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SHORT COMMUNICATION

Assessment of donkey milk chemical, microbiological and sensory attributes in Greece and Cyprus

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This study aimed to assess the nutritional, hygienic and sensory characteristics of donkey milk produced in Greece and Cyprus. The average values for pH, fat, protein and lactose were 7.14, 0.52 g/100 mL, 1.22 g/100 mL and 7.01 g/100 mL, respectively, whereas aflatoxin M1 and beta-lactam residues were not detected in any sample. The microbiological analysis revealed very low somatic cell counts and total microbial counts, while Escherichia coli, Salmonella *spp and* Listeria monocytogenes *were not detected in any sample. The sensory evaluation classified the milk as white, thin, with a slightly sweet pleasant taste, pleasant milky aroma, sweet flavour and no persistent aftertaste.*

Keywords Donkey milk, Milk composition, Hygiene, Greece, Cyprus.

INTRODUCTION

Donkey milk (DM) use dates back to antiquity with numerous references for its virtues by Greek and Roman historians. Over the last decade, research has been directed towards the properties and uses of DM that has a distinct chemical composition and consequently particular nutritional properties, similar to breast milk (Guo *et al.* 2007;). In Greece and Cyprus, there are ethnographic reports about using donkey's milk (DM) to feed infants either as a replacement of breast milk or as a means to treat specific illness, that is whooping cough. The notion is that the latter was due to the composition and the biological activity of DM; such assumption has only recently been partly justified (Tidona *et al.* 2011).

The status of donkeys in Greece was recently investigated by Arsenos *et al.* (2010). They reported an increased interest in donkeys in Greece due to their potential for milk production. Based on the latest census, donkeys' population in Greece and Cyprus was 16 443 and 2000. In both countries, there is enhanced interest from consumers for DM, while there are no official reports or published data with reference to its quality and hygiene.

The evidence in the literature suggests that DM contains less fat, less protein but more lactose, when compared to cow's milk, and therefore, it is easily digestible, palatable and rich in nutrients (Salimei and Fantuz 2012). More specifically, the available research data summarise DM composition as follows: 8–10 g/100 mL total solids, 1.5–1.8 g/100 mL protein, 6–7 g/100 mL lactose and 0.28–1.82 g/100 mL fat (Salimei *et al.* 2004; Piccione *et al.* 2008; Tidona *et al.* 2011). Moreover, DM has been classified as hypoallergenic (Monti *et al.* 2007; Restani *et al.* 2009; Bertino *et al.* 2010) and also rich in natural antimicrobials (Zhang *et al.* 2008). The latter has been erroneously interpreted by some raw DM consumers, constituting an alerting issue for both scientists and consumers (Zhang *et al.* 2008). The microbiological

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status of DM is characterised by very low microbial load, which is attributed to the antimicrobial activity of lysozyme (Zhang *et al.* 2008). The lack of comprehensive evidence regarding the level of safety and the nutritional value of DM in Greece and Cyprus, as well as its potential uses, have been the main drivers for this study. Hence, the objective was to study the chemical, microbiological and sensory attributes of DM produced in Greece and Cyprus.

MATERIALS AND METHODS

Sample collection

A total of 90 DM samples (79 from 6 Greek farms and 11 from a Cypriot farm) were randomly collected. All animals used to obtain milk samples belonged to the indigenous Greek Donkey and Cypriot Donkey breeds, respectively. All donkeys selected for sampling were thoroughly examined for any signs of clinical disease, and there was no medication administered.

Compositional analysis

All milk samples were analysed by applying the ISO 2446:2008 for the determination of fat, using the CH-8307 Gerber centrifuge (Gerber Instruments AG, Effretikon, Switzerland), ISO 8968-2:2001 for total nitrogen, using the Kjeltec 8100 (FOSS Analytical A/S, Hillerod, Denmark), and the AOAC 984.15-1985 for lactose, using a UV–vis spectrophotometer DR 5000 (Hach Lange, Düsseldorf, Germany), while pH was measured with the Hanna Instruments pH210 (Woonsocket, RI, USA).

Chemical contaminants determination

Thirty samples were tested for aflatoxin M1 (BIOOScientific Corp.-1060-01) and beta-lactam (BIOOScientific Corp.-1065-1) residues by ELISA (ASYS EXPERT 96; Biochrom Ltd, Cambridge, UK), strictly following the kit's instructions.

