma samples taken prior to CIDR-G insertion were 0.49 ± 0.06 ng/ml among ewes of the two groups, indicating probably seasonal anestrus and/or transitional period from estrus to anestrus in some ewes. Following CIDR-G insertion P_4 concentrations increased sharply and maximum values were reached 2 days post insertion (day –6) and were 5.7 ± 0.8 and 5.9 ± 0.6 ng/ml in the P4-treated and control groups, respectively. Differences in maximum P_4 concentrations on day –6 were not significant ($P > 0.1$). Progesterone gradually decreased by day from day –6 until day –1 and concentrations on day –1 were similar ($P > 0.05$) between P4-treated and control groups 3.1 ± 0.3 and 2.9 ± 0.2 ng/ml, respectively .

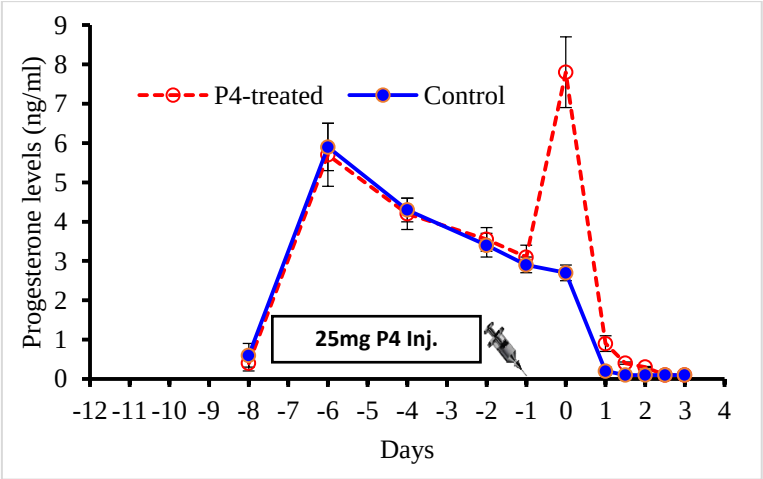

Figure 1. Plasma progesterone concentrations in Expt. 1 during the period in which CIDR-G were in place in P4-treated and Control groups from day –8 to day 3.

In the P_4-treated ewes, P_4 concentrations increased abruptly in response to the P_4 supplementation on day –1 from 3.1 ± 0.3 to 7.8 ± 0.9 ng/ml on day 0, then decreased sharply to 0.9 ± 0.2 ng/ml on day 1. Progesterone concentrations in the control group decreased from day –1 values of 2.9 ± 0.2 to 2.7 ± 0.2 ng/ml on day 0, then gradually declined to a value of 0.2 ± 0.1 ng/ml on day 1. Plasma P_4 concentrations between days –1 and 0 differed ($P < 0.001$) significantly between P_4-treated and control groups (Figure 1).

Estrus Responses and the Preovulatory LH Surge

Reproductive parameters occurring following day 0 among ewes of the two groups are presented in Table 1. The overall estrus expression rate was 72.3% (26/36 ewes). Cyclic activity was induced in 67.7 and 77.8% in P4-treated and control groups, respectively. There was no treatment effect ($P > 0.05$) on the incidence of estrus between P4-treated and control ewes. Intervals from 0 h to onset of estrus differed ($P < 0.05$) significantly between the two groups and were shorter (42.4 ± 2.5 h) in control than P4-treated (51.0 ± 2.6 h) ewes.

Occurrence of the preovulatory LH surge was similar (P > 0.05) between the P4-treated (44.4%) and control (27.8%) ewes.

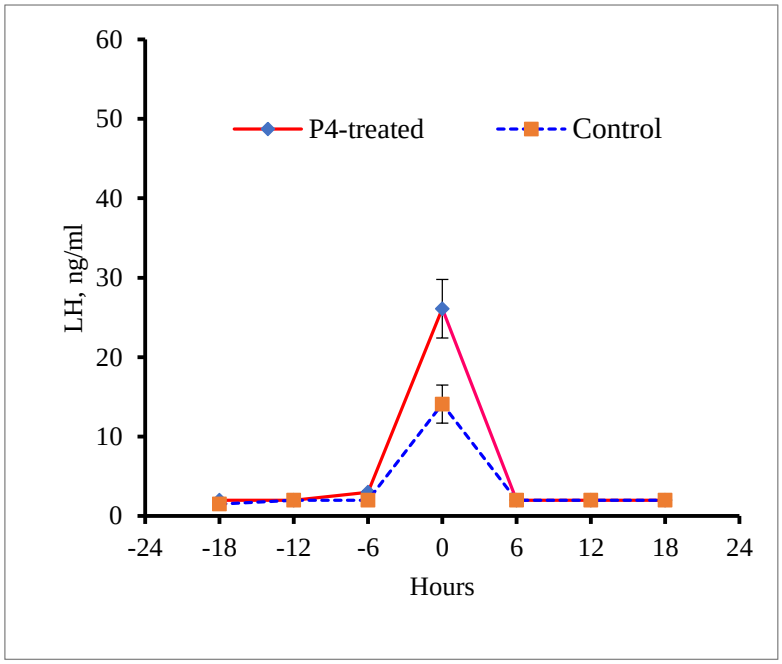

Figure 2. Plasma LH concentrations and magnitude of the preovulatory LH surge in P4-supplement-treated and control ewes in Expt. 1. The time (0 h) represents the aligned peak LH levels in both groups.

Intervals from 0 h to onset and peak LH surge were shorter (P < 0.05) in the control (44.4 ± 4.5 h) group than the P4-treated (51.8 ± 3.4 h) group ewes. Occurrence of estrus and LH surge coincide and intervals from onset of estrus to onset or peak LH surge were similar between the two groups and averaged 1.0 ± 1.1 h in the P4-treated and 2.0 ± 0.9 h in the control. The amplitude of the preovulatory LH surge was greater in the P4-treated (26.1 ± 2.8 ng/ml) than the control (14.1 ± 1.9 ng/ml) group (Figure 2).

Progesterone Profiles after CIDR-G Removal, Pregnancy and Lambing Rates

Progesterone levels in the P4-treated and control groups dropped after day 1 to basal values and remained low until day 5 and then rose gradually until day 15. Differences in P4 rise between days 5 and 15 were not significant (P > 0.1). Progesterone levels remained elevated through day 19 in 8/18 P4-treated and 11/18 control ewes. These ewes were confirmed pregnant by ultrasonography performed on day 30. Differences in pregnancy rates were not significant (P > 0.05) between the two groups. Progesterone levels among ewes that did not become pregnant in groups dropped spontaneously after Day 15 and concentrations were typical of those detected during the process of luteal regression. Of the ewes that became pregnant from mating at induced estrus 7/18 (P4-treated) and 11/18 (control) lambed on day 148.6 ± 0.3. The number of lambs born and the multiple birth rates were similar (P > 0.1) between the P4-treated and control ewes (Table 1).

Experiment 2

Progesterone Concentration during the 12-Day Period in Which CIDR-G were in Place:

Ewes maintained CIDR-G devices during the period of insertion until they were pulled out. Progesterone concentrations in plasma samples of ewes during the period from day –12 through day 3 are illustrated in Figure 3. Initial P4 concentrations in plasma samples taken prior to CIDR-G insertion were <0.2 ng/ml among ewes of the two groups, indicating seasonal anestrus. Differences between the two groups in initial P4 values on day –12 were not significant (P > 0.5). Following CIDR-G insertion P_4 concentrations increased sharply and maximum values were reached 2 days post insertion (day –10) and were 5.7 ± 0.8 and 5.9 ± 0.6 ng/ml in the P4-treated and control groups, respectively. Differences in maximum P_4 concentrations on day –10 were not significant (P > 0.1). Progesterone gradually decreased by day from day –10 until day –1 and concentrations on day –1 were similar (P > 0.05) between P4-treated and control groups 1.9 ± 0.1 and 1.8 ± 0.1 ng/ml, respectively.

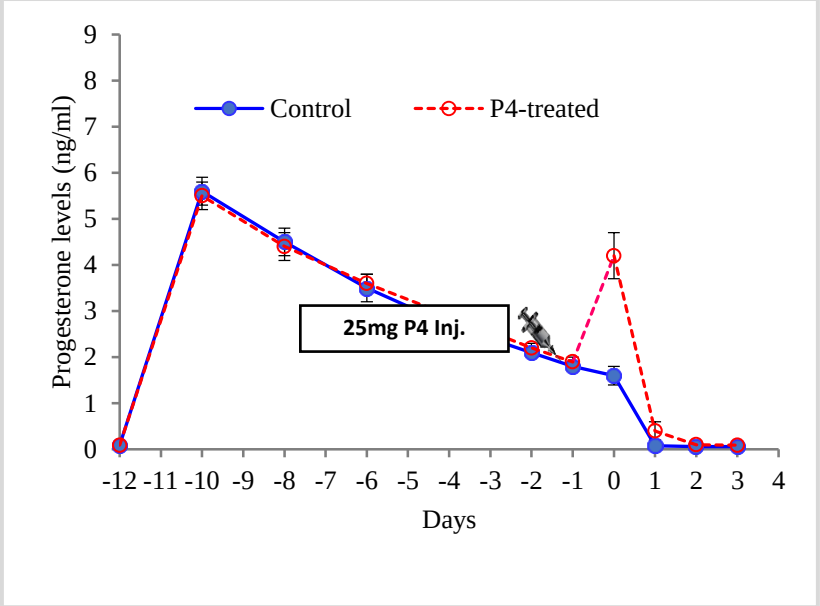

Figure 3. Plasma progesterone concentrations in Expt. 2 during the period in which CIDR-G were in place in P4-treated an d Control groups from day –12 to day 3.

In P4-treated group, plasma P4 concentrations increased sharply from 1.8 ± 0.1 on day –1 to 4.2 ± 0.3 ng/mL on day 0 in response to the 25 mg P4 injection given on day –1. Progesterone concentrations in the control group declined from 1.9 ± 0.1 ng/mL on day –1 to 1.7 ± 0.1 ng/mL on day 0. Plasma P4 concentrations between days –1 and 0 differed ($P < 0.001$) significantly among ewes of the two groups.

Estrus Responses and the Preovulatory LH Surge

Reproductive parameters occurring following day 0 among ewes of the two groups are presented in Table 1. Cyclic activity differed ($P < 0.05$) significantly and was induced in 100 and 64.3% in P4-treated and control groups, respectively. Intervals from 0 h to onset of estrus differed ($P < 0.05$) significantly between the two groups and were shorter (35.3 ± 1.9 h) in the control than in P4-treated (45.4 ± 2.4 h) groups. All ewes in the P4-supplement-treated group and 6/14 control ewes had identifiable surge release of LH. Intervals from 0 h to onset of the preovulatory LH surge were longer in P4-treated (46.6 ± 2.6 h) than control (37.0 ± 2.9 h) ewes, respectively. Differences in occurrence and onset of the preovulatory LH surges were significant ($P < 0.05$) between the P4-treated and control ewes (Table 1). Of the ewes exhibiting a surge release of LH, amplitudes of the preovulatory LH surges were greater ($P < 0.01$) in P4-treated (53.5 ± 4.3 ng/mL; range 33–72) than control (23.7 ± 2.4 ng/mL; range 16–33) ewes (Figure 4).

