

ASS'S MILK IN ALLERGY TO COW'S MILK PROTEIN: A REVIEW

PASQUALE MANSUETO¹, GIUSEPPE IACONO², GIUSEPPE TAORMINA¹, AURELIO SEIDITA¹, ALBERTO D'ALCAMO¹, FLORIANA ADRAGNA¹, GIUSI RANDAZZO¹, MIRIAM CARTA¹, GIOVAMBATTISTA RINI¹, ANTONIO CARROCCIO³

¹Internal Medicine, University of Palermo, Italy - ²Pediatric Gastroenterology, "Di Cristina" Hospital, Palermo, Italy - ³Internal Medicine, Hospital of Sciacca, ASP Agrigento, Italy

[*Il latte d'asina nell'allergia alle proteine del latte vaccino: revisione della letteratura*]

ABSTRACT

Several studies has recently enlightened therapeutic use of ass' milk (AM) due to its special composition and nutritional properties, which are very close to human one. This feature makes ideal substitute of breast milk, whenever the mother cannot or will not breastfeed, or the child is intolerant to cow's milk (CM), providing the nutritional and health needs of the infant. The authors reviewed the literature about AM tolerance, safety and efficacy in the treatment of infants and children with a food allergy, i.e. CM protein allergy (CMPA). In all the reviewed studies, AM was well tolerated and acceptable, due to its palatability. Researchers enrolled children over 6 months of age, who did not have an exclusive milk diet, and/or had medium-chain triglycerides added. Overall data showed an adequate increase in auxological parameters measured after several months of AM consumption. Finally, potential cross-reactivity between AM protein and CM proteins must be considered. These results suggest that AM might be considered nutritionally adequate in infants and children with CMPA or multiple food allergies, included CMPA.

Key words: Ass' milk; donkey's milk; cow's milk protein allergy.

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Introduction

Discovery of ass' milk (AM) properties find its roots in antiquity, when doctors recommended it to treat several afflictions, due to its healing and cosmetic virtues. Even Hippocrates (460-370 BC) prescribed it for several purposes ranging from poisoning to fevers and infectious disease, from edema to wounds healing, from nose bleeds to liver troubles⁽¹⁾. According to tradition, Cleopatra, Queen of Ancient Egypt (69-30 BC), took baths in AM to preserve her beauty and softness of her skin; no less than 700 she-asses (daily milk production of a female ass or donkey fluctuate between 0.2 to 0.3 liters) were needed to provide the quantity of milk necessary for her daily bath. Similar baths were also performed by Poppaea Sabina (30-65 AD), Roman Emperor Nero's second wife⁽²⁾.

Possible use of AM was proposed by Pliny the Elder (23-79 AD), in his encyclopedic work, *Naturalis Historia*, to fight fever, fatigue, eye strain, weakened teeth, face wrinkles, poisonings, ulcerations, asthma and certain gynecological troubles (2). Benefits of AM were also reported by Georges-Louis Leclerc, Comte de Buffon (1707-1788), in his *Histoire Naturelle*⁽³⁾. First reports as possible substitute for breast milk were datable at the beginning of the 20th century. Dr. Charles Porcher (1872-1933) of the Lyon National Veterinary Institution testimony, in 1928, showed that the practice was still used, to a lesser extent, in the interwar years⁽⁴⁾. Nowadays AM is largely used in the manufacture of soaps and moisturizers, but new evidence show its possible medical use, especially to treat infants and children with cow's milk (CM) protein allergy (CMPA).

We reviewed international literature, using PubMed, and the searching terms "ass' milk", "donkey's milk" and "cow's milk protein allergy", highlighting how AM may be a valid nutritional support for patients affected with CMPA, and it is able to ensure adequate increase in auxological parameters, filling nutritional deficiencies of these subjects.

