Effects of Large Volume Crocodile Blood Collection on Hematological Values of Siamese Crocodiles (*Crocodylus siamensis*)

Win Chaeychomsri[1], Sirilak Yamkong[2], Sudawan Chaeychomsri[3], and Jindawan Siruntawineti[1]
[1]Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
[2]Graduate School, Kasetsart University, Bangkok 10900, Thailand
[3]Central Laboratory and Greenhouse Complex, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand
Email: {fsciwcc, rdisuc, fscijws}@ku.ac.th, plathong.love@hotmail.com

***Abstract*—Freeze-dried blood from *Crocodylus siamensis* is a natural product which can serve as food supplement. The present study aimed to develop the process for a large volume blood collection without killing animals in order to extend their life and keep them healthy. The process was performed from 20 captive breeding crocodiles. They were randomly divided into control and 3 experimental groups. Ten milliliters of blood were collected weekly from control group for 12 weeks. Group 1, samples were withdrawn 150 ml on week 1, 12 and collected 10 ml weekly on week 2-11. Group 2, samples were withdrawn 150 ml on week 1, 12 and collected 10 ml on week 4 and 8. Group 3, samples were withdrawn 150 ml on week 1 and 12. The results showed that there were no significant differences (p<0.05) in hematological values among the blood samples taken from group 1, 2, 3 and those of the control group at all time intervals. The results of hematological values as well as the results obtained from the behavioral observation revealed that large volume blood collection up to 150 ml had no detrimental effect on crocodile health and behavior. The results from the present study offer a possible alternative to conventional way for commercial crocodile blood collection.**

***Index Terms*—crocodile blood, *Crocodylus siamensis*, Siamese crocodile, hematological values**

I. Introduction

Freeze-dried Crocodile Blood (FDB) is a natural product which can serve as an alternative medicine for curing illness such as allergy and asthma, and also for prolonging the life of the patients. It has been widely consumed not only for its nutritious composition, but also for its claimed medicinal value [1]-[3]. The practice of consuming crocodile blood for improving human health is found in many Asian cultures and traditions. Recently, the FDB production process has been developed and the safety for crocodile blood consumption has been reported in rat [1]. FDB consumption has increased and the market for FDB is growing rapidly in many countries. In order to serve this market properly, the production facilities should be developed.

For collecting a large volume of blood, the blood is usually collected from healthy crocodiles at the slaughterhouse before they are killed. However, the amount of blood taken by this method is not enough to supply the markets that will be opened to the new products of FDB. Therefore, the blood collection process that maintain a crocodile's life has been developed [4], [5]. It is known that large volume blood collection from animals may induce risk to donor's welfare and health [6]-[8]. The presence and severity of signs and symptoms are related to the amount of blood loss. Therefore, the purpose of this study was to evaluate the effects of blood collection on the health status of the crocodile blood donors and the quality of the products. This study will pave the way for developing the necessary guidelines for crocodile blood collection to produce at the appropriate quantity and time, to ensure product quality, and also safety of the animals.

II. Materials and Methods

A. Crocodile Samples

Twenty captive Siamese crocodiles (*C. siamensis*) used in this study were obtained from Rungtaweechai Crocodile Farm, Nakhon Pathom Province, Thailand. These crocodiles were 4-5 years of age, with an average weight 27kg, and 191 cm in length. The crocodiles of both sexes were randomly divided into control group and 3 experimental groups according to duration of time for hematological investigations. Each group contained 5 crocodiles (n=5) and retained in a single pond.

B. Blood Collection

The crocodiles were immobilized by 220 volts of electricity for 3 sec, and then snared with a catapult. Blood samples were collected from the supravertebral vein. Equipments needed for blood collection were dependent on the volume of blood taken at each time point. These include 21-gauge needle and 10ml syringe for 10 ml of blood collection, needle [9] and peristaltic pump connected to a sterile receiving bottle for 150 ml of

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blood collection. Ten milliliters of blood were collected from control group every week for 12 weeks. Experimental group 1, blood samples were withdrawn 150ml on week 1 and 12 and collected 10 ml weekly on week 2-11. Experimental group 2, blood samples were withdrawn 150 ml on week 1 and 12 and collected 10ml on week 4 and 8. Experimental group 3, blood samples were withdrawn 150ml on week 1 and 12. Two milliliters of blood was immediately placed into the tube containing EDTA anticoagulant, well mixed, stored at 4℃ and subjected to hematological analysis.