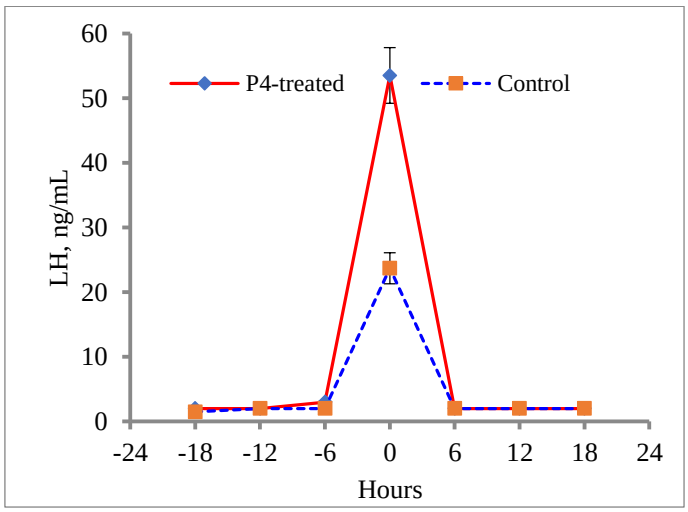

Figure 4. Plasma LH concentrations and magnitude of the preovulatory LH surge in P4-supplement-treated and control ewes in Expt. 2. The time (0 h) represents the aligned peak LH levels in both groups.

Progesterone Profiles after CIDR-G Removal, Pregnancy and Lambing Rates

Progesterone levels in the P4-treated and control groups dropped after day 1 to basal values and remained low until day 5 and then rose gradually until day 15. Differences in P4 rise between days 5 and 15 were not significant ($P > 0.1$). Progesterone levels remained elevated through day 19 in 14/14 P4-treated and 7/14 control ewes. These ewes were confirmed pregnant by ultrasonography performed on day 30. Differences in pregnancy rates were significant ($P < 0.05$) between the two groups. Progesterone levels among ewes that did not become pregnant in groups dropped spontaneously after day 15 and concentrations were typical of those detected during the process of luteal regression. Of the ewes that became pregnant from mating at induced estrus 14/14 P4-treated and 7/17 control lambed on Day 149.3 ± 0.3. Lambing and the multiple birth rates differed significantly ($P < 0.05$) between the P4-treated and control ewes (Table 1).

Table 1. Reproductive parameters following CIDR-G removal in P4-treated and control ewes

Parameter	Treatments[1]			
	Experiment 1 (n= 36 (mixed breed ewes)		Experiment 2 (n=28 Awassi ewes)	
	P4-treated (n = 18)	Control (n = 18)	P4-treated (n = 14)	Control (n = 14)
Body weight (Kg)	64.3 ± 2.5	66.1 ± 2.9	50.8 ± 2.5	49.7 ± 2.7
Ewes displayed estrus*	12 (66.7%)	14 (77.8%)	14 (100%)^{a}	9 (64.3%)^{b}
Intervals to estrus onset (h)*	51.0 ± 2.6^{a}	42.4 ± 2.5^{b}	45.4 ± 2.4^{a}	35.3 ± 1.9^{b}
Ewes displayed LH surge	8/18	5/18	14/14^{a}	6/14^{b}
Intervals to onset of LH surge (h)	51.8 ± 3.4^{a}	44.4 ± 4.5^{b}	46.7 ± 2.6^{a}	37.0 ± 2.9^{b}
Amplitude of LH surge (ng/ml)	26.1 ± 2.8^{a}	51.0 ± 2.6^{a}	53.5 ± 3.7^{a}	23.7 ± 2.4^{b}
Pregnancy rate	8 (44.4%)	11 (61.1%)	14 (100%)^{a}	7 (50%)^{b}
Lambing rate	7 (38.9%)	9 (50%)	14 (100%)^{a}	7 (50%)^{b}
Fecundity	1.4 (144%)	1.35 (135%)	1.29 (129%)^{a}	0.5 (50%)^{b}
Multiple birth (from lambed ewes)	5/7	6/9	4/14	0/7

[ab] means within row with different superscripts for each experiment differed significantly ($P<0.05$).

Discussion

The present study investigated the effectiveness of additional P4 supplement given 24 h before device removal in two estrus synchronization (8-day CIDR-G versus 12-day CIDR-G) protocols in two different geographical locations and to investigate whether or not results obtained are comparable. Reproductive responses resulted in Expt. 2 of the present study using a 12-day CIDR-G-P4 supplement were superior compared to those obtained in Expt. 1 using an 8-day CIDR-G-P4 supplement and were similar to the results reported previously by Husein and Ababneh (2008).

Results obtained from Expt. 2 demonstrate that P4 supplement given one day before device removal was successful in inducing fertile cycles in 100% of the ewes and capable of producing superior reproductive performance compared to those obtained from Expt. 1. Protocols implemented in the present study differed procedurally in the period of CIDR-G insertion (8 vs 12 days). The amounts of circulating P_4 concentrations in Expt. 1 during the 8-day period of pessary insertion protocol are relatively greater than those found Expt. 2 during the 12-day protocol. Progesterone profiles during the 12-day period of pessary insertion determined in Expt. 2 of the present study were typical of those previously reported in the literature (Husein and Kridli, 2002, Husein et al., 1998, Husein et al., 1996). In both experiments, P4 supplement was administered on day –1 at which time concentrations of P_4 increased sharply from about 3 to day 0 values of 7.8 ± 0.9 ng/ml among ewes of Expt. 1. In contrast, P_4 concentrations on day –1 of the 12-day (Expt. 2) protocol were about 1.8 ng/ml to day 0 values to 4.2 ± 0.3 ng/mL (Figure 5).

Therefore, higher and/or sustained P_4 concentrations during the 8-day period are important and maybe associated with down-regulation of LH receptors in a manner affecting dominance of follicles. Thus, follicular dominance is negatively influenced by sustained P_4 levels and therefore, follicular growth is disrupted. In contrast, sensitizing pituitary-ovarian axis with P_4 supplementation at the end of the 12-day protocol would, perhaps, reset recruitment of healthy follicles at the time when gonadotropins and estradiol concentrations are low. However, higher circulating P_4 levels powerfully inhibits LH release, thereby, preventing premature LH surges from occurring and are considered necessary for recruiting a greater number of follicles (Sharma et al., 1993; Caraty &Skinner, 1999).

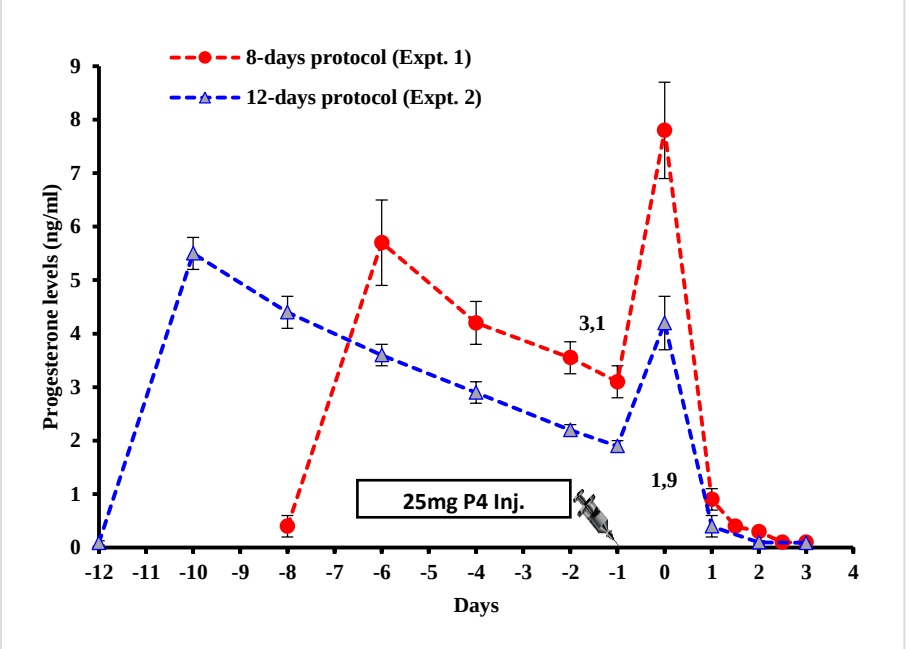

Figure 5. Plasma progesterone concentrations during the period in which CIDR-G were in place in P4-treated ewes in both experiments from day –12 or -8 to day 3.

Similarly, responses of estrus and the preovulatory LH surges were significantly different between the 8-day and the 12-day CIDR-G protocols. More ewes expressed estrus and displayed a surge release of LH in the 12-day CIDR-G than the 8-day CIDR-G protocol. Moreover, the magnitude of the LH surges in the 12-day CIDR-G (53.5 ± 4.3 ng/mL) protocol was greater than those occurring in the 8-day CIDR-G (26.1 ± 2.8 ng/ml) protocol. This evidence may particularly be one of the major contributing factors strengthening follicular dominance and oocyte quality. Likewise, the higher magnitude of LH surges occurring in the 12-day CIDR-G protocol in Expt. 2 may be a reflection of high pro-estrus estradiol levels caused by a sharp decrease in P4 concentrations following CIDR-G removal. Higher amplitudes of LH surges have been reported with high estradiol levels than those with persistently in ewes with persistent follicles (Joseph et al., 1994). In fact, ovulation of mature follicles on the ovary is induced by a large burst of the preovulatory LH surge. Residual cells within ovulated follicles proliferate to form corpora lutea, which secrete progesterone, necessary for the maintenance of pregnancy. In most mammals, LH is required for continued development and functionality of corpora lutea and subsequent embryonic development. However, low LH magnitude occurring in the 8-day CIDR-G protocol may be suboptimal for oocyte quality and subsequent embryonic development competence. It seems that lower magnitude of LH surges was slightly effective because the pituitary gland insufficiently reacted to the positive feedback of rising pro-estrus estradiol levels at the end of the follicular phase. This evidence may inactivate some follicular dominance factors, resulting in premature oocytes at the time of ovulation and may cause supraphysiological thecal androgen production associated with atresia. In addition, the 25 mg P4 injection given one day before device removal caused a significant delay in onset of estrus and the preovulatory LH surge by about 10 h in Expt.2 and 7 h in Expt. 1. The reason for the delay to onset of estrus and LH surge can possibly be attributed to the additional time required for the recruitment and maturation of the ovulatory follicles (Webb et al., 1989) and a slight delay in P4 clearance. These authors indicated that such a delay period is necessary for a large estrogenic follicle (>5 mm in diameter) to develop from a pool of small follicles (<2 mm in diameter) around the time of luteolysis.

