

Review

# Donkey Milk Bioactive Proteins and Peptides, Health and Food Applications – A review

Reda Derdak<sup>1§</sup>, Oana Lelia Pop<sup>2,3§</sup>, Souraya Sakoui<sup>1</sup>, Carmen Muresan<sup>2,3</sup>, Dan Cristian Vodnar<sup>2</sup>, Boutaina Addoum<sup>1</sup>, Ramona Suharoschi<sup>2,3\*</sup>, Romana Vultur<sup>4,5</sup>, Abdelaziz Soukri<sup>1</sup>, Bouchra El Khalfi<sup>1</sup>

<sup>1</sup>Laboratory of Physiopathology, Molecular Genetics & Biotechnology, Faculty of Sciences Ain Chock, Health and Biotechnology Research Centre, Hassan II University of Casablanca, B.P 5366 Maarif, Casablanca, Morocco  
[reda.derdakk@gmail.com](mailto:reda.derdakk@gmail.com) (RD), [sakouisouraya@gmail.com](mailto:sakouisouraya@gmail.com) (SS), [addoum636@gmail.com](mailto:addoum636@gmail.com) (BA),  
[a\\_soukri@hotmail.com](mailto:a_soukri@hotmail.com) (AS), [bouchra.elkhalfi@gmail.com](mailto:bouchra.elkhalfi@gmail.com) (BE).

<sup>2</sup>Department of Food Science, University of Agricultural Science and Veterinary Medicine, 3-5 Calea Mănăştur, Cluj-Napoca, Romania, [oana.pop@usamvcluj.ro](mailto:oana.pop@usamvcluj.ro) (OLP), [carmen.muresan@usamvcluj.ro](mailto:carmen.muresan@usamvcluj.ro) (CM)  
[dan.vodnar@usamvcluj.ro](mailto:dan.vodnar@usamvcluj.ro) (DCV)

<sup>3</sup>Molecular Nutrition and Proteomics Lab, CDS3, Life Science Institute, University of Agricultural Science and Veterinary Medicine, Calea Mănăştur 3-5, Cluj-Napoca, Romania,  
[carmen.muresan@usamvcluj.ro](mailto:carmen.muresan@usamvcluj.ro) (CM), (OLP), (RS)

<sup>4</sup>Department of Molecular Sciences, 'Iuliu Hațieganu' University of Medicine and Pharmacy, Cluj-Napoca, 8 Victor Babeş, Cluj-Napoca 400000, Romania, [romana.vultur@gmail.com](mailto:romana.vultur@gmail.com) (RV)

<sup>5</sup>Cognitive Neuroscience Laboratory, Department of Psychology, Babeş-Bolyai University, Cluj-Napoca, Romania, 1 Mihail Kogalniceanu, Cluj-Napoca, Romania, (RV)

§These authors contributed equally to this work

\*Correspondence: [ramona.suharoschi@usamvcluj.ro](mailto:ramona.suharoschi@usamvcluj.ro) (RS)

Received: date; Accepted: date; Published: date

**Abstract:** Due to its similarity with human milk and its low allergenic properties, donkey milk has long been used as an alternative for infants and patients with cow's milk protein allergy. In addition, this milk is attracting growing interest in human nutrition because of presumed health benefits. It has antioxidant, antimicrobial, antitumoral, antiproliferative and antidiabetic activity. Also, it stimulates the immune system, regulates the gastrointestinal flora and prevents inflammatory diseases. Although all components of donkey milk can contribute to functional and nutritional effects, it is generally accepted that the whey protein fraction plays a major role. The purpose of this review is to highlight the active proteins and peptides of donkey milk in comparison with other types of milk, emphasizing their properties and their roles in different fields.

**Key words:** Donkey milk, donkey colostrum, mammal's milk, bioactive peptides, biologic activity, health benefits.

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## 1. Introduction

Since ancient times donkey milk is known for its therapeutic properties and it was used to treat various diseases such as bronchitis, wound healing, asthma, joint pain, gastritis. Today, it is available on the market as a commercial product for the benefit of newborns, of people with allergies to cow's milk proteins and for the elderly people [1, 2]. Donkey milk has become increasingly attractive due to its biological activities, such as antimicrobial, antioxidant activity. In addition, other activities have been reported such as antiviral, anti-inflammatory and antiproliferative activity [1, 3].