Biochemical properties of ass'milk

AM chemical composition and protein content, together with its low allergic potential, have made it, since a long time, a great replacement of breast milk. AM digestibility is better than CM and similar to human one, due to the high whey protein and the low casein content, so it may be used in infants and children suffering from CMPA. A Northwest China study, taken on by Guo et al., aimed to investigate the chemical composition, nitrogen fraction distribution, and amino acid (AA) profile of milk samples obtained during lactation from donkeys. The study pointed-out that AM contained 9.53% total solids, 1.57% protein, 1.16% fat and 6.33% lactose, and 0.4% ash; all these data makes it more similar to human and mare milk than to other mammals (Table 1). pH and density proved constant in all the samples collected during lactation, whereas protein and ash content displayed an apparent negative trend. Moreover, it has been proved that lactose content exhibited an increase during first 120 days postpartum, followed by a decrease. Fat content showed wide variability, whereas a small one was pointed-out to casein, whey protein and AA. A casein to whey protein ratio of 52:37 was evidenced, between the lower human milk and the higher CM value. AM proved rich in β -lactoglobulin and lysozyme at Sodium dodecyl sulfate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE). The percentage of 8 essential AA in AM protein was 38.2%, higher than those of cow and mare milk. Moreover AM had higher levels of serine (6.2%), glutamic acid (22.8%), arginine (4.6%), and valine (6.5%) and a lower level of cysteine (0.4%) (5-8). Lately an extensive proteomic study and a detailed comparative analysis among the protein fractions (i.e. casein and whey proteins) of AM, CM and human milk has been conducted in Italy. These studies reported detailed protein composition and structural features and explained molecular reasons of AM hypoallergenic quality. Analyzing AM allergenic properties with those of CM, it seems that the difference lies

in the significant differences between primary structures of proteins, which determine deep divergence between the amino acid sequences of IgE-binding linear epitopes of CM allergens and the corresponding domains present in donkey's milk proteins⁽⁹⁻¹⁴⁾. In regard to lipid fraction, AM has been indicated as a nutraceutical food due to some bioactive compounds, which are able to modify, directly or indirectly, the intestinal environment and immunity playing a role in the prevention and treatment of some pathologies. Chiofalo et al., analyzing AM triacylglycerol (TAG) composition, identified 72 TAGs, and examined similarities and differences among ass and human milk TAGs fraction; a partition number values from 30 to 50 was enlightened. Short-chain fatty acids (FAs) are not well represented in human milk while the PN values range between 36 and 52.

Furthermore $\omega 3$ and $\omega 6$ FAs amount in AM is larger than human milk ones, this last contains only significant amounts of $\omega 6$ FA (linoleic). AM high polyunsaturated fatty acids (PUFA) n-3 content, and especially its low n-6/n-3 ratio, acquires particular interest in subjects with CMPA. In addition, both donkey and human milk present the saturated FA preferably on the sn-2 position. Aforesaid, together with the relatively high content of medium-chain triglycerides, explain the increasing interest toward AM as an alternative food for a hypoallergenic diet in humans: as a matter of fact all this results in high bioavailability and digestibility of AM lipids, despite their low amount⁽¹⁵⁻¹⁸⁾.

A late study of La Torre et al. studied amines composition of 13 AM samples by high performance liquid chromatography atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS): 8 bioactive amines (histamine, tyramine, tryptamine, 2-phenylethylamine, cadaverine, putrescine, spermidine and spermine) were found: among these, putrescine, spermine and spermidine proved to be the most represented even if their concentration were lower than the respective found in mature human, cow and sow milk⁽¹⁹⁾.

Ass'milk and food hypersensitivity

One of the most frequent causes of poor absorption and growth retardation in unweaned children during the first months after birth is definitely food hypersensitivity. CMPA alone seems to affect 2-7.5% of the general population and its diagnostic incidence is increasing (1:200 versus

Composition of donkey's, mare's, human and cow's milk (g/100 g)				
<i>composition</i>	<i>donkey</i>	<i>mare</i>	<i>human</i>	<i>cow</i>
pH	7.0-7.2	7.18	7.0-7.5	6.6-6.8
Total Solids g/100 g	8.8-11.7	9.3-11.6	11.7-12.9	12.5-13.0
Protein g/100g	1.5-1.8	1.5-2.8	0.9-1.7	3.1-3.8
Fat g/100g	0.3-1.8	0.5-2.0	3.5-4.0	3.5-3.9
Lactose g/100g	5.8-7.4	5.8-7.0	6.3-7.0	4.4-4.9
Casein Nitrogen g/100 g	0.64-1.03	0.94-1.2	0.32-0.42	2.46-2.80
Whey protein g/100 g	0.49-0.80	0.74-0.91	0.68-0.83	0.55-0.70
Non-protein nitrogen g/100 g	0.18-0.41	0.17-0.35	0.26-0.32	0.1-0.19
Casein Nitrogen %	47.28	50	26.06	77.23
Whey protein %	36.96	38.79	53.52	17.54
Non-protein nitrogen %	15.76	11.21	20.42	5.23

Table 1: Composition of donkey's, mare's, human and cow's milk

From Guo HY, Pang K, Zhang XY, Zhao L, Chen SW, Dong ML, Ren FZ. Composition, physiochemical properties, nitrogen fraction distribution, and amino acid profile of donkey milk. *J Dairy Sci* 2007; 90: 1635-43 (modified).