The superior pregnancy and lambing rates produced in the 12-day CIDR-G protocol in Expt. 2 of the current study compared to those obtained from the 8-day CIDR-G protocol are believed, perhaps, to be mainly due to eliminating the aged persistent follicles. Thus, our results may provide an indirect evidence for the deleterious effect of aged follicles on fertility in the 8-day CIDR-G protocol due to higher or sustained P4 levels during the 8-day period. In beef cattle, an injection of P4 given 2 days before the end of progestogen treatment induces atresia of persistent follicles and significantly improved pregnancy rates (Anderson a& Day, 1994; McDowell et al., 1998). As a consequence of P4 decline following pessary removal, healthy follicles destined to ovulate, grow and secrete estradiol (Wehrman et al., 1993), which, along with the male effect, induces the preovulatory LH surge leading to ovulation of potential ova. These factors may be considered the key feature for initiating the expression of superior pregnancy and lambing rates in Expt. 2.

In addition, factors including, the sharp P4 decline following pessary removal and the delay in LH surge occurrence and higher amplitude may have participated in resetting the hypothalamic–pituitary–ovarian axis, establishing ovarian-uterine synchrony and thus, superior reproductive performance. It has been indicated that the use of sponges impregnated with high P4 (750 mg) was associated with higher amplitudes of LH surges and higher pregnancy rates in anestrous Finncross ewes (Husein et al., 1996; Husein et al., 1998). In contrast, other studies in sheep associated low P4 concentrations with abnormal follicular development, persistent follicle and reduced fertility (Johnson, 1996; Leyva et al., 1998; Vinoles et al., 1999; Flynn et al., 2000).

Another difference between the two experiments was that ewes were either prolific mixed-breed (Expt. 1) or non-prolific Awassi breed (Expt. 2). Plasma concentrations of P4 play an important role in the control of follicular turnover. This effect is most probably mediated by LH pulsatility, as has been postulated for cattle (Savio et al., 1993). High P4 concentrations lead to lower levels of stimulation of the developing follicles by reducing pulsatile LH secretion (Mann et al., 1992; De Castro et al., 1999). In prolific ewes with multiple ovulations, the ovarian recruitment of follicles is enhanced by the relatively low P4 concentrations. This may limit the subsequent ovulatory outcome and therefore, caliber of oocytes in turn may be influenced by the ewes' exposure to adequate P4 in the few days period prior to ovulation. With higher P4 concentrations being administered to prolific ewes with higher ovulation rate, it is expected that LH receptors will be down-regulated. Thus, the dominance factors responsible for follicles selection are inhibited. Such observation may be beneficial and work better in non-prolific breeds such as Awassi. In this regard the dominance factors are enhanced in selected follicles over the subordinate ones. Thus, a P4 injection given 24-h prior to pessary removal will down-regulate growth of follicles destined to ovulate in prolific ewes, and would rather enhance the dominance of the ovulatory follicles over the subordinate ones in non-prolific ewes (Gonzalez-Bulnes et al., 2004).

The current study also was conducted in two geographical locations. Latitude and day length and breed are not the only factors involved in controlling reproduction in sheep. Some sheep breeds exhibit short seasonal anestrus, particularly when social (ram effect) or nutritional factors are handled appropriately (Quirke & Hanrahan, 1985). Some breeds of sheep in temperate latitude exhibit a seasonal variation in reproductive activity during the year. Despite the fact that P4 profiles in Expt. 1 and Expt. 2 behaved the same, results therefore were not comparable and did not confirm repeatable reproductive performance outcomes. This evidence may eliminate the breed and location effects and establish season of breeding (early April in Expt.1 versus late May in Expt. 2). From this perspective, ewes in Expt. 1 may have been in seasonal transitional period, a crucial factor that may alter reproductive responses compared to ewes in Expt. 2 which were in deep anestrus period.

From another prospective, the amount of P4 rather than the change in magnitude differed between the 8-day and the 12-day protocols and may have played a vital role in producing better reproductive performance. Plasma concentrations of P4 play an important role in controlling follicular turnover. The overall amount of P4 in the 8-day protocol on days –1 and 0 (3.1 ± 0.3 and 7.8 ± 0.9 ng/ml) were greater ($P < 0.05$) than the corresponding values in the 12-day protocol (1.9 ± 0.1 and 5.7 ± 0.8 ng/ml). The higher P4 levels by more than 150% on day –1 in the 8-day protocol in Expt. 1 in comparison to the 12-day protocol in Expt. 2 might have played an important down-regulatory effect on the P4 injection, preventing better suppression of premature LH secretion. Premature LH surge is linked to lower oocyte quality and subsequent lower pregnancy rates. In fact, it is noteworthy that at the time of P4 injection, ewes of Expt. 1 may have had a totally different follicular wave stage than those in Expt. 2. In conjunction with the higher P4 levels arising from CIDR-G in the 8-day protocol, this might have counteracted the desired suppression of endogenous P4 levels to prevent premature LH surge through the expected negative feedback on the hypothalamic-pituitary-ovarian axis. Therefore, the 12-day but not the 8-day protocol with lower P4 levels at day 0 may have permitted better synchrony of estrus occurrence and subsequent LH surge release and, consequently, better overall reproductive performance. To insure effectiveness of the P4 supplement in CIDR-G protocols to produce better LH magnitude and subsequent reproductive responses, mean circulating P4-levels should be < 2 ng/ml, corresponding to normal 12-day protocol P4 profiles. Assuming this is not the case, then failure of the 8-day to produce higher reproductive performance may be attributed to unknown reasons.

Conclusion

In conclusion, there was variability in effectiveness of P4-suppliment to ewes synchronized to estrus with CIDR-G pessaries. Progesterone injection one day before CIDR-G removal is effective in improving LH surge magnitude and superior reproductive responses including pregnancy and lambing rates in 12-day estrus synchronization protocols. However, such improvement is not seen among ewes exposed to the 8-day

progesterone pessary protocol. Such variability in responses between the two protocols might be due to follicular dynamics variations, P4 level at the time of P4 supplement, geographical location and seasonal status.

Recommendations

We recommend that 25 mg progesterone injection can be given to ewes synchronized to estrus for 12 days using CIDR-G protocols 1 day before CIDR-G removal, and would produce superior pregnancy and lambing rates. Nonetheless, the same is not true for ewes synchronized using the same protocols but for only 8 days. Further research is encouraged to incorporate ultrasonographic study of ovarian follicular dynamics concurrent with hormonal profiles in both protocols to ascertain possible follicular dynamic variations between the two protocols.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

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Analysis of the Effects of State Aid on the Development of Algerian Dairy Farms: The Case of National Unemployment Fund (CNAC)

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Abstract: This study is aimed at demonstrating the importance of public investment in developing the dairy sector through support schemes such as the CNAC (national unemployment fund). Fifty-five dairy farms benefiting from CNAC funding were surveyed. The results show that 63% of the beneficiaries have difficulties with the submission and processing of their administrative files. The lack of technical control by the CNAC services was reported by the vast majority of beneficiary farmers (83%). There has been an increase in the number of wage labourers from nine to 20% of the total farm labour force. There has been an increase in the number of farms with a small forage area (0-5 ha). Bovine livestock increased by 12%, the most important breed being the Montbeliarde. More than half of the farmers have increased the amount of concentrates distributed, from an average of 7 to 10 kg per animal per day. Green fodder is also appearing in the form of wrapped silage distributed throughout the year. Milk production increased by 7.3%, from 17.7 litres per cow per day to 19.1 litres per cow per day. Natural breeding is still practised by 42% of farmers, despite the prevalence of artificial insemination. Finally, 54 % of the farmers benefiting from the programme say that dairy production has become unprofitable because of higher production costs. It would be preferable for this mechanism to improve and simplify the administrative procedure and ensure effective follow-up of beneficiary farmers in order to improve the efficiency and effectiveness of CNAC funding.

Keywords: State aid, Dairy cattle, Development, Dairy Farms

Introduction

Since the independence of Algeria, the agricultural policy of the government has been aimed at ensuring the food security of the population through an increase in agricultural production (Bessaoud et al., 2019).

Agricultural subsidies are one of the public policy responses to the various distortions in the world market that can have an impact on food security. State aid policies have long been applied to the various stages of producing, collecting, processing and consuming milk (CNAC, 2018). As part of these policies, instruments have been in place to support investments in farms and the growth of production. However, since the creation of aid schemes like CNAC, state aid also focuses on creating production units, in our case dairy farms (Boubker, 2016). The aim is to increase the national production of milk in order to meet the growing demand in society and, as far as possible, to reduce the amount of milk powder that has to be imported.

This paper attempts to show the impact of the CNAC scheme on the process of dairy unit creation/expansion and milk yield improvement, also assessing the effectiveness of the scheme's administrative procedure. A normative approach is adopted (Butault, 2004). It consists in analysing the effectiveness of the CNAC compared to the initial objectives set.

Method

Presentation of the Study Region

Tizi-Ouzou covers an area of 2,957.93 km2, or 0.13% of the national territory. 80% is mountainous, with an average altitude of 800m (DPSB, 2007). It is a coastal wilaya. It has 70 km of coastline. The landscape of the wilaya is mountainous with more than 50%. Slopes exceed 12% and sometimes reach 25% (DPAT, 2010). The region is known for its low forage production levels. According to DPSB (2019), the Utilised Agricultural Area (UAA) of the wilaya is 98,842 ha. Only 2% of these are irrigated.

The Methodology Used

The study was carried out on 55 dairies that benefit from CNAC subsidies. These 55 farms are located in six (6) municipalities. These communes are dominated by dairy farming. The aim of the survey was to analyse the beneficiaries' assessment of the flexibility of the CNAC scheme and the impact of the funding on the development of the farms. Descriptive statistics were used. The mean values, standard deviations and percentages have been used.

Results and Discussion

The Administrative Procedure

The results show that the financing is triangular and divided as follows: personal contribution: 2%; CNAC: 28% and the bank: 70%. There is no requirement for guarantees on the part of project promoters. Most of the farmers (63%) said that they struggled when trying to apply. In their view, the application process was difficult and time consuming. 50% of project promoters reported waiting between six and ten months to have their proposal validated.

Livestock Buildings

It is worth noting that 80% of the livestock buildings on the farms studied before the programmes were funded had a shed that met livestock standards, while 20% of the farms did not meet livestock standards. Today, there has been a 98% improvement in the quality of livestock housing. Only 2% of the farms do not comply with the standards for animal husbandry.

The Labor Force

Before the programme was funded, only 9% of the enterprises had recruited permanent staff. 8% employed 1 or 2 people and only 1% employed up to 3 people. In 91% of the holdings, however, the workforce is essentially family-based. After CNAC funding, 20% of the holdings reported an increase in the number of permanent employees (1 to 4 employees).

