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DONKEY MILK- BENEFITS AND HYPOALLERGENIC PROPERTIES

Vințe Cezara Georgiana\*, Coroian Aurelia\*

\*University of Agriculture Sciences and Veterinary Medicine, Cluj-Napoca, Faculty of Animal Science and Biotehnology, Mănăștur Str 3-5, 400272, Cluj-Napoca, Romania Romania, e-mail:<u>cezaragrama20@gmail.com;</u> corresponding author: aurelia.coroian@usamvcluj.ro

#### Abstract

Lately, the interest in donkey milk has been steadily increasing, and the attention has been focused on the milk of this species due to the food allergies related to the products obtained from other farm animals. Also, the similarity shown by donkey milk with breast milk, both in terms of protein profile and its lactose content, palatability and assimilation at intestinal level, concur to this concern. Moreover, since the milk yielded from this species was not used in the past for such purposes, it is still on debate, unveiling its properties, such as the antimicrobial, immunomodulatory and hypoallergenic activity, which are having special benefits for human health. The nutritional profile as well as the chemical and microbiological safety have been discussed lately, various studies being carried out regarding it. The donkey milk is especially recommended for infants and elderly, and it can be successfully used as a substitute for cow's milk, by people suffering from allergies. From a protein point of view, its composition is similar to that of human milk, also the high amount of whey protein is an advantage for consumption, being indicated for human health. In this review it is highlighted the hypoallergenic proprieties of donkey milk and the benefits it presents, thus encouraging the population to consume this product.

Key words: donkey milk, hypoallergenic activity, protein profile

#### **INTRODUCTION**

It is well known that donkey milk composition is very similar to the one of human milk, especially with regard to lactose and protein content, being also a good substitute for dairy products obtained from other species, mainly in people suffering from milk intolerance (Vincenzetti S., et al. 2017).

Various studies have shown the importance of milk from this species, which shows a higher nutritional value compared to cow's milk, being an important source of minerals, fatty acids, with a protein profile similar to that of the human milk (Polidori P., et al., 2019).

Milk is one of the main allergens, especially in case of children, milk production in donkey being still a subject of interest (Polidori P., et al., 2010).

The highest allergen potential, in the case of milk and dairy products, is determined by casein, one of its main proteins, but allergies have also been reported in the case of proteins such as  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin (Polidori P., et al., 2010).

Various clinical studies carried out on infants have indicated donkey milk as a valuable alternative when lacking breast milk feeding in humans, especially in case of milk intolerance prevention, based on its similarities with human milk (Polidori P., et al., 2010).

Regarding the protein composition of donkey milk compared to cow's milk it shows differences related to its quantities, namely a lower protein content of 13-28 % mg/ml, meanwhile whey proteins show a 25-50% ratio of the nitrogen fraction (Polidori P., Vincenzetti S., 2012).

Another known reason, supporting the importance of milk production in donkey, is represented by the low number or even the lack of certain bacteria such as *Salmonella*, *Lysteria monocytogenes* or *Staphilococcus*, that are able to cause health issues, such advantages standing for its consumption, due to its nutritional properties and qualities, that proved no posed danger to human health (Sarti L., et al., 2019).

Data on genetic polymorphisms are currently not fully known in donkey milk, genetic variants of  $\alpha$ -lactalbumin being reported (Cunsolo V., et al. 2007).

Compared to the cow's milk, in which the amount of casein shows values of 2.6 g/100 ml, 3 times lower quantities, next to  $\alpha$ -S1,  $\alpha$ -S2,  $\beta$ -casein and k-casein fractions were found in donkey milk (Martini M., et al., 2017).

In the last years, various studies have shown that whey proteins of donkey milk had higher values compared to the milk of other species, moreover beta-lactoglobulin is more digestible compared to betalactoglobulin in cow's milk (Martini M., el al., 2017).

Studies assessing protein stability highlighted no considerable differences related to the influence of various processes as heating, fermentation and ultrasonic treatments (Miao W., et al., 2020).

In addition to its special chemical properties, donkey milk shows antibacterial, antioxidant, immunomodulatory properties and intense antitumor activity, scientists assessed milk proprieties in various forms i.e. liquid and lyophilized, along with spray dry powder and fermented (Miao W., et al., 2020), but also for the presence of lysozyme and lactoferrin, which gives its main antimicrobial properties (Prasad B., 2020).

This study shows the importance of chemical proprieties and benefits of donkey milk and also shows the result of thermal processes.

### DONKEY MILK PRODUCTION AND CHEMICAL COMPOSITION

Milk production in donkeys was determined, as in the rest of the calving mammals and also related to the influence of various factors based on the farming system, meanwhile the local latitude is an important element, which seems to be responsible for the appearance of a photoperiodic oscillation between the seasons, thus showing effects on the sexual cycle (Aspri M., et al., 2016).

Donkey milk, based on its nutritional value, which is an important feature of it, is successfully used for infants and elderly, but also for sensitive people (Aspri M., et al., 2016).

