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Cataracts and strabismus associated with hand rearing using artificial milk formulas in Bengal tiger (*Panthera tigris* spp *tigris*) cubs

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Abstract
The aim of this investigation is to describe the potential contributing nutritional factors involved in the development of ophthalmic and dermatologic changes in four Bengal tiger (*Panthera tigris* spp *tigris*) cubs fed an artificial milk formula. The affected animals were compared with two other tiger cubs that had been nursed by their dam naturally. After the first clinical signs appeared, the tiger cubs underwent ophthalmic evaluation. Severe symmetric generalized alopecia over the trunk, sparing the head and distal portion of the front and rear limbs, bilateral cataracts and strabismus were noticed. Milk and blood from the mother, as well as blood from the healthy and affected cubs were collected in order to evaluate complete blood counts, serum chemistry values, and amino acid levels. The amino acid concentrations in the artificial formula were also evaluated for comparison to the milk from the dam. The concentration of taurine, arginine, phenylalanine, tryptophan and histidine were very low in the artificial formulas as compared to the dam´s milk. The tiger cubs that received the artificial formula had lower levels of the amino acids listed previously as compared to those that nursed from the dam naturally. Taurine, as well as arginine, phenylalanine, tryptophan and histidine deficiency appeared to be possible causes of the development of skin problems, cataracts and strabismus in the tiger cubs fed with these particular artificial milk replacers. In the future, special attention should be given in order to make sure that adequate levels of these amino acids are present in artificial milk for tiger cubs.
Keywords: Alopecia, Amino acids, Lens opacity, Wild felid, Zoo animal.

Introduction

Wild felids are commonly kept in zoos and large felids such as lions, tigers and cheetahs are popular among visitors and attract the population's interest worldwide. Large felids breed commonly in captivity and occasionally neonates of these species are not able to be raised by their dams. Optimum nutrition for suckling neonates is provided by the natural milk from the dam (Baines, 1981; Remillard *et al*., 1993).

When species-specific milk is not available, nutritional formulation are extrapolated from the milk of related species (Baines, 1981; McManamon, 1993; Remillard *et al*., 1993). The milk composition of the following felids has been investigated: domestic cat (*Felis catus*) (Adkins *et al*., 1997; Jacobsen *et al*. 2004), lion (*Panthera leo*) (BenShaul, 1962; De Waal *et al*., 2004), cheetah (*Acinonyx jubatus*) (Osthoff *et al*., 2006), serval (*Felis serval*) (Osthoff *et al*., 2007) and clouded leopard (Senda *et al*., 2010).

However, no information is available regarding the composition of tiger (*Panthera tigris*) milk. These previous publications show that protein and fat content vary considerably among different species of wild felids.

In zoos, an option frequently used is to provide cow´s milk or even other non-related species' milk-replacer formulas (Tilson and Seal, 1988; Osthoff *et al*., 2006, 2007). There are numerous protocols for hand rearing tigers using natural milk from another species (Tilson and Seal, 1988), artificial formulas and even mixtures of both (Baines, 1981; Remillard *et al*., 1993; Adkins *et al*., 1997).

Cataracts, dietary intolerance and reduced weight gain have been anecdotally attributed to differences in protein, fatty acid and carbohydrate composition between natural tigress milk and milk replacers' formulas (Tilson and Seal, 1988; Remillard *et al*., 1993; Osthoff *et al*., 2007). Specific nutritional requirements or optimal surrogate milk composition for tiger neonates have not yet been characterized.

The aim of this article is to describe the potential contributing nutritional factors involved in the development of the dermatologic and ophthalmic changes in tiger cubs fed artificial milk formulas.

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Case details

Clinical history

Six Bengal tigers (*Panthera tigris* ssp. *tigris*) cubs and two adult tigresses living at Pomerode's Zoo (Pomerode City, Santa Catarina State, Brazil) were investigated. The cubs were conceived naturally and born in three different litters, two successive litters from Tigress 1 (Fig. 1a) and a litter from Tigress 2. Two out of three litters were separated from the dam by the Zoo employees due to the dam´s history of killing its cubs.

