HIGH PROTEIN MILK REPLACER FOR THE HAND-REARING OF A GIANT ANTEATER (MYRMECOPHAGA TRIDACTYLA) AT ZOO MIAMI

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Abstract
A giant anteater pup was hand-reared at Zoo Miami after being rejected by its mother. Based on high protein maternal milk composition in giant anteaters the pup was started on a high protein milk replacer (Zoologic 42/25) but after several days of weight loss was switched to a high fat milk replacer (Zoologic 30/52). Although apparently healthy, the pup continued to have a lower growth rate despite high energy intake. Once the pup was stable, higher protein was reintroduced to the formula as Zoologic 42/25 and then whey protein powder to better reflect expected maternal milk protein. These adjustments were associated with higher growth rates and lower apparent energy requirements. Increasing protein, particularly whey protein, in anteater hand-rearing formulas may promote more natural growth and metabolism.

Introduction
On December 8th, 2020, a neonatal giant anteater pup was found at morning welfare check. The pup appeared to be abandoned by its dam and was hospitalized for emergency supportive care. The pup's weight was 1045 g, which is below the expected birthweight of ~1400 g and well below the weight of a half-sibling that was successfully parent-reared and 1760 g at 7 d of age. It was suspected that the abandoned pup may have been premature. Once stable, the pup was tube fed with a combination of Pedialyte, Zoologic 42/25 Milk Replacer (PetAg.com) and ~1% maternal milk as available from manual milking. On December 10th, an attempt was made to reunite the pup with his dam but she rejected him again and full hand-rearing was initiated.

Milk Composition
Published milk compositions for anteaters present conflicting data. In a recent survey, 37 milk samples were evaluated from 3 captive giant anteaters and found to be high in protein, moderate in carbohydrate, and low in fat (Power, 2015). Except for the low fat, this is consistent with the milk of other Xenarthran species (Power *et al*., 2018) and insectivores (Wenker et al., 2019; White *et al*., 1985). However, the Handbook of Biological Data from 1956 reports a very high fat, lower protein, and low carbohydrate composition for anteater milk (Spector, 1956). No additional information is provided on these milk values including the anteater species, whether the subject was wild or captive, whether this was a single or pooled sampled, or the point of lactation. Nevertheless, this reference has been used to support the use of high fat milk replacer formulas for anteaters.

The protein requirement for growth in kittens is ~23% of ME kcals (NRC, 2006). Giant anteater, armadillo, and aardvark milk are approximately 60, 39, and 37% of kcal from protein, respectively (Power, 2015; Power *et al*., 2018; Wenker *et al*. 2019). High protein in the milk of giant anteaters, armadillos, and aardvarks may reflect the high protein content of insectivore diets. Excess milk

protein likely serves as a primary energy source (Power *et al*., 2018). High protein milk may also represent an evolutionary strategy for Xenarthrans to deliver calcium and phosphorous (bound to milk casein protein) to facilitate the development of specialized armor and whey proteins to deliver the sulfur amino acids for the di-sulfide bonds in hair for members of this clade (Power *et al*., 2018). Zoologic 42/25 milk replacer provides 35% of kcal from protein while Zoologic 30/52 provides only 20% of kcal from protein.

The wide range of fat contents in Xenarthran and insectivore milks is difficult to interpret. Studies on the maternal milk in Xenarthrans have found a protein:fat ratio as high as 5.63:1 in giant anteaters (Power, 2015), though fat concentration apparently increases substantially after 90 days (Power, unpublished data). Armadillo milk had a protein:fat ratio of 2.23 decreasing to 1.46 by day 50 (Power *et al*., 2018). One study of aardvark milk (White *et al*., 1985) found a protein:fat of 1.46 decreasing to 1.16 by about 30 d while the other had a protein:fat ratio decreasing from ~1.3 to ~0.6 across 114 d lactation (Wenker *et al*., 2019). Decreasing protein:fat over lactation increases the energy density of the milk, which may be necessary to meet energy requirements for late growth (Cabana *et al*., 2019). Pangolins reared on KMR (protein:fat ratio 1.55) have lower growth rates starting around 80 d compared to parent-reared pangolins, although these observations were confounded by weaning (Cabana *et al*., 2019). The authors suggested that increasing the formula fat level at this point might be beneficial. Similarly, a giant anteater reared on KMR had heavy cream added to boost formula fat and calories at 28 d of age (Bickel *et al*., 1975), and a mixed formula with a protein:fat of 1.02 was successful for rearing aardvark pups (Cabana *et al*., 2019).