The characterization of the main constituents of milk has a fundamental importance according to the correlation between health and nutrition. In this context, proteins are considered as an important nutrient because some of them show bioactivity when they are native [2, 4].

According to their different solubility, milk proteins are classified into three classes: milk fat globule membrane, caseins and whey proteins [3, 4]. The protein content of milk and the relative protein composition may vary among species, among breeds within the same species, and even among individual animals within the same breed. Furthermore, it is well known that there is a strong qualitative resemblance between the principal classes of proteins (i.e. caseins and whey proteins) in all types of milk. These whey proteins and caseins are biopolymers which means they are biologically active agents with biomedical applications [5, 6]. This review aims to report published data about the proteins and peptides from donkey milk in comparison with other kinds of milk (cow, goat, camel and, human milk) and on the other hand, in comparison with donkey colostrum. And also, to show their biological activities such as anti-microbial, anti-oxidant, anti-inflammatory, anti-allergic, anti-tumoral, anti-obesity and anti-diabetic activity and finally their applications in different fields and the benefit of ingesting donkey milk proteins and peptides.

## **2. Global composition of donkey milk compared to other types of milk**

Due to its chemical composition, milk is considered the most perfect food. It consists of water, carbohydrates, fats, proteins and other minor components such as hormones, vitamins, minerals, cytokines [1, 4]. Among the constituents of milk, proteins vary between mammalian species ranging from 1% to 24%. These proteins exist under two main categories of proteins which are defined by their chemical composition and their physical properties: caseins and whey proteins. Also, the carbohydrate (lactose) content of milk varies from 0.7% to 7.0% between different species of mammals. Regarding the fat content, not only the concentration varies but also the chemical composition [3, 4]. Table 1 represents the global composition of donkey, cow, camel, goat and human milk. The amount of donkey milk components, such as whey protein, lactose and caseins, are similar to that of human milk, although they differ significantly from cow, goat and camel milk. The only major difference between donkey milk and human milk is the fat content, which is very low in donkey milk. But regarding the casein-to-whey protein ratio, in donkey milk this is intermediate between human milk and cow milk.

**Table 1:** Milk composition – different species [4, 7, 8].

	Donkey (%)	Goat (%)	Cow (%)	Camel (%)	Human (%)
<b>Proteins</b>	1.74	3.41	3.43	1.8	1.64
<b>Fat</b>	1.21	4.62	3.46	1.8	3.38
<b>Lactose</b>	6.23	4.47	4.71	2.91	6.69
<b>Dry Matter</b>	9.61	13.23	12.38	11.3	12.43
<b>Ashes</b>	0.43	0.73	0.78	0.85	0.22
<b>Water</b>	90.39	86.77	87.62	90.6	87.57
<b>Energy (KJ/Kg)</b>	1939.4	3399.5	2983	2745.8	2855.6

### 3. Bioactive proteins and peptides in donkey milk compared to other types of milk

Table 2 shows the different protein fractions identified in cow's, donkey's, goat's, camel's and human's milk, as well as their amount in g/L. Camel's milk, cow's milk and the goat's type have high levels of proteins compared to human and donkey milk. Cow's milk, camel's milk, and goat's milk have more caseins (80%) and less whey proteins (20%) [5] compared with donkey milk which has more whey proteins (60%) and less caseins (40%) [9]. Donkey's milk has a quantity of  $\alpha$ -lactoglobulin resembling that identified in human milk, and has a high level of  $\beta$ -lactoglobulin, which is not found in human milk. This  $\beta$ -lactoglobulin is the major allergen of cow's milk, besides caseins [4, 5].

**Table 2:** Main proteins of donkey milk compared to other types of milk [4, 5, 9].

	Cow (g/L)	Donkey (g/L)	Goat (g/L)	Camel (g/L)	Human (g/L)
<b>Total protein content</b>	31-38	13-28	25-39	25-45	9-17
<b>Total casein</b>	27.2	6.6	25	26.4	5.6
<b>Total whey protein</b>	4.5	7.5	6	6.6	8
<b><math>\alpha</math>s<sub>1</sub>- casein</b>	10--15	0.2-1	0-7	5	0.3-0.8
<b><math>\alpha</math>-s<sub>2</sub>- casein</b>	3-4	0.2	4.2	2.2	n.d.
<b><math>\beta</math>-casein</b>	9-11	3.9	11-18	12.8	1.8-4
<b>casein <math>\kappa</math></b>	3-4	n.d.	4-4.6	0.8	0.6-1
<b><math>\alpha</math>-lactalbumin</b>	1-1.5	1.8-3	1.2	3.5	1.9-2.6
<b><math>\beta</math>-lactoglobulin</b>	3.3-4	3.2-3.7	2.1	1.45	n.d.
<b>Lysozyme</b>	0.00007	1	Trace	0.00015	0.04-0.2
<b>Lactoferrin</b>	0.1	0.08	0.02-0.2	0.22	1.7-2
<b>Immunoglobulin</b>	1	n.d.	1	1.54	1.1
<b>Albumin</b>	0.4	n.d.	0.5	0.4	0.4