The daily milk yield in donkeys is lower, compared to those of other farm animals, consisting in about 1 litre of milk/day, thus explaining its high selling price (Danahy A., 2020).

Also, the studies carried out have proved that milk yields obtained in case of the Ragusana breed of donkeys are higher in the first 3 months of lactation, the largest amount being registered in the first month, then decreasing even by half in the ruminants 10 months (Salari F., et al., 2019).

From a chemical point of view, both raw and powdered milk have a high content of proteins, fats, water and ash, their values making it similar to breast milk (Li L., et al., 2017).

The main source of energy in milk is determined by lactose, which according to the studies carried out was detected in the composition of donkey milk, in proportions similar to human milk (Li L., et al., 2017).

### Tabel 1.

Protein	Lactose	Whey protein	Casein	References
g/100g	g/100g	g/100g	g/mg	
1.34	6.07	0.78	0.56	Malacarne
				M., et al.,
				2019.
1,5-1,8	5,8-7,4	0,49-0,80	0,64-1,03	Polidori P.,
				Vincenzetti
				S., 2012
2.14	5.9	0,49	0,41	Li L., et al.,
				2017
1.5-1,8	5.8-7.4	0,36	0.64	Guo H.Y., et
				al., 2007

The chemical composition of raw donkey milk

The protein profile in donkey milk was determined by using different protein separation techniques and methods, including chromatography and electrophoresis (Poldiri P., Vincenzetti S., 2012).

Another way to determine the protein profile was achieved in China, where milk was subjected to various technological processes, among which are listed ultrasonic heating and treatments, electrophoresis, hydrophobicity and RP-HPLC analysis for peptide separation (Miao W., et al., 2020).

The amount of protein found in donkey milk range between 15-18 g/L of which the casein fraction represents 25-45%, thus being in lower quantities compared to ruminant milk, but in a proportion similar to breast milk, a complete characterization of milk in this species is complicated due to heterogeneity, partly due to post-translational processes and genetic polymorphism (Marletta D., et al., 2016).

Another difference, in the case of this species, is represented by the distribution of caseins, more precisely the largest quantity being represented by  $\beta$ -casein, followed by  $\alpha$ s1- casein,  $\alpha$ s2-casein, while, k-casein is found in lower quantities (Marletta D., et al., 2016).

Compared to the milk of other farm species, donkey milk has been shown to be weaker in protein, but rich in lactose and rich in whey protein, the casein content is also low, and in terms of amino acids, the percentage is much higher compared to cow's and mare's milk (Guo H.Y., 2007).

In the case of donkey milk, lactose is found in values similar to human milk, it is constant throughout lactation, being also once responsible for the osmotic pressure of milk, being noticed in various studies differences in its quantity depending on the breed, nutrition and lactation stage (Salimei E., et al., 2004).

Various vitamins important for the human body are found in the composition of donkey milk, among which, vitamin D is listed, which has been detected in significant quantities in it, respectively 1.68  $\mu$ g/100 ml in raw milk and 0.60  $\mu$ g, being influenced to some extent by the season, a greater amount being found in summer and spring (Martini M., et al., 2018).

Vitamins B2, B6 and B9 have also been identified in raw milk, lacking vitamins B1 and B12, which are not affected in heat treatments (Matera A., et al., 2022).

Chemical processes can influence the nutritional quality of donkey milk, even in pasteurization processes at temperatures of about 62 °C for 30 minutes (Matera A., et al., 2022).

As a result of studies carried out by using high temperature protocols for a short time, it was shown that the power of lysozyme,  $\beta$ -lactoglobulin and antioxidants was affected due to heat treatments, also the content of lysozyme was substantially reduced (20-60%) (Matera A., et al., 2022).

In whey, three major proteins have been determined, namely  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and lysozyme, demonstrating the antiviral, antitumor and anti-stress properties they confer, in particular  $\alpha$ -lactalbumin (Polidori P., Vincenzetti S., 2012).

#### HYPOALLERGENIC PROPERTIES OF DONKEY MILK

The current prospects, regarding the nutritional requirements at a global level, especially in the case of children or sick people, are increasing, that is why solutions have been sought to remedy the problems of food intolerances, that is why donkey milk is of increasing interest in the market, having a special chemical and nutritional composition (Sarti L., et al., 2019).

The allergies that occur in the case of cow's milk are abnormal immunological reactions of the body to the proteins found in their milk, leading to reactions mediated by immunoglobulin E, clinical manifestations may be represented by gastrointestinal as well as respiratory states (Vincenzetti S., et al., 2014).

By using an HPLC system, the casein fraction in donkey milk was determined, and the concentrations of lysozyme,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin in different phases of lactation were determined by RP-HPLC analysis (Poldiri P., Vincenzetti S., 2012).

