61st Annual Meeting of the European Association for Animal Production
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IN VITRO DIGESTION OF DONKEY MILK WITH HUMAN GASTROINTESTINAL ENZYMES

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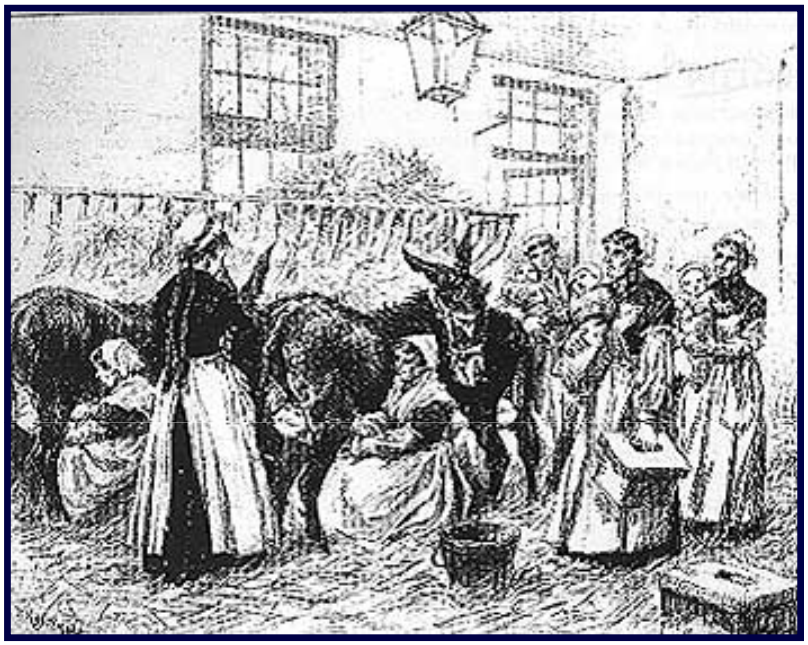

- **Donkey milk has good palatability and attractive nutritional and functional properties.**
- **Clinical studies proved it an adequate alternative for the dietotherapy of CMPA (Carroccio et al., 2000; Monti et al., 2007; Tesse et al., 2009).**
- **The good digestibility of donkey milk is commonly accepted, but so far no direct data are available.**

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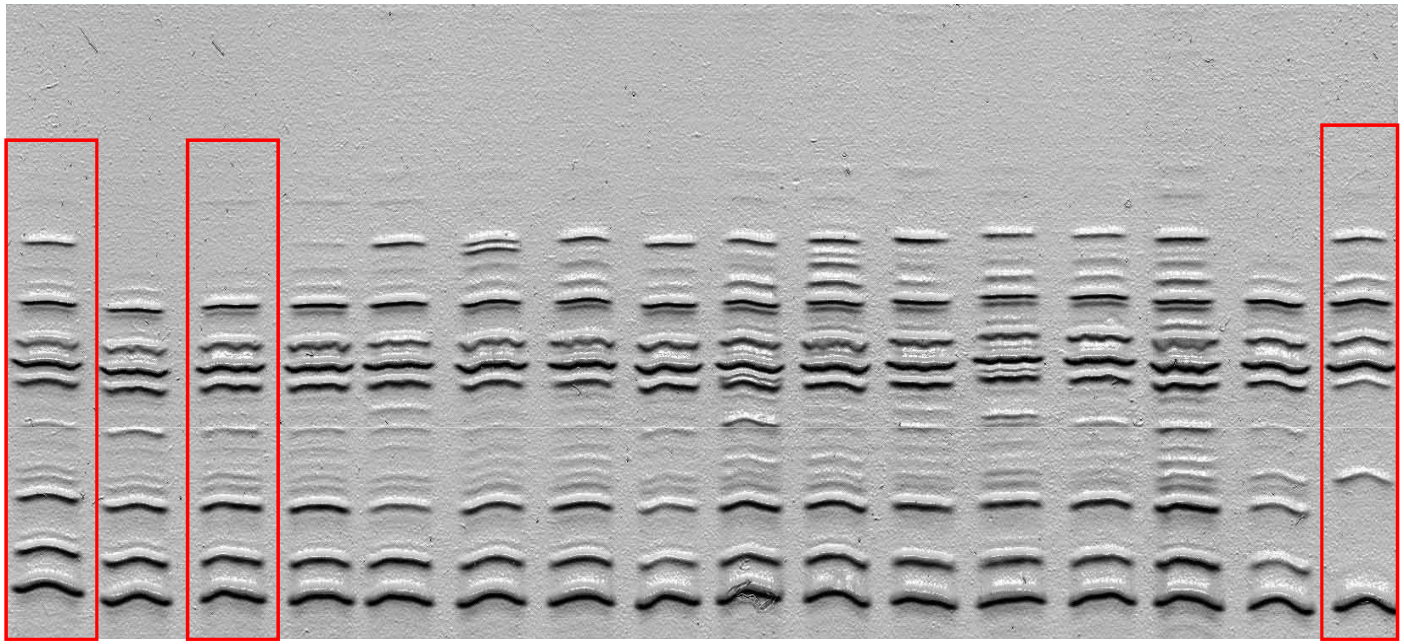