n.d.- not detected

Several research groups have been able to characterize the protein fractions of whey in donkey milk and have demonstrated their nutraceutical properties and their beneficial properties for human health. These proteins will be described in detail below.

### 3.1. Caseins

The caseins are organized into micelles (supramolecules of colloidal size) whose diameter varies from 30 to 600 nm. In particular casein  $\alpha$ 1, casein  $\alpha$ 2, casein  $\beta$ , casein  $\kappa$  and traces of casein  $\gamma$  can be found. These micelles are made up of different types of proteins (94%) and 6% of colloidal calcium phosphate made of calcium, phosphate, magnesium and nitrate. These different caseins have hydrophilic regions and other hydrophobic regions that are different from one casein to another [10]. In addition, the caseins are phosphoproteins, therefore they have phosphorylated regions at the level of the serine residues. The proline residues, uniformly distributed into the casein structure prevent the formation of secondary structures such as  $\alpha$  helices or  $\beta$  sheets, hence the so-called open or "random coil" conformation of casein [11]. Caseins  $\kappa$  have a particular role, they are first of all glycoproteins and have only one phosphoserine group but above all, they are stable in the presence of calcium ions and thus protect all of the caseins against precipitation and stabilize the micelles [10, 12].

In mature cows' milk, caseins make up 80% (w/w) of all proteins, whereas in humans [13] and equines [14], they represent only 35% and 50% of the total protein content, respectively. The donkey milk essentially comprises  $\alpha$  s1- and  $\beta$ - casein while  $\alpha$  s2- and  $\kappa$ - casein are minor components.  $\beta$ -casein can represent up to 80% of the total casein in human milk [15], and is also the predominant protein in the casein fraction of donkey milk [13].

### 3.2. $\beta$ -Lactoglobulin

$\beta$ -lactoglobulin, a globular protein containing 162 amino acids and belongs to the family of lipocalin proteins, has a molecular mass of 18.36 kDa. Lipocalin molecules have pockets capable of hosting iron complexes. Iron binds to protein through iron chelators called "siderophores" [4]. This protein is known for its richness in lysine, leucine, glutamic acid and aspartic acid. Its secondary structure is mainly composed of  $\beta$  sheets ( $\approx$ 50%), but there are also  $\alpha$  helices (10%),  $\beta$  elbows (8%) and a high proportion of disordered structures (35%) [16]. Its structure is also reinforced by two disulfide bridges and by a tertiary structure mainly composed of antiparallel  $\beta$  sheets. Studies showed that two different isoforms of  $\beta$ -lactoglobulin can be found in donkey milk: the major isoform is  $\beta$ -lactoglobulin I (80%), while the isoform  $\beta$ -lactoglobulin II is encountered in lower quantities.

In donkey milk, the  $\beta$ -lactoglobulin content is 3.75 g/L resembles that found in cow's milk, and is lower than that found in camel's and goat's milk, while it is absent in human's milk.

$\beta$ -lactoglobulin is known to have several functions, both nutritional and functional. One of the functions most studied at the moment is the ability of the protein to bind certain molecules of nutritional interest and to serve as a protective matrix during digestion.  $\beta$ -lactoglobulin was shown to bind certain vitamins (D2, D3), cholesterol, certain catechins and even mercury [16, 17]. These

interactions occur mainly in a central area of the protein, denominated calyx (also known as  $\beta$ -barrel), which is formed of  $\beta$  sheets. This hydrophobic cavity, which makes it possible to fix a large variety of ligands, is regulated by an EF loop, which is working as a gate to the site of binding. At low pH, this loop is in the “closed” position and interactions are impossible. When the pH increases, the loop opens allowing the ligands to insert into the hydrophobic cavity [16]. This change in structure, called the Tanford transition, generally occurs between pH 6.5 and 7.5 [18].