The first litter (litter 1) from Tigress 1 yielded cub 1A (male) and cub 1B (female) that were separated from the dam at 3 days of age and hand-raised with a homemade artificial milk replacer (MR1) and subsequently developed clinical abnormalities. The second litter (litter 2) from Tigress 2 yielded cub 2A (male) and cub 2B (male), which also were separated from the dam at 3 days of age and hand-raised with a different artificial milk replacer (MR2) that was formulated in an attempt to avoid the clinical abnormalities noted in cubs 1A and 1B. A third litter (litter 3) from Tigress 1 yielded cub 3A (male) and 3B (male). This time, a successful attempt to leave the cubs to be raised by the dam was made and the cubs received natural tiger milk.

Litter 1

The tiger cubs 1A and 1B were fed MR1 for the first 60 days after birth. During the first days of life, the cubs received a volume of MR1 via bottle-feeding (Fig. 1b), which was calculated based on body weight as recommended in the literature (Binczik *et al*., 1988; Tilson and Seal, 1988). The cubs' eyelids opened by 7 days of age (cub 1B) and 10 days of age (cub 1A). During the first 30 days of age, cubs 1A and 1B developed dermatological abnormalities; generalized erythema, dry skin, and alopecia. Both of these cubs developed all these signs but the alopecia was more severe in the cub 1A.

The alopecia was severe (about 60% of the body surface), symmetrical, generalized over the trunk but sparing the head and distal portions of the front and rear limbs (Fig. 1c), which began to resolve around 60 days of age (Fig. 1d). At the end of the second month, cub 1B weighed 7.0 kg and cub 1A 6.0 k. A set of sharp and fine teeth started to appear after the first seven days of age. At about 60 days of age, the teeth were notably thicker and larger and the cubs started to receive a supplement of raw meat (without bones).

When cubs 1A and 1B were 70 days of age, the Comparative Ophthalmology Laboratory of the Veterinary Teaching Hospital of Federal University of Paraná (LABOCO-HV-UFPR), Curitiba, Paraná State, Brazil, performed an ophthalmic evaluation in the cubs to assess suspected visual impairment, noticed when they were about 30 days of age.

Fig. 1. Sequential photographs of some of the *Panthera tigris* spp. *tigris* involved. (A): Tigress 1 in the external zoo enclosure after giving birth. (B): Male cub (1A) at 14 days of age receiving milk replacer from a bottle. (C): Cub 1A at 30 days of age demonstrating severe symmetric generalized alopecia over the trunk, sparing the head and distal portion of the front and rear limbs. (D): Cub 1A (right) and Cub 1B (left) with cataracts and strabismus at 70 days of age. Note that the fur was already starting to grow at this time point.

During ophthalmic evaluation, bilateral mature (cub 1A) and immature (cub 1B) cataracts were diagnosed in addition to unilateral exotropia (also known as divergent strabismus) in the right eye of cub 1A (Fig. 2a) and bilateral exotropia for cub 1B. The eyes were examined with a slit lamp biomicroscope (Hawk Eye; Dioptrix, L'Union, France) to evaluate the anterior segment. Neither cub had a menace response. Pupillary and consensual light reflexes were normal in both eyes. The cornea, anterior chamber, aqueous humor and iris were normal in both cubs. The cataracts in both cubs showed no anterior capsular opacities and there were no signs of lens subluxation. The cataracts precluded posterior segment evaluation. With the exception of cataracts and strabismus, no other ocular abnormalities were noted.

Litter 2

Cubs 2A and 2B also were separated from the dam at 3 days of age and fed with a different artificial milk formula (MR2). At this point a nutritional problem was already a suspicion. MR2 was developed empirically by the staff of another zoo who claimed to have successfully used it to hand-rear tiger cubs in the past. So the information for the MR2 formula was communicated and this formula was tried in cubs 2A and 2B, in an empiric attempt to prevent the clinical signs seen in Litter 1.

MR2 formula, nevertheless, was similar to MR1. Basically it is MR1 formula with a multivitamin supplement and a commercially available milk powder

for small animals added. Further details of MR2 are discussed below. However, cubs 2A and 2B developed similar clinical signs to the cubs from the first litter (cubs 1A and 1B).

