Journal of Dairy Research (1988), **55**, 57–66 *Printed in Great Britain*

Interspecies variation in milk composition among horses, zebras and asses (*Perissodactyla: Equidae*)

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(*Received 31 December 1986 and accepted for publication 29 June 1987*)

SUMMARY. Milk samples of four species of wild equids (onager, *Equus hemionus onager*; mountain zebra, *E. zebra hartmannae*; plains zebra, *E. burchelli*; Przewalski horse, *E. caballus przewalskii*) and two domesticated equids (ass, *E. asinus*; pony, *E. caballus*) were analysed. At mid to late lactation the milks of all species were very similar, containing on average 10–12 % total solids, 1–2 % fat, 1·6–1·8 % true protein, 6–7 % 'lactose', 0·3–0·5 % ash, 0·08–0·12 % calcium, 0·04–0·07 % phosphorus and a calculated energy content of 2·0–2·4 kJ/g. Milk samples collected in the first 2 weeks after birth showed elevated levels of total solids and protein, and some had reduced 'lactose' levels, but there were no observable trends in milk composition during mid to late lactation (1–12 months post partum). It was concluded that these closely related species produce milks that are nearly identical in gross composition and that the domestic horse is a representative model for the study of equid lactation.

Milks from different species differ greatly in composition, although species in the same taxonomic order tend to produce milks of somewhat similar composition (Jenness & Sloan, 1970; Oftedal, 1984). Comparisons of closely related species are hindered by the fact that most species hitherto investigated are represented by only one or a few samples of milk, and rarely are these samples from equivalent lactation stages. Observed differences among species may stem as much from sampling error or effects of lactation stage as from true interspecies variation.

Species of the family Equidae (horses, zebras and asses) are a case in point. Although much research has been devoted to milk composition in the domestic horse (Linton, 1931, 1937; Neseni *et al.* 1958; Oftedal *et al.* 1983; Schryver *et al.* 1986*a*), relatively little is known about other species. Human consumption of ass milk has stimulated some interest in this domestic species (Wagner, 1908; Jackson & Rothera, 1914; Anantakrishnan, 1941; Gonzalez-Diaz & Cravioto, 1947), but the few reports on wild equids refer to only one or two milk samples per species (Masek, 1939; Ben Shaul, 1962; King, 1965; Linzell & King, 1966; Jenness & Sloan, 1970). Available data suggest that the milks of asses, zebras and wild horses are similar to domestic horse milk in being low in total solids (TS) (8–10 %) and protein (1–2 %) but high in 'lactose' (6–8 %). By contrast the reported levels of fat range from 0·6 % in ass milk

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(Anantakrishnan, 1941) to 4·7 % in plains zebra milk (King, 1965; Linzell & King, 1966). King (1965) concluded that different equids should be fed formulas differing in fat content when foals are bottle-fed in zoos.

The equids are a relatively homogeneous group of moderately large, cursorial grazers adapted to grassland and semi-arid conditions (Groves & Willoughby, 1981; Berger, 1986). As all belong to a single genus (*Equus*), they represent an opportunity to test the hypothesis that closely related species produce milks of very similar composition. In this paper we examine the gross composition and Ca and P contents of milk from five of the seven extant equid species (Corbet & Hill, 1980), including two species for which milk composition has not previously been reported, the mountain zebra (*Equus zebra*) and the onager or Asiatic wild ass (*E. hemionus*). Milk analyses are also provided for the Przewalski horse, a wild Asian horse that is sometimes accorded full specific rank as *E. przewalskii* (Honacki *et al.* 1982).

METHODS

Milk samples

Equid milk samples ($n = 51$) were obtained from zoos (San Diego Wild Animal Park, Escondido, CA; National Zoological Park, Washington, DC; Como Zoo, St. Paul, MN), from a feral population of asses in California, and from a privately owned domestic ass, a Welsh pony and a Shetland pony (Table 1). Most samples were collected by manual expression from animals that had been chemically immobilized for hoof trimming or medical examination. Two to nine mares were milked per species (Table 1). For the Przewalski horse and mountain zebra, repeated samples were collected from individuals during different lactations, but for one of the ponies repeated samples were collected during the same lactation. Samples averaged 91 ml (range 8–500 ml); as oxytocin was not used in most cases, they probably did not represent entire mammary contents. Samples were frozen in air-tight vials immediately after collection, but a delay in freezing some samples from feral asses may have permitted fermentation to occur before freezing. All samples remained frozen until thawed for analysis.

