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ORIGINAL ARTICLE

Predicted metabolizable energy density and amino acid profile of the crop contents of free-living scarlet macaw chicks (*Ara macao*)

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Summary

Hand rearing of neonates is a common practice for the propagation of psittacines. However, nutritional requirements for their growth and development are not well understood, and malnutrition is common. We analysed the amino acid (AA) profile of the crop contents of 19 free-living scarlet macaw (*Ara macao*) chicks, 19–59 days old. Predicted metabolizable energy (PME) density was 16.9 MJ/kg DM and true protein (total AA protein) 8.3 g/MJ PME. Crude protein (CP) was 10.0 g/MJ PME, lower than the requirements of 0- to 12-week-old leghorn chicks but not different than the requirements of growing budgerigars (*Melopsittacus undulatus*) and lovebirds (*Agapornis spp.*). The mean concentrations of leucine, isoleucine, threonine, lysine (Lys) and methionine on a PME basis were below the minimum requirements of 0- to 12–week-old leghorn-type chicks. The calculated PME density of the samples did not vary with age. However, there was a significant negative correlation between the average age of the chicks and the Lys concentration. We conclude that the lower CP and AA densities compared with poultry could result from a combination of (i) differences in the essential AA composition of the body tissues, (ii) adaptations that allow the birds to grow on low-protein food sources and (iii) suboptimal nutrition of these free-ranging chicks.

Introduction

Despite the increasing volume of research on the nutritional requirements of psittacines (Roudybush and Grau, 1991; Koutsos et al., 2001; Pryor et al., 2001), malnutrition is still a major concern for the care and propagation of this group (Koutsos et al., 2001; Petzinger et al., 2010). Studies of the diet and nutrition of free-ranging psittacines are scarce (Snyder et al., 1987; Gnam, 1991; Crowley and Garnett, 2001; Carciofi, 2002; McDonald, 2003; Cottam et al., 2006; Ndithia and Perrin, 2006; Renton, 2006), in part, because the birds' extensive food manipulation and processing make it very difficult to gather quantitative food intake data.

Hand rearing of neonates is a common practice for the propagation of psittacines, both for the pet industry (Low, 1980) and for conservation aviculture (Jones, 1998; Brightsmith et al., 2005; Groffen et al., 2008). However, the nutritional requirements for the growth and development of neonates are not yet well understood (Roudybush and Grau, 1991; Taylor et al., 1994; Koutsos et al., 2001; Wolf and Kamphues, 2003), and imbalances are still common (Koutsos et al., 2001). Only a few studies have looked at the nutritional content of diets consumed by parent-fed neonatal psittacines (Cottam et al., 2006; Brightsmith et al., 2010), and the diversity of food habits and ecology among psittacines makes it tenuous to extrapolate from these limited studies to requirements for the group as a whole.

In growing birds, dietary protein is used for tissue accretion and maintenance. The amino acid (AA) balance needed for growth closely mirrors the AA composition in tissues (Klasing, 1998). The tissue AA composition of different species is relatively similar, so the difference in AA requirements is mainly driven by the different fractional growth rates (Klasing, 1998). AA requirements have been studied extensively in commercial fowl (National Research Council, 1994; Baker et al., 2002; Baker, 2009). However, psittacines have received limited attention with most studies focused on adult maintenance requirements (Earle and Clarke, 1991; Taylor et al., 1994; Koutsos et al., 2001; Pryor et al., 2001), and only a few data are available on growth requirements (Roudybush and Grau, 1991; Taylor et al., 1994; Koutsos et al., 2001; Wolf and Kamphues, 2003). In the absence of controlled studies of requirements or comprehensive data on the diets of wild birds, nutritional prescriptions for psittacine growth and maintenance are generally extrapolated from dietary recommendations for poultry (Koutsos et al., 2001). However, psittacines are not closely related to poultry and differ both developmentally (O'Connor, 1984) and ecologically (Collar, 1997), so it is questionable whether the available data adequately model their dietary requirements.

The metabolizable energy (ME) densities of diets is the primary factor that determines the amount of food an animal will consume (Klasing, 1998). Expressing nutrient concentrations on a per energy basis allows for more meaningful comparison among diets even when the ingested amounts are not known (Klasing, 1998). Brightsmith et al. (2010) studied the nutritional content of 30 crop samples of scarlet macaw chicks 28–60 days post-hatch at the same study site. Crude protein (CP), crude fat and mineral concentration were reported, but neither the AA profile nor the ME.