Microbiological analysis

A total of forty-one samples were analysed by applying the ISO 4833:2003 for total viable count (TVC) enumeration, ISO 6611:2004 for yeasts and moulds, ISO 15214:1198 for lactic acid bacteria, ISO 21528-2:2004 for Enterobacteriaceae, ISO 11866-1:2005 for *Escherichia coli,* ISO 6888-1:1999 for *Staphylococcus aureus*, ISO 11290-1-1996 for *Listeria monocytogenes* and ISO 6579:2002 for *Salmonella* spp. Somatic cells counts were also determined by microscopic enumeration (ISO 13366-1) using the XSZ-107BN Binocular Microscope (Jiangsu Zhengji Instruments, Jin Tan, China).

Sensory analysis

Thirty pasteurised samples were submitted for sensory evaluation by ten panellists (four females and six males, aged 22–45 years). The panellists were selected according to ISO 8586-1. Samples were described using quantitative descriptive analysis. The sensory evaluation tests were performed in individual booths with controlled temperature and lighting conditions. The panellists received approximately 10 mL of each sample at temperatures between 7 and 8 °C in disposable plastic cups, coded from 1 to 30, respectively. Water was available to panellists during the test. On a standardised sensory test form (with a 5-point hedonic scale), panellists presented the perceived intensities of each of the attributes checked, namely appearance, taste, aroma, flavour, aftertaste and texture. Reference standards were used to develop the appropriate descriptive abilities of the panel and to also calibrate the panellists in using the intensity scale, as described in Chapman *et al.* (2001).

Statistical analysis

Data were analysed using the statistical package SPSS 15.0 (SPSS Inc, Chicago, Illinois, USA). Quantitative data are presented as mean with standard deviation (SD). Kruskal–Wallis test was used to examine differences between categories of qualitative variables and quantitative variables. Results were considered statistically significant when the P was <0.05.

RESULTS AND DISCUSSION

Chemical composition and residues

The chemical composition of DM is presented in Table 1, and results are in line with other studies (Guo *et al.* 2007; Medhammar *et al.* 2011; Salimei and Fantuz 2012). It is confirmed that DM composition is similar to human milk and could be used for a variety of purposes (Vita *et al.* 2007; Tesse *et al.* 2009). However, any suggestion to use DM as alternative milk for infants should take into consideration its low fat content. When sample's results from the different areas were compared, there were statistically significant differences regarding pH ($P < 0.001$), protein ($P < 0.001$) and fat ($P = 0.005$) (Table 2), which could be attributed to the fact that the sampled population belonged to different breeds and it was reared under different conditions. There is evidence in the literature that DM is influenced by the feeding regime, breed and age (Salimei 2011). This study also assessed the presence of beta-lactam residues and aflatoxin M1 and results showed that the milk tested was clear. Beta-lactams have been selected to be tested in this study, as they represent the most common antibiotics applied in farming. Thus, there is no literature on the pharmaceutical residues in DM. Passantino *et al.* (2011) have reported, however, the challenges of ivermectin residues in DM. With reference to aflatoxin M1, Caloni and Cortinovis (2011) reported the occurrence of aflatoxins in feeds for equines, while there is no report on aflatoxin M1 occurrence in DM. Aflatoxin M1 and antimicrobial residue

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Table 1 Donkey milk chemical composition (n = 90) and microbial counts (n = 41)

Parameter	*Min*	*Max*	*Mean*	*SD*
pH	6.68	7.60	7.14	0.15
Fat g/100 mL	0.02	2.05	0.52	0.40
Protein g/100 mL	0.21	2.88	1.22	0.58
Lactose g/100 mL	3.54	8.46	7.01	0.59
Enterobacteriaceae (cfu/mL)	9.0×10^2	4.5×10^3	1.8×10^3	1.5×10^3
TVC (cfu/mL)	1.1×10^3	6.3×10^4	6.7×10^3	1.1×10^4
Staphylococcus (cfu/mL)	5.0×10^1	3.6×10^3	1.6×10^3	1.4×10^3
Yeasts and Moulds (cfu/mL)	4.2×10^2	1.7×10^4	4.9×10^3	5.6×10^3
Lactic acid bacteria (cfu/mL)	3.0×10^2	1.1×10^4	2.3×10^3	2.7×10^3
Somatic cells/mL	5.0×10^3	1.3×10^4	8.1×10^3	2.5×10^3

Table 2 Chemical composition of donkey milk in the different sampling areas (n = 90)

	Central Greece (n = 49)				*Cyprus* (n = 11)				*North Greece* (n = 30)				
Parameter	*Min*	*Max*	*Mean*	*SD*	*Min*	*Max*	*Mean*	*SD*	*Min*	*Max*	*Mean*	*SD*	*P*-value*
pH	6.68	7.30	7.07	0.16	7.03	7.60	7.30	0.15	6.71	7.35	7.14	0.13	<0.001
Fat g/100 mL	0.15	1.20	0.62	0.34	0.15	1.20	0.60	0.27	0.02	2.05	0.44	0.44	0.005
Protein g/100 mL	1.43	1.81	1.69	0.09	0.30	0.64	0.47	0.08	0.21	2.88	1.10	0.58	<0.001
Lactose g/100 mL	6.64	8.46	7.06	0.32	6.82	7.33	7.04	0.19	3.54	8.29	6.97	0.76	0.945

*Kruskal–Wallis test.