Thawed samples were thoroughly mixed before subsampling for analysis. As analyses were conducted in several laboratories over a number of years, methods of analysis differed among samples, as noted within parentheses in the following:

Total solids. The samples were dried to constant weight in a vacuum oven at 70 °C (one plains zebra, all ass and all pony milks) or for 5 h in a forced convection oven at 100 °C (all other samples).

Fat. Fat was determined using the Babcock volumetric method (Horwitz, 1980; one plains zebra, one ass and some pony milks) or by extraction with petroleum and diethyl ether using the Roese-Gottlieb method (Horwitz, 1980; all other samples).

Total nitrogen (TN). This was determined by digestion with H_2SO_4 and H_2O_2 followed by direct Nesslerization (Belec & Jenness, 1962; some ass and some pony milks) or by micro-Kjeldahl digestion with SeOCl but without $HClO_4$ (Pepkowitz & Shive, 1942) and distillation into H_3BO_3 (Ma & Zuazaga, 1942; one plains zebra, some ass and some pony milks), or macro-Kjeldahl digestion with $CuSO_4$ and distillation into H_3BO_3 (Horwitz, 1980; all other samples).

Non-protein nitrogen (NPN). Samples were analysed by the Kjeldahl procedure or by Nesslerization (as above) of filtrate after protein precipitation in a solution of 12 % trichloroacetic acid.

Table 1. *Sampling information for equid milks*

Species	n/N*	Lactation stage† (d post partum)	Source
Ass (*Equus asinus*)	9/9	35, 62, 120, 180(3) unknown(3)	California (feral) Private farm (domestic)
Onager (*E. hemionus onager*)	3/3	0, 1, unknown	San Diego WAP‡, National Zoo
Mountain zebra (*E. zebra hartmannae*)	10/5	5, 12, 85, 93, 109, 123, 149, 241, 336, 371	San Diego WAP
Plains zebra (*E. burchelli*)	5/5	90, 151, 157, 199, 248	San Diego WAP, National Zoo, Como Zoo
Przewalski horse (*E. caballus przewalskii*)	16/5	6, 93, 112, 119, 127, 175 197, 233, 265, 268(2), 281, 315, 337, 352, 651	San Diego WAP
Pony§ (*E. caballus*)	8/2	25, 28, 64, 76, 93, 106, 116, 180	Private farms

* No. of samples/no. of mares.
† Numbers in parentheses refer to no. of samples collected at a given lactation stage
‡ San Diego Wild Animal Park.
§ Welsh and Shetland breeds.

'*Lactose*'. Samples were analysed by the picric acid method (Perry & Doan, 1950; one plains zebra, some ass and some pony milks) or the phenol-sulphuric acid method (Marier & Boulet, 1959; all other samples). Results are expressed as 'lactose', signifying the anhydrous lactose equivalent of the observed reducing power or chromogenic power; lactose monohydrate (reagent grade) was used in preparation of standard curves.

Ash. Samples were incinerated in a muffle furnace overnight at 600–650 °C.

Calcium. Ca was determined by EDTA titration of milk ash solution (Jenness, 1953; one plains zebra, all ass and pony milks) or by atomic absorption spectroscopy in the presence of lanthanum chloride (all other samples).

Phosphorus. The colorimetric method of Sumner (1944; one plains zebra, all ass and pony milks) or the procedure involving $SnCl_2$–hydrazine reduction of phosphomolybdic acid (Schryver *et al.* 1986*a*; all other samples) was used.

Sodium. Na was determined by flame emission spectroscopy.