The present study provides the first estimates of the ME density and AA profile of free-ranging Neotropical parrot chicks. The objectives are (i) to characterize the AA profile and ME concentration of the crop content of wild scarlet macaw (*Ara macao*) chicks and (ii) to compare the AA and ME levels of the crop contents with nutritional information from other psittacines and the domestic chicken.

Materials and methods

Crop samples

We collected crop contents from free-living scarlet macaw chicks 19–59 days post-hatch from Tambopata Research Center in the lowland forests of southeastern Peru (13°07′ S, 69°36′ W; 250 m elevation). In this region, parrots and macaws consume a diverse mixture of seeds, fruit, flowers, tree bark and soil from river edge 'clay licks' (Brightsmith et al., 2008). Once every 7–10 days, crop contents were collected from chicks in nests of wild macaws following the procedures described by Enkerlin-Hoeflich et al. (1999). Samples were placed in refrigeration at 4 °C within 30 min of collection. In the 2006 breeding season, a total of 38 samples were collected from 10 chicks (mean dry weight per sample 2.4 ± 2.3 g). During the 2008 breeding season, a total of 18 samples were obtained from nine chicks (mean dry weight per sample 1.2 ± 1.1 g). All sampled chicks appeared in good health and fledged at appropriate ages for the species (Vigo Trauco, 2007). Owing to the small quantity of each sample, we pooled samples for analysis. For 2006, a total of 15 composite samples were created by combining samples from chicks in the same nest collected on the same day or from chicks of similar age. The 2008, samples were scanned with a near infrared reflectance spectroscope [Perten DA 7200 IR; Perten Instruments AB, Sweden, (more details in Cornejo et al., in preparation)], and pooling was done according to the similarity of their spectra.

Chemical analysis

Samples were freeze-dried and ground. The crude nutrients were analysed at the Palmer Research Center at the University of Alaska. N was determined by the Kjeldahl method, crude fat was calculated using the ether extraction method (Randall, 1974), neutral detergent fiber (NDF) was calculated by Van Soest's detergent analysis system (Goering and Van Soest, 1970), and ash by high-temperature ashing (Association of Official Analytical Chemists, 1996). True protein was determined as the total AA concentration (Aurand et al., 1987) and CP by multiplying total N by a 6.25 factor (Pellet and Young, 1980). Soluble carbohydrates were calculated by difference following the formula: % soluble carbohydrates = 100 − % CP − % crude fat − % ash − % NDF. True protein, CP and AA concentration are presented as g/MJ predicted metabolizable energy (PME) for comparison among diets and species. PME values of the crop contents of the scarlet macaws and the kakapos, as well as the diet of Leghorn chickens, budgerigars (*Melopsittacus undulatus*) and lovebirds (*Agapornis spp*.), were calculated using the formula PME (kJ/100 g DM) = (18.4 × CP) + (36.4 × crude fat) + (16.7 × soluble carbohydrates) (National Research Council, 1994; Harper, 2000). Average true and CP level are also presented as % DM.

Macaws at the study site feed soil to their chicks (Brightsmith et al., 2010), but the amount of soil varied among samples (Brightsmith et al., 2010), and the soil may have led to inflated NDF values because of filtration issues (Randall, 1974). To calculate PME using the equation above, we corrected the NDF values for each sample by subtracting the estimated amount of soil ash from the original NDF value. The percentage soil ash in each sample was estimated as the total ash minus the average ash content from samples known to contain no soil (6.2 ± 1.4%, N = 8). We took into consideration that the average ash content of 6.2% is similar to the average ash for natural foods consumed by scarlet macaws in Peru (5.7 ± 3.4%, N = 17, Brightsmith, unpublished observation).