- **Individual variability in donkey's milk proteins, analyzed by isoelectrophoresis (IEF), SDS-PAGE and HPLC combined with mass spectrometry (Criscione et al., 2009) reported that might play a crucial role in its properties**

- **The different protein composition can influence the digestive utilization of milk nutrients (Malacarne et al., 2002).**

- **Individual milk samples from 14 multiparous Ragusano donkeys**

- **Qualitative protein analysis by isoelectric focusing (IEF) (Criscione et al., 2009).**

- **Distribution of nitrogen fractions by Kjeldahl analysis (IDF 1993).**

- **Size of the casein micelles by photon correlation spectroscopy (PCS) (Aabøe Inglingstad et al., 2010)**

In Vitro digestion with human proteolytic enzymes (Almaas *et al.*, 2006)

HCl **HGJ** NaOH **HDJ**

37° C

Milk
10 mL

Step 1:
pH 2 o pH 4
30 mins

Step 2:
pH 7.5
30 mins

Stop
on ice

Aliquot Aliquot Aliquot Aliquot Aliquot

- **HGJ and HDJ were collected from 6 healthy volunteers.**

- **The pepsin activity of HGJ and total proteolytic activity of HDJ were measured (Sanchez-Chiang et al., 1987; Krongedal and Holm, 1979).**

- **Each individual sample was run twice.**

- **The protein profiles have been visualized on SDS-PAGE gels (15% acrylamide for resolving gel and 4% for stacking gel).**

- **Image Quantity 1D software was used for the semi-quantitative analysis of the protein bands.**

- **Statistical analysis (ANOVA, General Linear Model) was performed to study the protein degradation during the two-steps digestion assay.**