Both cubs developed alopecia at 25 days of age, however, alopecia was milder than the first litter. At the end of the second month, cub 2A weighed 5.8 kg and cub 2B weighed 7 kg. When these animals were about 50-days-old their teeth also were evident and they started receiving raw meat in order to empirically supplement the MR2 possible diet deficiencies, since a nutritional cause was suspected. The alopecia began resolving on its own at approximately 60 days of age, like cubs 1A and 1B. Ophthalmic findings of cubs 2A and 2B included bilateral immature cataracts and mild bilateral esotropia (convergent strabismus) (Fig. 2b).

Fig. 2. Bengal tiger (*Panthera tigris* spp. *tigris*) cubs affected with cataracts. (A): Cub 1A, fed with artificial milk replacer 1 (MR1) demonstrating unilateral divergent strabismus (OD) and bilateral mature cataracts. (B): Cub 2A, fed with MR2 showing bilateral immature cataracts and mild bilateral convergent strabismus. Note the faint blue tapetal reflex and nuclear and cortical lens opacity.

Litter 3

The cubs from the third litter (cubs 3A and 3B) were parent raised and received natural tigress milk for eight weeks. These cubs did not develop dermatological or ophthalmic signs. At the end of the second month of life, the weight of cub 3A was 5.3 kg and 3B was 4.75 kg.

Milk replacer formulas

Two milk replacer formulas (MR1 and MR2) were prepared based on previous supposedly effective experiences by other Brazilian zoos. There were small differences between the components of MR1 and MR2. Tables 1 and 2 list the ingredients used in the MR1 and MR2 recipes. The cubs were fed these diets from the third day of life and the composition of MR1 and MR2 did not vary over time.

Table 1. Ingredients used in MR1 recipe.

- 500 ml of 4% fat whole milk - ultra-high-temperature-pasteurized (UHT) cow's milk (Leite Líquido NINHO Forti+ Integral, Nestlé Ltda, PR, Brazil).
- 500 ml of 90% reduced lactose cow's milk (Leite Líquido NINHO Forti+ Zero Lactose, Nestlé Ltda, PR, Brazil).
- 10 ml of cod-liver oil (Emulsão de Scott Regular, Laboratório GlaxoSmithKline, RJ, Brazil). Each 10 ml also contains vitamin A (2,530 IU) and vitamin D (253 IU)
- 75 mg – equivalent of 1 ml of dimethicone oral emulsion (Luftal, Bristol-Myers Squibb, SP, Brazil).
- 1 ml of a probiotic supplement for dogs and cats containing *Saccharomyces cerevisiae*, *Lactobacillus acidophillus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Lactobacillus plantarum* (Probiótico Vetnil, Vetnil Indústria e Comércio, PR, Brazil).
- 15 g of canned cat food (Hill's a/d Science Diet, Hills Pet Nutrition INC, SP, Brazil).
- 10 ml of a multivitamin oral suspension (Clusivol, Wyeth Indústria Farmacêutica Ltda, SP, Brazil). Each 10 ml contained: Retinyl Palmitate (vitamin A) 2,500 IU; Cholecalciferol (vitamin D3) 200 IU; Ascorbic Acid (vitamina C) 32.50 mg; Cyanocobalamin (vitamin B12) 3.00 mcg; Thiamine Chloride (vitamin B1) 0,75 mg; Riboflavin (as riboflavin 5'-phosphate) (vitamin B2) 0.85 mg; Pyridoxine Hydrochloride (vitamin B6) 1.00 mg; Nicotinamide 10.00 mg; Dexpanthenol (d-panthenol) 6.00 mg; Inositol 5.00 mg. Amino acids: L-lysine Hydrochloride 25.00 mg; Choline Tartrate 5,00 mg. Mineral salts: Iron (as ferrous gluconate) 3.00 mg; Calcium (as calcium lactate and calcium hypophosphite) 40.00 mg; Phosphorus (as calcium hypophosphite) 30.00 mg; Iodine (as potassium iodide) 75.00 mcg; Potassium (as potassium gluconate) 2.50 mg; Manganese (as manganese gluconate) 0.52 mg; Zinc (as zinc lactate) 0.50 mg; Magnesium (as magnesium gluconate) 3.00 mg.

Table 2. Ingredients used in MR2 recipe.