Complete AA analysis was performed in the AA Laboratory of the Department of Molecular Biosciences, School of Veterinary Medicine, UC Davis. The majority of the essential AAs were analysed using the Association of Official Analytical Chemists (AOAC) modified method 994.12 (Association of Official Analytical Chemists, 1996). Protein hydrolysate was prepared by treating 10 mg of finely ground sample with 2.0 ml of 6 N HCl in a 10-ml evacuated ampule (Wheaton Prescored Gold-Brand, Fisher Scientific Cat No. 12-009-38, Wheaton Industries Inc., Millville, NJ, USA) for 24 h at 110 °C. After flash evaporation using nitrogen gas, the dried residue was dissolved in Biocrom loading buffer. Aliquots were analysed by ion-exchange chromatography using an LKB Biochrom 30 automatic AA analyzer. Methionine (Met) and cystine (Cys) were analysed separately (AOAC Method 994.12). After performing acid oxidation and subsequent hydrolysis with 6 N HCl, Cys and Met were determined by measuring cysteic acid and methionine sulfone using a Biochrom 30 AA analyzer. Tryptophan (Trp) was determined by AOAC method 988.15. After alkali (LiOH) hydrolysis, the quantification was performed using the Biochrom AA analyzer. As part of the QA/QC procedure, 1000 nmol/ml of norleucine was included in 6 N HCl as internal standard, and casein powder of known AA value was used as reference sample to evaluate the hydrolysis and the chromatography procedures. To determine the reproducibility of the assay, four replicates were assayed using a casein control sample. On average, 99.2% of the sample was recovered, and the CV of the means was below 5% for most of the AAs, except for serine (Ser), proline (Pro) (6%), glycine (Gly) (8%), aspartic acid (Asp) (9%) and Cys (15%).

Published requirements and crop content of other species

For comparison with our results, we compiled the published CP and AA requirements for optimal growth of leghorn chickens age 0–6 and 6–12 weeks (National Research Council, 1994), broiler chicks 0–3 weeks old (Baker and Han, 1994; Baker et al., 1996) and 3–8 weeks old (Emmert and Baker, 1997), and budgerigars and lovebirds estimated using the factorial method (Wolf and Kamphues, 2003). We compared our average protein and AA concentrations from crop samples with the pooled crop contents of 15 free-ranging kakapo (*Strigops habroptilus*) chicks from 10 nests (age 10–43 days). The kakapos are phylogenetically far from the macaws (Collar, 1997) and very specialized herbivores, who feed their chicks almost exclusively rimu fruits (*Dacrydium cupressinum*), but it is the only other species of psittacine in which AA profile of the crop contents of parent-reared chicks have been published (Cottam et al., 2006).

Statistical analysis

Mann–Whitney *U*-tests were used to compare the levels of total protein and AAs in the scarlet macaw crop samples between years, and to compare the concentration of ash with the findings of a previous study. One-sample *t*-test was used to compare the crop nutrient levels with the published requirements for poultry and other psittacine species, and with the average levels found in the kakapo crop contents (Roudybush and Grau, 1991; National Research Council, 1994; Wolf and Kamphues, 2003; Cottam et al., 2006). Linear correlation was used to determine the relation between the average age of the chicks in each sample with the concentration of

PME, protein and AAs. Linear correlation was also used to look at the relation between the concentration of protein and of each AA. Statistical tests were conducted by using JMP software (version 8.02; SAS Institute Inc., Cary, NC, USA) with $\alpha = 0.05$. Data are presented as mean ± standard deviation (minimum – maximum).

Results

PME

The scarlet macaw chick crop contents contained, on average, 16.3 ± 4.3% DM (10.4–23.8) CP, 22.0 ± 5.6% DM crude fat and 37.7 ± 17.3% DM soluble carbohydrates. The PME density was 16.9 ± 2.6 MJ/kg DM (10.2–19.7). Energy density was not significantly different from commercial diets offered to Leghorn chicks ($p > 0.05$) (National Research Council, 1994), nor the average of 11 parrot hand feeding formulas (16.2 MJ PME/kg, $t = 2.60$, df = 14, $p < 0.05$) (Wolf and Kamphues, 2003), but was significantly higher than the 7.7 MJ PME/kg received by kakapo chicks ($t = 5.56$, df = 14, $p < 0.001$) (Cottam et al., 2006).