C. Temperature and Relative Humidity Measurement

Temperature and relative humidity of crocodile captive pond were recorded daily because these factors can potentially affect hematological values of crocodile blood.

D. Behavioral Observation

Crocodile behaviors including feeding, basking, diving as well as social behaviors were observed after blood collection. The food intake of crocodile was determined and recorded weekly.

E. Hematological Measurement

The hematological tests including total cell counts, packed cell volume or hematocrit (Hct), and hemoglobin (Hb) concentration were performed. Total cell counts providing the number of Red Blood Cell (RBC), White Blood Cell (WBC), and thrombocyte were determined by using hemocytometer. Hct was determined by using microhematocrit centrifugation. Hb concentration was measured by using the cyanmethemoglobin method. In addition, red blood cell indices as Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated by using established formulas.

F. Statistical Analysis

The results were expressed as mean ± SE. The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). P values <0.05 were considered statistically significant.

G. Ethical Considerations

The experimental process in animals has been approved by the local animal ethics committee at Kasetsart University, following the Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes [10].

III. Results and Discussion

A. Effect on Hematological Values

Effects of blood collection volume up to 150 ml on the hematological variables of crocodile blood were determined over a period of 12 weeks by a complete blood count. The comparison of hematological values between the experimental groups and those of the control group is shown in Table I. Mostly, there were no significant differences in hematological values between the control and experimental groups after the first blood collection (time zero) until 12 weeks ($p<0.05$). Significant difference in the values of RBC count and Hb between the control and experimental groups was observed at week 4 after time zero while significant difference in the Hct value was found at weeks 1 and 12 after time zero ($p<0.05$). The values of MCV and MCH in the control and experimental groups were not significantly different over a period of 12 weeks ($p<0.05$) whereas the MCHC value had significant difference between the control and experimental groups at weeks 4, 7 and 9 after time zero ($p<0.05$).

B. Environmental Information

The environmental information including the temperature and relative humidity of crocodile captive ponds at weeks 1 to 3 was lower than the later weeks. These values were obviously increased in week 4, compared to those of the previous weeks (Fig. 1).

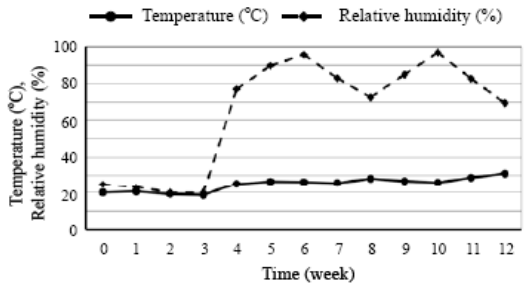

Figure 1. Temperature and relative humidity of captive pond during the experimental period.

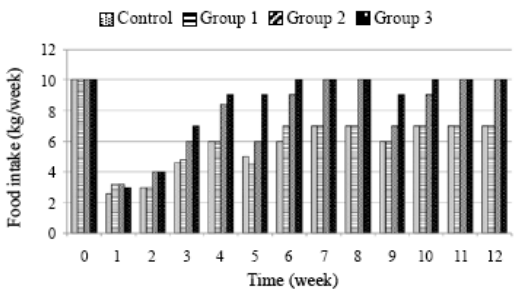

Figure 2. Food intake monitoring in crocodiles during the experimental period.