- 500 ml of 4% fat whole milk - ultra-high-temperature-pasteurized (UHT) cow's milk (Leite Líquido NINHO Forti+ Integral, Nestlé Ltda, PR, Brazil).
- 500 ml of 90% reduced lactose cow's milk (Leite Líquido NINHO Forti+ Zero Lactose, Nestlé Ltda, PR, Brazil).
- 10 ml of cod-liver oil (Emulsão de Scott Regular, Laboratório GlaxoSmithKline, RJ, Brazil). Each 10 ml also contains vitamin A (2,530 IU) and vitamin D (253 IU).
- 75 mg – equivalent of 1 ml of dimethicone oral emulsion (Luftal, Bristol-Myers Squibb, SP, Brazil).
- 1 ml of a probiotic supplement for dogs and cats containing *Saccharomyces cerevisiae*, *Lactobacillus acidophillus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Lactobacillus plantarum* (Probiótico Vetnil, Vetnil Indústria e Comércio, PR, Brazil).
- 4 g of a commercially available milk powder for dogs and cats (PetMilk, Vetnil Indústria e Comércio, PR, Brazil).
- 5 ml a multivitamin suspension (Supre Gatos, Syntec, SP, Brazil) Each 5 ml contained: Folic Acid 0.045 mg; Choline Tartrate 35 mg; Copper 0.114 mg; Pantothenic Acid 4 mg; Zinc 0.35 mg; Vitamin A 1479 IU/g; Iron 0.75 mg; Magnesium 0.004 mg; Vitamin B1 1.5 mg; Phosphorus 0.04 g; Manganese 0.24 mg; Vitamin B6 1.51 mg; Biotin 0.012 mg; Vitamin B2 1.50 mg; Vitamin B12 5.886 mcg; Calcium 0.061 g; Niacin 7.4 mg; Vitamin K 0.77 mg; Iodine 0.28 mg; Potassium 0.09 mg; Vitamin D3 148 IU/g; Sodium 0.001 g; Taurine 1 mg; Vitamin E 6.7 mg; Inositol 1.5 mg.

Sampling

Blood samples from two of the affected cubs (2A and 2B) were collected in order to evaluate the amino acid levels. Additionally, milk and blood from Tigress 1 and blood from the two healthy cubs (3A and 3B) were collected to serve as controls. Furthermore, complete blood counts (CBC), serum biochemical analyses of these animals and amino acid analysis of MR1 and MR2 were evaluated. Milk was obtained from Tigress 1 during the eighth week of lactation. The tigress was five years old and fed 5 kg per day of a beef and chicken meat mixture which was supplemented with a commercial mineral and vitamin C veterinary supplement (Aminomix pet performance; Vetnil Indústria e Comércio de Produtos Veterinários LTDA, SP, Brazil). To collect milk samples, Tigress 1 was anesthetized by darting with an intramuscular combination of ketamine (10 mg/kg) (Ketamin S(+), Laboratório Cristália – Produtos Químicos e Farmacêuticos, SP, Brazil) and midazolam (0.5 mg/kg) (Midazolam; Eurofarma Laboratórios LTDA, SP, Brazil). Once anesthetised, blood samples were collected from the cephalic vein, and a bolus of oxytocin (0.25 IU/kg [ocitocina; Vetnil Indústria e Comércio de Produtos Veterinários LTDA, SP, Brazil]), was injected intravenously. The milk sample (150 mL) was collected immediately after oxytocin injection by manual compression and then divided into three 50-ml plain tubes (Falcon, Becton Dickinson, Lincoln Park, NJ). The milk was then frozen for one week at -20 C until the amino acid analysis was performed.

Blood samples of all cubs were collected from the femoral veins into plain 3 ml tubes using manual restraint when the cubs were 60 (cubs 3A and 3B) and 58 (cubs 2A and 2B) days old. Whole blood was allowed to clot and then immediately centrifuged. The serum was then collected and frozen until analyzed. Blood samples were processed by the Clinical Pathology Laboratory of the Federal University of Paraná (Curitiba City, Paraná State, Brazil), and milk and blood serum amino acid analyses were performed by the State University of Campinas, Center of Science and Food Quality (Centro de Ciência e Qualidade de Alimentos ITAL, Campinas, SP, Brazil).

Milk and serum analyses results

Concerning the CBC and serum biochemistry, no significant findings or pertinent differences between the cubs were observed on the blood work.