1:7.500 recorded in 1948). Nevertheless, CM proteins are just some of the many possible allergens triggering food hypersensitivity reactions: rice, soy and hen's egg are the most reported ones. These rarer food hypersensitivities are reported to be more frequent in patients allergic to CM protein first, creating a multiple food hypersensitivity. Remarkable progress have been made in parenteral nutrition leading to substantial prognosis improvement; nevertheless the need to reintroduce alimentation per os as soon as possible is universally accepted, to stimulate the functional recovery of the damaged intestine. Breast-feeding seems the safest way to gain this goal, even if, obviously, human milk is not often readily available; other solutions are needed. On the other hand, severe hypersensitivity reactions might be evoked by formulas containing soy protein or by hydrolyzed protein ones. Consequently, multiple food hypersensitivity, including CMPA could be treated by the use of AM: this might offer an important and satisfactory solution. Its above reported composition and nutritional properties make it a valid alternative especially considering its ability to integrate fat intake in CMPA patients.

Infants seems to like it more than other substitute due to its high lactose concentration and, qualitatively, this is preferable to semi-elemental formulas, protein hydrolysates or soy formulas, which contain carbohydrates other than lactose. This high lactose content stimulates calcium intestinal absorption leading to better bone mineralization in the infant. Other point which makes AM a eligible substitute is the renal solutes load, mainly determined by the dietary amount of proteins and inorganic substances, which is substantially very similar in both breast-fed infants and AM fed ones. This represents a considerable advantage, considering the lower fat, and obviously caloric, content of AM compared to CM^(20,21). Considering the above mentioned, it could be concluded that, in areas where it is readily available, AM is certainly preferable to a lactose-free artificial dietary milk.

In 1992, Iacono et al., analyzed the clinical data of 9 patients with multiple food hypersensitivity, including CMPA, treated over the last 2 years, and initially re-fed exclusively with AM (Table 2). The patients who referred severe symptoms of CMPA did not improve their clinical condition, at

successive attempts using milk containing soy protein and/or a semi-elemental formula due to the onset of hypersensitivity also to these allergens. After a short period of parenteral alimentation, the infants were re-fed per os with AM (250 mL/kg/day) plus medium chain triglycerides (40 mL/L milk). All patients tolerated this kind of alimentation. No negative clinical reaction was recorded and during hospitalization average weight increase was 39.8 g/day. The follow-up of patients showed that AM was well tolerated up to an age ranging from 15 to 20 months⁽²²⁾. The same authors in another retrospective study (Table 2), evaluated the clinical characteristics and the long-term outcome of treatment with AM of patients affected with CMPA and/or multiple food hypersensitivity, including CMPA, and hydrolyzed protein (HP) intolerance, focusing their attention on its nutritional value. In the past, intolerance to HP formulas has been considered, a very rare event, but late reports hypothesized that it might not be so uncommon; however, very few data have been published about the natural history of CMPA subjects intolerant to HP milk formulas too. In the study the authors reported clinical characteristics and the follow-up (median period of about 4 years) of 21 CM- and casein hydrolysate (CH) formula-intolerant infants, treated with an AM-based diet, and, as controls, 70 CM-intolerant infants, treated with CH milk-based diet. Double-blind placebo-controlled challenge positivity have been considered the preliminary point to define CH formula-intolerance and intolerance to other foods. Formal CM-challenges were conducted at yearly intervals until tolerance was demonstrated. The study demonstrated that the patients intolerant to extensively CH formula had a more severe clinical framework than the patients successfully treated with this same formulas. CM-tolerance at the end of the study, after a median follow-up period of 4 years, was achieved by 52% of the CM- and CH-intolerant patients, whereas 78% of CH-tolerant patients became CM-tolerant at the end of the study. Furthermore, the median age CH-intolerant patients achieved CM-tolerance was significantly higher than the CH-tolerant ones. These data could be explained valuing the higher hyperactivity of CH-intolerant subjects, which seems to be confirmed by the higher frequency (100%) of a coexisting multiple food hypersensitivity (i.e. to soy, goat's and sheep's milk, soy, oranges, tomatoes and fish). Intolerance to extensively hydrolyzed protein seems to be just the epiphénoménon of an elevated