### 3.3. $\alpha$ -Lactalbumin

The  $\alpha$ -lactalbumin, protein composed of 123 amino acid residues, with a molecular weight of 14.2 kDa, has in its tertiary structure four disulfide bridges. Native  $\alpha$ -lactalbumin is made up of two distinct domains, a large section made up of  $\alpha$  helices and a small  $\beta$  sheet domain. The two sections are connected by a calcium fixation loop. This protein found in donkey milk in two isoforms with different isoelectric points (pI) values: 4.76 and 5.26.  $\alpha$ -lactalbumin content in donkey milk is 1.8 g/L, a value very close to that found in cow and human milk [3, 4].

$\alpha$ -lactalbumin is a protein recognized for its nutritional qualities, mainly in infants. First,  $\alpha$ -lactalbumin plays an important role in milk production in mammals, because it is binding to the enzyme  $\beta$ -1,4-galactosyltransferase and creates the lactose synthase essential for lactose formation. Another important nutritional element of this protein is its high content of tryptophan since it is an essential amino acid. This amino acid has demonstrated positive effects on the development of the brain and nervous system of newborns, in addition to playing an important role in the functioning of these systems as a direct precursor of serotonin or niacin (also known as vitamin B<sub>3</sub>). Also, studies have shown that regular intake of  $\alpha$ -lactalbumin in adult subjects makes it possible to increase the plasma quantities of tryptophan, thus improving certain neurological functions (attention, cognitive performance, morning alertness, etc.) [19, 20]. This protein also has good digestibility and a low allergenic capacity [21, 22].

### 3.4. Lysozyme

Lysozyme, or muramidase, is a globular enzyme consisting of 129 amino acids and belonging to the class of hydrolases [23]. The latter consists of two domains: a domain composed essentially of  $\alpha$  helices and the other consisting of a  $\beta$  anti-parallel sheet and two  $\alpha$  helices. Three disulfide bridges provide the three-dimensional configuration of the molecule: two are found in the  $\alpha$ -helix domains, while one is located in the  $\beta$  sheet. Lysozyme is able to catalyze the hydrolysis of the glycoside 1 $\rightarrow$ 4 bond of peptidoglycans in the bacterial wall, as well as chitin present in the walls of fungi [1, 4]. Two isoforms of lysozyme which differ by an oxidized methionine at position 79 were described in donkey milk: lysozyme A with a molecular weight of 14.631 kDa and lysozyme B with a molecular weight of 14.646 kDa.

Compared to human milk, donkey milk has a higher content of lysozyme (1 g/L), while in goat and cow milk lysozyme is missing [3, 4]. This high amount of lysozyme gives donkey milk the particularity of preserving these organoleptic and microbiological properties for a long time.

### 3.5. Lactoferrin

Lactoferrin is a glycoprotein which belong to the transferrin family and has a molecular weight of 80 kDa. Its structure is built by two homologous domains which are binding ferric and carbonate ions. The antimicrobial activity of lactoferrin applies to a wide range of Gram-positive and Gram-negative bacteria and is, on one hand, partly dependent on its capacity to bind iron, resulting in an environment scarce in iron which is limiting the bacterial growth; on the other hand, on its capacity to bind to the lipopolysaccharides of bacterial cell walls via its N-terminus, resulting in the permeabilization of the bacterial cells [4, 24].

### 3.6. Lactoperoxidase

Lactoperoxidase (LPO) is an oxidoreductase enzyme and has a protective function against infections by microorganisms. It is found in low concentrations in fresh donkey milk, as well as in human milk [1]. LPO is able to catalyze the oxidation of diverse substrates by using hydrogen peroxide. The oxidation products possess a bactericidal activity against bacteria, viruses, parasites, fungi and mycoplasmas, and a bacteriostatic effect against *Listeria monocytogenes* [3, 4].

