A COMPARATIVE STUDY OF BLOOD LIPIDS, ELECTROLYTES AND STRESS LEVELS BETWEEN CAPTIVE AND SEMI-CAPTIVE ORANGUTANS (*PONGO PYGMAEUS*) UNDER TROPICAL CONDITIONS

Choy Foon Seng,* Goh Yong Meng, DVM., PhD, and Rasedee Abdullah, MSc, PhD

Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400, Serdang, Selangor Darul Ehsan, Malaysia.

Abstract

Blood samples were collected from 27 orangutans (*Pongo pygmaeus*) (14 captive and 13 semi-captive) and were analyzed for triglycerides, total cholesterol, high density lipoprotein cholesterol (HDLC), low density lipoprotein cholesterol (LDLC), sodium, potassium, chloride, calcium, phosphate, glucose, cortisol levels, total white blood cells (WBC) and WBC differential count. There were significant differences ($P<0.05$) in total cholesterol and HDLC level between groups where semi-captive males had the highest level while the lowest were the captive male orangutans. When the blood lipid values were compared across age groups, the triglyceride, total cholesterol and HDLC levels of adult animals differ significantly ($P<0.05$) from the younger age groups. As for the blood electrolytes, there were significant differences ($P<0.05$) in sodium, potassium, chloride and calcium levels between captive and semi-captive orangutans. Finally, captive orangutans had higher ($P<0.05$) serum cortisol level and neutrophil:lymphocyte ratio as compared to semi-captive orangutans suggesting that captive orangutans are in more stressful states. Based on the results, captive orangutans tend to have "less" desirable blood lipids and are "potentially" more stressful than semi-captive orangutans. In this study, it is evident that nutrition plays a sizable role in determining the blood lipids and electrolyte levels. Other factors that affect blood lipids are gender and age. As for the stress levels, it was postulated that the lack of stimulating environment and enrichment programs in captive environment, may have lead to the increase in stress indicators.

Keywords: Orangutan, *Pongo pygmaeus*, lipid, cholesterol, electrolytes, stress

Introduction

The major challenge that is faced by keeping any animal in captivity is to simulate a diet and habitat that is as natural as possible. However, limited knowledge of the physiology and natural diet of the orangutan has led to the feeding of a diet that is quantitatively and qualitatively different from their natural diet.[10] Many captive orangutans have been fed an urbanized diet that is typically high in fat, sodium and carbohydrate.[11] In addition, captive orangutans are also subjected to an unenriched and non-stimulating environment that may result in stressful states. Consequently, many captive orangutans end up being overweight and suffer from excessive boredom. Obesity, hypercholestremia, high density lipoprotein cholesterol (HDLC)/ low density lipoprotein cholesterol (LDLC) imbalance and chronic stress are among the risk factors for atherosclerosis, coronary heart disease, hypertension, diabetes mellitus and myocardial infarction that have been identified in human populations.[12] However, these risk factors have never been studied or identified in orangutans. Therefore, this study was conducted to assess and compare

the blood lipid profile, electrolytes and stress levels of captive and semi-captive orangutans as well as to contribute towards the database of the measured parameters in captive orangutans kept under tropical conditions.

Methods

Animals & Blood Collection

A total of 27 Bornean orangutans (*Pongo pygmaeus*) of various sex and age were included in this study, 14 animals sampled from captive location (National Zoo of Malaysia & Sabah Zoological and Botanical Garden, Malaysia) and 13 animals from a semi-captive location (Sepilok Orangutan Rehabilitation Center, Malaysia). General anaesthesia was induced by using tiletamine and zolazepam/ Zoletil 100® (Virbac Laboratories, NSW, Australia) at the dosage of 3 to 5 mg/kg intramuscularly via hand syringe, blowpipe or air-pressured pistols. Following immobilization, a physical examination was performed to ensure the good health of the animal. 10 ml of blood was collected from either the brachial, cephalic or femoral vein and 3 ml of blood was placed in EDTA (Vacutainer® K_2 EDTA, Becton-Dickinson & Co, Franklin Lakes, NJ, USA) and the remaining 7 ml in serum separator tubes (SST™, Becton-Dickinson & Co, Franklin Lakes, NJ, USA), stored at 4°C until processed within 24 hours. Serum was obtained after centrifuging blood samples at 5000 G for 5 minutes. The serum samples were maintained at -20°C in 1.5 ml microcentrifuge tubes (Eppendorf®, Hamburg, Germany) for subsequent serum biochemistry and cortisol assay.