C. Behavioral Information

The results obtained from the food intake monitoring of crocodiles after blood collection are presented in Fig. 2. Crocodiles in the control and 3 experimental groups scarcely ate the food after the week 1, and this behavior remained in crocodiles of the control group and the experimental group 1 until in the week 3. The increasing of food consumption by these crocodiles was observed from weeks 4 to 12. However, the intake was still less than the intake of crocodiles of the experimental groups 2 and 3. While the crocodiles of the experimental groups 2 and 3 initially ate only a small amount of food, then gradually increased their intake from week 2 and reached the normal level at week 6 (Fig. 2).

In the present study, the technique for collecting large blood volumes from crocodiles without killing animals

has been established. The results of this study showed that there were no statistically significant differences ($p<0.05$) in hematological values between the crocodiles in the experimental groups and those of the control group during the experimental period for 12 weeks. Although, there were significant differences in some of the hematological values between groups reported in this study (Table I), the data were within the normal ranges for healthy animals [11]. Thus, though some of the statistically significant differences were identified, biological significance of these results probably was inconsequential.

Interestingly, the results of the present study demonstrated that there were no significant changes in RBC count, Hb, MCV, MCH, and MCHC values between the control and experimental group 1 in week 1 after time zero. These data indicated that the withdrawal of 150ml of crocodile blood or approximately 25% of the total blood volume had no effect on the health status of these crocodiles. They were able to replenish their erythrocytes as well as hemoglobin in erythrocyte rapidly and their RBC count had returned to normal levels within a week. Limit volumes and recovery periods after blood collection are dependent on animal species. Effects of blood loss on animals and humans were documented [12]. With approximately 20% or less of the total blood volume, can be safely harvested each time from the blood donor. In horses, the removal of approximately 20% of the total blood volume has a marked adverse effect on RBC count, Hb and Hct during the first week after blood collection, followed by a gradual increase in values and returned to values comparable to those measured at time zero within 3 weeks [13]. Therefore, references to horse studies a recovery period of 3 weeks after blood collection is recommended. In dogs, a recommendation for good laboratory practice is to limit total blood collection in dogs to no more than 10% of their blood volume [12]. However, removal of as much as 15% of the total blood volume can be acceptable in laboratory situations [14]. Therefore, crocodiles are more tolerant of larger volumes of blood removal and up to 25% of total blood volume can be collected. They appear to survive and recover from blood loss more rapidly than other animals.