Lactose was measured in milk and MR samples and the results are expressed in mg/100 mL. The natural tigress milk had 2.77 lactose, compared to the 2.46 of MR2 and 1.55 of MR1. All amino acids evaluated in tigress milk and MRs are presented in Table 3, and the content of free amino acids in serum of Tigress 1 and cubs (cubs 2A, 2B, 3A and 3B) can be seen in Table 4.

Table 3. Qualitative and quantitative amino acid analysis of tigress milk and milk replacers (MR1* and MR2**).

Amino acids	Total amino acids (mg/100 mL of sample)		
	MR1*	MR2**	Tigress milk
Alanine	146.96	121.87	413.92
Arginine	144.62	137.53	732.14
Aspartic acid	301.02	287.83	1092.02
Cystine	91.00	89.29	306.29
Glutamic acid	737.85	763.39	2638.78
Glycine	112.97	74.73	151.35
Histidine	98.46	94.40	426.54
Isoleucine	188.96	186.82	631.67
Leucine	360.94	354.67	1558.20
Lysine	333.09	316.60	944.97
Methionine	72.83	67.09	219.10
Phenylalanine	185.98	177.81	503.05
Proline	308.75	321.14	1085.74
Serine	197.52	193.11	542.71
Taurine	3.58	0.06	90.00
Threonine	169.97	165.09	808.36
Tryptophan	121.38	124.96	276.94
Tyrosine	179.70	177.71	670.49
Valine	225.47	219.72	741.86
Total	3981.07	3873.83	13834.15

*MR1 – Milk Replacer 1.
**MR2 –Milk Replacer 2.

All of the amino acids measured in MR1 and MR2 were present in lower quantities as compared to tigress milk. Compared to tigress milk, taurine levels were particularly low in both MRs but levels of arginine, phenylalanine, tryptophan and histidine were also low. Inferential statistical analyses were not performed due to the small sample numbers.

In Table 4, the comparison between free amino acids in serum of the cubs fed with MR2 (2A and 2B) and cubs fed with tigress milk (3A and 3B) revealed that the levels of some amino acids were higher in the cubs fed MR2 as compared to those fed tigress milk, but a lower level of taurine. Nevertheless, the level of serum taurine in affected cubs was similar to that in the tigress.

Discussion

Cataracts, strabismus and alopecia were diagnosed in four tiger cubs that had been fed with different artificial formulas during the initial weeks of development.

Table 4 – Content of free amino acids in serum of Tigress 1 and cubs.

Amino Acids	Free amino acids (mg/100 mL of sample)				
	Tigress 1	Cub 3A*	Cub 3B*	Cub 2A**	Cub 2B**
Alanine	5.51	3.33	2.44	5.19	6.63
Arginine	2.70	2.98	2.77	3.43	3.49
Aspartic acid	0.01	0.39	0.21	0.43	0.43
Cystine	3.59	4.67	3.39	3.67	3.78
Glutamic acid	0.92	1.92	1.51	3.49	3.57
Glycine	3.12	2.19	2.03	3.84	4.05
Histidine	1.93	2.73	2.13	2.86	3.60
Isoleucine	0.82	1.49	0.88	1.18	1.03
Leucine	1.19	2.92	1.69	2.00	1.66
Lysine	1.83	1.68	1.22	1.87	1.67
Methionine	0.91	1.14	0.97	1.89	2.31
Phenylalanine	0.63	1.02	1.06	1.28	1.14
Proline	1.10	3.31	1.80	3.17	3.09
Serine	5.9	6.69	5.65	7.91	11.47
Taurine	3.87	6.31	5.92	3.75	2.81
Threonine	0.93	2.68	1.59	1.42	1.36
Tryptophan	1.49	1.96	1.86	2.10	1.97
Tyrosine	0.72	1.67	1.04	1.31	1.02
Valine	1.44	2.66	1.72	2.13	1.89
Total	38.61	51.74	39.88	52.92	56.97

*Cubs 3A and 3B were raised by their dam and received only natural tiger milk.
**Cubs 2A and 2B were raised by hand and received milk replacer 2 (MR2).

Amino acid analyses of the milk formulas and natural milk from the dam were performed. The concentration of taurine, arginine, phenylalanine, tryptophan and histidine were very low in the artificial formulas as compared to the dam´s milk.