Mechanization

We found that only 32% of farmers owned a tractor before the programmes were funded. For farmers who own large plots of land and grow fodder, tractors are an essential tool. In comparison, 68% of the farmers did not own a tractor due to a lack of financial resources. Mechanisation increased by 15% after the programme was funded, bringing it to 47%. This was due to the purchase of equipment through the scheme and the purchase of equipment by the farmer. The remainder (53%) of farmers do not have a tractor. In general, due to the very high purchase prices, they have either bought another vehicle or have no mechanisation at all.

Farm Surface Area

The changes in agricultural land in different municipalities of the Wilaya of Tizi-Ouzou are shown in table 1. In the Wilaya of Tizi-Ouzou that have been in receipt of funding under the various programmes. According to the results in Table 1, the agricultural area in hectares in the Wilaya of Tizi-Ouzou is equal to 686 ha. Kadi et al, (2007), indicate that the foraging base is low. This is an average of 5.15 ha before the finance of the programmes, then increased by an average of 0.18 after the programmes were financed, giving a total of 710.5 ha.

Table 1. Useful agricultural area per hectare of farms before and after CNAC scheme funding

	0 to 5 ha	5 to 10 ha	10 to 15 ha	15 to 20 ha	20 to 50 ha
% of farms before scheme funding	48	42	6	3	1
% of farms after scheme funding	50	36	9	3	1

In the wilaya of Tizi-Ouzou, the municipalities of Freha, Iflissen, Timizart and Mekla have the largest areas of agricultural land. When compared with the other municipalities, the situation is similar both before and after programme funding. Before programme funding, 48% of the farms had an agricultural area between 0 and 5 hectares; after programme funding, the number of farms had increased by 2%. Some holdings have reduced the number of hectares in use: 42% of holdings with 5-10 hectares have reduced the number of hectares in use by 6%. The sale of land and the change of activity of several farmers are responsible for this reduction. However, it should be noted that 4% of holdings did not report any change after receiving support. It is also worth noting that some farmers do not own any agricultural land.

Cattle Numbers

According to the respondents, we note that the average cattle herd before the finance from the schemes is estimated at 8.96 head, and the average herd in 2021 after the aid from the schemes is estimated at 11.49 head. An average increase by 2.53. In Sétif region, an average of 7.85 animals per farm was reported by Lazereg and Brabez (2019) in a survey of small livestock farmers. Compared to the other regions, the number of cattle in the Iflissen, Feha, Tizi-ouzou and Timizart regions is high. The availability of agricultural land suitable for livestock farming may explain this. Both before and after the programmes, the commune of Freha had the highest number of cattle. The number of cattle per holding before receiving support from CNAC is shown in Table 2.

Table 2. Cattle numbers of farms before and after CNAC scheme funding

Cattle numbers	0 to 20 head	20 to 40 head	40 to 60 head
% of farms before scheme funding	86	10	4
% of farms after scheme funding	75	22	3

The table above shows that 86% of the holdings with between 0 and 20 head of cattle before the programmes were financed have decreased to 75%. This represents a decrease of 11%, which can be explained by the sale and/or death of animals, but also by the change of activity of several farmers. 10% of the holdings which had between 20 and 40 head of cattle prior to the programme experienced an increase of 12% in cattle numbers after the programme. Explained by the ownership of agricultural land that can be used for livestock farming. Also explained by receiving public aid. On the other hand, 4% of the holdings before support showed a decrease of 1%. This can also be explained by the sale or death of animals and the change of activity of several livestock farmers.

Dairy Cows Numbers

Dairy cows are cows bred to produce milk for human consumption. Beneficiaries had an increase in the number of dairy cows from 690 before the programmes to 1,033 after. The average number of dairy cows per beneficiary was 7.76. From these results it can be seen that 83% of the farms which had between 0 and 10 dairy cows before the schemes were funded reduced the number of dairy cows by up to 59%, i.e. a reduction of 24% (Table 3). The sale of dairy cows or their death from disease may explain this.

The sale of dairy cows for slaughter due to reduced milk production is another explanation. There may also have been a change in the type of activity of a number of holdings. Nevertheless, 17% of farms with between 10 and 40 dairy cows have increased the number of dairy cows by up to 41% since receiving funding. This is due to the large areas of arable land in these regions, which enable them to produce enough feed to meet the needs of dairy cows and thus achieve better production. This is also due to the financial support provided by the programmes.

Table 3. Dairy cows numbers of farms before and after CNAC scheme funding

Cattle numbers	0 to 20 head	20 to 40 head	40 to 60 head
% of farms before scheme funding	83	12	5
% of farms after scheme funding	59	35	6

The breed most chosen by farmers before the programmes were funded was the Montbéliard with 40%, followed by the local breed with 17%. Fleckvieh with 6 % and Holstein with 5 %. 8% of the farms surveyed had a mix of Montbéliard, local breed, Holstein and Friesian. 4% had Montbéliard, pie noir, pie rouge and brune de l'atlas.

Feeding

Dairy cow feeding varies from farm to farm. It depends on the forage types available and the season. The basic ration of "green fodder" is distributed in spring on all the farms surveyed. The amount of fodder distributed is higher than at other times of the year.

Concentrates

The concentrates are distributed by farmers to supplement their livestock. An increase in the amount of concentrates distributed by the surveyed farmers before and after the support is shown in Table 4. 54% of the respondents distributed between 70 and 250 kg per day per herd (average of 10 animals). Before the CNAC support, only 34% of the farmers distributed this amount. Before the support, 8% of the farmers distributed more than 250 kg/day per flock. After support, 15% of farmers distributed more than this amount. 13% and 9% of beneficiaries before and after support respectively do not distribute concentrates to their herds because of the high cost of feed or because they do not have dairy cows.

Table 4. The quantities of concentrate distributed before and after the CNAC scheme funding.

Quantity of concentrate distributed per kg/day/herd (10 head on average)	% of farmers before scheme funding	% of farmers after scheme funding
0 kg	13	9
4 to 30	16	10
30 to 70	29	12
70 to 250	34	54
More than 250	8	15

Wrapped Silage

From our survey of farm visits, almost half of the farmers do not distribute wrapped silage to their cattle. This can be explained by the fact that it is not available in certain regions and that it is too expensive.

The Daily Amount of Feed

On the survey farms, the average amount of feed per day before the schemes was funded was 250 kg per day per flock and 25 kg per day per cow. Quantities increased to 350 kg per day per flock and an average of 35 kg per day per cow after the schemes was funded.

Milk Production

Milk production on the surveyed farms varies according to the season and the feed given to the herd. The average varies from 8 litres to 40 litres (figure 1). Analysis of the results obtained on milk production shows that before the schemes were funded, around 14% of farmers had no livestock and therefore no production, 78% of farmers had an average production of 2 to 25 litres and only 8% of farmers had an average production of 25 to 40 litres.

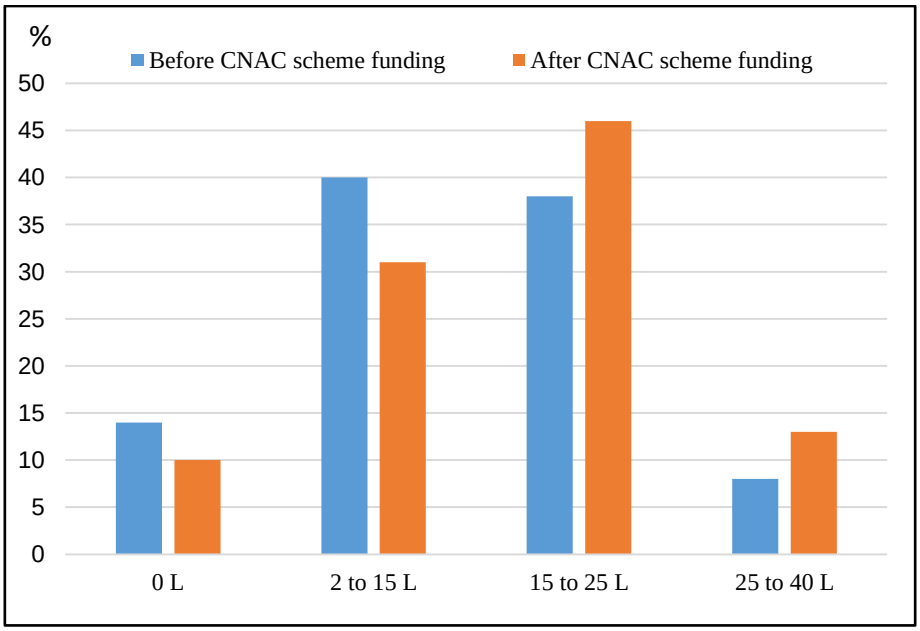

Figure 1. Average milk production on farms surveyed before and after CNAC scheme funding

After the schemes were funded, 10% of farmers stopped farming. In addition, 77% of the farmers have an average production of 7 to 25 litres, and 13% of the farmers have an average production of 25 to 37 litres. This is evidence of the positive impact of scheme funding on agricultural value addition (Ferroukhi et al., 2021).

Breeding Method

Breeding is an important factor in livestock management. It ensures the maintenance and improvement of the farmer's activity. Natural breeding (NB) is the first method of reproduction before the funding of the programmes (Table 5). It is used by 41% of the farms surveyed, followed by both methods (Artificial insemination (AI) and Natural breeding (NB)) with a rate of 38%. After the finance, 42% of the farmers used both methods (AI + NB), while 38% of the farmers used natural reproduction on their farms.

Table 5. Method of reproduction used by farmers

Method of reproduction used by farmers	% of farmers before scheme funding	% of farmers after scheme funding
Natural breeding	41	38
Artificial insemination	21	20
Both methods AI+NB	38	42

Artificial insemination, with a rate of 21% and 20% respectively, is the last reproductive method used both before and after funding. This can be explained by the low success rate of the cows that are mated and by the

cost of this method, according to the farmers questioned. On all the farms surveyed, we found that the average age of the heifers at first service was 18 months, both before and after the funding of the schemes. 27 months is the average age of heifers at first calving.

Profitability of Financing Schemes

Dairy farming is on the rise. According to the 55 respondents and the results of the analysis, 44% of investments are profitable. Despite the 54% whose activity is not profitable, this percentage really shows that livestock farming is developing. This can be explained by the high cost of feed, which is the main problem for all farmers. This is followed by the high cost of veterinary consultations and vaccines, the lack of professionalism and, finally, the unavailability of arable land for livestock farming.

Conclusion

The study shows that the administrative procedures are still relatively slow and difficult to use. This is particularly true for the processing of applications. These difficulties are also reflected in waiting times and the lack of ex-post monitoring of projects by the CNAC services. However, the scheme has a number of advantages, not least the fact that it is open to all project applications from promoters who can demonstrate that they have the necessary skills. The fact that the promoter's contribution to the funding of the project is negligible is also a strength of the scheme. Cattle numbers have increased in the study area. This is reflected in the creation and/or expansion of dairy farms. Milking performance has also improved in relative terms. Increased use of artificial insemination has been observed in reproduction practices. However, the structural problem of a lack of fodder in the area under study continues to be a problem.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as a poster presentation at International Conference on Veterinary, Agriculture and Life Sciences (www.icvals.net) held in Antalya/Turkey on November 16-19, 2023.