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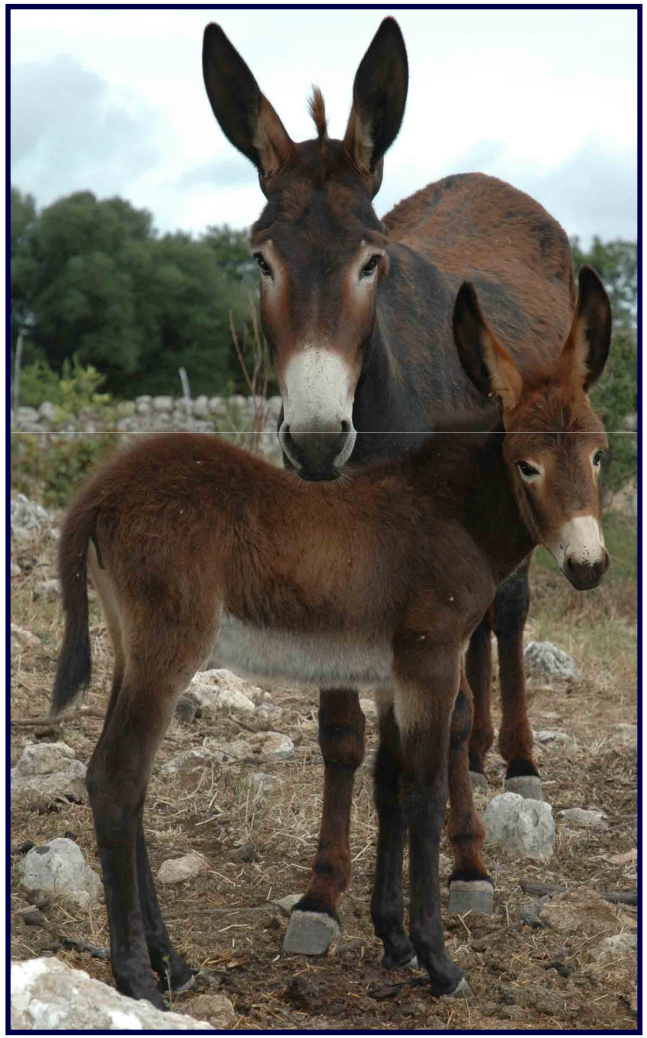
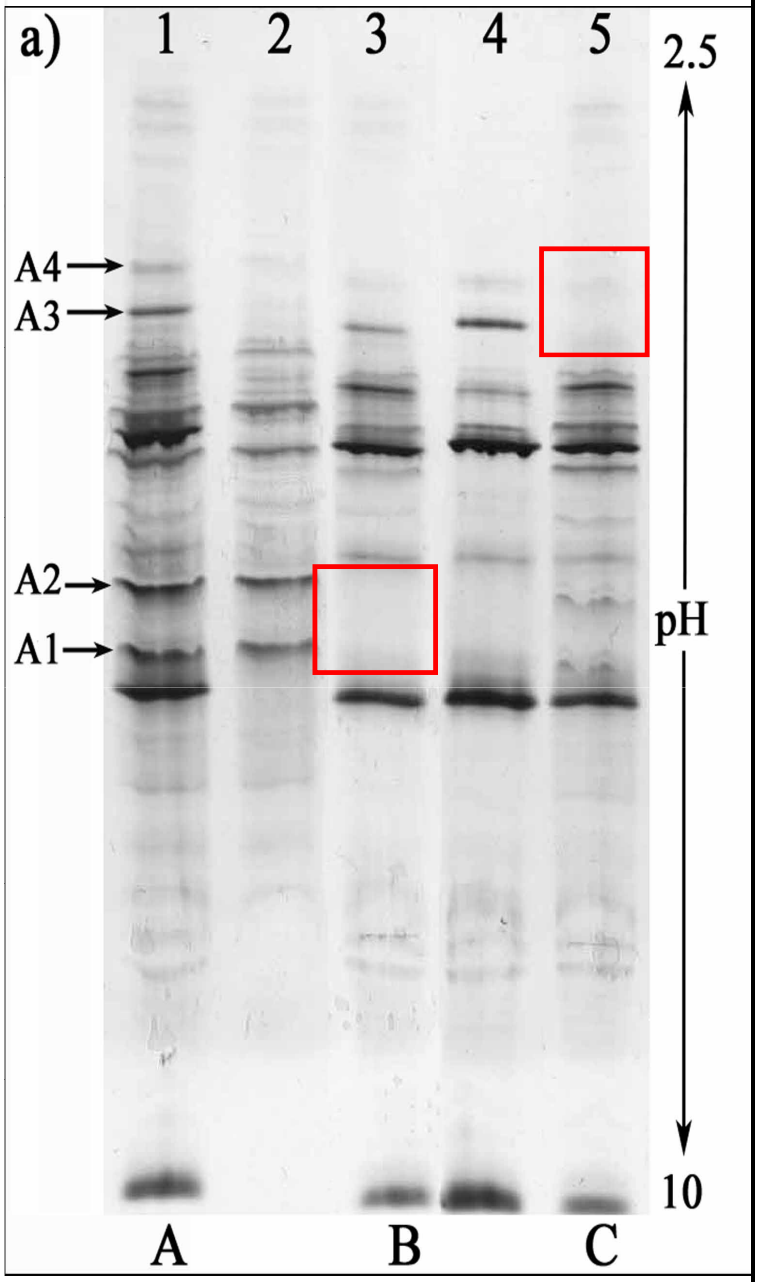


RESULTS



QUALITATIVE PROTEIN ANALYSIS

IEF protein patterns of the tested samples

a)

1: Reference pattern A (11)

2: *Casein fraction of pattern A*

3: Defective pattern B (1)

4: *Whey protein fraction of pattern A*

5: Defective pattern C (2)

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Table 1.
Nitrogen fraction contents and IEF pattern for each individual milk sample.