reactivity which is the basis of a more prolonged and severe food intolerance history. As a matter of fact high serum IgE levels can be detected in two-thirds of the CH-intolerant patients. In addition, the authors pointed out a higher frequency and more elevated levels of total serum IgE and specific IgE to CM antigens than in CH-intolerant patients than in their tolerant counterpart. Whenever treated with an AM diet, the subjects gained satisfying weight and height and, just in 1 year of CM-free diet, the more common blood nutritional parameters fall back to the normal range. Besides, the authors did not observe any difference in growth parameters during the follow-up period between the AM and the CH-milk treated group. This must be considered in the light of multiple food intolerance coexistence: in the CH-milk treated group only 14% patients showed multiple food intolerance, whereas all infants treated with AM suffered from this comorbidity; this greatly limited food choice. The study demonstrated that AM is a safe solution even in infants in whom hydrolyzed-milk formulas had failed. For the latter, L-aminoacid based formulas have been recently used and achieved good results, providing satisfactory growth recovery. Finally, but not of minor importance is the taste and the cost: hydrolyzed formula products have an unpleasant taste and are quite expensive, so the use of AM might be encouraged also in CMPA CH-tolerant subjects⁽²³⁾.

A fairly recent prospective study by Monti et al. (Table 2), investigated in vivo tolerance, palatability and nutritional adequacy of AM in a population of 46 infants and children with CMPA and other food allergies (mainly soy, wheat, egg and fish), for whom maternal milk was not available and no available CM substitute could be used. CMP elimination diet, followed by double-blind, placebo-controlled food challenge (DBPCFC) have been used to diagnose CMPA. Before food challenge, CM proteins skin prick tests (SPT) and RAST were performed too. An IgE-mediated CMPA was proved in 33 children which were CM protein SPT- and/or RAST-positive. The remaining 13 were classified as non-IgE-mediated CMPA. AM challenge proved positive in 8 children (17.4%), whereas the remaining 38 (82.6%) both liked and tolerated AM at the challenge and throughout the follow-up period. AM was tolerated by 26 (78.8%) of IgE-mediated CMPA children and by 13 of non-IgE-mediated CMPA ones. Catch-up growth (in terms of length/stature and weight and Z-scores for

Reference number in the text	Author	Year of Publication	Clinical presentation	Cases number	Age (month range)	Cow's milk hypersensitivity	Hydrolyzed formula hypersensitivity	Soy milk hypersensitivity	Goats' milk hypersensitivity	Ass' mil hypersensitivity	Auxological response after ass's milk treatment
22	Iacono G. et al.	1992	Diarrhea, abdominal pain, vomiting and growth retardation	9 unweaned infants with CMPA and other food allergies	0 - 3	9/9 (100%)	9/9 (100%)	9/9 (100%)	Not determined	0/9 (0%)	9/9 (100%)
23	Carroccio A. et al.	2000	Diarrhoea, abdominal pain, vomiting	Group A: 21 cow milk and hydrolyzed protein intolerant patients Group B: 70 cow milk intolerant patients Total: 91	2	Group A: 21/21 (100%) Group B: 20/70 (29%) Total: 41/91 (45%)	Group A: 21/21 (100%) Group B: 20/70 (29%) Total: 41/91 (45%)	Not determined	Not determined	Group A: 3/21 (14%) Group B: not determined	Group A: 18/21 (86%) Group B: not determined
26	Monti G. et al.	2007	Cutaneous symptoms, gastrointestinal symptoms and growth retardation	46 CMPA patients	1 - 146	46/46 (100%) 39/46 refused hydrolyzed formula	7/46 (15%) 35/46 (76%) 11/46 not determined	Not determined	8/46 (17%)	33/46 (72%) 8/46 (17%): not determined due to AM hypersensitivity 5/46 (11%): not determined due to follow-up drop-out	
27	Vita D. et al.	2007	Atopic dermatitis	28 CMPA patients	6 - 36	28/28 (100%)	Not determined	Not determined	23/26 (88%) Drop-out: 2	1/26 (4%) Drop-out: 2	Not determined, but improvement of atopic dermatitis
28	Tesse R. et al.	2009	Cutaneous symptoms, gastrointestinal symptoms and respiratory symptoms	25/25 CMPA patients	6 - 11	25/25 (100%)	Not determined	Not determined	Not determined	1/25 (4%)	24/25 (96%)

Table 2: Ass's milk in the treatment of cow's milk protein allergy and others food allergies.