Lactation of the giant anteater dam at Zoo Miami was maintained for 10 d by manual milking and oxytocin stimulation. Three samples of maternal milk collected on days 5, 7, and 10 were sent to the Nutrition Laboratory at the Smithsonian National Zoo for macronutrient analysis. The maternal milk compared well to the data from Power (2015), until day 10 when there was an unexpected increase in fat content. At this point, the female was drying up due to the lack of a nursing neonate, so the difference in this final sample could have been a reflection of the cessation of lactation. This could also just be normal variability in early milk samples as observed in aardvark milk (Wenker *et al*., 2019).

Based on targeting a high protein level and to avoid an extreme fat level, it was determined to hand-rear the anteater pup using the high protein Zoologic 42/25 milk replacer. This milk replacer was intermediate between the published anteater milk compositions and met the nutrient requirements for cats, which was presumed to be the most appropriate model species. PetAg KMR which is comparable to Zoological 42/25 has been used successfully to hand-rear giant anteaters in the past (Bickel *et al*., 1975). If targeted growth rates were not achieved it was proposed to mix in Zoologic 30/52 and gradually increase the fat level but not to reduce protein:fat ratio below 1.0.

Growth and Energy

Growth rate was targeted to the average growth rate reported for giant anteaters in the Species360 database (including hand-reared and parent-reared individuals; www.species360.org). Individual growth curves for 19 hand-reared giant anteaters were also provided by the Nashville Zoo at Grassmere. Nashville Zoo's hand-rearing protocol suggested a growth rate of 2-5% BW/d. Per the Species360 data the average growth rate of captive giant anteaters is lower at approximately 1.1-2.6% BW/d, decreasing over the course of growth (Figure 1).

The energy requirement for maintenance in a giant anteater is $82.9*BW_{kg}^{0.75}$ (Stahl *et al*., 2012). This agrees with expectations for silky anteaters (Nagy & Montgomery, 2012) and tamandua (McNab, 2008). The energetic cost of growth for anteaters, however, is unknown. Other species range from 1.8 kcal/g growth in cats (Hendricks & Wamberg, 2000) to >8 kcal/g growth in horses (NRC, 2007). Conversion cost of protein, fat, or carbohydrate and composition of growth affects the caloric cost. Using detailed formula energy estimations and weight data for hand-reared sloths (Minnesota Zoo, personal communication) a cost per g of growth was calculated to be ~3 kcal. Based on this the following target energy level was proposed:

Giant Anteater kcal/d = $82.9*BW_{kg}^{0.75} + 3*ADG_{g}$

This expected requirement agreed reasonably with the recommended hand-rearing formula amounts from Nashville Zoo calculated from their formula composition, feeding schedule, and consumption as %BW. Energy estimates served as rough guidelines for target intake. Actual offering was based on consumption, feeding behavior, apparent fullness, and observed/desired growth rate.

Hand-rearing

Throughout the hand-rearing process the Zoo Miami anteater pup, 20M056, remained active, curious, responsive, eager for feeding, and in apparently good health with no extremes in body condition observed. Weights were collected daily initially and then every few days (Figures 2 and 4), and morphometrics were collected initially weekly and then every few weeks (Figure 5) throughout hand-rearing to monitor growth. A minimum of 7 feeds per 24 h were required initially to achieve the estimated energy requirement. However, it was found to be difficult to provide more than 2.5% bodyweight per feeding based on visible fill, satiation, and formula clearance between meals. Anteaters have a specialized stomach for masticating ants, and stomach content was 2.5 ± 1.5% of carcass weight (Mean ± SD) in wild giant anteaters (Bissell, personal communication).