Sample	Tot Protein g/100g	Caseins g/100g	Whey P. g/100g	NPN g/100g	Cns / WP	Pattern IEF
1	1,83 ±0,01	0,98 ±0,01	0,61 ±0,01	0,24 ±0,02	1,61	A
2	1,44 ±0,01	0,74 ±0,01	0,43 ±0,01	0,27 ±0,02	1,70	C
3	1,54 ±0,01	0,42 ±0,02	0,85 ±0,01	0,27 ±0,02	0,49	B
4	1,81 ±0,01	0,96 ±0,02	0,58 ±0,02	0,26 ±0,01	1,65	A
5	1,73 ±0,01	0,79 ±0,02	0,66 ±0,02	0,27 ±0,01	1,19	A
6	1,40 ±0,01	0,60 ±0,01	0,57 ±0,01	0,22 ±0,01	1,05	A
7	1,74 ±0,01	0,83 ±0,01	0,67 ±0,01	0,24 ±0,01	1,24	A
8	1,38 ±0,01	0,65 ±0,01	0,47 ±0,01	0,27 ±0,01	1,38	A
9	1,49 ±0,01	0,65 ±0,01	0,60 ±0,01	0,23 ±0,02	1,07	A
10	1,31 ±0,02	0,57 ±0,09	0,52 ±0,10	0,22 ±0,02	1,08	A
11	1,35 ±0,01	0,63 ±0,06	0,47 ±0,06	0,24 ±0,01	1,33	A
12	1,52 ±0,03	0,72 ±0,02	0,59 ±0,02	0,22 ±0,01	1,21	A
13	1,54 ±0,04	0,75 ±0,01	0,55 ±0,01	0,25 ±0,01	1,37	A
14	1,43 ±0,01	0,69 ±0,03	0,50 ±0,03	0,25 ±0,02	1,38	C

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Casein micelles' size

146 ± 0.7 nm

Human

183,9±0.4 nm

Cow

220,4±2.8 nm

Goat

311, 5±1,1 nm

Horse

(Aaboe *et al.*, 2010)