TABLE I. COMPARISON OF HEMATOLOGICAL VALUES OF THE CROCODILES IN THE EXPERIMENTAL GROUPS AND THOSE OF THE CONTROL GROUP

Variables	Group	Time (week)/Values												
		0	1	2	3	4	5	6	7	8	9	10	11	12
RBC count ($\times10^6/mm^3$)	Control	1.03 ± 0.04	0.91 ± 0.11	1.05 ± 0.13	0.84 ± 0.08	1.10 ± 0.06[a]	1.19 ± 0.85	0.86 ± 0.08	1.15 ± 0.15	1.03 ± 0.08	0.95 ± 0.07	0.99 ± 0.09	0.95 ± 0.09	1.03 ± 0.07
	Gr 1	1.03 ± 0.04	0.91 ± 0.08	1.01 ± 0.13	0.90 ± 0.09	1.44 ± 0.09[b]	1.25 ± 0.25	0.94 ± 0.09	1.02 ± 0.10	1.67 ± 0.11	0.99 ± 0.09	1.11 ± 0.10	0.83 ± 0.06	1.24 ± 0.14
	Gr 2	1.03 ± 0.04	ND	ND	ND	1.13 ± 0.09[c]	ND	ND	ND	0.94 ± 0.11	ND	ND	ND	1.13 ± 0.06
	Gr 3	1.03 ± 0.04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.38 ± 0.22
WBC count ($\times10^3/mm^3$)	Control	6.40 ± 0.38	5.35 ± 1.06	5.60 ± 1.03	5.43 ± 1.04	6.00 ± 0.64[a]	6.96 ± 1.00	6.35 ± 1.26	6.79 ± 0.84	6.04 ± 0.67	6.50 ± 1.08	6.53 ± 1.07	6.48 ± 0.73	5.63 ± 0.93
	Gr 1	6.40 ± 0.38	8.35 ± 0.85	7.67 ± 0.78	7.48 ± 0.63	8.33 ± 0.65[b]	8.15 ± 1.29	6.43 ± 0.34	6.74 ± 0.83	6.18 ± 0.11	6.58 ± 0.90	5.97 ± 0.37	6.14 ± 0.62	7.85 ± 0.39
	Gr 2	6.40 ± 0.38	ND	ND	ND	4.84 ± 0.17[c]	ND	ND	ND	5.61 ± 1.38	ND	ND	ND	7.81 ± 0.92
	Gr 3	6.40 ± 0.38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	9.29 ± 1.15
Thrombocyte count ($\times10^4/mm^3$)	Control	2.90 ± 0.18	2.43 ± 0.16[a]	3.44 ± 0.31	3.33 ± 0.32	3.08 ± 0.33	4.12 ± 0.28	3.00 ± 0.46	3.20 ± 0.52	3.34 ± 0.16	3.32 ± 0.32	3.66 ± 0.20	3.61 ± 0.32	3.23 ± 0.16
	Gr 1	2.90 ± 0.18	3.44 ± 0.37[b]	3.68 ± 0.42	3.78 ± 0.33	3.97 ± 0.55	4.26 ± 0.76	3.56 ± 0.15	3.79 ± 0.16	4.42 ± 0.16	3.78 ± 0.63	4.68 ± 0.45	3.10 ± 0.04	3.85 ± 0.26[a]
	Gr 2	2.90 ± 0.18	ND	ND	ND	2.68 ± 0.45	ND	ND	ND	3.85 ± 0.65	ND	ND	ND	2.70 ± 0.28[b]
	Gr 3	2.90 ± 0.18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.01 ± 0.26[c]
Hb (g/dl)	Control	7.00 ± 0.33	6.33 ± 0.37	7.40 ± 0.49	7.30 ± 0.70	8.12 ± 0.45[a]	8.01 ± 0.48	7.21 ± 0.72	8.71 ± 0.75	8.20 ± 0.65	7.61 ± 0.45	7.68 ± 0.51	7.38 ± 0.40	7.65 ± 0.44
	Gr 1	7.00 ± 0.33	6.78 ± 0.25	7.54 ± 0.26	7.40 ± 0.58	8.36 ± 0.36[b]	7.69 ± 0.53	7.53 ± 0.37	8.29 ± 0.55	8.78 ± 0.37	7.78 ± 0.21	8.82 ± 0.60	7.34 ± 0.94	9.29 ± 0.50