Hematology and Serum Biochemistry

Total White Blood Cells (WBC) were manually quantified using haemocytometer techniques. Thin blood films were also made, air dried and stained with Wright's stain (Sigma®, Sigma-Aldrich Corporation, MO, USA). WBC differential count was performed after Wright staining. The neutrophil:lymphocyte (N:L) ratio was calculated from the differential counts. Serum biochemistry results were obtained by processing the thawed frozen serum on an automated analyzer (Hitachi® 902, Roche Diagnostics, GmBH, Germany). The parameters that were analyzed included total cholesterol, triglyceride, HDLC, LDLC, sodium, potassium, chloride, calcium and phosphate. Serum cortisol was measured using a cortisol test kit (Coat-A-Count®, Diagnostic Product Corporation, Los Angeles). Glucose was measured using a portable glucometer immediately after the blood samples were collected.

Statistics

Differences in blood lipid values were compared across management status, gender and ages using one way ANOVA procedure and differences in stress indicators were compared using two tailed t-test. When significant, means were separated using Duncan's New Multiple Range test. Correlations of lipid values with age were investigated using Spearman's rank correlation coefficient. All statistical analyses were performed using SPSS version 14.0 with a 95% confidence level.

Results

Lipid

The blood lipid values of orangutans segregated according to management system and sex are shown in Table 1. There were no significant interactions between sex and captivity status for all the blood lipid parameters observed in this study ($P<0.05$). There were significant differences ($P<0.05$) in total cholesterol and HDLC levels between groups with semi-captive males had the highest level and those with the least were the captive male orangutans. When the blood lipid values were compared across age groups, the triglyceride was found to be significantly higher ($P<0.05$) while the total cholesterol and HDLC levels were significantly lower ($P<0.05$) in adult orangutans. In fact, there was a strong correlation of triglyceride that increases with age of the animal (Pearson's correlation = 0.493, P = 0.009). A trend was seen for the blood lipid profiles of male and female orangutans. Male orangutans had higher levels of triglyceride, total cholesterol, LDLC and Cholesterol/HDLC ratio compared to female orangutans.

Electrolytes

Blood electrolytes for orangutans based on management system are shown in Table 2. There were no significant interactions between sex and captivity status for all the electrolyte parameters observed in this study ($P<0.05$). The sodium and chloride level for semi-captive orangutans was lower than the captive orangutans where the semi-captive male orangutans were significantly different ($P<0.05$) than the female captive orangutans. The male captive orangutans had the highest potassium level followed by female captive, male semi-captive and female semi-captive. All of them were significantly different ($P<0.05$). As for the calcium level, the female semi-captive had the lowest levels and was significantly different from the rest of the groups. There were no differences for phosphate level.

Stress Levels

Captive orangutans had significantly higher ($P<0.05$) levels of serum cortisol and neutrophil: lymphocyte ratio (N:L ratio) as compared to semi-captive orangutans (Table 3). The serum cortisol level of captive orangutans was almost two times greater than semi-captive specimens. However, there were no differences for glucose and cholesterol levels.

Discussion

Lipids

The differences of the lipid profile for the captive and semi-captive orangutans may be due to the differences in nutritional management. One of the captive locations fed the animal high carbohydrate and triglyceride food items such as coconut, cocoa bread, milk and sugar cane. Carbohydrates tend to raise serum triglyceride and decrease LDLC levels especially if it comprises at least 60% of the total calories.[5] The cocoa bread and milk contain butter and milk fat which can potentially elevate the triglyceride and cholesterol levels. Furthermore, coconut is a major source of myristic and lauric acid, both potent cholesterol-raising fatty acids.[4] Only bananas and milk are offered as a supplementary feed at the semi-captive location and the amounts offered were designed to not fulfill daily caloric requirements. This would promote more foraging activities and help achieve the objectives of the rehabilitation program.