Intrinsic limitations in this investigation are absence of a controlled environment and availability of these animals to be examined at matched ages in a controlled environment. Nevertheless, taurine, as well as arginine, phenylalanine, tryptophan and histidine deficiency appeared to be possible causes of the development of skin problems, cataracts and strabismus in the tiger cubs fed with these particular artificial milk replacers.

Cataracts associated with nutritional deficiencies in immature animals have been reported in dogs (Vainisi *et al*., 1981; Martin and Chambreau, 1982; Glaze and Blanchard, 1983; Ranz *et al*., 2002), cats (Frankel, 2001), wolves (Vainisi *et al*., 1981), rabbits (Devi *et al.*, 1965), guinea pigs (VonSallman *et al*., 1959), rats (Albanese, 1952; Bagghi, 1959; Bunce *et al*., 1978, 1984; Koch *et al*. 1982), and fish (Poston *et al.*, 1977; Poston and Rumsey, 1983; Richardson *et al*., 1985). In these species, there are different nutritionally related causes involved in cataract development. Amino acid imbalances can alter the physiological milieu of the body's tissues and cells leading to severe pathophysiologic states (Raju *et al*., 2007). Deficiencies of valine, histidine, arginine and phenylalanine amino acids may cause cataracts in dogs, cats and wolves hand-reared on commercial or empirically produced milk replacers (Vainisi *et al*., 1981; Martin and Chambreau, 1982; Glaze and Blanchard, 1983; Frankel, 2001). Tryptophan, phenylalanine and histidine deficiency also were associated with cataract formation in immature rats (Pike, 1951; Albanese, 1952).

Nutritional cataracts resulting from a deficiency of essential amino acids and certain vitamins or an excess of particular carbohydrates, have been observed in different species in the last several decades (Malone *et al*., 1993).

The mechanism of cataract development for most amino acid deficiencies observed in rats was apoptosis of lens epithelial cells due to lowered intracellular pH or to disruption of lysosomes (Wegener *et al*., 2002; Raju *et al*., 2007). Nutritional cataracts caused by artificial milk replacers in large felids are not a rare occurrence in zoos and most experienced zoo workers are at least aware of this possible complication. Paradoxically, there are several anecdotal reports and non-scientific publications on the subject but not very many scientific publications about this apparently common phenomenon.

A report links the use of milk replacers and the occurrence of cataracts in an African lion (Sardari *et al*., 2007). Yet, the exact deficient amino acid involved in the development of cataracts in large felids has not been identified (Magrane and VanDeGrift, 1975; Benirschke *et al*., 1976). Heritable cataracts have not yet been demonstrated in tiger cubs, making amino acid deficiency a likely important cause for cataract development in these animals. Nevertheless, some authors have suggested the occurrence heritable cataracts in lions and clouded leopards (Cooley, 2001). Although these are not the same species, they are large wild felids facing similar breeding challenges in captivity such as low genetic diversity and consequently more chances to demonstrate genetic diseases.

In fact, interesting progresses have been made in the area of heritable cataracts in large felids bred in zoos. For instance the genomic sequences of 4 crystalline genes CRYAA, CRYAB, CRYBB2, and CRYBB1 was analyzed in inbred Angolan lions kept in German zoos. In addition, 10 candidate genes were analyzed using adjacent microsatellites (Philipp *et al*., 2010). As a result, these genes were excluded as responsible for the familial primary cataract in these Angolan lions.

Nutritional cataracts caused by inappropriate formulation of artificial milk formulas were studied in laboratory rats (Sonnenber *et al*., 1982). Twenty percent of rat pups fed an artificial milk substitute developed cataracts. These rat pups also had atypical amino acid levels and low concentrations of several amino acids in the blood. In particular, only traces of taurine were found, (Sonnenber *et al*., 1982) and it was speculated that taurine deficiency leads to the development of cataracts in these rats.