Initial energy requirement was calculated to be 183 kcal/d allowing for 33 g/d growth. However, both the cost of growth and the rate of growth were expected to be overestimated for the perinatal period. Per the hand-rearing protocol provided by Nashville Zoo their estimated energy offered did not reach 183 kcal/d until Day 13.

During this initial period, keepers continued to milk the dam post oxytocin stimulation, and this maternal milk was added to the formula. Initial feedings were diluted, gradually working towards a target of 12% DM (137 kcal/d) by day 4. At this point, the Nashville Zoo protocol was estimated to provide around 100 kcal/d. The neonate continued to drop weight so formula was concentrated to 14% DM (153 kcal/d) on the evening of day 4. On day 5 of continued weight loss, Zoologic 30/52 was added at 25% of the formula dry matter to boost calories to 171 kcal/d, and on day 5, it was decided to rapidly switch to entirely Zoologic 30/52 because this was the milk replacer used successfully by Nashville Zoo. This transition was completed by day 7 (206 kcal/d), and the animal also transitioned smoothly from tube feeding to a bottle from day 7 to 8. Weight loss appeared to stop around 140 h (5.8 d), and it is unclear if this was the result of the increased calories or the change in formula from high protein to high fat (Figure 2).

On the initial Zoologic 42/25 formula, 20M056 tended to have watery stool and notable gasiness. Simethicone (20 mg/0.3 mL; 1 drop per 167 mL formula) was added to the formula as this was

used successfully in hand-reared aardvarks (Bissell, personal communication). After transitioning to the Zoologic 30/52 formula the feces became more formed and defecation reduced to every few days. Although not a significant concern, some gasiness continued and possible mild constipation was observed. Taurine was also added to the Zoologic 30/52 formula to meet the requirement for cats (NRC, 2006).

For the first month on the Zoologic 30/52 formula 20M056 met targets for growth based on Species360 data but remained low in weight and at the bottom of the range for average daily gain and %BW gain per day compared to giant anteaters hand-reared on Zoologic 30/52 at Nashville Zoo, despite 20M056 apparently consuming more kcal per day. Interestingly, shifting the growth curve for 20M056 back 2 weeks puts him closely in line with the smaller Nashville anteaters, possibly supporting a 2-week delay in growth/functional age. 20M056 also continued to have hard fecals with straining at defecation so at 29 d, the formula was diluted to 12% DM.

On day 37, to promote growth and possibly improve formula digestibility and fecal consistency, 25% of the Zoologic 30/52 was replaced by Zoologic 42/25. The new formula, 38/53, provided a protein:fat of 0.72. It was hypothesized that increased protein might boost lean growth and satiation. Around the time of this change, growth rate was observed to increase to meet targets. Defecation remained every few days but straining was no longer observed. Although more feedings were required due the lower energy density of the 38/53 formula, energy levels to achieve growth appeared more consistent with the data from Nashville Zoo and slightly below the expected energy requirement calculated from the proposed energy equation.

On day 84, an additional growth spurt was anticipated so it was decided to try increasing the protein content of the formula further to boost the protein:fat and better reflect the expected maternal milk composition. It was also noted that the Zoologic formulations are almost exclusively casein milk protein which might have been a factor in the hardness of the stools. Giant anteater milk is expected to be higher in whey protein to provide sulfur amino acids for hair growth although actual data are not available. Whey protein is more rapidly digestible, and it was thought that this might benefit energy efficiency and boost consumption while and benefit targeted growth, such as specialized anteater hair.

To boost protein, whey protein powder (Nuts.com) was added to the formula to replace the Zoologic 42/25 portion and achieve 75% Zoologic 30/52 + 25% whey protein powder on a dry matter basis. This resulted in a formula with a protein:fat ratio of 1.0 with protein providing 30% of kcal.

Because the whey protein powder was not nutritionally complete, the formula was reviewed for nutrient deficiencies and several were found. Besides being deficient in taurine, the Zoologic 30/52 just meets the protein requirement for cats and is deficient in zinc, copper, and especially iron at less than 1/3rd of the expected iron requirement. Dicalcium phosphate and a feline multivitamin (Vetriscience NuCat Multivitamin Everyday Health) were added to the new formula transition to meet NRC requirements for growing cats (NRC 2006).