Semi-captive orangutans have easier access to food items that are high in dietary and structural fibers. There is a strong inverse correlation between dietary fiber and coronary heart disease. Soluble fibers such as β-glucan (found in oats, barley and yeast), psyllium (found in husks of blonde psyllium seed) and pectin (found in fruits) have been demonstrated to effectively lower blood cholesterol levels.[5] When compared across age groups, older orangutans have higher triglycerides, LDLC and Cholesterol/HDLC ratio and lower HDLC/LDLC ratio. It is believed that the age of the patient reflects the cumulative exposure to atherogenic risk factors throughout its life.[12]

Electrolytes

All blood electrolyte values of the captive orangutans in this study were similar to the ISIS[6] reference values, obtained mostly from orangutans in captivity. The values of captive orangutans in this study were somewhat lower than the captive orangutan in the study by Crissey.[3] These differences could be associated with diet variation and the frequency of mineral supplementation at both locations. Due to the absence of mineral supplementation to semi-captive and wild orangutans, these animals tend to have lower blood electrolyte levels as seen in this study and Kilbourn.[7] None of the semi-captive orangutans manifested obvious signs of diseases associated with mineral deficiency, therefore this study may suggest that by eating a balanced diet based on food items found in the forest, mineral supplementation may not be necessary.

Stress Levels

Responses to stressors often involve changes in physiologic function (biochemical, neuroendocrine, metabolic), immunity, reproductive, histopathology, psychological state, behavior and toxicology status.[1,8] In this study, only endocrine (cortisol), immunology (N:L ratio) and to a certain extent, metabolic (glucose and cholesterol) and behavioral parameters were measured and evaluated. However, behavioral changes were only subjectively accessed by comparing the natural behavior between semi-captive and captive orangutans and therefore were not conclusive in this study. One of the factors that may have elevated the stress indicators in captive orangutans is the lack of stimulating environment and activities. By nature, orangutan's feet are adapted to life in trees than on the ground.[2] Many zoo managements fail to provide orangutans with sufficient vertical space and structures that allow arboreal locomotion. In addition, captivity has removed much of the complexity and seasonality of food sources. Without understanding the importance of seasonal availability of food in the feeding ecology of an animal, captive animals are typically fed discrete meals at predictable times, year in and year out.[9] The influence of a diet that differs dramatically from a more natural diet in terms of quantity and quality on the stress levels of the animals has yet to be investigated.

Conclusion

In conclusion, captive orangutans had "less" desirable blood lipids and are "potentially" more stressed than semi-captive orangutans. This study suggests that nutrition plays a role in determining blood lipid and electrolyte levels. Other factors that affect blood lipids are gender and age. It is postulated that the lack of a stimulating environment and the limited enrichment programs may have lead to the increased stress indicators seen among captive orangutans in this study.

LITERATURE CITED

1. Balm, P.H.M. 1999. Preface In Stress Physiology in Animals. England: Sheffield Academic Press.
2. Benneth, E.L. 2002. The Natural History of Orang-utan, 2nd Ed. Borneo: Natural History Publication.
3. Crissey, D.S., K.A. Slifka, J.E. Barr, P.E. Bowen, M. Stacewics-Sapuntzakis, C. Langman, A. Ward, G. Meerdink, and A. Kimberly. 2000. Circulating Nutrition Parameters in Captive Apes at Four Zoos In The Apes: Challenges for the 21st Century Conference.
4. Grundy, S.M. and M.A. Denke. 1990. Dietary influences on serum lipids and lipoproteins. Journal of Lipid Research 31: 1149-1172.
5. Hilpert, K.F., A.E. Griel, T. Psota, S. Gebauer, Y. Coa, and P.M. Kris-Etherton. 2006. New Insights on the Role of Lipids and Lipoproteins In Cardiovascular Disease: The Modulating Effects of Nutrition *In* Lipid Metabolism and Health, Moffatt R.J and Stamford B (Eds), 1st Ed. London: Taylor and Francis Group, pp 211-264.
6. International Species Information System (2002). Physiological Reference Value for *Pongo pygmaeus*. International Species Information System, Apple Valley, Minnesota.
7. Kilbourn, A.M, W.B. Karesh, N.D. Wolfe, E.J. Bosi, R.A. Cook, and M. Andau. 2003. Health Evaluation of Free-Ranging and Semi-Captive Orangutans (*Pongo pygmaeus pygmaeus*) In Sabah, Malaysia. Journal of Wildlife Diseases, 39(1): 73-83.
8. National Research Council. 1992. Recognition and Alleviation of Pain and Distress in Laboratory Animals. Washington D.C. National Academy Press.
9. Oftedal, O.T. and M.E. Allen M.E. 1996. Nutrition and Dietary Evaluation in Zoos In Wild Mammals in Captivity: Principles and Techniques, Kleiman D.G, Allen M.E, Thompson K.V and Lumpkin S (Eds), 1st Ed. Chicago:The University of Chicago Press. pp 109-128.
10. Robbins, C.T. 1993. Wildlife Feeding and Nutrition, 2nd Ed. Orlando. Academic Press Inc.
11. Schmidt, D. 2004. Orangutan Husbandry Manual: Nutrition Chapter. URL: www.nagonline.net/orangutan.htm. Accessed on 20th October 2006.
12. Third Report of the National Cholesterol Education Program (NCEP). 2002. Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Circulation 106: 3143-3422.