Crude protein and true protein were calculated as $TN \times 6{\cdot}38$ and $(TN - NPN) \times 6{\cdot}38$ respectively. Sample volume did not allow NPN determination in 16 samples. For these samples, species mean values for NPN were used in calculation of true protein content. Gross energy (GE) was calculated by adaptation to kJ (1 kcal = 4·184 kJ) of a formula developed by Perrin (1958):

$$GE\,(kJ/g) = [(38{\cdot}1 \times \text{fat}) + (24{\cdot}5 \times \text{true protein}) + (16{\cdot}5 \times \text{'lactose'}) + (31{\cdot}0 \times NPN)] \div 100.$$

For purposes of comparison, the lactation stage of each sample has been calculated to the nearest post partum (PP) month, with a month assumed to be of 30 d duration. Actual collection dates are listed in Table 1. Data for one sample each of ass and plains zebra milk have been reported in an earlier review (Jenness & Sloan, 1970). Mineral assays for most milk samples from onager, mountain zebra, plains zebra and Przewalski horse have been reported by Schryver *et al.* (1986*b*); Ca and P values are included herein for comparison with additional data on asses and ponies. Interspecies differences were tested by one-way analysis of variance; samples were assumed to be independent observations since all samples except some pony milks were collected from different mares or during different lactations.

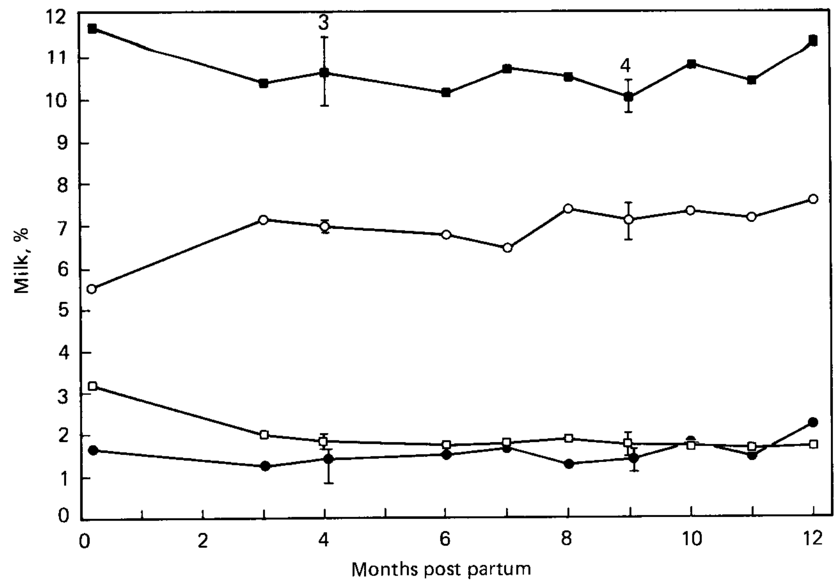

Fig. 1. Composition of Przewalski horse milk in relation to lactation stage. Total solids, ■; fat, ●; protein, □; 'lactose', ○. Vertical bars indicate ±1 s.d. if more than one sample was analysed at a given lactation stage; no. of samples is indicated above bar for total solids.

RESULTS

One sample of onager milk was available for which lactation stage could not be determined. This sample contained 12·0% TS, 3·67% fat, 2·75% crude protein, 6·52% 'lactose', 3·2 kJ/g GE, 0·35% ash, 0·037% Ca and 0·32% P. In comparison to other equids (see below) Ca and P levels were unusually low while fat and crude protein levels were atypically high, indicating that this sample may not have been representative. A sample of Przewalski horse milk collected 651 d PP and a sample of mountain zebra milk collected 336 d PP had low 'lactose' values (3·4% and 3·0% respectively) and high Na levels (0·16 and 0·17% respectively) indicating mammary involution. Normal Na levels for these species are 0·01–0·03% (Schryver *et al.* 1986*b*). These three samples were considered anomalous and excluded from data analyses.