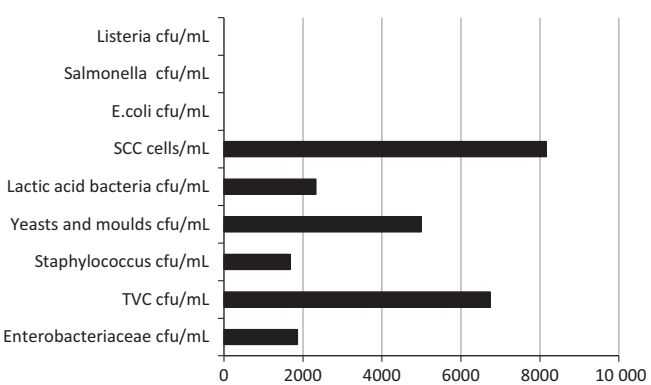

Figure 1 Donkey milk microbiological status (n = 41).

testing should be evaluated when milk is intended for human consumption.

Microbial flora

Microbiological analysis showed very low microbial counts (Table 1), while *Escherichia coli, Salmonella* spp and *Listeria monocytogenes* were not detected (Figure 1). Somatic cells were found to be low (8.1×10^3 cells/mL). These results are in agreement with other studies (Ivankovic *et al.* 2009; Pilla *et al.* 2010; Salimei 2011), implying the antimicrobial capacity that is present in DM (Zhang *et al.* 2008; Mao *et al.* 2009). Many consumers prefer raw DM because they believe it maintains all the beneficial attributes, and

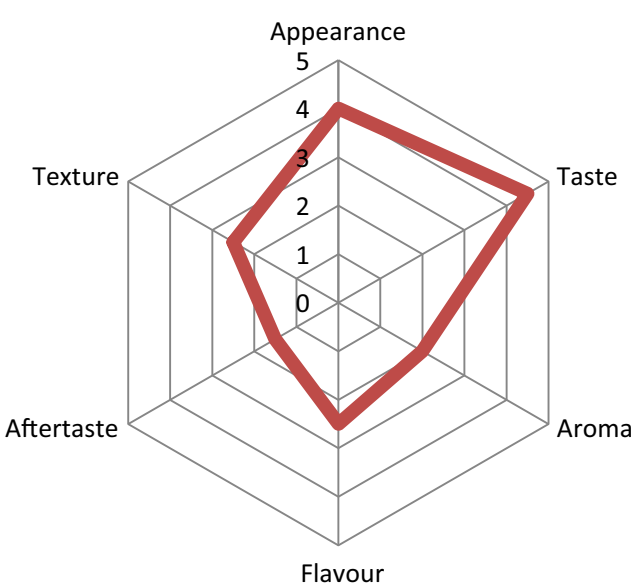

Figure 2 Donkey milk sensory profile using a 5-point hedonic scale (n = 30).

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thus, it is important to stress that several zoonotic agents (e.g. *Brucella*) have not been yet investigated in depth in DM. Hence, the Greek government recently announced a decision (314/15074-FEK 363, 17/2/2014) that requires DM sanitisation prior to consumption.

Sensory analysis

Panel members defined DM as white, thin, with a slight sweet pleasant taste, pleasant milky aroma, sweet flavour and no persistent aftertaste. The mean scores (Figure 2) obtained for the sensory attributes were similar among the assessed milk samples ($P > 0.05$). The sensory analysis conducted in this study confirmed consumer's perception that DM is a tasty food, as it has a slight sweet taste due to the high lactose content as reported by other researchers (Salimei 2011; Gubic *et al.* 2014).

To the best of our knowledge, this is the first study in Greece and Cyprus in relation to DM, which allows us to make an initial assessment and to reflect the potential for exploitation. Thus, there is need to systematically assess the impact of feeding regime on milk's quality and to investigate in depth donkeys' health profile.

CONCLUSION

The examined DM had similar composition to breast milk, had extremely low microbial counts and highly acceptable sensory characteristics scores. Given the interest of consumers and farmers in Greece and Cyprus for DM production, it seems that there are grounds for further research to investigate any possible health and nutritional effects it may have.

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