Table 1. Blood lipid values of orangutans based on management system and sex. (All values are expressed as mean ± SEM).

Blood Lipid Values	Captive		Semi-captive	
	Male (n=4)	Female (n=10)	Male (n=9)	Female (n=4)
Triglyceride				
mmol/L	1.70 ± 0.36	1.01 ± 0.22	0.92 ± 0.09	0.84 ± 0.31
(mg/dL)	(150.40 ± 31.76)	(89.40 ± 19.08)	(81.81 ± 8.37)	(74.20 ± 27.27)
Total Cholesterol				
mmol/L	4.58 ± 0.50[a]	5.16 ± 0.28[ab]	5.69 ± 0.24[b]	4.74 ± 0.19[ab]
(mg/dL)	(177.15 ± 19.41)	(199.50 ± 10.67)	(220.12 ± 9.28)	(183.44 ± 7.37)
HDLC				
mmol/L	1.38 ± 0.10[a]	1.95 ± 0.17[ab]	2.16 ± 0.15[b]	1.74 ± 0.25[ab]
(mg/dL)	(53.50 ± 3.73)	(75.50 ± 6.56)	(83.46 ± 5.95)	(67.14 ± 9.70)
LDLC				
mmol/L	2.65 ± 0.45	2.75 ± 0.19	3.08 ± 0.24	2.59 ± 0.19
(mg/dL)	(102.56 ± 17.31)	(101.46 ± 7.19)	(119.07 ± 9.20)	(100.33 ± 7.36)
HDLC/LDLC ratio	0.57	0.72	0.75	0.70
Cholesterol/HDLC ratio	3.31	2.73	2.72	2.89

[a,b] Means in the same row with different superscripts are significantly different ($P<0.05$)

Table 2. Blood electrolytes level of orangutans based on management system and sex. (All values are expressed as mean ± SEM).

Electrolytes	Captive		Semi-captive	
	Male (n=4)	Female (n=9)	Male (n=10)	Female (n=4)
Sodium Mmol/ L	139.60 ± 0.67[ab]	140.22 ± 0.62[a]	137.01 ± 2.08[b]	137.20 ± 1.30[a]
Potassium Mmol/ L	5.10 ± 0.36[a]	4.45 ± 0.11[b]	4.09 ± 0.07[b]	3.48 ± 0.40[c]
Chloride Mmol/ L	98.35 ± 1.49[ab]	101.28 ± 0.89[a]	97.97 ± 0.57[b]	99.93 ± 1.39[ab]
Calcium Mmol/ L	2.37 ± 0.03[a]	2.38 ± 0.03[a]	2.25 ± 0.03[ab]	2.23 ± 0.08[b]
Phosphate mmol/ L	1.38 ± 0.16	1.48 ± 0.07	1.69 ± 0.07	1.33 ± 0.22

[a,b,c] Means in the same row with different superscript are significantly different (P<0.05).

Table 3. Stress indicators of orangutans based on management system. (All values are expressed as mean ± SEM).

Stress Indicators	Captive (n=14)	Semi-captive (n=11)
Serum Cortisol µg/ dL	11.60 ± 1.62[a]	6.93 ± 0.94[b]
Neutrophil: Lymphocyte ratio	1.71 ± 0.24[a]	1.08 ± 0.18[b]
Glucose mmol/ L	5.26 ± 0.24	5.15 ± 0.19

[a,b] Means in the same row with different superscripts are significantly different (P<0.05).