Following the addition of whey protein powder and the vitamin and mineral supplement, the feces from 20M056 became greenish in color and more frequent. The color likely reflected the increased iron from the supplement. Occasionally, a small amount of liquid stool was noted though always

in conjunction with formed feces, and overall fecal consistency was considered appropriate. Growth increased to a point that exceeded both Species360 estimates and Nashville Zoo averages, which may be attributable to a combination of higher protein in the formula while still being high fat and stage of growth/maturity.

At 132 d of age, formula was dropped to 3 feeds per day. At this point, formula alone no longer met the caloric requirement, and consumption of solids was inconsistent so the growth rate decreased. From day 165-193, Zoologic 30/52 was gradually replaced with Mazuri Insectivore 5M6C in the formula. Over this period, 20M056 began to eat the formula better as a slurry. On day 197 the whey protein powder was removed, and 20M056 was officially weaned onto a slurry of 92% Mazuri 5M6C + 4% ground dried mealworms + 4% ground dried black soldier fly larvae. Increased solids consumption resulted in an increase of average daily gain.

Conclusion

Since this was a single case with many confounding factors throughout, it is impossible to define optimal recommendations for hand-rearing giant anteaters. High protein Zoologic 42/25 formula appeared to be unsuccessful during the first week, and increasing the fat level may have helped overcome this failure. It is also possible that in the perinatal period the high casein content of the Zoologic 42/25 may have been poorly digested or that deficiency of sulfur-containing amino acids may have been limiting during this phase of rapid hair growth. It is also possible that the animal was compromised by being premature, hypothermic, or traumatized around birth and required several days to recover normal function. The animal appeared to thrive acceptably on Zoologic 30/52, but the addition of Zoologic 42/25 and then whey protein later in growth may have potentiated increased growth rates and improved digestibility. For future cases, incorporating whey protein during early growth may promote growth and development most closely resembling that of parent reared anteaters.

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Table 1. Composition of maternal milk from anteaters compared to other species and milk replacer formulas suggested for hand-rearing anteaters.

	DM, %	CP, %DM	Fat, %DM	Carbohydrate, %DM
Anteater Milk[1]	37	30	54	1
Giant anteater Milk[2]	11	50	9	28
Armadillo Milk[3]	17-25	44-48	21-31	9-18
Aardvark Milk[4,5]	26-38	35-43	31-55	5-10
Felid Milk		30-66	21-57	8-28
Zoologic 30/52 Milk Replacer[6]		34	60	6
Zoologic 42/25 Milk replacer[6,7]		48	31	21

[1]Spector 1956
[2]Power 2015
[3]Power *et al*., 2018
[4]Wenker *et al*., 2019
[5]White *et al*., 1985
[6]PetAg.com
[7]Same as PetAg KMR (kitten milk replacer)

Table 2. Macronutrient analysis of maternal giant anteater milk at Zoo Miami. Lactation was being maintained by daily oxytocin stimulation and manual milking which ended following the sample on day 10.

