Whey proteins in donkey milk

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REVIEW

Abstract
In recent years, by-products obtained from farm animals are of increasing interest to farmers and once again to the population, which have properties that are not to be neglected by consumers. Milk is one of the main sources of essential compounds, and the by-products obtained from its processing are important elements in human nutrition, bringing an important contribution of elements to the body. Whey is a complex by-product with a high protein content, and in the case of donkey milk, important benefits have been highlighted, such as antimicrobial, antiviral and anti-allergenic properties. The cost of whey from donkey milk is currently low, which is of interest in terms of its capitalization. In the case of whey obtained from donkeys, the amount of protein is very high, thus, it can be successfully used as a substitute in people with allergies to milk of other species and can be harnessed in the form of protein supplements of interest to the population.

Keywords: Donkey milk, by-products, antimicrobial, anti-allergenic, milk substitute.

INTRODUCTION
As a result of the coagulation process, whey is left over after milk is processed intro curd (Robinson et al., 1994). Whey occurs from the precipitation of milk proteins, which is either facilitated by microorganisms or by the addition of acid casein for the manufacture of dairy products or some enzymes (Kilara, 2018). Of the total solid parts found in whey, a high proportion is attributed to lactose, this by-product being considered an important source of proteins, peptides, lipids, minerals and vitamins, this causing the whey to be transformed from a rejected and wasted product into a product used in food, biotechnology and also once in medicine (Smithers 2008). Due to the relatively low quantity during lactation of donkey milk (100-150 kg of milk), the amount of whey obtained from this species is also lower compared to other species used in the direction of milk and dairy production (Ling et al., 2017). In the case of donkey milk, the nitrogen fraction is very rich in whey protein, which is a percentage of about 35-50%, compared to those in cow's milk, where this percentage is only 20% (Gallina et al., 2016), donkey milk being much more similar to human milk (Marelatta et al., 2013).

WHEY PROTEINS
The nitrogen fraction in the donkey whey represents about 40-50%, a higher percentage compared to the nitrogen fraction in the cow's whey, where it represents only 20%, and the found serum albumin was determined from the DNA sequence in the milk structure (Cunsolo et al., 2007). Of the total level of protein in donkey milk, a percentage of 35-50% is represented by whey proteins, with a very high similarity with proteins in human milk (Gubic et al., 2020).
Together with milk casein, whey comprises the main essential proteins found in this product (Marshall 2004). In the case of donkey milk, as in human milk, 50% of the total proteins existing in milk are found in whey (Vincenzetti et al., 2021).

Due to the fact that the proteins in donkey whey belong to the soluble protein class, they form small and soft clots in the stomach, thus being easy to digest and absorb, compared to the milk casein (Ling et al., 2017). Due to the biological value that whey holds, even exceeding the biological value of egg proteins by 15%, this by-product, is a valuable choice for performance athletes, bodybuilders and people in poor health (Smithers, 2008).

In donkeys, compared to milk from other species, 3% of all milk compounds is determined by whey proteins (Zhang et al., 2018).

Protein levels in donkey milk are low, with levels between 1.36 g/100g milk and 1.9 g/100g-1, while whey protein levels are between 0.49-0.80 g/100g-1 (D’Alessandro et al., 2011).

Compared to the chemical composition of whey, obtained from other species, donkey whey contains less β-lactoglobulin, which is the major protein in milk (Zhang et al., 2022) and a higher level of α-lactalbumin and immunoglobulins, but the production of milk, and also a whey date, is much lower in the case of this species (Akan 2022).

In the whey of donkey milk, proteins such as β-lactoglobulin, α-lactalbumin, lysozyme, serum albumin, lactoferrin and immunoglobulin were found (Brumini et al., 2016), β-lactoglobulin is found as a monomer in this case (Li et al., 2021). β-lactoglobulin in donkeys milk contains two components, namely the major β-globulin I of 162 amino acids residues (Brumini et al. 2016) and the minor β-lactoglobulin II of 163 amino acids residues (Brumini et al., 2016), which present genetic polymorphism (Gallina et al., 2016).

As regards the composition of β-lactoglobulin II, the structure found at the level of donkey whey is very close to that determined in pony milk, compared to mare’s milk, where its level is different (Miranda et al., 2004), also, this compound is considered the main source of allergy (Brumini et al., 2016).

Due to the low content of β-lactoglobulin as the main whey protein, in the case of donkey milk compared to cow’s milk, the beneficial properties in case of allergies are obvious, most of these allergies are caused by this milk protein, which is absent in human milk (Polidori and Vincenzetti 2012).

Dependent on the species, β-lactoglobulin, it can be more or less resistant to gastrointestinal enzymes of humans, because of the fatty acids, so in the case of β-lactoglobulin from the ovine species, its digestibility is much higher, compared to that of cattle, and in the case of β-lactoglobulin from the donkey, it is very degraded in vitro by gastric and duodenal juice, this is responsible for the higher tolerability in milk of this species (Brumini et al., 2016).