## 4. Comparison between donkey colostrum and donkey milk

Several studies have shown that, the composition of milk is different not only between the species but also between the different stages of lactation (colostrum and mature milk) [25, 26]. Colostrum is the first form of milk obtained directly after the birth of the mammal until the 7th day. Studies of whey proteins from different mammals (human, bovine, camel) have shown that they generally have differences between colostrum and mature milk. Yet, changes in the composition of donkey milk in the course of lactation were not sufficient studied. Recently, the compositions, comparisons and alterations of the proteome in milk of mammals at various stages of lactation have been studied using advanced proteomics technologies [27, 28]. Li et al. [27] were able to identify 300 proteins in donkey milk and mature milk colostrum, including 13, respectively 12 whey proteins expressed only in donkey colostrum and mature milk (Table 3). They also showed that in the two types of milk,  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, lysozyme and the constant region of the heavy chains of immunoglobulins gamma 1 were the main whey proteins.

The same study showed that among the proteins identified, 18 were expressed differentially between colostrum and mature milk, of which neural epidermal growth factors like type 2, perilipin, thymosin beta 4, cathepsin B, transforming factor beta, induced and fatty acid binding proteins had higher levels in mature milk, while tetraspanin, amine oxidase, immunoglobulin gamma 1 heavy chain

constant region, apolipoprotein B, prothrombin, major histocompatibility complex (MHC) class I antigen, beta-lactoglobulin II and alphas 2 casein B were higher in colostrum [27].

Other studies have also shown that there are differences not only between proteins, but also between other metabolites (including lipids) composition which reveals that the composition of donkey milk changes during lactation [29-31].

**Table 3:** Uniquely expressed proteins in donkey colostrum and donkey milk [27, 29-31].

Types of Milk	
Donkey Colostrum	Donkey Milk
Zinc-alpha-2-glycoprotein	Histone H3
Immunoglobulin lambda light chain variable region	Myristoylated alanine-rich C-kinase substrate
Thrombospondin 4	Histone H4
Peptidoglycan recognition protein 1	Multiple coagulation factor deficiency 2
Cartilage acidic protein 1	C-C motif chemokine
Peptidyl-prolyl isomerase	Transcription factor AEBP1
L receptor related protein 1	Follistatin like 1
Insulin like growth factor binding protein 7	ST6 beta-galactoside alpha-2,6-sialyltransferase 1
MHC class II associated invariant chain	Uncharacterized protein
DNA J-like protein subfamily B member 11-like protein	Multiple coagulation factor deficiency protein-like protein
Cathepsin Z	Uncharacterized protein
Uncharacterized protein	Glutathione peroxidase (Fragment)
Amino peptidase	

## 5. Antimicrobial activity of donkey milk

For donkey milk various properties were demonstrated, such as anti-bacterial, anti-viral and anti-fungal activity. Several studies showed that donkey milk has an anti-bacterial property against a wide range of pathogenic bacteria such as *Escherichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Shigella dysenteriae*, etc. [5, 32-35], which make donkey milk a safe milk, without food-borne pathogenic bacteria, and gives it a long natural life. This anti-bacterial activity is due to its high value of anti-bacterial components [36-38], mainly some whey proteins such as lysozyme and lactoferrin [34, 35]. Since Gram negative bacteria resist to lysozyme due to its lipopolysaccharide membrane, the anti-bacterial activity of donkey milk can be explained by two mechanisms; firstly by the specific structure of lysozyme of donkey milk (similar of equine's lysozyme), which is able to bind to calcium ions which improve its activity against Gram negative bacteria [32, 39-41], secondly by a synergistic activity of lysozyme and lactoferrin, because the latter has the ability to bind to membrane proteins of Gram negative bacteria which disrupt the membrane and opens the pores to lysozyme which destroys the glycosidic linkage (N-acetylglucosamine and N-acetylmuramic acid) of peptidoglycans [24, 34, 42]. Other studies have shown that the immunoglobulins also contribute to the

inhibition of bacterial growth, acting in synergy with lysozyme [43, 44]. Saric et al 2014 [33], have shown that in addition to the immunoglobulins, some fatty acids such as linoleic acid, lauric acid and oleic acid, when they act in synergy with lysozyme, show an important antibacterial activity against gram-negative and gram-positive bacteria.

In addition to its antibacterial activity, donkey milk and its whey proteins were tested for their antiviral activity. Brumini et al 2013 [45] have demonstrated that they have the ability to inhibit the replication of *Echovirus type 5*; an enterovirus that affects the human gastrointestinal tract. This activity is due to high molecular weight whey proteins such as lactoferrin, LPO and immunoglobulin.