Crude and true protein

The mean true protein content, calculated as the sum of the AAs (Association of Official Analytical Chemists, 1996), was 13.6 ± 3.9% DM (7.5–20.6) and 8.3 ± 2.9 g/MJ PME (4.1–13.7, N = 15 pooled samples) (Table 1), with no significant differences between years ($U = 10.0$, $p > 0.05$). The CP content was 10.0 ± 3.5 g/MJ PME (5.8–16.9, N = 15 pooled samples). The mean CP content, on a PME basis, was lower than the requirements of 0- to 6-week-old leghorn chicks (16.6 g/MJ PME; $t = 7.33$, df = 14, $p < 0.001$), and 6- to 12-week-old leghorn chicks (14.7 g/MJ PME; $t = 5.23$, df = 14, $p < 0.001$) (National Research Council, 1994). It was not different than the requirements of budgerigars (10.2 g/MJ ME; $t = 0.24$, df = 14, $p > 0.05$) and lovebirds (9.5 g/MJ ME; $t = 0.54$, df = 14, $p > 0.05$) (Wolf and Kamphues, 2003), nor the concentration found in the crop of wild kakapo chicks (10.9 g/MJ PME, $t = 1.01$, df = 14, $p > 0.05$) (Cottam et al., 2006).

AA profile

The concentrations of the AAs in the crop contents of the scarlet macaws did not differ significantly between years except for Cys, which was 0.25 g/MJ PME in 2006 and 0.05 g/MJ PME in 2008 ($U = 0.00$, $p < 0.01$). As a result, values for all AAs are combined across years for the remaining analyses. The AAs present in the highest concentrations were glutamic acid (Glu 1.32 g per MJ ME), arginine (Arg 0.88 g), aspartic acid (Asp 0.73 g) and leucine (Leu 0.60 g) (Table 1). There was a strong positive correlation ($p < 0.001$) between the concentration of each essential amino acid (EAA) and the total protein content (as g per MJ PME). The mean concentrations of five EAAs were below the minimum requirements established for leghorn-type chicks aged 6–12 weeks: Leu (76% of recommended), isoleucine (Ile 79%), threonine (Thr 69%), Lys (65%) and Met (75%). In addition, phenylalanine was also below the minimum requirement for leghorn-type chickens 0–6 weeks old (75%) (National Research Council, 1994) (Table 1). None of the EAAs were found in concentrations below the minimum requirements of budgerigars and lovebirds (Wolf and Kamphues, 2003). The kakapo chick crops had higher concentrations of all the EAAs except for Thr (Cottam et al., 2006).

Age variations

There was no significant correlation between the age of the chicks and the PME of the samples, the total protein content as percentage DM or the protein in g/MJ PME ($R < 0.24$, $p > 0.05$, N = 12). However, a significant negative correlation was found between the age of the chicks and the concentration of Lys ($R = 0.56$, $p = 0.005$, N = 12) and Met ($R = 0.34$, $p = 0.044$, N = 12).

Discussion

The PME density of the regurgitate fed to the scarlet macaw chicks was equivalent to a starter poultry feed (National Research Council, 1994) and higher than the low-quality food used by the kakapo to feed their chicks (Cottam et al., 2006). The daily energy requirements of growing birds change as a function of weight gain rate and body composition (Kamphues and Meyer, 1991; Klasing, 1998). As anticipated, the PME density of the diet fed to the scarlet macaw chicks did not change with age (Taylor et al., 1993).

Our samples contained 16.3% CP on a DM basis. A previous study of scarlet macaw chick crop samples from the 2005 breeding season in the same study site found 23.5 ± 5.6% CP (DM basis) (Brightsmith et al., 2010), nearly twice what we found here. We suspect that the difference was not because

Table 1 Crude protein (CP) and amino acid (AA) composition in g/MJ PME of crop contents of free-living scarlet macaw chicks (*Ara macao*), crop contents of kakapo (*Strigops habroptilus)* chicks, and the nutritional requirements of budgerigars (*Melopsittacus undulatus*), lovebirds (*Agapornis spp.*) and leghorn chickens (*Gallus gallus*) chicks