In our investigation, amino acid analysis of the blood showed higher concentration of arginine, glutamic acid, glycine and methionine and a lower level of taurine in the cubs fed MR as compared to those fed natural tigress milk. Higher concentrations of any of these amino acids has not been associated with cataract formation in other animal species; however, since taurine deficiency has been associated with cataract development in other species (Poston *et al*., 1977; Bunce *et al*., 1978, 1984; Sonnenber *et al*., 1982; Poston and Rumsey, 1983; Richardson *et al*., 1985; Raju *et al*., 2007), it is possible that the lower level of taurine is involved in the cataract development in the tiger cubs.

Quantitative analysis of rat eye tissues revealed that taurine was the most abundant amino acid in the retina, vitreous, lens, cornea, iris, and ciliary body (Ripps and Shen, 2012). However, physiological mechanisms mediating the actions of taurine in the eye are not fully known (Ripps and Shen, 2012). Results of an *in vitro* study using intact lenses of rabbits incubated in tissue culture media containing galactose and taurine, and galactose alone, showed that galactose without taurine significantly increased the rate of cataract development compared to galactose with taurine. In this study, taurine appeared to protect the lens against the development of sugar-induced cataracts and may have exerted this effect by decreasing oxidative damage (Malone *et al*., 1993; Rathore and Gupta, 2010). The role of taurine deficiency in cataract development has been studied by a number of other groups (Zhang and Chen, 1998; Son *et al*., 2007; Hsu *et al*., 2012). It is possible that the deficiency of taurine in the cubs receiving the two different artificial milk formulas may have exposed their lenses to an oxidative effect. However, levels of lipid peroxidation products, galactose and lactose in the blood were not measured.

A thorough investigation of retinal function was not performed in the cubs, though taurine deficiency is known to cause retinal degeneration in domestic cats. A single case report of a white Bengal tiger with a central retinal defect compared the affected animal's taurine levels with those of orange Bengal tigers and taurine-deficient domestic cats with resulting central retinal degeneration. Nonetheless, the authors concluded that though the affected white Bengal tiger's taurine was lower, it was not as low as affected domestic cats and thus, the low taurine levels were unlikely to have caused that animal's lesion (Pickett *et al*., 1990).

Although the cubs fed with MR2 and cubs fed with tigress milk revealed lower levels of taurine, the level of serum taurine in affected cubs was similar to that in the tigress. However, the converted tigress´ serum value (309.25µmol/L) was lower than the only previous report of a normal range for Bengal tigers of (320–620 µmol/L) (Hedberg *et al*., 2007). While the domestic cat appears to be an acceptable physiologic model for wild felids, it is often difficult to assess taurine status in zoo feeding programs owing to scattered data on feed taurine content as well as a lack of normal ranges for assessment of taurine in biological tissues (Hedberg *et al*., 2007). Additionally, it is known that individual domestic cats vary greatly in their capacity to regulate taurine metabolism and occasionally do well with diets containing less than ideal taurine levels (Hedberg *et al*., 2007). According to Hedberg *et al*. (2007) one blood sample might not be enough to confirm inadequate taurine levels unless the diet offered is severely deficient, which is the case in the present report.

The characteristics of nutritional cataracts in tiger cubs have not been previously described in the literature. We believe this is because these animals are not frequently examined in zoos until they develop severe ophthalmic changes (like vision loss and/or intense lens opacity). In our cubs fed with milk replacer, the ophthalmic evaluation revealed an initial nuclear opacity of the lens that appeared to progress quickly in a matter of few weeks to involve the cortex during the cataract development. This pattern is very different to that observed in domestic puppies fed with diets deficient in arginine and phenylalanine, which are both essential amino acids. The opacities in this case were described as a nuclear–cortical junction ring with some vacuolization of the equatorial fibers and Y-sutures (Ranz *et al*., 2002). This pattern was not observed in the tiger cubs presently described.

Some authors do not recommend surgery in tigers with nutritional cataracts because they considered these cataracts to be self-limiting (Tilson and Seal, 1988). However, cataract development certainly varies according to the type and intensity of the nutritional imbalance in question. The opacities observed in these cubs did not appear to be self-limiting, as in other descriptions, and instead were unquestionably progressive.

It is important to determine the possible origin of lens opacities when a veterinary ophthalmologist evaluates any animal, but it is especially important for zoo animals because the diagnosis of nutritional cataracts may have a different clinical course compared to those due to metabolic, inflammatory, traumatic, hereditary

or congenital causes. In some zoos, the diagnosis of a hereditary or congenital cataract may result in castration, exclusion from reproduction and even euthanasia (Tilson and Seal, 1988). This is important for the protection of the species by eliminating affected animals from the gene pool (Seitz and Weisse, 1979; Tilson and Seal, 1988).