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Characteristics of Algerian Goat's Milk

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Abstract: Goat milk, which is very popular especially in France where many famous cheeses are made, could meet the needs of people living in the mountains, and can be eaten fresh or processed. However, for the latter case, it is established that only milk with α S1-casein expressed with a high percentage could result in the manufacture of cheese. For this, we conducted a study that aims in a first step to assess the physical and chemical characteristics of goat milk collected in three regions of Tizi-Ouzou. The analysis performed has carried on the pH, acidity, total solids, proteins, fat, lactose and vitamin C. The results obtained for these parameters, including total solids (109.3 g / l), fat (30.7 g / l), lactose (39.1 g / l) and proteins (26g / l), are state of the good nutritional value of milk collected locally. However, the levels obtained were below those of bovine milk analyzed under the same conditions. In a second step, we performed the isolation and characterization of proteins. For this, we used the precipitation of the casein at their isoelectric pH (pH 4.2) followed by ion exchange chromatography on DEAE-cellulose. The fractions were then checked by polyacrylamide gel electrophoresis under different conditions (native, in the presence of urea, in the presence of SDS). The resulting electrophoretic profiles have identified similarities between goat and cow milk as they have helped to highlight some particularities including both caseins (αS) as serum proteins (PP3, α-La and β-Lg). The use of weak anionic resin in a single chromatographic separation step is advantageous in that it led to the isolation of goat protein (casein β, casein α S, PP3, SA and β-Lg) with a high degree of purity.

Keywords: Goat milk, Proteins, Phenotyping, Electrophoresis, Chromatography.

Introduction

The nutritional benefit of milk lies in its richness in basic nutrients (proteins, lipids and carbohydrates) but also in calcium, vitamins and trace elements. It is one of the rare foods that is suitable for different age groups where it can be consumed as is, fresh or in the form of a processed product, notably in cheeses and yogurt. In addition, the different ingredients constituting milk (proteins, peptides, lactose, fat, etc.) have been used wisely by the food industry for recent decades to produce products with new functionalities and best suited to the demands of the consumer. It is precisely for these reasons that the need for this material continues to increase around the world while global milk production is unable to follow this trend.

Thus, over the last quarter of a century, the milk consumption of the world population has increased by 32% while production per capita has fallen by 9%. In these ratios, cow's milk occupies the largest proportion (around 80%), the rest is made up of buffalo, goat, sheep and camel milk. This situation of deficit in milk produced is even more accentuated when we look closely at the case of our country which is rightly considered as the first

Maghreb consumer of milk (100 l/year/inhabitant) but whose milk production (1 billion l/year) does not cover needs estimated at more than 3 billion l/year. Here too, other dairy species (goat, sheep, camel) only cover around 10% of needs which are met by resorting each year to the importation of milk powder (250,000t/year) and fat. anhydrous. In order to stem this trend somewhat and restore balance, our country has put in place a strategy for developing and encouraging national production by allowing breeders in particular to import appropriate dairy breeds and to establish themselves in breeding cooperatives while the milk collection circuit has been improved by the introduction of collection centers and means of early milk refrigeration. This development plan has also recorded an increase in the production and collection of fresh milk. But until then, the bulk of efforts have been focused on the beef sector. The other sectors (sheep, goats and camels) remain marginal with production intended mainly for self-consumption. Furthermore, in mountain agriculture, the goat, renowned for its hardiness and its adaptation to this particular terrain, has always constituted an ideal solution for local populations who obtained practically all of their milk needs from this animal, which is known and prized in other countries, particularly in France for the reputation of the guaranteed appellation cheeses that are made exclusively from its milk. If the development effort in our country continues, the tonnages of goat milk, will be revised upwards, which will provide interesting prospects for the sale and consumption of this milk in its fresh state or its processing, particularly into cheese.

In this section in particular, it is established that only milks having αS1 casein expressed with a high percentage can be transformed into cheeses. Therefore, it seems obvious that the analysis of this milk and its characterization on the protein level can help to better guide technologists on the possibilities of industrial exploitation of this collection milk. From this perspective, the present study aims, on the one hand, to evaluate physico-chemical plan the goat milk collected in 3 three regions of Tizi-Ouzou (Azazga, Mekla and Larbaâ Nath Irathen) and, on the other hand, to carry out the isolation and purification of the major proteins of these milks before characterize in terms of their electrophoretic behavior.

Material And Methods

Raw Material

The milk samples analyzed are fresh, large-mix milks from herds of healthy goats, located in the Tizi-Ouzou region.

Analysis Methods

Milk Collection

Milk samples were collected from healthy goats. The milk is milked cleanly and is immediately added with 0.3 g/l of sodium azide (NaN3), in order to avoid any microbial development. The samples are transported in a cooler to the laboratory where they are immediately analyzed. On arrival, a pH measurement is immediately carried out. Depending on the experimental objective, the milk is fractionated. One part is intended for physicochemical analyzes and another for protein phenotyping. For the rest, it is divided into small fractions and frozen thus for later use.

Physico-Chemical Analyzes

The milk comes from healthy goats from the localities of Mekla, Azazga and Larbaâ Nath Irathen. As soon as it arrives at the laboratory, the milk is analyzed. Parameters such as pH and acidity (°D) are measured. The other parameters were analyzed: determination of the total dry extract (FIL 21 standard, 2010), fat content (FIL 22B, 1987), lactose content (according to the method described by AUDIGIE et al, 1978) and finally vitamin C following the AOAC 967.21 method (2006). The protein content was estimated according to the method of LOWRY et al (1951).

Protein Isolation and Purification

The proteins were separated by acid precipitation at pH 4.2 followed by centrifugation at 3500 g/20 min. The supernatants (serum proteins) were checked by polyacrylamide gel electrophoresis (PAGE-native) according to

the method of HILLIER (1976); with porosity of the gel (T = 12% and C = 2.7%); gel buffer (TRIS, 0.75 M, pH 8.9) and electrode buffer (TRIS, 5 mM; glycine, 77 mM; pH 8.3). The pellets (caseins) were analyzed in PAGE-Urea by applying the protocol described by NG-KWAI-HANG and KROEKER (1984) using a concentration gel (T = 4% and C = 2.7%, buffer: urea , 0.8 M, TRIS, 0.49 M at pH 6.8) and a separation gel (T = 13% and C = 2.7%, buffer: urea, 4 M, TRIS, 1.5 M for a pH of 8.8). The determination of the molecular weights of these proteins in PAGE-SDS is carried out following the protocol of LAEMMLI and FAVRE (1973), with a concentration gel (T = 4% and C = 2.7%, buffer: TRIS - HCl, pH 6.8) and the separation gel (T = 15% and C = 2.7%, with a buffer: TRIS - HCl, pH 8.8). For the fractionation of individual caprine caseins we applied a step•wise according to the protocol of WEI and WHITNEY (1985) (pH 7 buffer: 0.02 M, urea at 3.3 M and 2-mercaptoethanol at 0, 3% (v/v). The fractionation of individual caprine serum proteins is carried out according to the conditions established by MAUBOIS (1964) (0.01 M Tris-HCl buffer pH 6.8.

Results and Discussion

Physico-Chemical Analyzes

The pH recorded for the goat milk samples analyzed with an average of 6.61 remains relatively close to that recorded for bovine milk at 6.69. These values generally agree with those reported in the bibliography (REMEUF et al, 2001; IMRAN, 2008). The acidity follows these trends in the two milks (average: 15.5°D), this reveals their good health status in reference to the fact that certain dairies give the upper limit of acceptance of milks at 16°D (QUELLETTE, 2004). On average the contents of dry extract (109.3 g/l), fat (30.7 g/l), lactose (39.5 g/l), vitamin C (13.7 mg/l) and proteins (26.7 g/l) of goat's milk, follow a similar fluctuation consistent with the literature (VENOUGLOU et al, 1982; JAUBERT G, 1997), but remain lower than those of bovine milk (respectively: 119 g/l, 35. 7 g/l, 45.4 g/l, 18.5 mg/l and 28.5 g/l).

Table 1. Protein concentrations of LC goat's milk compared to LV cow's milk. PT: Total protein. PLS: Whey proteins CN: Caseins.

Paramètres	LC1	LC2	LC3	LV
pH	6,65 ± 0,04	6,58 ± 0,02	6,61 ± 0,06	6,69 ± 0,03
Acidité titrable (°D)	15,3 ± 1,43	16,5 ± 0,40	14,7 ± 0,85	15,5 ± 1,22
EST (g/l)	115,7 ± 3,29	110 ± 1,41	102,3 ± 1,73	119 ± 2,16
MG (g/l)	34 ± 1,24	30,4 ± 1,28	27,7 ± 0,35	35,7 ± 0,92
Lactose (g/l)	40,6 ± 1,88	42,7 ± 2,20	34,2 ± 1,53	45,4 ± 0,73
Vitamine C (mg/l)	13,9 ± 0,95	10,8 ± 1,25	16,4 ± 1,45	18,5 ± 1,25
PT (g/l)	28 ± 0,52	27,4 ± 0,85	24,8 ± 1,19	28,5 ± 1,60
PLS (g/l)	6,5 ± 1,14	6,8 ± 0,27	5,5 ± 0,85	6 ± 1,34
CN (g/l)	21,5 ± 1,03	20,6 ± 0,66	19,3 ± 0,78	22,5 ± 1,23

However, sample 3 apart from vitamin C (16.4 mg/l) stands out for the other parameters mentioned above by relatively lower averages (EST: 102.3 g/l, MG: 27.7 g/l , lactose: 34.2 g/l, proteins: 24.8 g/l) in comparison to the other two samples.

Characterization of Proteins

The serum fraction of goat milk in native PAGE presents the same protein species as its bovine counterpart. Referring to the work of MATI (1992), we can see the same level of migration of immunoglobulins and serum albumin from both milks and a different level for PP3 (bovine PP3 migrates further forward than that of goats). B-lactoglobulin and α-lactalbumin have relatively lower levels of migration.