Furthermore, the antifungal activity of donkey milk has been tested and found to be effective against fungal strains which are pathogenic for human. A preliminary study on four samples of donkey milk shown that it inhibits mycotic growth, mainly of *Microsporum canis* and *Trichophyton mentagrophytes*, which are more sensitive than *Microsporum gypseum* to donkey milk [46]. Another study showed an antiviral activity of donkey milk against 2 dermatomycotic fungus: *Trichophyton rubrum* and *Trichophyton mentagrophytes*, which are the main cause of inflammatory tinea corporis [47].

It should be mentioned that these antimicrobial factors (lysozyme, LPO and lactoferrin) are relatively identical in different species (Table 4), still their quantity and importance can differ considerably. Indeed, in human milk and donkey milk the content of lysozyme is substantially higher compared to that of camel, cow and goat milk, while the LPO is present in small quantities in donkey milk, as well as human milk, but abundant in cow's milk. Regarding the lactoferrin, its content is higher in human milk, camel's and goat's milk, respectively [4, 5].

**Table 4:** Quantity of the major antimicrobial proteins found in human, bovine, camel and donkey milk [4, 5].

Milk type	Lysozyme (g/L)	Lactoperoxydase(g/L)	Lactoferrin (g/L)
Donkey	1.0	0.11	0.08
Human	0.12	0.77	0.3-4.2
Goat	Trace	Trace	0.02-2
Camel	0.00015	n.d.	0.22
Cow	0.00007	30-100	0.10

n.d. – not detected

## 6. Antioxidant activity of donkey milk

Donkey milk is known to have an antioxidant activity which gives it an oxidative stability providing consumer protection. A study which consisted in comparing the donkey milk, cow milk, as well as donkey milk powder in terms of antioxidant activity, has shown that donkey milk has a higher antioxidant capacity than cow milk. It has a high ability to remove anionic superoxide radicals and to eliminate hydroxyl radicals, which are free radicals generated by body metabolism [48]. Simos et al [49] were able to determine the antioxidant activity of donkey milk using the method of oxygen radical



absorbance capacity, and have shown that the principal contributors of this activity are caseins and the hydrophilic antioxidant compounds, such as uric acid and vitamin C.

#### 7. Anti-inflammatory and anti-tumoral activity of donkey milk

Donkey milk is a matrix rich with mediators such as lactoferrin which has antimicrobial and anti-tumoral activity, interferon  $\gamma$  which stimulates macrophages, natural killer cells and cytotoxic T cells [50, 51]. It has the ability to induce the release of anti-inflammatory cytokines, maintaining a condition of immune homeostasis [50]. Yvon et al 2018 [52] have demonstrated that the treatment of C57BL/6 mice (Crohn's disease model) with donkey milk has an anti-inflammatory effect by restoring the levels of antimicrobial peptides such as  $\alpha$ -defensin and lysozyme, which help to reduce the imbalance of the microbiota. Moreover, other studies have shown that the lactic flora of donkey milk has an anti-inflammatory effect, for example by the production of nitric oxide by *Lactobacillus farciminis*. In addition, this anti-inflammatory activity can be due to the synergy between this flora and the antimicrobial peptides [53, 54]. Another study has shown that donkey milk and colostrum stimulate the secretion of nitric oxide, which is a potent vasodilator, and therefore prevents atherosclerosis. They demonstrated that donkey milk stimulates the secretion of immunoglobulins G and interleukins (IL) IL-1 $\beta$ , IL-10 and IL-12, while colostrum stimulates the secretion of immunoglobulins A, and they also showed that the two types of milk stimulates the expression of CD25 and CD69 on human peripheral blood mononuclear cells and thus, may be useful in the treatment of human immunological diseases [55].

Furthermore, other studies have shown that the administration of human milk or donkey milk improves the anti-inflammatory state of the liver by improving the mitochondrial hepatic functions [56]. Trinchese et al 2018 [57] have shown that the levels of TNF- $\alpha$  and IL-1 decreased, while IL-10 levels have increase in the serum and tissues of rats fed with donkey milk, compared to control rats and rats fed with cow milk. The same team showed that oral supplementation with human and donkey milk influences the metabolism of glucose and lipids, by modulating pro- and anti-inflammatory serum and tissue mediators.