	Gr 2	7.00 ± 0.33	ND	ND	ND	6.36 ± 0.66 [c]	ND	ND	ND	7.36 ± 0.84	ND	ND	ND	8.29 ± 0.54
	Gr 3	7.00 ± 0.33	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	9.98 ± 0.76
Hct (%)	Control	21.65 ± 0.71	17.20 ± 0.74 [a]	20.00 ± 1.38	18.00 ± 1.64	19.40 ± 1.03	19.00 ± 1.14	16.60 ± 1.78	19.80 ± 1.66	18.60 ± 1.50	17.60 ± 0.93	18.00 ± 1.45	18.20 ± 1.02	18.00 ± 1.48 [a]
	Gr 1	21.65 ± 0.71	19.60 ± 0.68 [b]	21.20 ± 1.07	18.80 ± 1.28	20.60 ± 1.21	19.20 ± 1.02	18.40 ± 1.21	20.20 ± 1.77	20.80 ± 0.97	19.20 ± 0.37	20.60 ± 0.93	18.00 ± 2.27	22.50 ± 0.87
	Gr 2	21.65 ± 0.71	ND	ND	ND	17.40 ± 1.86	ND	ND	ND	17.20 ± 1.59	ND	ND	ND	20.80 ± 1.24 [b]
	Gr 3	21.65 ± 0.71	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	25.40 ± 1.97 [c]
MCV (fl)	Control	214.25 ± 7.74	199.63 ± 20.83	198.02 ± 15.10	216.85 ± 14.88	177.17 ± 11.08	161.05 ± 10.54	193.83 ± 11.65	176.52 ± 10.78	185.15 ± 19.89	187.84 ± 7.89	185.23 ± 14.45	196.38 ± 12.27	174.14 ± 7.93
	Gr 1	214.25 ± 7.74	221.94 ± 19.69	224.18 ± 28.37	213.26 ± 13.97	145.80 ± 12.64	168.65 ± 20.47	201.25 ± 16.49	199.34 ± 9.02	181.59 ± 9.03	199.41 ± 17.83	189.91 ± 12.37	214.60 ± 15.58	188.57 ± 20.14
	Gr 2	214.25 ± 7.74	ND	ND	ND	154.51 ± 13.20	ND	ND	ND	184.65 ± 8.29	ND	ND	ND	184.61 ± 10.04
	Gr 3	214.25 ± 7.74	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	194.18 ± 16.71
MCH (pg)	Control	69.73 ± 4.15	72.44 ± 5.90	73.39 ± 5.72	88.74 ± 8.05	74.39 ± 5.58	68.07 ± 5.22	84.96 ± 6.01	77.48 ± 4.00	81.60 ± 8.93	81.12 ± 3.72	79.76 ± 5.31	81.12 ± 3.72	74.71 ± 3.88
	Gr 1	69.73 ± 4.15	76.76 ± 6.87	80.40 ± 11.13	83.76 ± 5.73	59.44 ± 5.47	67.25 ± 7.77	82.85 ± 7.48	82.52 ± 5.07	76.90 ± 4.68	80.62 ± 6.80	87.13 ± 6.47	81.12 ± 3.72	78.89 ± 12.18
	Gr 2	69.73 ± 4.15	ND	ND	ND	57.04 ± 6.16	ND	ND	ND	78.45 ± 4.66	ND	ND	ND	73.35 ± 3.22
	Gr 3	69.73 ± 4.15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	76.23 ± 6.57
MCHC (g/dl)	Control	32.19 ± 1.02	36.72 ± 1.11	37.04 ± 0.51	40.63 ± 1.43	41.94 ± 1.44	42.19 ± 0.90	43.76 ± 1.26	44.00 ± 0.50 [a]	44.17 ± 1.29	43.16 ± 0.41 [a]	40.58 ± 0.53	43.16 ± 0.41	42.95 ± 1.51
	Gr 1	32.19 ± 1.02	34.58 ± 0.59	35.68 ± 0.87	39.32 ± 1.12	40.82 ± 1.57	39.97 ± 1.25	41.09 ± 0.84	41.31 ± 0.94 [b]	42.29 ± 0.80	40.52 ± 0.62 [b]	40.72 ± 2.07	43.16 ± 0.41	41.34 ± 2.06
	Gr 2	32.19 ± 1.02	ND	ND	ND	36.69 ± 1.34	ND	ND	ND	42.46 ± 1.47	ND	ND	ND	39.90 ± 1.36
	Gr 3	32.19 ± 1.02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	39.29 ± 0.77

Gr = Group. ND = Non-detected. Values were indicated as mean ± SE. Different letters indicate significant differences ($p<0.05$).
Reference ranges [11]: RBC count = 0.36-2.20 $\times 10^6/mm^3$, Hct = 15.0-29.0%, Hb = 3.9-14.7 g/dl.