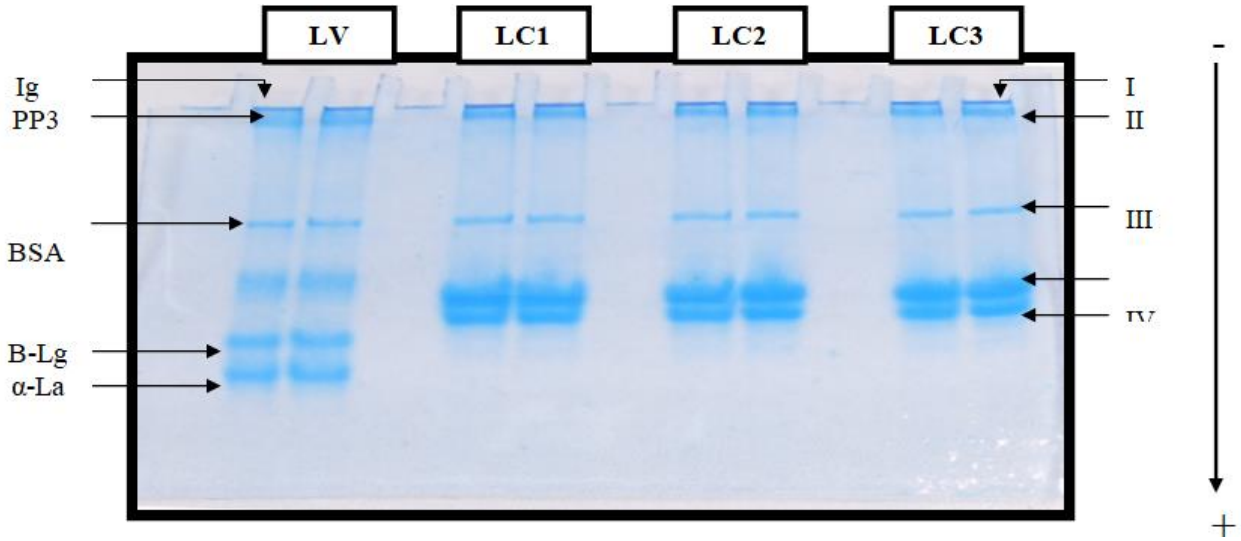

Figure 1. Electropherogram of serum proteins from goat milk compared to bovine milk (T = 12%; C = 2.7%); LV: cow's milk; LC1, LC2, LC3: goat's milk. Ig: immunoglobulin. PP3: proteose-peptone 3. BSA: Bovine Serum Albumin. B-Lg: B-lactoglobulin. α-La: α-lactalbumin.

PAGE-SDS determines three migration bands I, II and III (figure 2), the MW of these is respectively 66221 Da, 17005 Da and 13558 Da and would correspond to caprine serum albumin, B-lactoglobulin and with α-lactalbumin (Jovanovic et al., 2007).

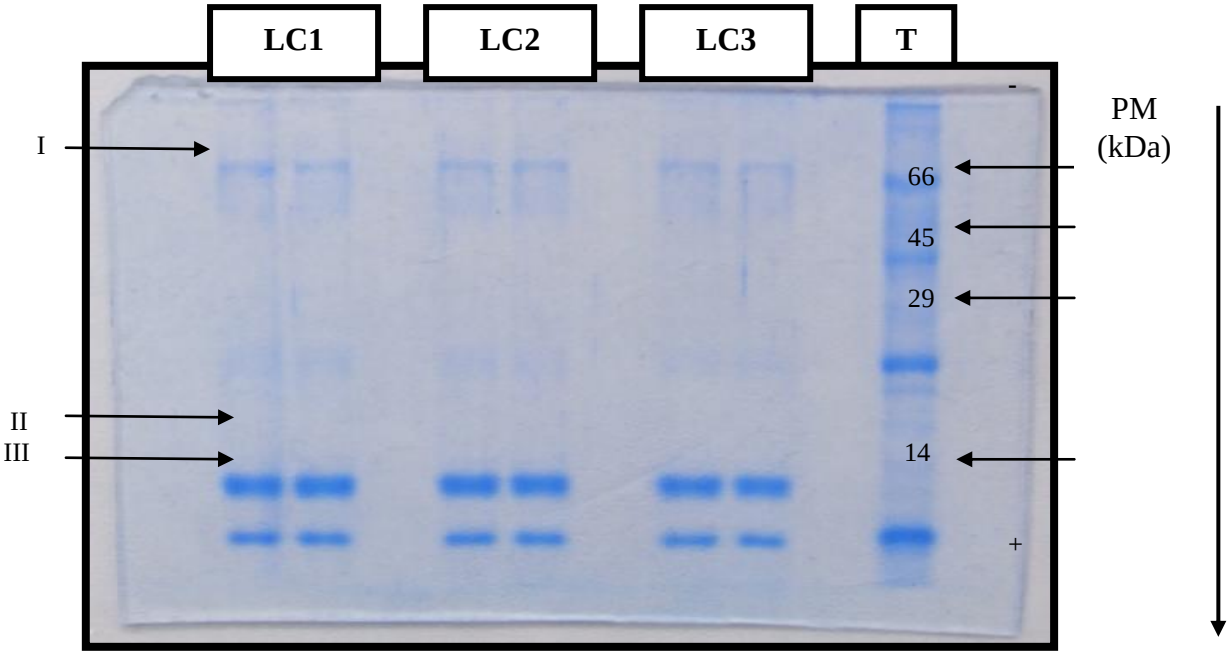

Figure 2. Electropherogram of goat milk serum proteins in PAGE-SDS; separation gel (T = 15%; C = 2.7%) and concentration gel (T = 4%; C = 2.7%); T: standard proteins; LC1, LC2 and LC3: goat's milk. CSA: Caprine Serum Albumin

The behavior of caprine caseins in PAGE-urea presents some similarities compared to their counterparts in reference milk. β-casein has the same level of migration (figure 3), while α S-caseins are more delayed, which is in line with the conclusions of LE BARS and GRIPON (1993). The presence/absence of α S1 casein has been the

subject of numerous studies in the literature which have shown that the presence of certain variants of this protein are correlated with good technological capabilities of goat milk (Piacer &Elsen, 1992; Remeuf, 1993; Remeuf et al., 2001) In PAGE-SDS, we obtain 4 migration bands (I to IV) with respective PMs of 33674 Da, 32270 Da, 28398 Da, and 27214 Da. These would correspond to α s2, α s 1, β and κ casein (Hiroyouki et al., 2006).

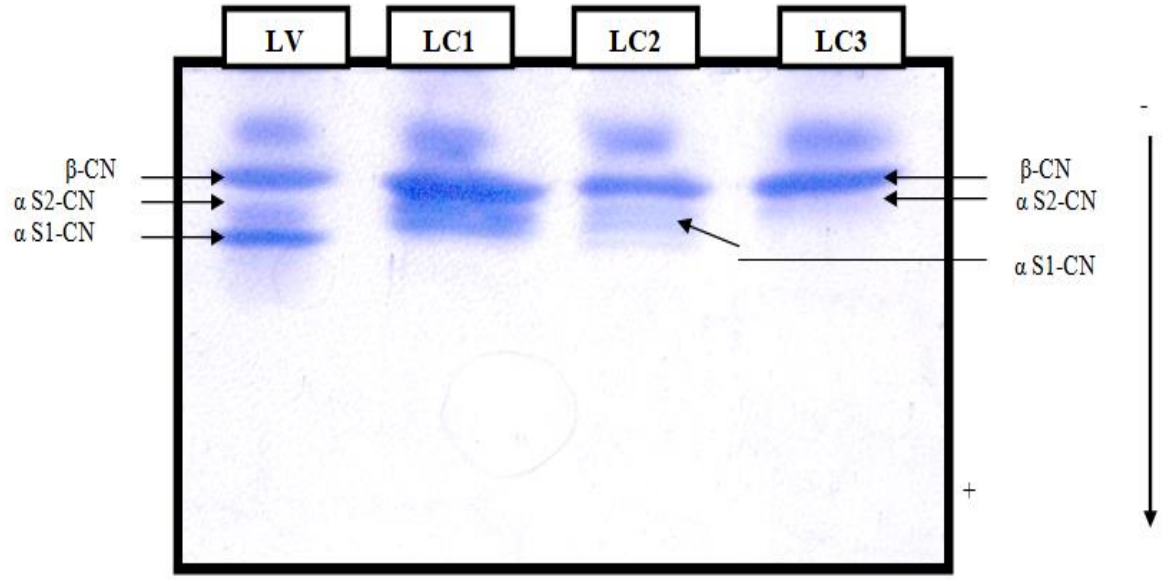

Figure 3. Electropherogram of Caseins in PAGE-urea (5.7 M); separation gel (T =13%; C =2.7%) and concentration gel (T = 4%; C = 2.7%); LV: cow's milk; LC1, LC2 and LC3: goat's milk; CN: Caseins.

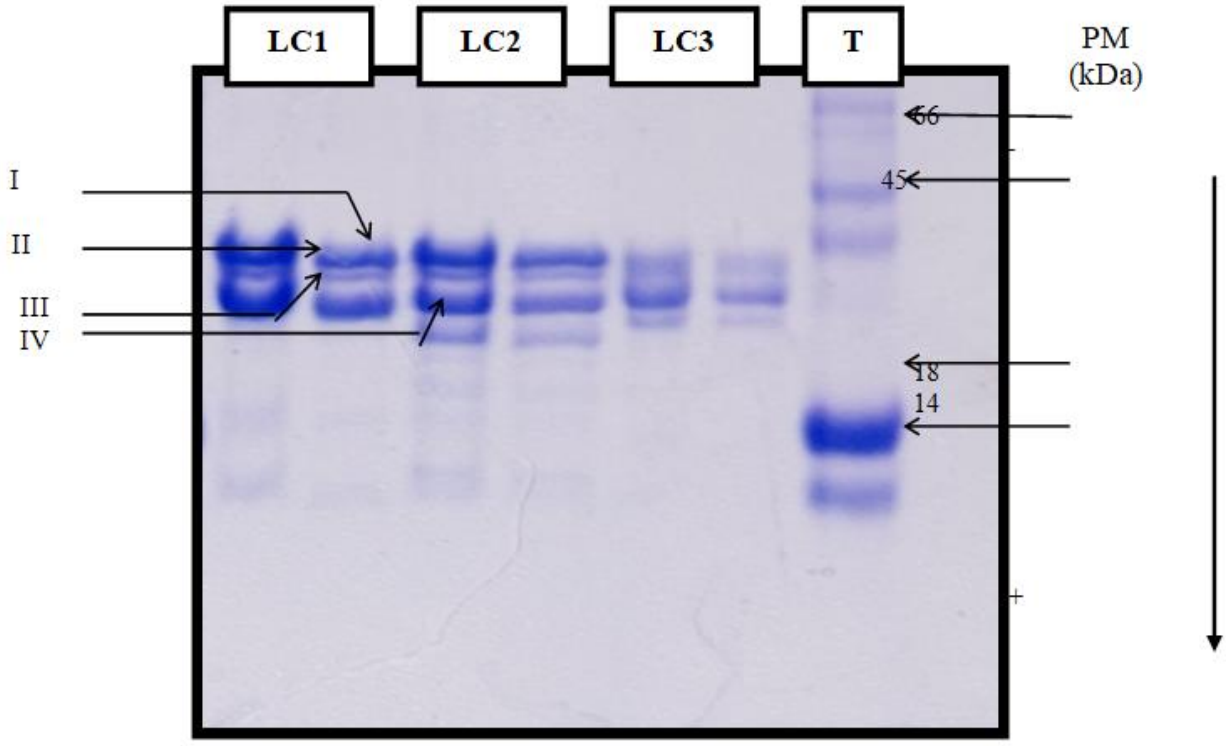

Figure 4. Electropherogram of Caseins in PAGE-SDS; separation gel (T = 15%; C = 2.7%) and concentration gel (T = 4%; C = 2.7%); T: standard proteins; LC1, LC2 and LC3: goat's milk.