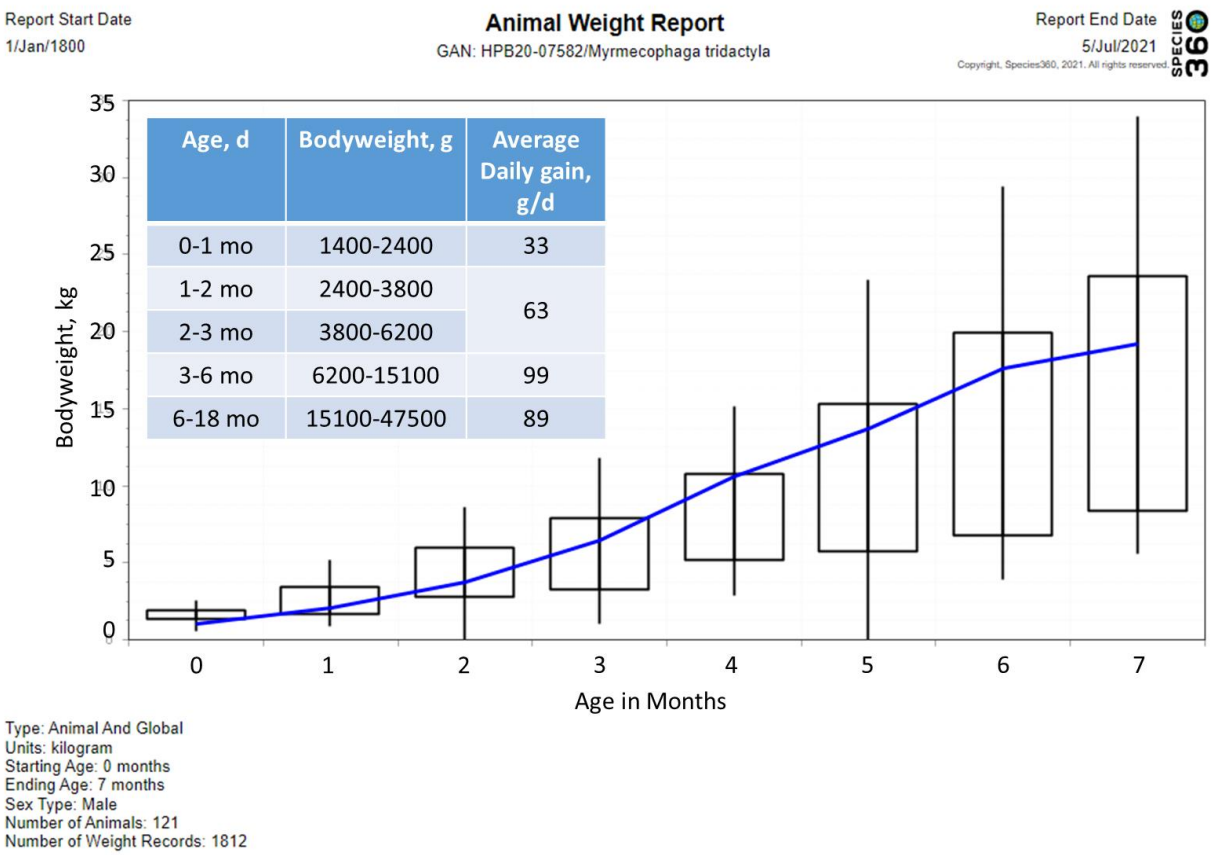
Days Post-Partum	Protein, %	Fat, %	Sugar, %	DM, %	Protein/Fat DMB[1]	Protein:Fat	kcal from Protein, %
5	4.61	0.85	3.03	9.88	54/10	5.4	57
7	5.24	1.67	2.24	11.04	57/18	3.2	57
10	5.81	5.36	2.24	15.57	43/40	1.1	37
Zoologic 42/25[1]					48/31	1.5	35
Zoologic 30/52[1]					34/60	0.6	20

[1]Dry Matter Basis
[2]Milk replacer PetAg.com

Table 3. Giant anteater formulas with Zoologic 30/52 supplemented with Zoologic 42/25 or whey protein powder, dicalcium phosphate, and Vetriscience NuCat Multivitamin Everyday Health.

		Formula 38/53	**Formula 41/41**	**Recommendation[1] (4 kg, 9% mature, 545 kcal/d)**	
	Units	**75% 30/52 25% 42/25**	**75% 30/52 25% Whey**	**Based on BW**	**Based on kcal**
Dry matter/day	g	94.6	98.4		
Wet weight/day:	g	100	104		
Water	g	5.4	5.6		
Energy	kcal	567	545	548	548
Protein	g	**32.6**	40.6	30	31
Fat	g	45	40.6	12	12
Ash	g	7.3	6.8		
Calcium, Ca	mg	1560	1579	1038	1089
Iron, Fe	mg	**3.8**	15.4	10.6	10.9
Magnesium, Mg	mg	87.5	86.0	50.6	54.5
Phosphorus, P	mg	1032.5	1016.5	942	981
Potassium, K	mg	997.5	999.4	529	545
Sodium, Na	mg	282.25	262.75	187	191
Zinc, Zn	mg	**9.175**	21.4	9.9	10.1
Copper, Cu	mg	**0.75**	1.24	1.1	1.1
Manganese, Mn	mg	0.95	1.79	0.63	0.65
Selenium, Se	ug	0	101.5	40.0	40.8
Taurine	mg	**25**	126	53.2	54.5
kcal from protein	%	23	30	22	22