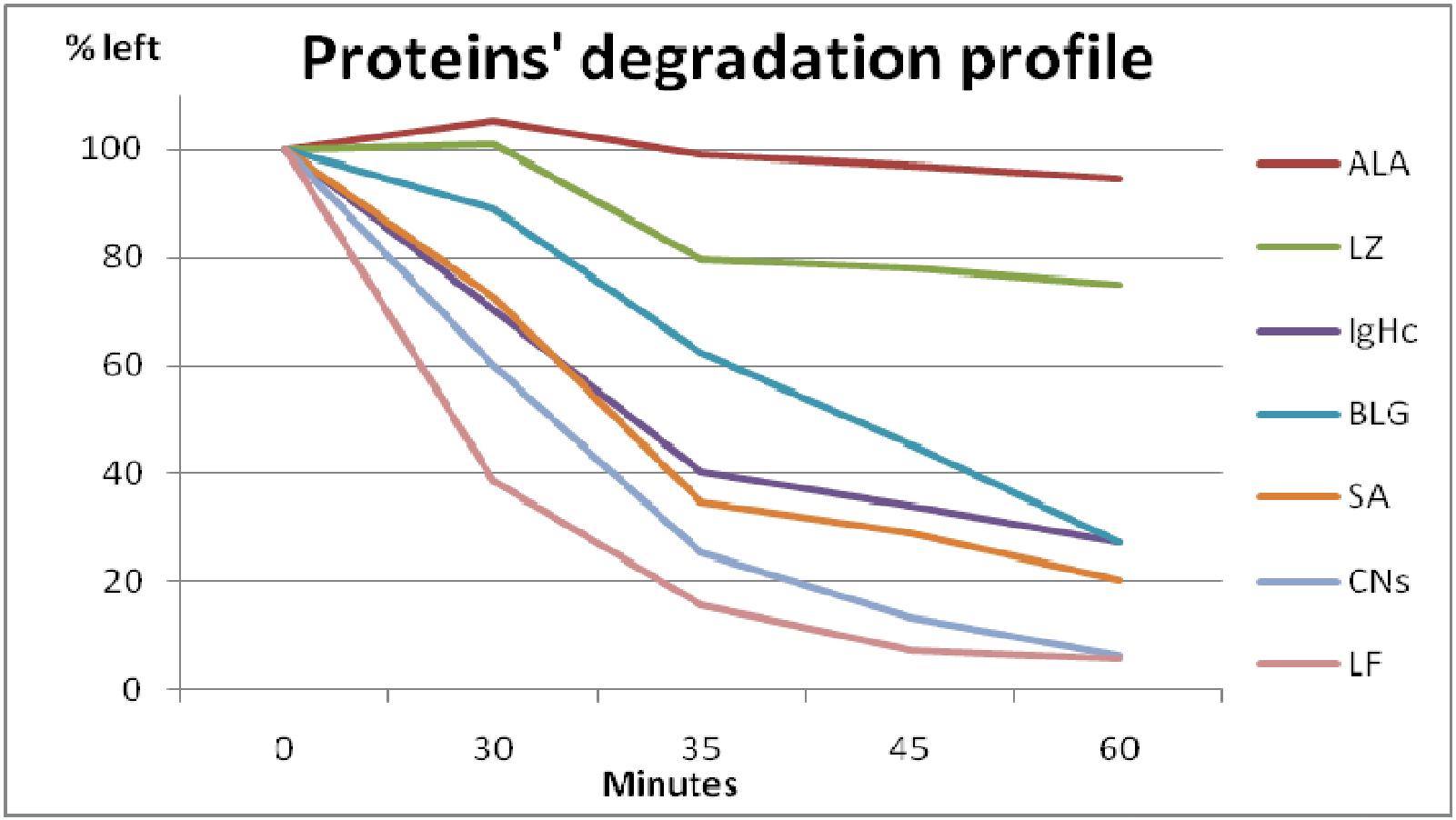
323.7 ± 4.9 nm

DONKEY

This work

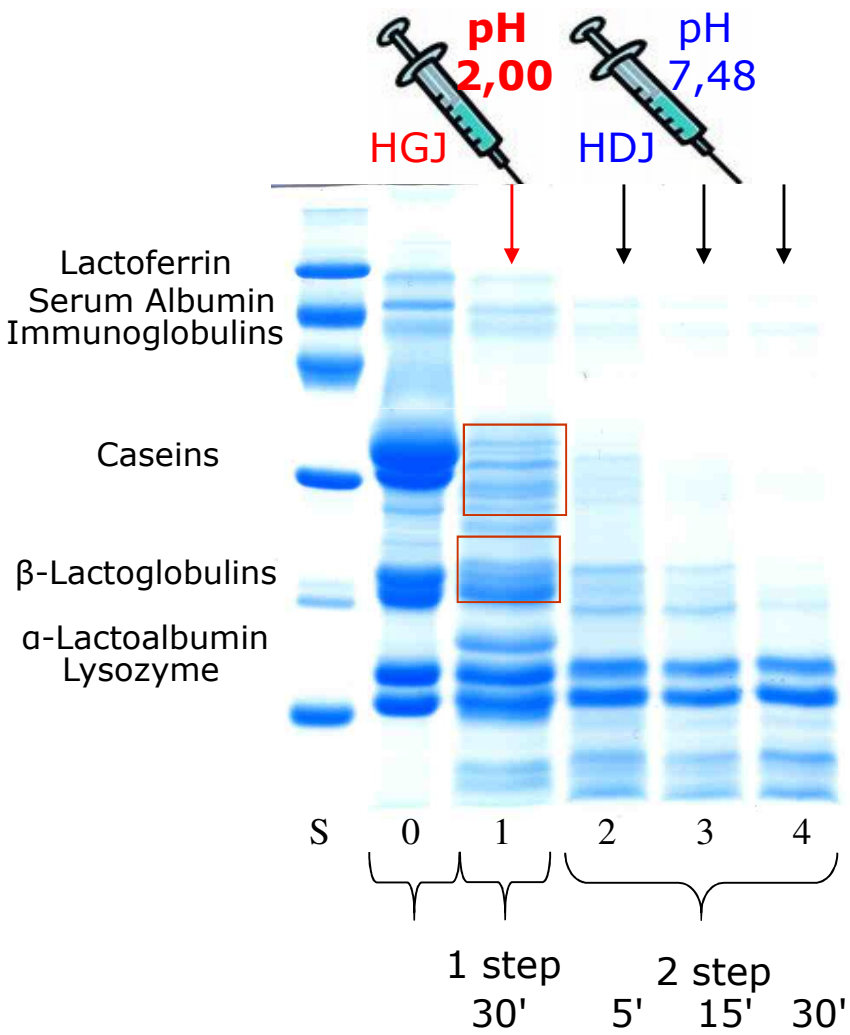
Table 2. Degradation degree of Total Proteins during digestion, from time 0 (100% of native proteins) to 60 minutes. Values show the average of relative proteins left undigested.