Chromatographic Fractionation

The elution of caseins from goat milk is carried out by a discontinuous NaCl gradient, the elution buffer contains NaCl concentrations varying from 0.06 to 0.18 M. It is also composed of imidazole (0.02 M, pH 7; 3.3 M urea

and 2-Mercaptoethanol. We obtained five fractions (F1 to F5) (figure 5). β-casein is eluted in fraction F1, α S caseins in fraction F5 with an appreciable degree of purity . For serum proteins, the electrophorgram (figure 6) obtained by native PAGE of the collected fractions (F1 to F5) shows that alpha-lactalbumin is eluted in fraction F1. However, it remains slightly contaminated by traces of PP3. The latter is eluted in fraction F3 at a high degree of purity. Serum albumin is eluted in fraction F3 while β - Lactoglobulin is found in fraction F4.

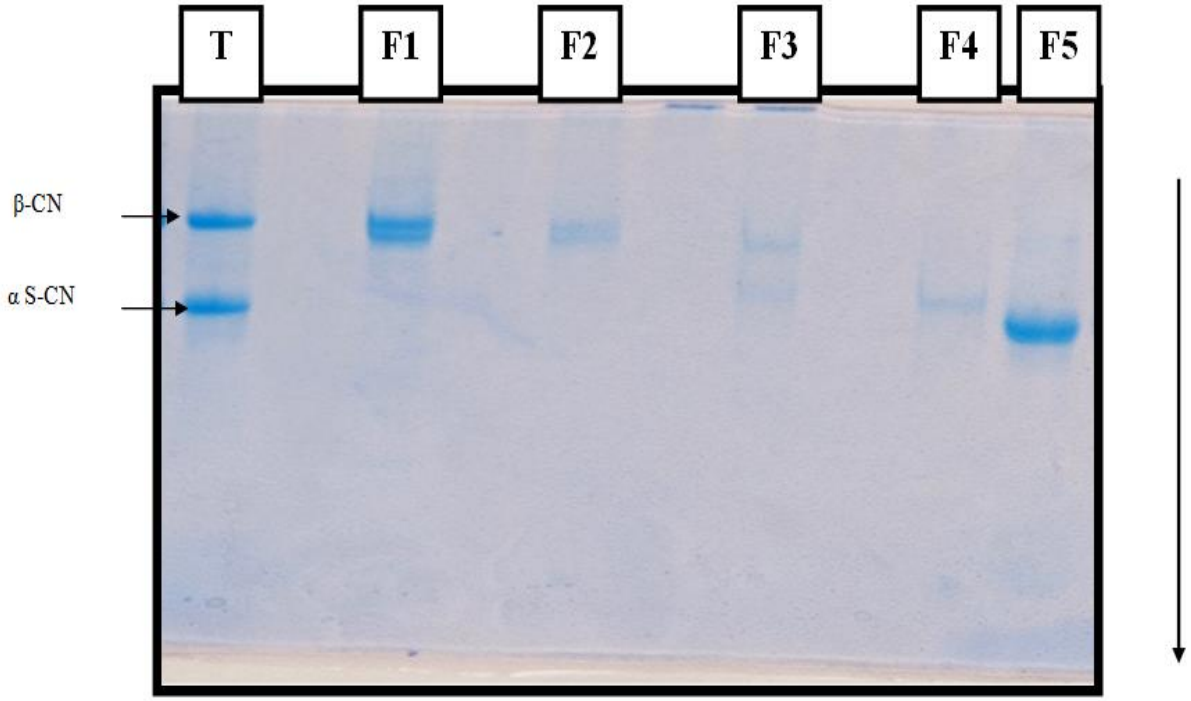

Figure 5. PAGE-urea electropherogram of the fractions collected after separation of the caprine caseins by chromatography on DEAE-cellulose; T: control (whole caseins) F1 fraction eluted at 0.06 M NaCl F2 fraction eluted at 0.09 M NaCl F3 fraction eluted at 0.12 M NaCl F4 fraction eluted at 0.15 M NaCl and F5 fraction eluted at 0.18 M NaCl.

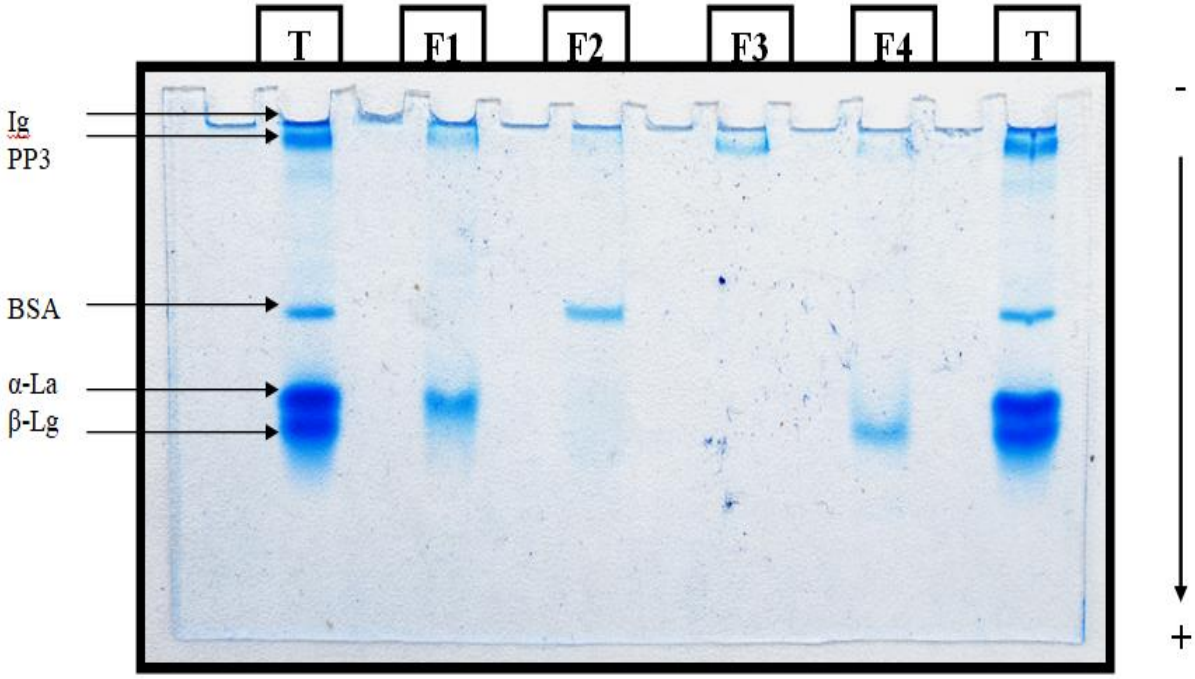

Figure 6. Native PAGE electropherogram of the fractions collected after separation of the Caprine serum proteins by chromatography on DEAE-cellulose; T: control (whole serum proteins) F1 fraction eluted at 0.08 M NaCl F2 fraction eluted at 0.11 M NaCl F3 fraction eluted at 0.13 M NaCl and F4 fraction eluted at 0.19 M NaCl.

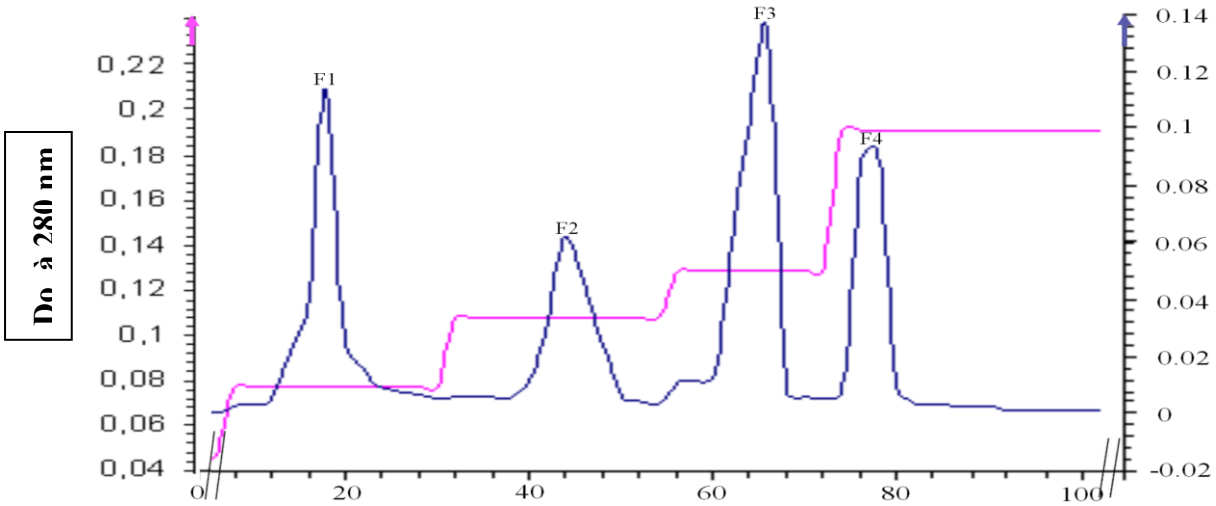

Figure 7. Chromatogram of goat milk serum proteins isolated on DEAE cellulose in 0.01 M Tris-HCl buffer, pH 6.8; flow rate 30 ml/h. F1 fraction eluted [illegible] at 0.11 M NaCl F3 fraction eluted at 0.13 M NaCl and F4 fraction eluted at 0.19 M NaCl.