Cataract surgery might be considered an option to improve the quality of life of affected individuals as in the case reported in a Siberian tiger (Seitz and Weisse, 1979). The tiger cubs that developed cataracts had normal globe positions until they reached 30 days of life. Divergent strabismus was noted after 45 days of age.

In another case report of a white Bengal tiger cub born with convergent strabismus and poor vision, the possible causes considered for strabismus were an adaptation to genetically abnormal visual pathways linked to lack of pigment, abnormalities of the abducens nerve and mechanical restricting conditions of the medial rectus muscles (Bernays and Smith, 1999; Pachaly and Montiani-Ferreira, 2003). In this Bengal tiger, no nutritional causes were speculated as it was born with strabismus.

The occurrence of cataracts and strabismus in the cubs fed with MRs was the main focus of the present study. Nevertheless, transitory alopecia also was a conspicuous clinical sign observed in these animals. Protein and amino acid deficiency is well known to cause loss of body hair in wild animals in captivity (Novak and Meyer, 2009). Although vitamin and mineral imbalances have been postulated as a possible cause of body hair loss (Novak and Meyer, 2009), the nutritional parameters that might regulate hair production in animals and humans were not completely characterized. Moderate to severe zinc deficiency has been associated with alopecia in children (Alhaj *et al*., 2007). Zinc, vitamin D and vitamin A deficiencies are known to cause alopecia in animals and humans (Rushton, 2002; Ginn *et al*., 2007).

Interestingly, deficiency of tryptophan also has been reported to lead to a very rapid formation of cataracts and alopecia in rats (Wegener *et al*., 2002). The amino acid deficiency presented here as the main candidate for the development of cataracts and strabismus, possibly contributed to the occurrence of alopecia as well. Vitamin and mineral levels were not objectively assessed in the cubs reported here. Nevertheless, the authors believe that imbalances of vitamins and minerals also might have contributed to the transient alopecia observed in these animals.

The tiger cubs presently reported were born with normal globe position, and strabismus became evident after other dermatological and ophthalmological clinical signs were also evident. The influence of amino acids in the pathophysiology of the tiger strabismus is still unknown and it must be studied in the future.

Considering nutrition of captive tigers, some zoos formulate diets from basic ingredients so the components are relatively constant. However, nutritional analysis on the finished product is rarely conducted. Diets should be weighed and daily records kept as to how much is offered to each individual tiger and how much is consumed. It is possible that the tiger cubs consumed a different quantity of milk replacer than the quantity calculated for the size of the cub. Because of this, it is difficult to say if in the early stages of growth, the quantity of amino acids absorbed by the tiger cubs were the same as those measured in the amino acid analysis at 60 days of age.

In the literature, there is no amino acid requirement information for growing cubs or even adult tigers. Even though taurine deficiency was believed to be involved in the cataract formation in these cubs, it is possible that altered levels of other amino acids, vitamins and even fat, carbohydrates or protein, may have contributed to the pathophysiology as well. In fact, a previous evaluation of two milk replacers fed to hand-reared cheetah cubs indicated that both formulas were low in the majority of essential amino acids compared with domestic cat maternal milk (Bell *et al*., 2011).

The difficulty in evaluating wild animals, particularly in large felids, during lactation, the quantity of milk samples to collect and the high costs of a complete milk analysis are factors that understandably inhibit investigations such as this. Limitations of our study include a small number of animals investigated and the analysis of a milk sample from only one tigress. However, it can be used in the future as a general guideline, reference and as a catalyst for other nutritional investigations with tigress milk and artificial formulations, including other tests like fat, carbohydrate and protein analysis.

Taurine deficiency appeared to be a possible cause of the development of cataracts in the tiger cubs fed with these particular artificial milk replacers. In the future, special attention should be given in order to make sure that adequate levels of taurine, arginine, phenylalanine, tryptophan and histidine are present in artificial milk for tiger cubs.

We believe that the present report provides important information that could help zoo veterinarians prevent the development of nutritional cataracts in neonatal tiger cubs.

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