In addition to its anti-inflammatory activity, donkey milk has other physiological functions such as immunoregulatory and anti-tumor activity [58]. Mao and his collaborators have shown that many donkey milk fractions are able to stimulate the production of cytokines IL-2, IFN- $\gamma$ , IL-6, TNF- $\alpha$  and IL-1 $\beta$  from lymphocytes and macrophages. These cytokines influence anti-proliferation by inducing apoptosis of A549 tumor cells (human lung cancer cells) and the differentiation of these A549 tumor cells into normal cells. They also showed that lysozyme has strong anti-proliferative effect, and therefore could be promising molecule in the treatment of lung cancer [59].

#### 8. Anti-diabetic and anti-obesity activity of donkey milk

In addition to its antioxidant, antimicrobial, anti-inflammatory and anti-tumoral activity, donkey milk has also an anti-diabetic effect (Table 5) [49, 59, 60].

Type 2 diabetes, also known as non-insulin dependent diabetes is a metabolic disease characterized by chronic excess blood sugar (hyperglycemia). The main causes of type 2 diabetes include obesity, dysfunction of  $\beta$  cells and resistance to insulin by peripheral tissues and cells [61]. Due to its higher whey protein content, donkey milk could help prevent and treat diabetes by improving glucose metabolism and insulin resistance. Besides the fact that donkey milk and fermented donkey milk has a low caloric intake, Trinchese and colab. have shown that in animals (rats fed with human or donkey milk) was identified an improved glucose and lipid metabolism with modified mitochondria in adult rat skeletal muscle (compared to untreated control animals); these studies found increased muscle and liver levels of a known regulator of lipid metabolism (OEA: N-oleoylethanolamine), and this could contribute to burning fat and protect the animals against developing certain obesity-associated metabolic and inflammatory sequelae. These animals had higher energy expenditures and decreased body lipid accumulation via the mild augmentation of mitochondrial uncoupling pathway. In addition, several authors speculated that diet-associated changes in microbiota and increased levels of butyrate (a short chain fatty acid) in human and donkey milk-fed rats (compared with cow milk fed rats) contributed to the differences in metabolism and mitochondrial function through several unknown, yet, signaling pathways [56, 57, 62].

Li et al 2020 [60] have shown that donkey milk improves the viability of damaged pancreatic  $\beta$  cells, but does not stimulate the secretion of insulin by damaged  $\beta$  cells, and that the  $\alpha$ -lactalbumin increases the insulin sensitivity of the target organs. Donkey milk has shown a better effect than metformin which is an anti-diabetic drug for the treatment of type 2 diabetes. In addition, they showed that donkey milk decreased the level of glycosylated hemoglobin and it acted positively in the treatment of diabetes by inhibiting the expression of phosphoenolpyruvate carboxykinase 1 and glucose-6-phosphatase which are key enzymes in hepatic gluconeogenesis.

Table 5. Biological activity of donkey milk proteins/peptides

Proteins/Peptide	Biological activity	Reference
$\alpha$ s1 and $\beta$ -casein	Antihypertensive	[63]
	Inhibitor activity of Angiotensin converting enzyme (ACE) and antioxidant activity.	[4]
$\alpha$ -S1-casein	Transport calcium phosphate	UniProtKB - P86272
$\alpha$ -S2-casein	Transport calcium phosphate	UniProtKB - B7VGF9
Lysozyme	Anti-inflammatory properties	[52]
	Anti-tumoral properties	[59]
	Reduced incidence of diarrhea	[64]
	Antimicrobial properties	[24]
Lactoferrin	Antimicrobial properties	[45]
	Antitumor	[63]
	Antithrombotic	[65]
Lactoperoxidase	Antioxydant properties	[49]
	Antimicrobial properties	[45]
$\alpha$ -lactalbumine	Anti-diabetic properties	[60]
	Antihypertensive	[65]
$\beta$ -lactoglobuline	Antihypertensive	[65]

## 9. Food and other applications of donkey milk

Considering its functional properties and its nutritional values, donkey milk becomes an attractive product for health, technology, cosmetic industry and others. Donkey milk is supplied in different forms: liquid milk, fermented products (with higher peptide content and bioavailable calcium source), freeze dried and spray dried powders [66]. Application of new technologies such as freeze drying and microencapsulation allow a better exploitation of this product [24]. Several studies have revealed that donkey milk is an adequate alternative to children suffering from allergy to cow milk proteins [67, 68], due to its low composition on caseins which constitute the main allergenic components of milk. Sarti et al 2019 [67] have shown that donkey milk has no negative influence on infants and children, and have assessed its ability to manage the “Food Protein Induced Enterocolitis Syndrome” caused by cow’s milk. However, cow’s milk proteins cause immune reactions mediated by immunoglobulins E (IgE), which is also managed with donkey milk [67, 68]. Donkey milk is also an important source of vitamin D, which can be obtained only from diet or from exposure to sun-light. The oral source of vitamin D can be important in winter and for people who cannot be exposed to sun-light [68].