[1]NRC, 2006



Type: Animal And Global
Units: kilogram
Starting Age: 0 months
Ending Age: 7 months
Sex Type: Male
Number of Animals: 121
Number of Weight Records: 1812

Figure 1. Average bodyweights with standard deviation for giant anteaters estimated from Species360 weight date for captive hand-reared and parent-reared anteaters. Average daily gain estimated from changes in weight averages. Growth curve for 20M056 overlaid in blue.

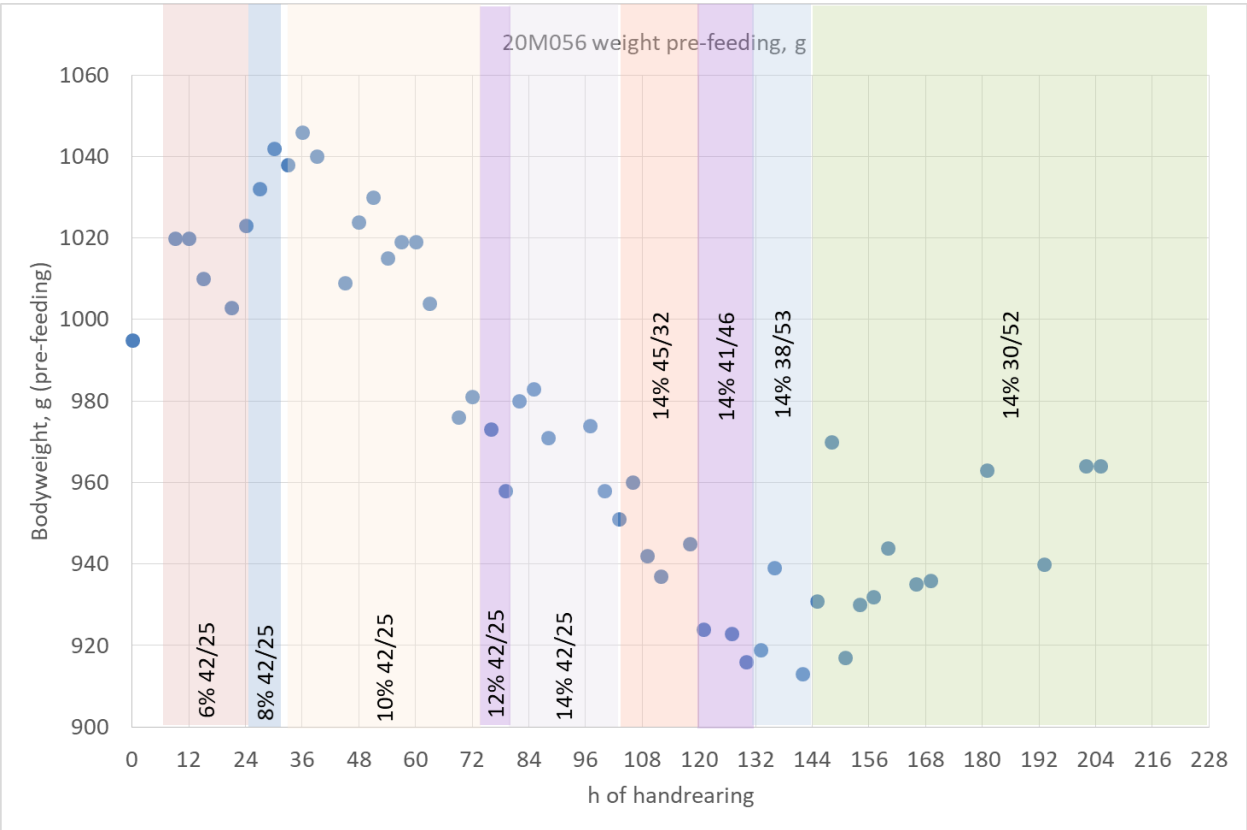


Figure 2. Early weight curve for hand-reared giant anteater 20M056. Times are overlaid with each formula %DM and approximate protein/fat content on a DM basis.