Conclusion

This study, undertaken with the aim of contributing to a better knowledge of milk goat collected in the Tizi-Ouzou region, is divided into two parts, one focused on phenotyping of the protein fraction whether serum or casein and the other on the physicochemical characteristics of goat's milk, thus aiming for better exploitation of it in the cheese industry. As for physicochemical analyses, these mark a notable difference between goat's milk and cow's milk. The latter being taken as the reference milk. Thus we note relatively lower levels of dry extracts (on average 109.3 compared to 119 g/l for cattle). This trend is thus verified for fat (30.7 versus 35.7 g/l) and lactose (39.1 versus 45.7 g/l). These variations are independent of the origin of milk collection. Concerning the protein fraction, this, the importance of which in technology has been proven, is at a rate of 26g/l below the content obtained for the bovine milk sample (28g/l) which itself is quite deficit, because it is below the required standards (32g/l). In this count, and even if the rate of caseins recorded is appreciable (20g/l), this alone cannot predict an interesting cheese transformation given that precisely in this aspect it is the presence and nature of the αS1 casein which is decisive in this technology. It is in this sense that we set out to qualitatively evaluate this milk by isolating and characterizing its major proteins. For this, we implemented and adopted ion exchange chromatographic isolation techniques on DEAE-cellulose followed by electrophoretic separations on polyacrylamide gel under several conditions (native, in the presence of urea, in the presence of SDS) . This is how the application of DEAE as a separation support for caprine proteins gave good results in the sense that we were able to obtain αS and β caseins with a high degree of purity. Serum proteins, notably PP3, serum albumin and beta-lactoglobulin were also obtained with few contaminants using this technique. Concerning the electrophoretic behavior, in native PAGE, we noted a different migration of caprine PP3, α-lactalbumin and β-lactoglobulin compared to their bovine counterparts. PAGE-urea highlighted the particularity of the migration of caprine caseins where in particular the absence of αS1-CN in the case of sample № 3 suggests a low use of this milk for cheese technology purposes. These preliminary results obtained call for the implementation of more specific investigations for protein identification and phenotyping as well as the development of an exhaustive mapping between the nature of the milk produced and its technological capabilities.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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Analysis of the Technical and Economic Performance of Dairy Farms in Algerian Conditions: The Case of the Wilaya of Tizi-Ouzou

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Abstract: The aim of this work is to carry out a comparative study of the costs of milk production for the two zones, plain and mountain, in the region of Tizi-Ouzou. Sixty-four farmers living in both areas were interviewed and surveyed. With an average of 12.5 cows per farm, there was no difference in the number of dairy cows between the two zones (P=0.586). Fodder consumption was 24.8 and 30.9 kg/cow/day in the mountain and plain areas respectively, a significant difference (P<0.0001). Milk productivity was not significantly different (P = 0.202) between farmers in the two areas. The average was 21.7 litres per cow per day. The total cost of producing one litre of milk was 41.05 AD/L and 35.5 AD/L respectively, a significant difference (P < 0.0001) between the two areas. The cost of feeding differs significantly (P < 0.0001) between the two areas. Feed is more expensive in the mountains than in the lowlands (37.62 vs. 30.81 AD/L). The cost of buying hay was the most significant difference in feeding costs (P = 0.002). These costs were 17.43 vs. 12.73 AD/ha, respectively. There is a small difference in the cost of farm labour (P=0.095). It is lower in the mountain area (0.64 vs. 1.19 AD/L) than in the plain area. The main item in the total cost of milk production is the cost of feed. It represents 90 % and 85 % respectively in the mountain and in the plain areas. Forage area is a limiting factor for the development of milk production in the Tizi-Ouzou region. In the context of territorial development, it is proposed to develop fodder and silage production in regions of Algeria where there is an abundance of arable land and to transfer this fodder to dairy basins such as Tizi-Ouzou to feed dairy farms.

Keywords: Dairy cattle, Milk production, Production cost, Development

Introduction

In Algeria, milk is considered to be a strategic product. This is due to the importance of milk in the Algerian model of consumption. It has a very high nutritional value. It can be a substitute for other expensive products such as meat (Amellal, 1995). With a consumption of 05 billion litres of milk per year, the dairy sector shows a weakness in the production of milk and in the collection of milk (Bellil, 2018). Cattle farming accounts for most of the national milk production. However, it is still not sufficient to meet the demand for milk and dairy products. Despite the potential of the existing farms, the yield of milk is still very modest (Belhadia, 2016). Disparities between farms in terms of production factors and management practices characterise the dairy sector in Algeria. The majority of farms are small-scale family farms that produce food, while other farms are large-

scale industrial farms that are more structured. Differences in dairy farm performance can result from this disparity between farms.

In this sense, our work is aimed at calculating the cost price of a litre of milk in the two milk-producing zones: the plains and the mountains of the Wilaya of Tizi-Ouzou. The factors determining the economic performance of these farms will also be identified. There will also be a comparison of the production costs of a litre of milk between the two areas and the identification of constraints.

Method

The study area (wilaya of Tizi-Ouzou) is located in the northern part of the country (www.tiziouzou-dz.com/). It covers an area of 975.79 km². This represents 0.13% of the national territory. The region is mountainous par excellence. There are only a few plains. Almost 52% of the study area has a 12% slope (DPAT, 2010). There are more than 50,000 cows in the region. They produce an average of 100 million litres of milk per year (DSA, 2019). A survey targeted 64 farmers, equally divided between mountain and plains areas. For the analyses, the descriptive method (mean, standard deviation and percentage) was adopted. In addition, a Student's t test was performed. These analyses were carried out in order to compare production and economic performance between the two zones; mountain and plains.

Results and Discussion

The number of animals reared in the plains zone is 1152, compared with 605 in the mountain zone. The average number of dairy cows was 13.2±10.78 head in the plains zone and 11.8±9.73 head in the mountain zone. There was no significant difference between these two areas (P= 0.586), with an overall average of 12.5 cows/farm.

Feed

Concentrates are added to the forage ration to improve milk production. The quantities of concentrates distributed per cow per day varied considerably between farms in the two zones (Table 1). It ranged from 6 kg to 16 kg. However, the average amount of concentrate distributed per dairy cow per day was 9.81±2.59 kg in the plains and 9.24±2.12 kg in the mountains. There was no significant difference (P=0.257). However, in a study of high-performance dairy cattle farming, Si Tayeb et al (2015) report higher quantities of concentrates distributed, averaging 15 kg/head/day. Whereas Gupta et al. (2014) reports that cows receive small amounts of concentrate, on average less than 3 kg / head / day.

Table 1. Distribution of farms according to the quantity of concentrate distributed in kg/cow/day.

Area	Class (VL)	Number of farms	Concentrate type
Mountainous	[3.5-6]	32	Vache laitière, wheat bran, maize, jeune bovin
Plains	[7-10]	32	
Total	[8-16]	64	

Livestock diets are based on roughage in the form of straw, hay, oats, sorghum and meadow grass. The most common fodder on farms in the two study areas are: Sorghum, oats, clover. Straw and hay were also used throughout the year. Silage was only used on 7 farms out of the 64 farms visited (1 farm in the lowlands and 6 farms in the mountains). In addition, natural and natural and artificial grasslands are also an important source of forage. The quantities consumed from these meadows can reach 31±4.80 kg/cow/day and 25±6.16 kg/cow/day respectively in the plains and mountain areas. There was a significant difference between these two areas in terms of forage intake from grassland (P<0.0001).

Milk Production

As none of the farmers have their own milking parlour, most of them (80%) milk twice a day in their own barn. Milking takes place twice a day. The interval between the two milkings is 12 hours. The average milk yield per cow per day on the farms surveyed was 21±4.84 liters in the mountain zone and 22.41±3.80 liters in the plains zone. There was no significant difference (P = 0.202) between the two study areas. In addition, milk production

at peak lactation could reach 26.7 liters/cow/day in the mountain area and 29.8 liters/cow/day in the lowland area.

Milk Production Costs

In the sample surveyed (64 farms), milk is collected twice a day in most cases. This is because they do not have equipment suitable for collecting raw milk. The following table shows that there is a significant difference ($P<0.0001$) in the total cost of milk production between the two zones. They were 35.50 ± 4.64 AD/L and 41.05 ± 5.75 AD/L in the mountainous and in the plain areas, respectively. Mouhous et al. (2020), in a study on dairy cattle in the same study region, reported a similar production cost of 35 AD/L. This cost is similar to that reported by Yerou et al (2019) in the western region of Algeria, which was 37.1 AD/kg.

The largest item of expenditure in milk production is feed. There was a significant difference ($P < 0.0001$) between the two zones. The cost of feed was 37.62 ± 6.22 AD/L in the plans and 30.81 ± 3.95 AD/L in the mountains (Table 2). Feed accounts for 87% of the total cost in the plains due to successive increases in market feed prices. In mountainous areas, this figure rises to 92%. This situation has also been reported by Ghozlane et al. (2009) for large farms in the eastern part of Algeria and by Srairi et al. (2013) for Morocco.

Regarding feed costs, the significant difference was in hay purchase ($P=0.002$). In the plains, hay purchase costs were 12.73±4.83 AD/L, whereas in the mountains they were 17.41±6.43 AD/L. In plains and mountain areas, the purchase of hay accounted for 41% and 46% of the feed costs, respectively. There were no significant differences in the purchase of other feeds. These included concentrates, straw, oats, lucerne and silage. Paradoxically, concentrates are used more in plains than mountains. In these areas, it accounts for 56.4 % and 49 % of the feed costs, respectively.

Table 2. Cost structure of milk production (AD/L)

Type of cost (AD/L)	Plains area	Mountainous area	P value
Feed	37,62±6,22	30,81±3,95	< 0,0001
Labour	0,47±1,72	1,44±4,90	0,296
Crop work	0,64±1,26	1,19±1,30	0,095
Energy and water consumption	1,08±1,09	0,88±0,59	0,364
Hygiene and health	1,23±0,84	1,18±0,53	0,795
Total cost	41,05±5,75	35,50±4,64	< 0,0001

In addition, there was little difference ($P = 0.095$) in the costs of farm work in the two study areas (0.64±1.26 VS 1.19±1.30) in the plain and mountain areas. These costs include ploughing, seed purchase, treatments, etc. Breeders have small areas of land for cultivation or grazing. There was a significant difference between the two areas ($P=0.006$) with 7ha/farm in plains and 2.5ha/farm in mountains. It should be noted that arable and pasture land is the limiting factor in the study area. Although it is a mountainous area, it has significant potential for milk production skills.

Regarding labour, farms are mainly run by family labour. Paid labour is used on very few farms. Moreover, labour costs represent only 0.47±1.72 AD/L and 1.44±4.90 AD/L of the total cost of milk production in the plain and mountain areas, respectively.

Conclusion

Overall, dairy farms tend to be family-owned with little paid labour. The farms are small. In general, there are no more than 15 cows per farm. Production resources, in particular pasture and arable land, are not the same in the two zones (plains and mountains). There is a significant difference between the plains and the mountains (7 hours compared with 2.5 hours). The most important item in the total cost of milk production is animal feed. These costs (92% vs. 87%) are higher in mountainous areas than in plain areas. Most of the feed costs are for the purchase of concentrates and hay.

Other expenditure in the plain and mountain areas is negligible (8% vs. 13%). This means that there is a significant difference in the costs of producing milk between the two zones. For the plain and mountain areas these costs are 35 AD/L, 5 AD/L and 41 AD/L respectively. With a low fodder base, the study area cannot

develop its milk production. It has considerable potential for milk production skills, despite being a mountainous area.

Recommendations

In order to achieve this purpose of development, it is necessary to put in place sustainable mechanisms for the supply of fodder between mountainous areas and other regions with a high potential fodder base. In some regions with a high potential for arable land, dairy farming is not the main activity. However, it can be a source of fodder in mountain regions where dairy farming is predominant.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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