As for the β-lactoglobulin, it is a globular protein, soluble, and in donkeys, its determine molecular weight following some studies, was 18.4 kg/mol-1, with a content of 1.3-5.5 mg/mL-1, thus being reported a low level of it compared to the determined α-lactalbumin (Brumini et al., 2016).

α-lactalbumin is a small globular calcium metal-protein, being synthesized in the endoplasmic reticulum of the mammary gland, and in the case of donkeys this protein was determined in very large quantities throughout the lactation period (Brumini et al., 2016).

As regards the amino acid residues in the case of α-lactalbumin in donkey milk, they number 123 and the molecular weight determined is 14,215 g/mol-1 (Brumini et al., 2016), and due to α-lactalbumin and β-lactoglobulin, whey proteins have a high gastric and duodenal resistance (Inglingstad et al., 2010).

Donkey whey proteins are known to stimulate the production of cytokines while also having an antiproliferative effect in vitro (Cosentino et al., 2012).

The antimicrobial activity that donkeys milk presents is considered due to proteins that are found mainly in whey, namely lysozyme, lactoferrin, immunoglobulin and lactoperoxidase, thus being responsible for this important property of milk of this species (Brumini et al., 2016).

An important property of donkey milk is the low level of casein, being thus similar to the human one, also the total level of whey proteins being close to those of human milk and much higher compared to those in cow’s milk (Polidori et al., 2015).

Compared to other species, donkey milk shows a high level of lysozyme, while the content in casein is much lower (El-Hatmi et al., 2015, Consentino et al., 2016, Adduci et al., 2019). The high level of lysozyme, an antibacterial protein that plays an important role in immunological processes such as the binding capacity of calcium ions, makes donkey milk exhibit improved antimicrobial activity compared to human or cow’s milk (Zhang et al., 2022).

It is known that donkey milk is also once a natural antimicrobial, having a beneficial impact on the health and integrity of the gastrointestinal mucosa (Brumini et al., 2016).

Antimicrobial properties are due to the level of immunoglobulins, lactoperoxidase, lactoferrin and lysozyme, they are responsible for the low number of bacteria (Brumini et al., 2016).
Also, in addition to the high content of whey in proteins such as lysozyme, α-lactalbumin, β-lactoglobulin, a high content of serum albumin has also been found, the values found in whey in donkey’s milk being much increased compared to those reported in cow’s milk and human whey (Zhang et al., 2022).

In donkey whey compared to whey in cow’s milk, vitamin D, serum albumin and various binding proteins are found (Zhang et al., 2022). After milk processing, the level of whey protein is one of the main factors determining its quality (Vincenzetti et al., 2017).

**Figure 1.** Comparison of whey protein in donkeys, cow and humans.

<table>
<thead>
<tr>
<th>Donkey</th>
<th>Cow</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Whey protein</strong></td>
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</tr>
<tr>
<td>4.9-9.6 g/L</td>
<td>5.5-7.0 g/L</td>
<td>6.2-8.3 g/L</td>
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<td>(Brumini et al., 2015)</td>
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<tr>
<td><strong>β-lactoglobulin</strong></td>
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<tr>
<td>1.3-5.5 g/L</td>
<td>3.2-3.3 g/L</td>
<td>0 g/L</td>
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<td>(Brumini et al., 2015)</td>
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<tr>
<td><strong>α-lactalbumin</strong></td>
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</tr>
<tr>
<td>0.8-2.7 g/L</td>
<td>1.2-1.3 g/L</td>
<td>1.9-3.4 g/L</td>
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<td>(Brumini et al., 2015)</td>
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<tr>
<td><strong>Imunoglobulins</strong></td>
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<tr>
<td>1.30 g/L</td>
<td>0.5-1.0 g/L</td>
<td>0.96-1.3 g/L</td>
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<td>(Brumini et al., 2015)</td>
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<tr>
<td><strong>Lactoferrin</strong></td>
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<tr>
<td>0.005-0.05 g/L</td>
<td>0.02-0.5 g/L</td>
<td>1.5-2 g/L</td>
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<td>(Brumini et al., 2015)</td>
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<tr>
<td><strong>Lysozyme</strong></td>
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</tr>
<tr>
<td>0.67-4.00 g/L</td>
<td>(70-600) x 10^-6 g/L</td>
<td>0.1-0.89 g/L</td>
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**Table 1.** Donkey milk proteins

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<tr>
<td><strong>Casein/ whey protein</strong></td>
<td>4.9-9.6 g/L</td>
</tr>
<tr>
<td><strong>α-lactalbumin</strong></td>
<td>2730 mg/L in 45 day of lactation</td>
</tr>
<tr>
<td><strong>α-lactalbumin (mg/ml)</strong></td>
<td>0.81-1.63</td>
</tr>
<tr>
<td><strong>β-lactoglobulin</strong></td>
<td>1,30 g/L</td>
</tr>
<tr>
<td><strong>β-lactoglobulin</strong></td>
<td>260,2 ml/L in 45 day of lactation</td>
</tr>
<tr>
<td><strong>β-lactoglobulin (mg/ml)</strong></td>
<td>3.60-4.13</td>
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<tr>
<td><strong>β-lactoglobulin</strong></td>
<td>3.75 mg/ml</td>
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INFLUENCE OF THE LACTATION PERIOD AND SEASON ON DONKEY WHEY

Among the factors that influence the number of proteins in whey, lactation is also listed, so in the first part of it, the protein values are the highest (D’Alessandro et al., 2011).