Time (min)	0	30	35	45	60
Undigested proteins (%)	100	76,96	50,53	40,71	32,62

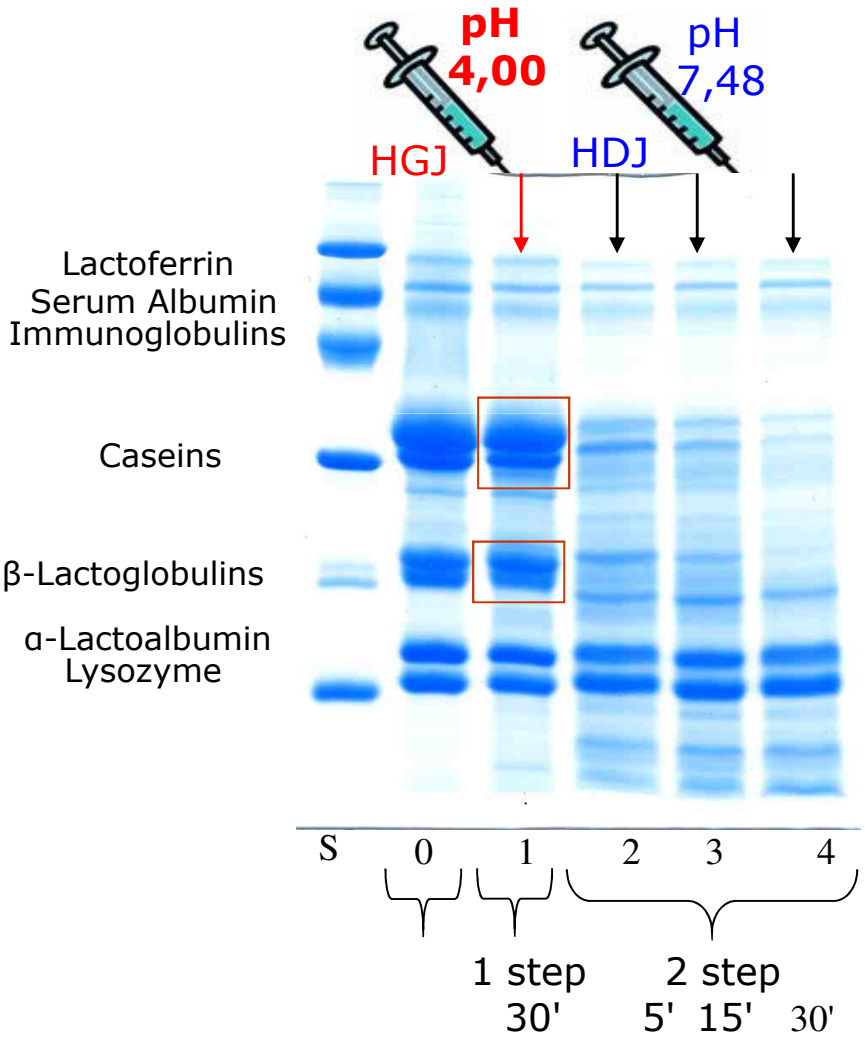


Digestibility of donkey milk proteins

Sample digested at the gastric **pH 2**
(stomach of adults)