The results of this study showed that there was no significant difference in value of RBC count between the experimental group 1 and control group in week 1 after blood removal. However, the Hct value of the experimental group 1 as shown in Table I was significantly higher than that of the control group. In general, Hct measurements are affected by the number of RBCs and their volume (mean corpuscular volume or MCV). The Hct will rise when the number of red blood cells increases. The observations from this study demonstrated that values for MCV had no significant difference in the experimental group 1 and control group in week 1 after time zero. These results implied that more reticulocytes might be present in the circulating blood of experimental group 1 than there were in the circulating blood of control group. Reticulocytes can be clearly distinguished from other red cells by microscopic blood film examination. Normally, reticulocytes are larger than erythrocyte. [15]-[17]. A high reticulocyte percentage reflects a marrow that is attempting to compensate for the loss of red blood cells [18]-[20]. However, a few reticulocytes were found on the blood film from crocodiles in the experimental group 1. Taken together, these results suggest that large volumes of blood can be obtained from crocodile without adverse clinical effects such as anemia to them. The crocodiles may be able to

produce and replace red blood cells fast enough when a large amount of blood is lost.

Besides in the week 1, the interesting results were obtained from the experiment in week 4 after time zero. There was concurrent increase in almost hematological variables, temperature and relative humidity in sample ponds, comparing these values to those obtained from the previous weeks. Since crocodiles are exothermic reptiles, unable to maintain a constant internal body temperature independently of the environment [21], they can easily adapt to different changes in their environment. The results of the present study showed that the increased values of almost hematological variables simultaneously occurred with the rise in temperature of the captive pond. According to the reports on thermoregulation in crocodiles and alligators [22]-[26], the growth rate of juvenile crocodiles is closely related to the temperature at which they are kept. Thus, juvenile crocodiles exposed to higher temperatures, will grow significantly faster. This phenomenon may be involved in the enhancement of a variety of compensatory neuroendocrine homeostatic mechanisms, facilitating rapid growth rate as well as hematopoiesis and other processes implicated in hematology of crocodiles.

Additionally, in the week 4 after time zero, all samples in this study including the control group and experimental group 1 increased their food intakes as compared with those observed in the previous weeks. Capture, disturbance and/or low temperature possibly make crocodiles to reduce feeding behavior in the weeks 1 to 3 after time zero [21]. Therefore, an increase in food intake in week 4 after time zero might be implicated in activation of hematopoiesis and other processes involved in hematology of crocodiles. Since the nutrients are necessary for red blood cell production and help constantly replenish the blood supply [6]. In the week 4 after time zero, the values of RBC and WBC count of the experimental group 1 were significantly higher than those of the control group and experimental group 2 whereas the Hb values of the experimental group 2 were significantly lower than those of the control group and experimental group 1. The implication of these studies is that the crocodiles are capable of adapting very well to any circumstances resulting in their survival. They are the sole survivors from the ruling age of reptiles, whose ancestry dates back to the Mesozoic, about 265 million years ago.

In conclusion, this study demonstrated that there was no adverse effect on donor crocodiles when 150 ml of blood (approximately 25% of blood volume) were withdrawn from them. Although significant difference of some hematological variables between a control group and experimental groups ($p<0.05$) was found in some weeks, the variations occurred within reference ranges [11] and did not represent a significant biological change. Therefore, the crocodiles can safely donate their blood in volume of 150 ml every 12 weeks with no adverse effects on hematological values and behavior. Under the conditions described in the present study, this recommendation is compatible with maintaining the crocodile health and welfare and can be acceptable. This study provides important information for developing the guidelines for collection of large volumes of blood from crocodile to ensure the product quality, and also safety of the animals.

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Win Chaeychomsri received Doctoral Degree in Agricultural Biotechnology (Animal Biotechnology) from Kasetsart University, Thailand. He is an outstanding lecturer in research and innovation. His current research interests focus on parasitology, immunology and animal biotechnology.

Siriluk Yamkong received Master Degree in Biology from Kasetsart University, Thailand. She has specialized in hematology of crocodile.

Sudawan Chaeychomsri received Doctoral Degree in Agricultural Science (Insect Virus Molecular Biology) from Nagoya University, Japan. Her current research interests focus on insect cell culture and insect virus molecular biology.

Jindawan Siruntawineti received Doctoral Degree in Agricultural Science from University of Tsukuba, Japan. She has specialized in applied biochemistry, cell and molecular biology, immunology, and animal physiology.