Milk samples spanning a full 1-year lactation period were available for Przewalski horse and mountain zebra (Figs 1 and 2). It appears that samples obtained in the first 2 weeks PP ($n = 3$) were somewhat higher in TS and crude protein content than subsequent samples; one of these samples was also rather low in 'lactose' content (Table 2). These trends were even more pronounced in two samples of onager colostrum which contained very high TS and crude protein levels, but only about 2% 'lactose' (Table 2).

Over the period of mid to late lactation (3–12 months PP) milk samples showed some variability in gross composition, but there were no significant trends in TS, fat, crude protein, 'lactose' or ash content in either the Przewalski horse or the mountain zebra (Figs 1 and 2), regression coefficients not being significantly different from zero at 0·05 level). Smaller series of milk samples from the ass (2–6 months PP; $n = 6$), plains zebra (3–9 months PP; $n = 5$) and pony (1–6 months PP; $n = 8$) also did not exhibit any consistent change in gross composition over the period of mid to late lactation. NPN content did not appear to be influenced by lactation stage in any equid species.

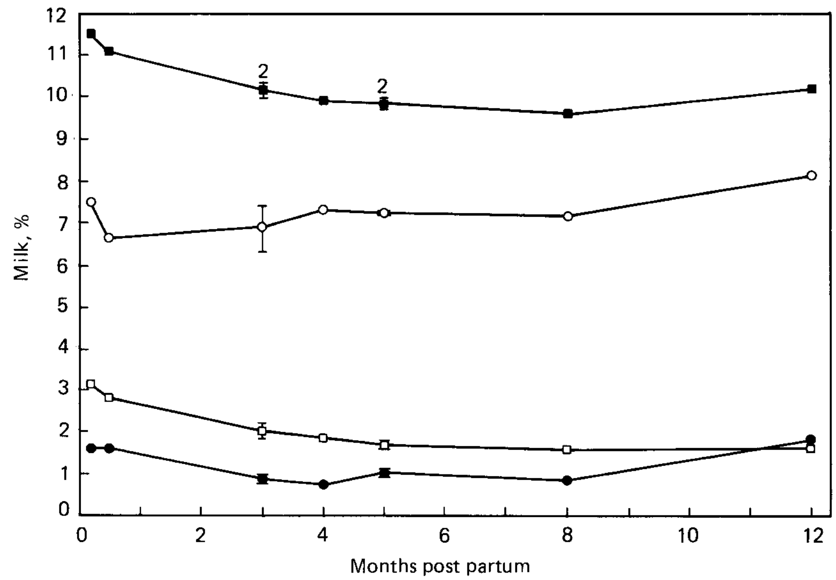

Fig. 2. Composition of mountain zebra milk in relation to lactation stage. Symbols as in Fig. 1.

Table 2. *Composition of colostrum and transitional milk**

Species	Stage, d post partum	Total solids, %	Fat, %	Crude protein, %	'Lactose', %	Ash, %	Ca, %	P, %	Gross energy, kJ/g
Onager	0	21·7	1·38	16·52	1·96	—	—	—	4·9
	1	31·8	2·28	25·32	1·84	0·78	0·132	0·068	6·9
Przewalski horse	6	11·6	1·65	3·18	5·23	0·64	0·151	0·087	2·3
Mountain zebra	5	11·5	1·65	3·18	7·14	0·56	0·117	0·085	2·6
	12	11·1	1·65	2·86	6·31	0·52	0·119	0·086	2·4

* Each result is for a single sample.