Sample digested at the gastric **pH 4**
(stomach of infants)



Digeribilità delle proteine del latte di asina

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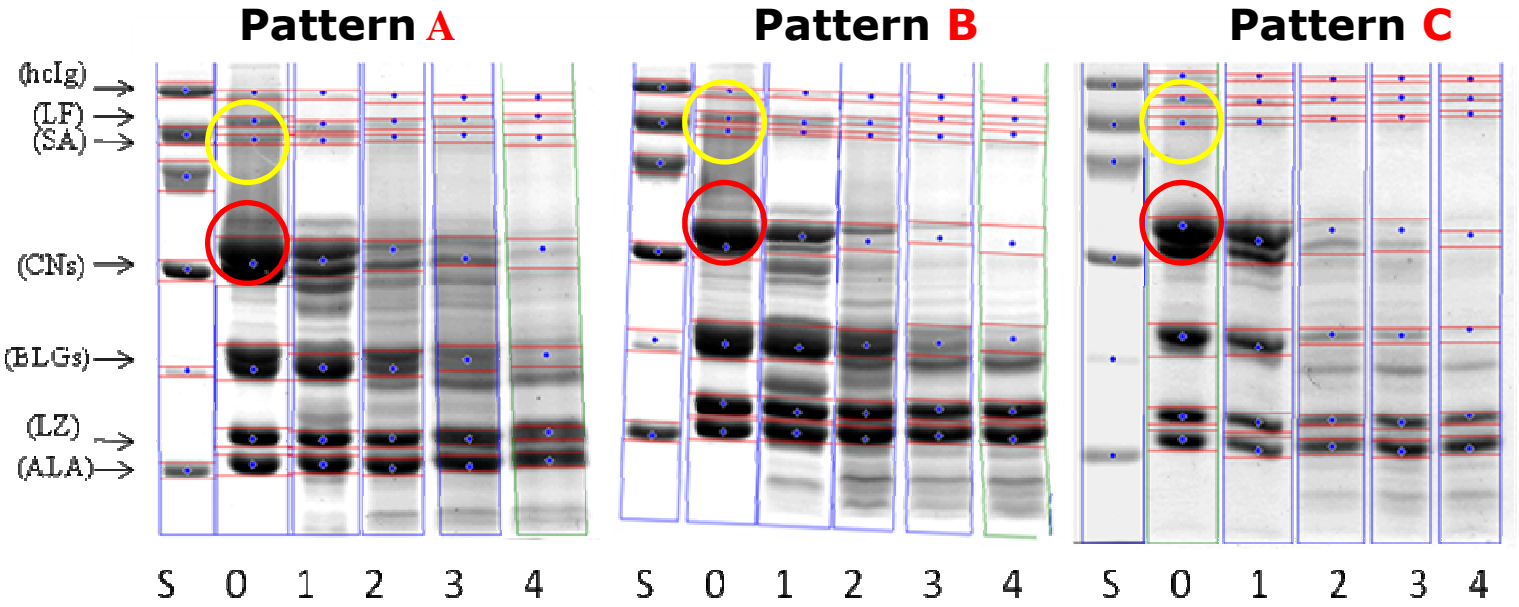

		Lactoferrin	Serum Albumin	Caseins	β-lacto globulins	Lysozyme	α-lacto albumin
Step 1	HG J pH2	38,5% *	72,8%	60,1% *	89,0% *	100,6%	105,2%
	HGJ pH 4	62,8% *	75,9%	89,5% *	95,3% *	96,5%	98,5%
Step 2	HDJ pH 2	5,7% *	22,6%	6,1%	30,5%	74,9%	94,7%
	HDJ pH 4	26,5% *	45,0%	8,2%	31,0%	84,9%	94,2%

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CONCLUSIONS

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Donkey milk showed a good digestibility in a two-steps degradation assay with HGJ and HDG.

The large size of the casein micelles seemed to facilitate enzymatic cleavage of this fraction even at gastric step. However in the duodenal step a more intense proteolic activity was observed.

The different gastric pH applied (pH 2.0 / pH 4.0) mainly affected the digestion of caseins and β-lactoglobulins.