The number of proteins found in donkey whey is approximately 4.9-9.6 mg/mL-1, a value determined mainly by β-lactoglobulin, α-lactalbumin, lysozyme, serum albumin, immunoglobulins and lactoferrin, with changes in each of them being observed during the lactation period (Brumini et al., 2016).

The stage of lactation can influence the protein composition of donkey milk, also the breed, it has been observed that it is an important factor in the composition of the protein content (Brumini et al., 2016). The level of immunoglobulins gradually increases during the lactation period, the highest level is determined on day 100 of lactation, after which it gradually decreases, and with regard to whey proteins, their level decreases linearly with the stage of lactation (Gubic et al., 2020).

Also, the season is a factor that affects the level of major whey proteins, in the spring period, when animals have access to pasture, the amounts of protein are positively influenced, the lowest amounts being reported in the summer-autumn period (D’Alessandro et al., 2011).

The quality of milk is determined by the level of major whey proteins, and this is influenced by the quality of the feed, which shows higher nutritional properties in the spring period (D’Alessandro et al., 2011).

WHEY IN HUMAN NUTRITION

Protein determination in the case of donkey whey and other species, was carried out to demonstrate their nutritional properties (Zhang et al., 2022).

In donkey whey, proteins that have immune responses are also found, such as phosphoprotein 1, which is a multifunctional protein, which is involved in various immune and anti-inflammatory responses, so due to the presence of these factors in whey and donkey milk, it can be a substitute in the nutrition of new-born with the ability to strengthen the immune system (Zhang et al., 2022).

Due to the state of satiety that it is able to create through consumption, whey, represents a functional food increasingly of interest, being economically accessible for its transformation into products beneficial to the human body (Luhovyy et al., 2007).

In addition to satiety, whey is an insulinotrophic and the peptides formed in it affect the renin-angiotensin system, being beneficial for people suffering from type II diabetes, obesity and hyper and dyslipidemia (Luhovyy et al., 2007).

As a result of the research carried out on donkey and camel milk, it has been proved that the digested whey fractions have no inhibitory effect on α-glucosidase, and the peptides released from the digested casein fraction of whey have antioxidant properties and also once antidiabetic properties (Akan 2022).

The content of whey proteins, make donkey milk much more favorable for human nutrition, so α-lactalbumin, plays an important role in the synthesis of lactose in the mammary gland, being also a source of essential amino acids (Polidori et al., 2015). Whey is also an important source of essential amino acids, rich in branched-chain amino acids such as leucine, isoleucine, and valine. It helps with metabolic processes in the body while contributing to the maintenance of body weight (Smithers 2008).

Since the level of casein is lower, compared to cow’s milk, and much more similar to the whey protein content of human milk, donkey milk is much more easily digestible by the human body (Li et al., 2022). Also, donkey milk, presents a low level of cholesterol, which makes it ideal for the elderly, this prevents metabolic diseases (Zhang et al., 2022).

The level of lysozyme is much higher compared to that found in whey obtained from cow’s milk, and β-lactoglobulin is present in donkey whey, its determined sequential homology is 60%, and due to the high similarity of proteins in donkey and human milk has led to the testing of donkey milk in the case of treating children affected from hypoallergenic disorders (Vincenzetti et al., 2021).

The content of β-lactoglobulin may vary depending on the variety of cheese obtained from processing (Robinson 1994), which is nevertheless an important source of calcium, lactose, protein, vitamins, and is described as a functional and nutritional food (Gonzalez-Martinez et al., 2002). Also, the high content of whey proteins, more specifically lysozyme, lactoferrin of serum albumin, determines the antitumor and anticancer characteristics of donkey milk (Shariatikia et al., 2017).

The proteins found in donkey whey, together with the minerals, vitamins, bioactive enzymes and coenzymes that this milk contains, have a beneficial effect on the processes of maintaining the skin, delaying the aging of the skin and leading to its hydration (Cosentino et al., 2012).

Within this species, there is a great interest in studying milk and whey proteins, due to the possibility of using it as a milk substitute in elderly people and children affected from allergies to milk produced by other species (D’Alessandro et al., 2011).
CONCLUSIONS
Following what has been reported, one can observe the importance of whey proteins on the human body, which are extremely important for the body due to the multiple benefits they bring. It is also noted that external and internal factors can contribute in terms of the level of these proteins and also in terms of their quality. Compared to other animal species, the level of proteins found in donkey whey has an important contribution being a substitute for breast milk, with multiple benefits especially in terms of children with various food problems.

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Conflicts of Interest
The authors declare that they do not have any conflict of interest.

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