AM: ass's milk

CM: cow's milk

CMPA: cow's milk protein allergy

length/stature and weight increases) was observed in all subjects characterized by growth deficit during CM protein challenge. No IgE crossreactivity versus AM proteins was enough strong or specific to be considered. Also Monti et al. identified AM as a valid alternative, both in terms of palatability and weight-height gain, in IgE-mediated and non-IgE-mediated CMPA⁽²⁴⁻²⁶⁾. Vita et al., in 2007 (Table 2), relying on the frequent association between CMPA and atopic dermatitis (AD), followed, in frequency, by urticaria/angioedema, gastrointestinal symptoms, wheezing and asthma, carried out a crossover randomized-controlled trial to objectively compare the tolerance of AM, with goat's milk (GM), used as the control. Their purpose was to analyze effect of AM based diet in the treatment of CMPA-related AD. As control diet GM was chosen because it is widely used as a CM substitute in clinical practice. Twenty-eight children suffering from CMPA and AD were enrolled in the study. Randomization was performed among patients to include in AM or GM diet group for 6 months, then they were switched to the other for further 3 months. The severity scoring of atopic dermatitis (SCORAD) index (SI) and a

visual analogue scale (VAS) were blindly evaluated. At the end of the study, food challenges with GM and AM were performed. Two children from the GM group dropped out after randomization and 26 completed the study. A significant improvement of SI and VAS symptoms ($p<0.03$ vs. baseline and inter-group) was always obtained by AM diet, whereas GM had no statistically significant clinical effects. At the end of the study 23 of 26 children had a positive food challenge with GM, while just one of 26 had with AM. The study proved that AM is tolerated by 88% of children with CMPA determining a significant improvement in AD. On the contrary, in all patients treated with GM, symptoms remained unchanged or even worsened. In particular, all children previously on AM diet had a relapse of AD after switching to GM. Extremely relevant, to our advice, is the sudden positivity to DBPCFC for GM, at the end of the study, in the most of the patient although none of them had been previously fed with GM-containing foods. We speculate that this may be due to the GM protein profile which is quite similar to CM, as confirmed by a SDS-PAGE analysis, performed on both kind of milk. Even if

this was not the first report documenting that AM could be an appropriate alternative to CM, this has surely been the first to demonstrate that AM is better tolerated than GM, which is still widely used. In conclusion, our revision results suggest that GM should not be used in children with CMPA and AD, whereas AM, if available, may be an effective and safe alternative⁽²⁷⁾.

Tesse et al., in 2009 (Table 2), evaluated 30 children with suspected CMPA. Skin prick tests, using fresh CM, AM, pear juice and other common food and aero-allergens, and DBPCFC to CM proteins were performed too. Patients, who were confirmed suffering from CMPA, received fresh AM in open challenge. Specific serum CM and AM protein IgE, as well as blood biochemical parameters were assessed. All subjects at entry and after 4-6 months of AM intake underwent to auxological evaluation (standing height, weight and BMI). Of the 30 subjects, 25 were considered suitable for the study, and 24 (96%) of this last tolerated AM at the food challenge. IgE-mediated CMPA was proved in 22 AM tolerant children, while 2 had non-IgE-mediated disease. AM was included in tolerant children diet balancing of age demand. Auxological data in all patients improved by the end of the study, while blood biochemical parameters did not vary during the follow-up. These data confirm a high rate of AM tolerance in children with moderate CMPA symptoms, and demonstrated that AM seems to be nutritionally adequate in subjects on a relatively free diet⁽²⁸⁾. Lately Pilla et al. examined 101 half-udder AM samples determining hygienic and health characteristics through somatic cell count (SCC), bacteriological analysis and total bacteria count (TBC). Antimicrobial susceptibility was tested in all the major pathogens, and *Staphylococcus aureus* isolates were further genotyped by nanoarray analysis. Whey lysozyme and N-acetyl-β-D-glucosaminidase (NAGase) activities were also assessed, they showed very low TBC (<250 CFU/ml) and SCC (<50.000 cells/ml) values and a minor prevalence of pathogens: *Staphylococcus aureus* was only isolated from 5 milk samples (3 animals), *Streptococcus equi* from 2 samples and *Streptococcus equisimilis* from a single sample. No resistance against the classes of antibiotics of veterinary use could be proved. None of the *Staphylococcus aureus* isolates was found positive to harbor genes coding for any enterotoxin, toxic-shock syndrome toxin, or antibiotic resistance. Lysozyme levels were always very high