The observed polymorphism (IEF patterns)

- **DETERMINED a different protein fractions distribution and a modified casein to whey protein ratio**

- **DID NOT SHOW any appreciable difference on the dimension of casein micelles**

- **AFFECTED digestibility of milk proteins only in the two defective samples lacking in β-lactoglobulin II, revealing a more rapid degradation especially in the gastric digestion.**

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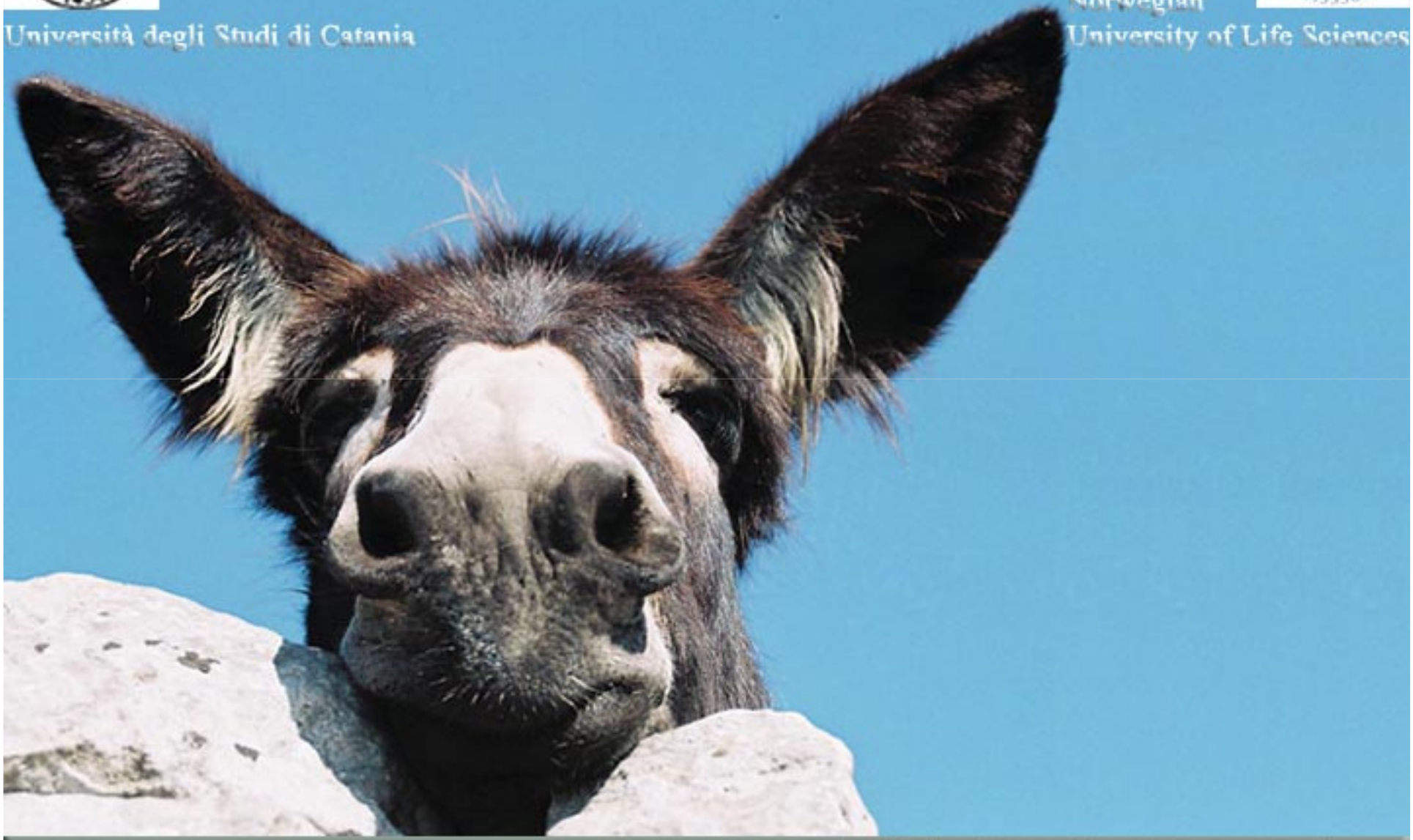

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