

## DETERMINATION OF WHEY PROTEINS PROFILE IN BALKAN DONKEY'S MILK DURING LACTATION PERIOD

Jasmina Gubić<sup>1\*</sup>, Tatjana Tasić<sup>1</sup>, Jelena Tomić<sup>1</sup>, Aleksandra Torbica<sup>1</sup>

<sup>1</sup>Institute for Food Technology, University of Novi Sad,  
Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

\*e-mail: jasmina.gubic@fins.uns.ac.rs

### Abstract

The aim of the present study was to investigate the whey proteins profile of raw donkey's milk from an autochthonous Domestic Balkan donkey breed during the lactation period from 45 to 200 days.

The following parameters were determined: whey proteins, alpha-lactalbumin, beta-lactoglobulin, lysozyme and lactoferrin. A technique use for determination of the protein profile is chip-based separations on the Agilent 2100 bioanalyzer.

The content of alpha-lactalbumin (2740 - 1300 mg/L), beta-lactoglobuline (260 - 139 mg/L) and lysozyme (2970 - 1670 mg/L) had a tendency to stabilize during mid-lactation period (100th - 150th day), after that it tended to decrease, in a straight line to the end of lactation. The immunoglobulin had an increasing trend, which was best manifested on 100th day (88 mg/L), but thereafter they showed decreasing trend (26 mg/L). The content of whey protein and lactoferrin decreased quite linearly (84 - 6mg/L), with the lactation progressed (80th - 125th day). The content of serum albumin (238 - 113 mg/L) showed a stabilizing trend observed in the middle period of lactation.

Based on results obtained in our study, more in-depth information on lactation and milk characteristics in donkeys are provided.

**Key words:** Whey protein, Donkey milk, Lactation stage.

### 1. Introduction

There is a particular worldwide interest for the conservation and breeding of the local breeds of animals due to their superior biological traits (Pelmus *et al.* [1]). Donkey produces nutritious milk for human consumption and this milk is naturally available in Serbia.

The protein concentration changes significantly during lactation, while the greatest change occurs in the whey protein fraction (Taddese [2]).

Approximately 35 - 50% of the proteins in donkey's milk are whey proteins, while the proportion in cow's milk is much smaller, only 20% (Herrouin *et al.* [3]).

Donkey's milk is characterized by a low ratio of casein/whey proteins (1.04 average) (Guo *et al.* [4]) and a key role in sensitizing the protein fraction of cow's milk, or low ratio reduces the occurrence of allergic to milk (Tidona *et al.* [5], Burumi [6]). The ratio of casein/whey protein, which changes during lactation decreases from (1.33 to 0.60) (Tidona *et al.* [5]; Burumi [6]). Donkey and human milk form a softer clot and the time of digestion in the stomach about 2 hours after intake of milk in the body, which increases the digestibility and absorption of soluble proteins, and the reason is more favorable ratio of whey protein and casein in the milk of these animals (Park *et al.* [7]).

In general, the composition of donkey milk whey proteins shows a 100% similarity to the human milk contained the amino acid sequences. Between, the alpha-lactalbumin and lactose is a protein subunit of the enzyme that catalyzes the biosynthesis of lactose (Bertino *et al.* [8]).

In equine milks, whey proteins are principally constituted by  $\alpha$ -lactoalbumins,  $\beta$ -lactoglobulins and lysozyme together with minor components such as serum albumin and lactoferrin (Cunsolo *et al.* [9]).  $\beta$ -lactoglobulin exists predominantly as monomer in equine milks, whereas this protein is dimeric in milk of ruminants (Herrouin *et al.* [3]).

70% of  $\beta$ -lactoglobulin from donkey's milk is digestible, which is twice more than in cow's milk. Thus a greater percentage of  $\beta$ -lactoglobulin digestible may also improve the yield of peptides in the stomach bioactivating (Inglistad *et al.* [10], and Tidona *et al.* [5]).

Although the donkey's milk present high levels of lysozyme 4 g/L, the content is reduced during lactation (Guo *et al.* [4]). This enzyme, together with other factors including immunoglobulin and lactoferrin may function in the infant's digestive tract to reduce the incidence of gastrointestinal infections (Businco *et al.* [11]).

The Domestic Balkan donkey's is an autochthonous breed primarily farmed in the Northern and Eastern regions of Serbia (Food and Agriculture Organization [12]; Kugler *et al.* [13]). In the present work, the content of whey protein fraction in donkey's milk was determined at different stages of lactation (45, 60, 80, 100, 125, 150, 170, 200 days after parturition) by using chip-based separations on the Agilent 2100 bioanalyzer.

