Casein composition and differential translational efficiency of casein transcripts in donkey's milk

Gianfranco Cosenza, Rosalba Mauriello, Giuseppina Garro, Barbara Auzino, Marco Iannaccone, Angela Costanzo, Lina Chianese and Alfredo Pauciullo

SUPPLEMENTARY FILE

Materials and methods

Chemicals

All chemicals were of the highest purity commercially available and were used without further purification. Hydrochloric acid, sulphuric acid, sodium hydroxide, boric acid, acetic acid, potassium sulphate were purchased from Carlo Erba (Milan, Italy) and trichloroacetic acid (TCA), was obtained from Aldrich (St. Louis, MO, USA).

The preparation of the nitrogen fractions

The nitrogen soluble fraction at pH 4.6 (SN) was determined after precipitation of the casein fraction (casein nitrogen, CN) with acetic acid/acetate buffer at pH 4.6. The CN content was calculated as a difference between the total nitrogen (TN) and SN. The non-protein nitrogen (NPN) was determined in the filtrate, obtained after precipitation of all proteins (caseins and whey proteins) by adding of TCA to 12% (w/v) final solution.

Sample preparation and conditions of the immunoelectrophoresis analysis

Individual donkey casein samples for electrophoretic analysis were dissolved in a 9 M urea solution (0.2 g L^{-1}), containing 2-mercaptoethanol (1 mL L^{-1}). Urea- PAGE at pH 8.6 was carried out with a vertical electrophoretic apparatus (Protean II, Bio-Rad, Richmond, CA 94804, USA) at 220 V and 10°C for 6 h. The gels used (0.75 mm thick, 180 x 140 mm) consisted of stacking gel containing 3.6 M urea, 7.5% (w/v) glycerol in 0.5 M Tris-HCl buffer at pH 6.8, layered on a running gel containing 6.1 M urea in 1.5 M Tris-HCl buffer at pH 8.6; tetra-methyl-ethylene-diamine and ammonium persulphate, at 0.04% (v/v) and 0.07% (w/v) final concentrations, respectively, were added to gels as polymerization agents. The electrode and migration buffers consisted of 0.19 M glycine and 0.024M Tris-HCl. Staining was performed with Coomassie Brilliant Blue R-250.

For immunoblotting analysis, Urea-PAGE profiles were transferred by capillary diffusion from the gel onto a nitrocellulose paper (0.45 µm, Trans - Blot, Bio-Rad).

Reverse-Transcription reaction mix, quantitative PCR amplification mix and thermal condition for donkey RNA analysis

1 µg of total RNA was processed for each 20 µl of Reverse-Transcription reaction using oligo-dT_{18} primer (15 mM) and Promega ImProm-II™ Reverse Transcriptase according to the Manufacturer's instruction (Promega Corporation, Madison, WI), and then it was dissolved in Nuclease-Free water (Promega Corporation). By means of Real Time quantitative PCR, the donkey *CSN1S1*, *CSN2*, *CSN1S2 I* and *CSN3* messengers were quantified using standard curves and the amount of each transcript occurring within each sample was expressed as relative to the amount of transcript measured for the single internal control (18S rRNA) used for normalization. This gene was chosen since it is expressed at the same level in the species, during the course of lactation.

The quantitative PCR amplification mixture (20 µl) contained 0.5 ng/µl single strand cDNA template, 10 µl of 2X QuantiTect SYBR Green PCR Master Mix (Qiagen) and 1.2 µl forward and reverse primers (5µM). The reaction was run (in triplicate) on an iCyclerIQ multicolor Real-Time PCR detection system (Bio-Rad). The thermal cycling condition was: 15 min polymerase activation at 95°C; 45 cycles at 94°C for 15s and 50°C for 30s. For the absolute quantification, the standard curves for each gene were obtained using serial dilutions (from 10 ngr to 0.01 ngr) of a pooled cDNA from the 8 samples a and the amplification efficiency was similar for all of them (data not shown). Primer sequences and amplicon size for *CSN1S1*, *CSN2*, *CSN1S2 I*, *CSN3* and 18S rRNA are reported in Supplementary Table S1. The sizes of the amplicons were confirmed by agarose gel electrophoresis (4%) (Biorad) in TBE 1× buffer and stained by ethidium bromide.

Supplementary Table S1. Primers used for Real-Time quantitative PCR. Each pair of primers amplifies the target cDNA (amplicon sizes ranging between 59-113 nucleotides).

Gene		Primers sequence (5' to 3')	Position, nucleotides	Amplicon size, bp	EMBL accession
CSN1S1	Forward	TTCCATTCTTGATTTTTCT	16384-16402	106	FN386610
	Reverse	AAGATTACCCTCAATTTG	Complementary to 16472-16489		
***CSN1S2* I**	Forward	GGCTGGAAAATCTATCTTCT	805-824	109	FM946022
	Reverse	GTCTTCATTTGGCTTGATGT	Complementary to 894-913		
CSN3	Forward	TAGGCCACATGTCCAAATTC	8822-8841	113	FR822990
	Reverse	GTTTCTTTGGAGGAATGGCA	Complementary to 8915-8934		
CSN2	Forward	TAAATTAATCTACATGCAAAAAT	8953-8975	90	FN598778
	Reverse	TATGAGTCAAATTCAAATTAAG	Complementary to 9021-9042		
18S rRNA	Forward	AGAAACGGCTACCACATCCAA	424-444	59	AJ311673
	Reverse	GGGTCGGGAGTGGGTAATTT	Complementary to 463-482		

Supplementary Table S2. Nitrogen fraction contents (total proteins, caseins, whey proteins, non-protein nitrogen and casein to whey proteins ratio (CN/WP), for donkey individual milk samples [a]

	Total proteins	**Caseins**		**Whey Proteins**		**Non-protein nitrogen**		**CN/WP**
	(g 100mL^{-1})	%	(mg mL^{-1})	%	(mg mL^{-1})	%	(mg mL^{-1})	%
Average	1.48 ± 0.21	34.61 ± 6.89	5.12	49.81 ± 7.50	7.37	15.55 ± 2.33	2.30	0.69

[a] Standard deviation is calculated on the average of three measurement repetition