Table 3. *Composition of equid milks at mid to late lactation*

Species, months post partum	No. of samples		Dry matter, %	Fat, %	True protein, %	'Lactose', %	Ash, %	Ca, %	P, %	Gross energy, kJ/g
Ass (1–6)	9*	$\bar{x}$	10·8	1·82	1·74	5·87	0·44	0·115	0·073	2·1
		s.d.	1·83	1·41	0·645	0·894	0·124	0·0388	0·0236	0·70
Mountain zebra (3–12)	7†	$\bar{x}$	10·0	1·02	1·56	6·92	0·32	0·084	0·055	2·0
		s.d.	0·23	0·368	0·217	0·448	0·034	0·0166	0·0067	0·18
Plains zebra (3–8)	5‡	$\bar{x}$	11·3	2·20	1·63	7·00	0·38	0·075	0·053	2·4
		s.d.	1·17	1·066	0·465	0·878	0·088	0·0132	0·0089	0·49
Przewalski horse (3–12)	14§	$\bar{x}$	10·5	1·50	1·55	6·72	0·33	0·082	0·043	2·1
		s.d.	0·53	0·385	0·171	0·374	0·048	0·0152	0·0064	0·16
Pony (1–6)	8‖	$\bar{x}$	10·4	1·46	1·82	6·74	0·47	0·084	0·053	2·2
		s.d.	1·02	1·098	0·205	0·513	0·163	0·0150	0·0090	0·46

* Ca and P on eight samples. † Ash, Ca and P on six samples. ‡ Ash, Ca and P on three samples. § 'Lactose' and gross energy on 13 samples. ‖ 'Lactose' and gross energy on seven samples; ash, Ca and P on six samples.

Table 4. *Distribution of N in equid milks*

Species, months post partum	No. of samples	Total N, %	Non-protein N, %	Non-protein N, as % of Total N, %
Ass (1–6)	9 $\bar{x}$	0·324	0·051	16·5
	s.d.	0·1124	0·0155	4·51
Mountain zebra (5–11)	3 $\bar{x}$	0·254	0·040	15·8
	s.d.	0·0133	0·0088	3·61
Plains zebra (3–7)	1 $\bar{x}$	0·259	0·043	16·6
Przewalski horse (3–12)	14 $\bar{x}$	0·279	0·036	13·1
	s.d.	0·0279	0·0031	1·36
Pony (1–6)	8 $\bar{x}$	0·331	0·046	13·9
	s.d.	0·0349	0·0114	2·94

Given the relative constancy in gross composition of equid milks in mid to late lactation, mean milk composition values were calculated for each species for this period (Table 3). Milk samples collected in the first 2 weeks PP have been excluded, as have samples obtained at the cessation of lactation (as indicated by 'lactose' levels < 4%). Three samples of ass milk for which lactation stage was not known were included because the analytical values were similar to samples known to be mid to late lactation in this species.

A remarkable similarity was found in gross composition of the milks of the ass, mountain zebra, plains zebra, Przewalski horse and pony (Table 3). The apparent variation in TS, from a mean of 10·0% in the mountain zebra to 11·3% in the plains zebra, reflected a similar variation in fat (1·0–2·2%), but interspecies differences in these constituents were not significant ($P > 0{\cdot}05$; Table 3). In all species the calculated SNF were virtually identical: 8·9% in the pony, 9·0% in the ass, mountain zebra and Przewalski horse, and 9·1% in the plains zebra. Mean values for true protein (range: 1·6–1·8%) also did not differ significantly ($P > 0{\cdot}05$; Table 3). Although 'lactose' content exhibited a significant species effect ($P < 0{\cdot}05$), this could be attributed to the lower 'lactose' level measured in ass milk. Six of the nine ass samples were suspected to have undergone fermentation; if these are excluded, the mean 'lactose' value for ass milk ($6{\cdot}90 \pm 0{\cdot}70$, $n = 3$) is similar to the other equid species (6·7–7·0). The sum of fat, true protein, 'lactose' and ash was equivalent to 96–101% of TS in all species, including the ass (if the suspect 'lactose' values are excluded). Calculated GE values were about 2·0–2·4 kJ/g for all species ($P > 0{\cdot}05$).

Although ash, Ca and P levels varied significantly among species ($P < 0{\cdot}05$), most of the species means were quite similar (Table 3). Ass milk appeared to contain somewhat more Ca and P than the other equid milks. All equid species produced milk containing about 0·04–0·05% NPN, equivalent to 13–17% of TN (Table 4).