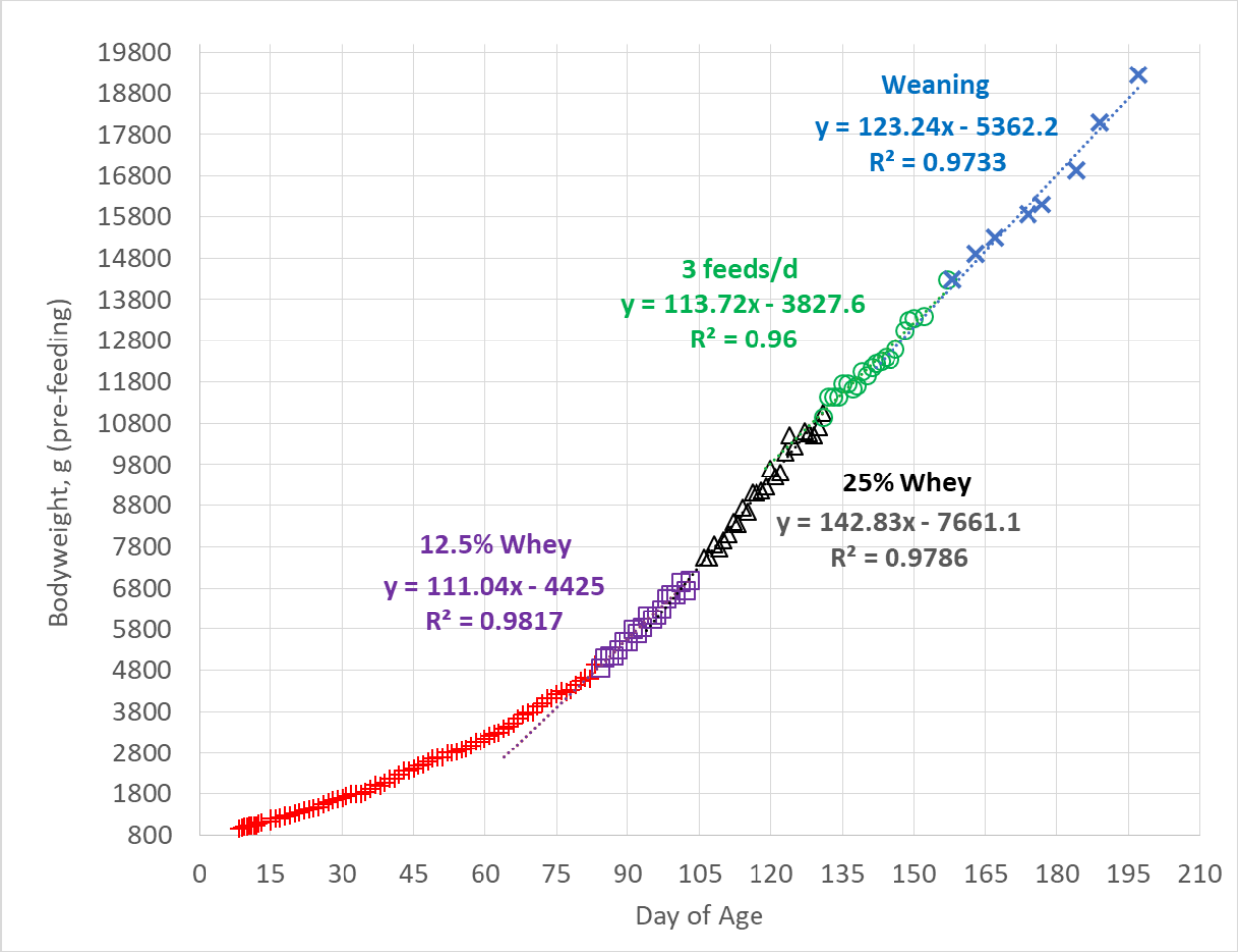


Figure 4. Growth curve and average daily gain (g/d) of hand-reared giant anteater 20M056 showing changes in growth rate with different formulations. Volumes and feeds per day were also adjusted based on growth with calories increasing steadily over time until weaning began (when feeds were dropped to 3 per day).