(4.000-5.000 U/ml), while, during the last part of lactation, NAG values were quite low (<50 U/ml). This study confirmed the low prevalence of intra-mammary infections in donkeys and the absence of food-borne pathogens, suggesting AM safeness profile, if the ass mammary gland is healthy and the animals are milked in proper hygienic conditions⁽²⁹⁾. In contrast to the above mentioned study, Conte et al., in 2008, described, in strains from 50 samples of AM in Sicily, isolation of two *Enterobacter sakazakii* (ES). Isolates revealed a multiple resistance profile, including fluoroquinolones, commonly used to treat animal infections. In 2002, the International Commission for Microbiological Specifications for Foods (ICMFS) ranked ES as a 'severe hazard for restricted populations, life threatening or substantial chronic sequelae of long duration'. ES ('yellow pigmented *Enterobacter cloacae*') has been found among the common foodborne pathogens, such as *Listeria monocytogenes*, etc. The genus *Enterobacter* was associated with the phytic flora and it was supposed that the principal environmental sources of ES are water, soil and vegetables, and a secondary contamination media may be vectors such as flies and rodents; nevertheless the organism is considered ubiquitous. Birth canal ES contamination or post-birth environmental sources might be responsible of neonatal infections.

Moreover, several neonatal meningitis cases may have some relationship with the most common newborn gastrointestinal disease associated with bacterial pathogens: necrotizing enterocolitis. Neonatal pathologies also include bacteraemia, wound exudates, appendicitis, and conjunctivitis; in adults the organism usually causes bacteraemia. A possible cause of infection and illness, including severe disease which can lead to serious sequelae and death, in infants can be due to intrinsic ES and *Salmonella* contamination of powdered infant formula. No link has been established between illness and other microorganisms in powdered formula, although such a link was considered plausible for other *Enterobacteria*. This is the first report of ES from AM and their recovery is noteworthy, especially because infants consume raw milk. Even this is a topic of great importance, the uncertainty about ES infectious dose and its antimicrobial susceptibility profile should be adequately valued. A full risk assessment of the organism will require greater knowledge of its presence in food, especially the ones used to feed neonates and infants⁽³⁰⁾.

Conclusions

As a result of careful analysis of literature, is beyond doubt the AM usefulness as substitute for breast milk in infants suffering from CMPA or multiple food allergies included CMPA. The authors have performed studies confirming the high tolerance of this product by the patients either with the IgE- and non-IgE-mediated CMPA. Another important factor is certainly its palatability, which make it more appetizing than hydrolyzed formulas. Despite AM protein composition is similar to human milk, is a low-calorie food. For this reason, in some studies the researchers enrolled children older than 6 months, who did not have an exclusive milk diet, and/or added medium chain triglycerides to the diet. Overall data showed an adequate increase in auxological parameters (i.e. weight, length/stature and Body Mass Index, BMI), measured after several months of AM administration. It is possible to argue that the effect of AM on growth is related to its ability to fill some nutritional gaps present in the diet of treated subjects. Moreover, during and after AM administration period, patients' biochemical and metabolic blood parameters did not vary. Nevertheless, all the studies suggest that a longer follow-up is needed in order to achieve reliable results. Finally, AM proteins potential cross-reactivity with CM proteins must be considered, suggested by the above mentioned studies that sometimes reported severe reactions to AM in their study cohorts. However, taken together, all these results suggest that AM might be considered nutritionally adequate in children with CMPA or multiple food allergies included CMPA. Another critical point which should be stressed, is lack of easy AM availability even in countries where donkeys are very common, turning its costs higher than CM and hydrolyzed formulas and making this food, especially for poorer patient families, difficult to access. For this, further studies would be needed to confirm AM usefulness in filling the nutritional gap of CMPA patients and make it considered among medicaments approved by public health.

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Request reprints from:

Prof. PASQUALE MANSUETO
Dipartimento di Medicina Interna e Specialistica
Azienda Ospedaliera Universitaria Policlinico 'P. Giaccone'
Via del Vespro, 141
90127 Palermo
(Italy)