							Leghorn chickens**	
	Scarlet Macaws‡			Kakapo§	Budgerigar¶	Lovebird¶	0–6 weeks old	6–12 weeks old
Item	g/MJ PME	SD	Range	g/MJ PME	g/MJ ME	g/MJ ME	g/MJ PME	g/MJ PME
CP	9.99	3.49	5.81–16.9	10.9	10.2	9.48	16.6*	14.7*
Arginine	0.88	0.32	0.43–1.49	1.30*	0.52†	0.46†	0.92	0.77
Leucine	0.60	0.22	0.31–1.01	0.82*			1.01*	0.78*
Valine	0.53	0.21	0.22–0.90	0.63*			0.57	0.48
Phenylalanine	0.41	0.15	0.18–0.63	0.50*			0.54*	0.41
Phe + Tyr	0.69	0.24	0.34–1.08	0.83			0.92	0.77
Isoleucine	0.36	0.14	0.18–0.61	0.51*			0.55*	0.46*
Threonine	0.36	0.14	0.17–0.61	0.38			0.63*	0.53*
Lysine	0.36	0.15	0.20–0.72	0.61*	0.33	0.32	0.78*	0.55*
Methionine	0.17	0.06	0.10–0.31	0.24*			0.28*	0.23*
Met + Cys	0.39††	0.14	0.21–0.72	0.51	0.50	0.37	0.57	0.48
Tryptophan	0.08	0.04	0.03–0.14					
Proline	0.42	0.15	0.18–0.68	0.54*			0.16†	0.13†,‡‡
Glycine	0.40	0.15	0.19–0.67	0.55*				
Histidine	0.23	0.08	0.12–0.36	0.38*			0.24	0.20
Glutamic acid	1.32	0.48	0.65–2.24	1.81				
Aspartic acid	0.73	0.35	0.39–1.33	1.23				
Alanine	0.47	0.17	0.24–0.82	0.58				
Serine	0.43	0.16	0.22–0.67	0.54				
Gly + Ser	0.83	0.30	0.41–1.34	1.09			0.65	0.53
Tyrosine	0.28	0.09	0.16–0.48	0.33				
Cystine	0.21††	0.11	0.04–0.42	0.26				
Hydroxyproline	0.02	0.03	0.00–0.10					
Citrulline	0.02	0.02	0.00–0.07					

PME, Predicted metabolizable energy.

*Values significantly higher than the scarlet macaw crops ($p < 0.05$).

†Values significantly lower than the scarlet macaw crops ($p < 0.05$).

‡Chick crop contents, $n = 15$, 3–8 weeks old, 16.87 ± 2.62 MJ PME/kg, total AA protein = 8.29 g/MJ PME.

§(Cottam et al., 2006) mean chick crop contents. 7.67 MJ PME/kg.

¶(Wolf and Kamphues, 2003) minimum growing requirements. 16.22 MJ ME/kg.

**(National Research Council, 1994) 11.93 MJ PME/kg.

††Significant differences between years ($U = 0.00$, $p < 0.001$). Cys average 2006 = 0.25 g/MJ PME, 2008 = 0.05 g/MJ PME.

‡‡(Graber et al., 1970).

of annual variations in the composition of available food items, as the weather patterns did not differ greatly between years (Brightsmith, unpublished observation). One possible explanation is that the samples from 2005 had lower clay content, which increased the relative concentration of CP on a DM basis. This is supported by the lower ash concentration (DM basis) of the 2005 samples vs. 2006 and 2008 samples (11.7 ± 8.7%, Brightsmith, unpublished observation, vs. 26.3 ± 18.9%, $U = 5.84$, $p < 0.05$ respectively). It was not possible to calculate PME of the diet in that original study; however, so we cannot determine whether there is also a difference on an energy density basis.

The CP values found in the scarlet macaw crop samples were lower than expected based on the estimated requirements of Leghorn chickens (National Research Council, 1994), but in line with the requirements estimated for cockatiels and lovebirds (Wolf and Kamphues, 2003) and the concentrations found in kakapo chicks (Cottam et al., 2006). We expected that macaw chick dietary protein levels would be higher than those of poultry because of their altricial development and higher growth rate (Koutsos et al., 2001). However, birds do not have a CP requirement *per se*; instead the requirements depend on protein quality, i.e. the concentrations of EAAs and protein digestibility (Izhaki, 1998; Klasing, 1998). The complexity of the AAs interrelationships makes it difficult to define a protein requirement for any species, and the evaluations should be limited to comparing typical protein intake values among species (Harper and Skinner, 1998).