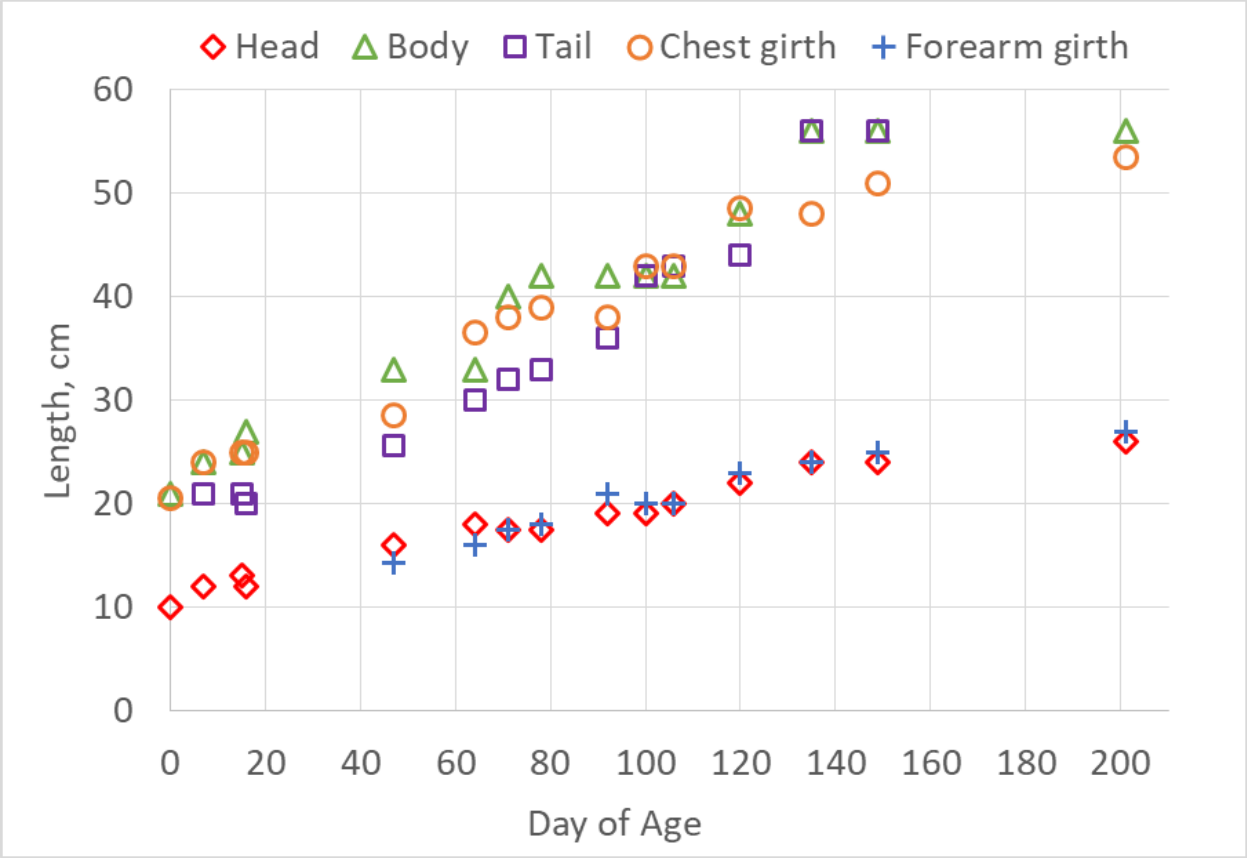


Figure 5. Morphometrics during growth of hand-reared giant anteater 20M056. Head was measured from base of skull to tip of snout. Body was measured from base of skull to base of tail. Tail was measured from tail base to tail tip. Chest girth was measured just behind the forelimbs. Forearm girth was measured as the widest part of the forearm.