DISCUSSION

The milk composition data reported herein confirm that equids in mid to late lactation produce a relatively dilute milk that is low in TS (10–11%), fat (1–2%) and protein (1·6–1·8%), but high in 'lactose' (6–7%). Among mammals, only the rhinoceroses are known to produce milks that are consistently lower in TS, fat and GE than the values reported herein for equids (Jenness & Sloan, 1970; Oftedal, 1984). The low energy content of equid milks implies that lactating mares must secrete large volumes of milk in order to supply the energy needs of the young (Oftedal *et al.* 1983; Oftedal, 1985).

Compositional data for the five species of domesticated and captive wild equids (Table 3) may be compared to reported values for domestic horses. In 12 studies of horse milk at mid lactation, mean values fell in the following ranges: TS, 9·9–11·2 %; fat, 0·7–2·0 %; true protein, 1·8–2·3 %; 'lactose', 6·0–7·0 % (Dittrich, 1938; Intrieri & Minieri, 1970; Antila *et al.* 1971; Oftedal *et al.* 1983 and seven studies cited therein; Pagan & Hintz, 1986). Since Shetland and Welsh ponies are breeds of small domestic horses, it is not surprising that gross composition values for pony milk fall within the range of domestic horse data. However data for the other four equids are also very similar. All are slightly lower in true protein (1·5–1·7 % *v.* 1·8–2·3 %), and plains zebra milk is slightly higher in fat (2·2 %), but these differences are minor. At mid to late lactation it appears that the various equid species produce milk very similar in gross composition to that of the domestic horse.

Earlier reports on non-domestic equids are restricted to analyses of two samples of Przewalski horse milk (Masek, 1939), a sample of 'zebra milk' (Ben Shaul, 1962), a sample of Grevy's zebra (*E. grevyi*) milk (King, 1965; Linzell & King, 1966) and a sample of plains zebra milk (King, 1965; Linzell & King, 1966). Of these, only the Przewalski horse milk analyses are similar to values in Table 3, namely 10·5 % TS, 2·2 % fat, 2·0 % crude protein, 6·1 % 'lactose' and 0·35 % ash. The zebra milks were all low in 'lactose' (3·8–5·8 %) and either very low (0·8 %) or very high (4·7–4·8 %) in fat (Ben Shaul, 1962; King, 1965; Linzell & King, 1966). The unidentified 'zebra' sample of Ben Shaul (1962) contained 3·0 % protein. Thus these samples appear to be atypical. The single samples of ass milk and plains zebra milk referred to by Jenness & Sloan (1970) are included in Table 3 and will not be discussed separately.

The fat content of ass milk has been reported to be as low as 0·6 % (Anantakrishnan, 1941) and as high as 2·5 % (Gonzales-Diaz & Cravioto, 1947). Some of the variation may be attributed to lactation stage. Wagner (1908) reported that ass milk contained 8·1 % fat on the day of birth, 2·7 % at 1–2 weeks PP, and 1·6 % at 3–5 weeks. Yet the mean value of 1·8 % fat obtained herein for 1–6 six months PP is considerably higher than the 0·6 % reported by Anantakrishnan (1941) for ass milk at 3–4 months PP. The reasons for this discrepancy are not clear. In domestic horses, incomplete mammary evacuation may cause underestimation of milk fat content (Doreau *et al.* 1986). The extent to which this error may have influenced the results of Anantakrishnan (1941) cannot be evaluated since sampling methods or volumes of milk obtained are not described. The ass milk samples obtained in the present study were only 15–30 ml and probably did not represent complete evacuation. If so, the expected bias would be towards low rather than high fat content. It is possible that some fat values reported in Table 3 are underestimates, but none approaches the 0·6 % reported by Anantakrishnan (1941).

The relatively low mean 'lactose' content of 5·9 % for ass milk (Table 3) is similar to the 6·1 % reported by Anantakrishnan (1941) and Gonzales-Diaz & Cravioto (1947), but distinctly lower than reports of 7·2 % and 7·4 % by Wagner (1908) and Jackson & Rothera (1914). It is also lower than estimates of the 'lactose' contents of the milks of other equids (Table 3). Three of the nine ass milk samples, known to have been preserved very carefully, averaged 6·9 % ± 0·70 'lactose' whereas six samples that may have had opportunity for fermentation before analysis averaged 5·35 % ± 0·35. We consider it likely that normal ass milk in mid to late lactation contains close to 7 % 'lactose'. The effect of earlier fermentation on analyses of other constituents would probably not be great.