Donkey milk is a basic ingredient for the production of high-value dairy products [69]. Donkey milk lysozyme is also used in the food industry due to its stability and resistance to various technological processes such as thermic treatment [70], and to digestive tract conditions such as acid pH and gastrointestinal enzymes [71].

In order to value the donkey, Coppola et al 2002 [72] have investigated donkey milk's ability for fermentation by *Lactobacillus rhamnosus*, and showed that donkey milk is a good base for probiotics and therapeutic food formulation. In this context, another team used *Lactobacillus casei* in addition to *Lactobacillus rhamnosus* as probiotics to produce a fermented drink made from donkey milk, and these probiotics were able to survive in this milk up to 30 days [73]. Indeed, donkey milk is also a base for producing yogurt products (Standard yogurt and probiotic yogurt), and yogurt supplied with probiotics showed a high antioxidant activity and a low content in lactose which is beneficial for consumers with allergy to cow milk proteins [74].

Because of its low fat and casein content, donkey milk constitutes a weak gel during cheese manufacturing. Several studies have faced this situation, for example, by the addition of MTGase- a microbial transglutaminase that can enhance the texture of the curd without any effect on moisture, proteins, fats, or cheese yield [75]. Saric et al 2015 [33] were able to overcome this limit by mixing donkey milk with goat milk, in order to produce a functional product with high quality [33].

Since recently, as Salimei 2016 points out, donkey's milk could thus be placed among the new generation of fermented milk drinks, such as koumiss derived from mare's milk, and would allow for an effective combination of the advantageous properties of the raw ingredient with lactic acid bacteria of probiotic interest. In these years, other products as ice cream, biscuits, cakes, desserts and liqueurs have been developed from pasteurized donkey's milk and its technological use (2%) has been successfully tested in hard cheese making, contributing to innovation in the dairy sector. Regarding the cosmetic industry, besides its potential roles in human nutrition, multipurpose applications of donkey's milk are reported in ethnomedicine and it is used in cosmetology, most likely due to its lysozyme content, effective in smoothing skin and scalp inflammations [76].

## 10. Conclusion

Starting with fundamental importance for the correlation between health and nutrition, this review outlines the importance of protein fraction of donkey milk. This type of milk was used since since ancient Egyptian and Roman times, not only for its nutritional value, but also for its beneficial properties in skin care. Later, it was recognized as a common remedy for many ailments, and in French orphanages during the late XIX<sup>th</sup> or early XX<sup>th</sup> century when infants receiving donkey milk grew well and with lower mortality than those given cow milk, as reviewed by Carminati et al 2017, Fantuz et al 2016 [23, 77]. Nowadays, donkey milk is considered a medicinal food (or "*pharma food*") because of its nutritional and functional properties, and because of having a composition similar to human milk when compared to other types of milk; is known that donkey's milk has casein-to-whey protein ratio intermediate between human milk and cow milk. Donkey milk has various biological activities such as: vasodilation (through the secretion of nitric oxide and therefore preventing atherosclerosis), stimulation

of the immune system, has anti-diabetic, anti-inflammatory, anti-allergic, anti-obesity, anti-proliferative and anti-microbial activities. These activities are specifically attributed to whey proteins such as lactoferrin, LPO, lysozyme and immunoglobulins. As Carminati et al 2017 mentioned, regarding the consumption of fermented donkey milk products (with higher peptide content) by elderly people, this should be encouraged due to this very good source of bioavailable calcium, low caloric intake, and the ability to modulate the aged immune system, including the intestinal mucosal immune response. In addition, the application of certain new technologies, such as lyophilization and microencapsulation allow better exploitation of this animal product.

**Author Contributions:** Conceptualization, R.D.; Writing – Original Draft Preparation, R.D.; Writing – Review & Editing, S.S., O.L.P., D.C.V., C.M., B.A., RV; Supervision, B.E., R.S. and A.S.; All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by ERANET 2/2018 (ProBone).

**Conflict of Interest:** The authors declare no conflict of interest.

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