The sequencing of a gene was determined, thus completely characterizing the structure of  $\beta$ -casein, by using  $\beta$ -casein derived from mare DNA, as a reference, highlighting the presence of 226 amino acids and the seven potential sites of phosphorylation (Marletta D., Tidona F., Bordone S., 2016).

As for  $\alpha$ -casein, it contains a number of 202 amino acids with lower molecular weight compared to  $\beta$ -casein, and by sequencing the alpha-s1casein protein, a rare mutation was also determined, namely the apparent absence of this fraction in certain breeds of donkeys, such as Ragusana (Marletta D., Tidona F., Bordone S., 2016).

Thus, due to the differences in amino acids between  $\alpha$ s1-CN in donkey milk and cow's milk, more precisely due to differences in the sequence of linear epitopes binding immunoglobulins, it may be one of the factors responsible for hypoallergenic properties (Marletta D., Tidona F., Bordone S., 2016).

Also, an important cause of allergies produced by milk, is determined by the  $\beta$ -lactoglobulin, which is the main protein in cow's milk, but in the case of donkey milk it is found in 40% proportions (Poldiri P., Vincenzetti S., 2012).

B-lactoglobulin in the case of donkey milk is a monomer, while in the case of ruminants this protein is a dimer, this protein being involved in the transport and capture of hydrophobic ligands, in the regulation of enzymes but also in the formation of folic acid complexes (Poldiri P., Vincenzetti S., 2012).

Studies have shown that donkey milk is a very valid alternative in case of allergies to cow's milk proteins, respectively, both in the case of immunoglobulin E-mediated proteins and in the case of non-mediated ones (Monti G., et al., 2007).

Better tolerability was found on the part of people who are prone to allergies, for donkey milk, a claim proven by in vitro analyses, compared to cow's and goat's milk (Vincenzetti S., et. al., 2014).

According to Souroullas K., et al., 2018, due to in vivo and in vitro analyses, in addition to the nutritional and functional characteristics of this milk and the immunological functions that it confers especially on infants have been demonstrated, being tolerated by them better than the milk of other species is tolerated.

In the case of children suffering from allergies to cow's milk, the use of donkey milk has proven to be a very good substitute, it can be successfully tolerated even by patients whose cow's milk protein produces enterocolitis syndrome (Barni S., et. Al., 2018).

## INFLUENCE OF THERMAL PROCESSES ON DONKEY MILK

The volatile compounds were measured by means of HPLC chromatography analysis coupled with mass spectrometry (Vincenzetti S., et al., 2017).

At the basis of the determination of the volatile compounds in donkey milk were the lyophilization methods, following the corresponding parameters continuously, both in terms of processing temperature and product temperature, also in the case of spray drying, an intake temperature of 190°C was used, while the exhaust temperature was 90°C (Vincenzetti S., et al., 2017).

Also, another study conducted in China, on freshly collected donkey milk subjected to four levels of heating, at temperatures of 65°C, 85 °C, 100 °C and 121 °C for 15 minutes and 5 levels of ultrasonic treatment at 200 Mpa, 300 Mpa, 400 Mpa, 500 Mpa and 600 Mpa for 15 minutes, the precipitation rate was observed, which is significantly different in the case of the two thermal processes (Miao W., et al., 2020).

The centrifugal precipitation rate showed the highest level at temperatures above 85°C, according to the study, resulting in its weaker stability at high heat (Miao E., et alt 2020).

Also, in the case of proteins found in donkey milk, following heating processes at temperatures above 80 °C, both casein, lactoferrin and  $\alpha$ -albumin completely disappear, and at temperatures above 100 °C, the lysozyme degrades in high proportions of 40.38 %,  $\beta$ -lactoglobulin instead maintains relative stability after terminal heating treatments (Miao E., et alt 2020).

As for casein, it disappears from ultrasonic processes at 200 W, but whey proteins show stability even from these ultrasonic processes, with only 12 % degradation observed (Miao E., et alt 2020).

Donkey milk is difficult to convert into products, due to the low level of casein, especially K-casein, so another study has achieved good results by using several types of bacteria including *Streptococcus thermophilus* leading to the production of a product from the milk of this species, more precisely, donkey milk cheese, in order to evaluate the effects of fortifying milk by microbial transglutaminase in relation to different types of added enzymes (D'Alessandro A. G., et al., 2019).

# CONCLUSIONS

As a result of the latest studies, a growing interest in donkey milk has been determined, one of the reasons being the high tolerability for people who have allergies to cow's milk proteins and also once again due to the chemical similarity with human milk.

Donkey milk is one of the products of interest at the moment, due to the hypoallergenic properties it owns.

The literature has reported a low number of bacteria in the case of milk of this species, this can be directly influenced by the high content of lysozyme and  $\alpha$ -lactalbumin found in it.

Another reason that has aroused the curiosity of scientists, is the increased digestibility of the protein fraction that donkey milk presents, in comparison with cow's milk.

Heat treatments influence the chemical composition of the milk of this species, but the differences detected were not very significant.

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