NPN content (0·036–0·051 %) accounted for a fairly large proportion of TN (13–17 %) in the equids studied (Table 4), as it does in domestic horses (Neseni *et al.*

1958; Oftedal *et al.* 1983). Use of TN assays to calculate crude protein overestimated milk protein content by 0·2–0·3 g/100 g milk in both domestic and wild equids.

In the present study, samples of equid colostrum and early lactation milk contained more TS and protein than milk obtained later in lactation (Tables 2, 3). A similar decline in TS and protein in early lactation occurs in domestic horses: the first 3 weeks PP constitute a transitional period between colostrum and mature, mid lactation milk (Oftedal *et al.* 1983). In the subsequent period (1–12 months PP), the equid species studied herein showed no evidence of substantial change in milk composition (Figs 1 and 2). In domestic horses, TS and fat have been variously reported to increase, decrease or remain unchanged in late lactation, while protein declines only slightly if at all (Oftedal *et al.* 1983). The relative constancy of milk composition over mid to late lactation in equids may indicate that milk yields remain relatively high into late lactation (Bouwman & Van der Schee, 1978; Oftedal, 1985). In cervids and bovids, declining yields in late lactation are accompanied by increases in TS and fat, and a decline in 'lactose' content (Oftedal, 1984, 1985).

Ash, Ca and P contents were relatively similar among equid milks at mid to late lactation, although ass milk appeared to be higher in Ca and P than the milks of other species (Table 3). Ash, Ca and P levels in domestic horse milk decline over the first 2–3 months of lactation (Schryver *et al.* 1986*a*), but by 3–4 months PP the levels of ash (0·3–0·4 %), Ca (0·07–0·08 %) and P (0·054–0·055 %) are similar to those found for ponies, Przewalski horses and the two zebra species (Table 3). Linzell & King (1966) found 0·10 and 0·07 % Ca in single samples of plains zebra milk (1 month PP) and Grevy's zebra milk (3 months PP) respectively. The relatively high Ca and P values of ass milk (Table 3) may reflect inclusion of milks from the first few months PP (Table 1; three samples were of unknown lactation stage). Anantakrishnan (1941) reported total Ca and P levels of 0·081 and 0·058 % in ass milk collected 3–4 months PP. Disparities among equid milks in levels of a number of major and trace minerals indicate that a larger number of milk samples need to be analysed to determine the extent of interspecies variation (Schryver *et al.* 1986*b*).

In conclusion, equid species secrete milks that are very similar with respect to TS, fat, true protein, NPN, 'lactose', ash, Ca and P. Earlier comparisons of equid milks have been misleading owing to the small numbers of samples, differences among samples in lactation stage, and possible sampling errors. Lactation in the domestic horse appears to be representative of other equid species. Milk formulas designed for feeding of domestic foals are probably appropriate for foals of other equid species given the close similarities in milk composition among equids.

We are particularly indebted to Don Janssen DVM and James Oosterhuis DVM of the San Diego Wild Animal Park for making available series of milks from Przewalski horses and Hartmann's mountain zebra, as well as samples of onager and plains zebra milk. We also thank: Mitchell Bush DVM and veterinary staff at the National Zoological Park for obtaining samples of onager and plains zebra milk; the staff of the Como Zoo, St. Paul, MN for a sample from plains zebra; Charles Douglas, University of Nevada at Las Vegas for specimens of feral ass milk; P. Gough and P. Bliss for pony milks.

Milk analyses were performed by Andrea Slosberg, Nutrition Laboratory, National Zoological Park; Claire Kotts, Dawn Buzzell and Michael Barone, Department of Biochemistry, University of Minnesota; and Janice Williams, Equine Research Program, Cornell University. We thank Dr Susan Crissey for assisting with statistical analyses, Dr Daryl Boness for comments on the manuscript and Friends of the National Zoo for providing a research traineeship to Andrea Slosberg.

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