## 2. Materials and Methods

### 2.1 Animals and biological samples

Donkeys' milk samples were collected immediately after morning milking from "Zasavica" Special Nature Reserve. To determine the milk quality parameters, as well as the milk protein profile, milk samples were obtained from 10 female Balkan donkey breed after parturition. Milking was done manually twice a day on 45th, 60th, 80th, 100th, 125th, 150th, 170th, 200th day of lactation.

### 2.2 Determination of whey protein profile

Milk proteins were further separated by SDS-PAGE electrophoresis on the Agilent 2100 bioanalyzer (Agilent Technologist, Santa Clara, CA, USA) in combination with the Protein 80 Plus Lab Chip kit and the dedicated Protein 80 software assay on 2100 expert software. Sample preparation was carried out according to Tidona *et al.* [5] with some modifications. Milk samples were diluted in 1 : 1.5 (v/v), sample : buffer (0.125m Tris-HCL, 4% SDS, 2% glycerol, 2%  $\beta$ -mercaptoethanol, pH 6.8) and heated at 100 °C for 5min.

### 2.3 Statistical analysis

Analysis of variance and Duncan's multiple range test were performed using the software package Statistica 9.1 for Windows, Stat Soft, Tulsa, Oklahoma, USA, 2009. Differences were considered significant at  $p < 0.05$ .

## 3. Results and Discussion

The changes of whey protein concentration from donkey's milk during the lactation period are shown in Table 1.

Whey proteins from donkey milk are characterized by the presence of high intensity  $\alpha$ -lactalbumin and lysozyme bands.  $\alpha$ -lactalbumin are the major whey protein in donkey milk and high content, obtained in this study, was very similar to that found in human milk 2200 mg/L (Vincenzetti *et al.* [14]). High concentration of lysozyme identified in donkey's milk, was very high compared to cow's milk (0.09 mg/L) and human milk (40-200 mg/L) (Polidori *et al.* [15]). Concentration of  $\beta$ -lactoglobuline in donkey milk was significantly lower than the concentration of  $\alpha$ -lactalbumin.

The  $\alpha$ -lactalbumin ranged from 2740 to 1300 mg/L,  $\beta$ -lactoglobuline from 260 to 139 mg/L and lysozyme from 2970 to 1670 mg/L, with tendency to stabilize during mid-lactation period (100<sup>th</sup> - 150<sup>th</sup> day). After that period, it tended to decrease, in a straight line to the end of the lactation period. The results obtained in this research were consistent with those obtained from other authors (Paolicelli [16], and Fox *et al.* [17]).

Results presented in Table 1 show that lactoferrin concentration range from 41 to 6 mg/L and decrease during the entire lactation period.

The immunoglobulin had an increasing trend, which was best manifested on 100th day (88 mg/L), but there after showed decreasing trend. Similar to immunoglobulin, serum albumin contains a high concentration to 100<sup>th</sup> days of lactation (237 mg/L), but there after showed decreasing trend. A similar trend was reported by (Guo *et al.* [4]). Data from this study are an important reference for an in-depth knowledge of donkey milk and in particular for the Domestic Balkan breed.

**Table 1. Changes of whey proteins fraction in the donkey milk throughout the lactation period**

Concentration (mg/L)	Time of milk sampling, lactation stage, day							
	45	60	80	100	125	150	170	200
$\alpha$ -lactalbumin	2730	2740	2450	2590	1700	1580	1980	1300
$\beta$ -lactoglobulin	260.2	263.3	207.4	211.2	200.2	212.7	139.4	141.7
Serum Albumin	113.1	115.2	238.4	236.8	202.1	191.7	217.8	192.2
Immunoglobulin	47.6	55.2	73.3	88.3	60.3	46.7	37.4	25.9
Lysozyme	2970	2490	2350	2490	2300	2210	1940	1670
Lactoferrin	41.4	30.6	25.5	21.9	16.1	13.2	12.6	6.4

#### 4. Conclusions

- Whey protein content in donkey milk varies through lactation period. The main components of donkey whey proteins are  $\alpha$ -lactalbumin and lysozyme respectively. The specific  $\alpha$  preferred whey protein fraction in donkey milk can be a good indicator of its importance of milk in the diet of children and adults.

- The results of whey protein concentrations from Balkan donkey's milk during lactation period, obtained in this study, can be used as a valuable aid to optimize the use of donkey milk for human feeding.