The wild diet seems to contain a moderate level of non-protein nitrogen, as indicated by the difference between the CP (10.0% ME basis) and the total AA concentration (8.3%). The CP estimation obtained by multiplying N by the widely used value of 6.25 (Pellet and Young, 1980) is 20% higher than the total AA concentration. If summed AAs are an accurate reflection of CP, this suggests that the appropriate N:protein conversion factor for true protein would be closer to 5.20, in the range of the 5.18–5.46 as proposed for nuts and seeds (FAO, 1982).

The concentrations of half of the EAA found did not fulfill the requirements of growing 6-week-old Leghorn chicks. Normally, the profile of EAAs required by birds corresponds to that found in the body tissues (Boorman, 1980). The lower densities in the diet of the scarlet macaw compared with the requirements of poultry could be driven by differences in the EAAs composition of the body tissues compared with precocial birds (Scott et al., 1982; Murphy, 1994). However, the bodies of adult budgerigars and chickens have similar AA profiles (Massey and Sellwood, 1960), and there is no reason to believe that the body of scarlet macaws should have a significantly different composition. Deviations from a one-to-one relationship between body and required dietary EAAs can be caused by (i) different turnover rates of individual tissue proteins (Baker, 2009), (ii) different digestibilities and efficiencies of reutilization of the EAAs (Heger and Frydrych, 1989), or (iii) alternative metabolic fates of different EAAs (Murphy, 1993; Wu, 1998). More studies on the AA metabolism of the scarlet macaw are needed to better understand the apparently low concentrations of EAAs in the diets found here.

We found a 38% decrease in Lys from age 19 through 59 days post-hatch. As the chick grows, protein gain as a percentage of total body weight gain decreases, as do the requirements for muscle accretion (National Research Council, 1994; Emmert and Baker, 1997; Baker, 2009). Around the fourth week post-hatch, when the chick starts to grow the flight feathers (Vigo Trauco and Brightsmith, 2007), the AA composition of the body protein changes, decreasing the relative concentration of total Lys and increasing Cys (Murphy and King, 1982; Kamphues and Meyer, 1991). If this relationship holds for scarlet macaws as well, this mechanism could help explain the decrease in Lys found here.

In summary, our research suggests that young scarlet macaws at our site are being fed a diet with a PME protein and EAA concentration similar to the requirements estimated for other psittacines, but lower than the requirements for poultry. Here we discuss two possible explanations for this finding. One possibility is that the birds evolved to raise chicks on low-protein food sources. Adaptations to low-protein food sources include low endogenous protein losses and low protein maintenance requirements. In psittacines, these mechanism have been found in strict frugivores including Pesquet's parrots (*Psittrichas fulgidus*) (Pryor et al., 2001), rainbow lorikeets (*Trichoglossus haematodus*) (Frankel and Avram, 2001) and kakapos. Adult kakapos are known to subsist during the non-breeding period on a diet of 3.7% DM CP (James, 1991). They feed their chicks a diet almost exclusively of *Dacrydium cupressinum* fruit, which is relatively high in indigestible matter and low in CP (10.9 g/MJ PME, 8.36% DM) and other essential nutrients (Table 1) (Cottam et al., 2006). Future research may look at the nitrogen balance of the scarlet macaws, to determine whether their physiology is adapted to a low-protein diet.

A second possible explanation for the low-protein and EAAs concentrations is that the chick diets we studied are not sufficient to promote optimal growth. Experiments with cockatiels have shown that optimum growth is achieved at 20% DM CP and 0.8% DM Lys. However, it was not until diets were below 10% DM CP that permanent damage or mortality occurred (Roudybush and Grau, 1991). The macaw chicks at our site fledge successfully and the populations are apparently not decreasing (Brightsmith, unpublished observation). However, chick growth rate does not reach the maximum possible for the species as evidenced by the higher growth rates found in other wild populations (Renton, 2002) and hand-raised birds (Abramson et al., 1995; Hanson, 1995; Vigo Trauco, 2007). Previous work in this population suggested that the concentration of Na in the chicks' diet may be deficient (Taylor et al., 1994). Our current study suggests that several EAA may also be acting as growth-limiting factors, as has been proposed for other free-ranging parrots (Stoleson and Beissinger, 1997; Renton, 2002; Pacheco et al., 2010). As a result, the AA profiles presented here should be used with caution when formulating hand-rearing diets for macaws and other psittacines.

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