#### Acknowledgement

This study was supported by the Ministry of Science and Technological Development of the Republic of Serbia, Project No. III 46012.

#### 5. References

- [1] Pelmus R. S., Pistol G. C., Lazar C. Marin D. E., Gras M., Radu M. Ghita E. (2012). *Preliminary study on milk composition and milk protein polymorphism in the Romanian local sheep breed Teleorman Black Head Tsigai*. Romanian Biotechnological Letters, 17, pp. 7582-7591.
- [2] Tadesse T. (2010). *Investigation on nutritional and microbiological properties of Abyssinian donkey milk from Adea woreda*. Theses in School of graduate studies of Addis Ababa University.
- [3] Herrouin M., Molle D., Fauquant J., Ballestra F., Maubois J. L., Le'onil, J. (2000). *New genetic variants identified in donkey's milk whey proteins*. Journal of Protein Chemistry, 19, pp. 105-115.
- [4] Guo H. Y., Pang K., Zhang X. Y., Zhao L., Chen S. W., Dong M. L. (2007). *Composition, physicochemical properties, nitrogen fraction distribution, and amino acid profile of donkey milk*. Journal of Dairy Science, 90, pp. 1635-1643.
- [5] Tidona F., Sekse C., Criscione A., Jacobsen M., Bordonaro S., Marletta D., Vegarud G. E. (2011). *Antimicrobial effect of donkeys' milk digested in vitro with human gastrointestinal enzymes*. International Dairy Journal, 21, pp. 158-165.
- [6] Burumini D. (2012). *Investigation on donkey milk protein fractions: in vitro antimicrobial, antiviral and anti-proliferative activities and casein separation by cation exchange chromatography*. Doctoral thesis, Università degli studi di Catania, Italy.
- [7] Park K., Song J., Beak K., Lee C., Kim D., Cho S. (2011). *Genetic diversity of a Korean echovirus 5 isolate and response of the strain to five antiviral drugs*. Virology Journal, 8, pp. 79.
- [8] Bertino E., Gastaldi D., Monti G., Baro C., Fortunato D., Perono G. (2010). *Detailed proteomic analysis on DM: Insight into its hypoallergenicity*. Frontiers in Bioscience, E2, pp. 526-536.
- [9] Cunsolo V., Saletti R., Muccilli V., and Foti S. (2007b). *Characterization of the protein of donkey's milk whey fraction*. Journal of Mass Spectrometry, 42, pp. 1162-1174.
- [10] Inglistad R. A., Devold T. G., Eriksen E. K., Holm H., Jacobsen M., Liland, K. H. (2010). *Comparison of the digestion of casein and whey proteins in equine, bovine, caprine and human milks by human gastrointestinal enzymes*. Dairy Science and Technology, 90, pp. 549-556.
- [11] Businco L., Gianpietro P. G., Lucenti P., Lucaroni F., Pini C., Di Felice G. (2000). *Allergenicity of mare's milk in children with cow's milk allergy*. Journal of Allergy and Clinical Immunology, 105, pp. 1031-1034.
- [12] Food and Agriculture Organization. (2009). *Domestic Animal Diversity Information system (FAO DAD-IS)* <URL:http://dad.fao.org/. Accessed 27 March 2014.
- [13] Kugler W., Grunenfelder H. P., Broxham E., (2008). *Donkey Breeds in Europe: Inventory, Description, Need for Action, Conservation*. In: Report 2007/2008, Monitoring Institute for Rare Breeds and Seeds in Europe, Save foundation, 62. <URL:www.donkeypover.donkecology.com/Alphrefs.doc. Accessed 27 March 2014.
- [14] Vincenzetti S., Polidori P., Mariani P., Cammertoni N., Fantuz F., & Vita A. (2008) *Donkey's milk protein fractions characterization*. Food Chemistry, 106, pp. 640-649.
- [15] Polidori P., Beghelli D., Mariani P., Vincenzetti S. (2009). *Donkey milk production: state of the art*. Italian Journal of Animal Science, 8, pp. 677-683.
- [16] Paolicelli F. (2005). *Impiego del latte di asina in campo medico, alimentare e cosmetico*. 1°Convegno Nazionale sull'Asino, Grosseto, pp. 28-29.
- [17] Fox P. F. (2003). *The major constituents of milk*. In: Dairy Processing, Smit G. (Ed.), CRC Press, Washington, DC, USA, pp. 7-19.