Proceeding
International Symposium in Veterinary Science 2018

Strengthening the Regional Veterinary Education and Research for the Future Excellent Veterinary Graduates

February, 28th 2018
Faculty of Veterinary Medicine, Universitas Gadjah Mada
PROCEEDING
INTERNATIONAL SYMPOSIUM IN VETERINARY SCIENCE
Strengthening the Regional Veterinary Education and Research for the Future
Excellent Veterinary Graduates

REVIEWERS:
Prof. Hiroshi Sato (Yamaguchi University, Japan)
Prof. Byeong Chun Lee (Seoul National University, South Korea)
Prof. Koichi Sato (Yamaguchi University, Japan)
Prof. Drh. Srihadi Agungpriyono, Ph.D, PAVet(K) (Institut Pertanian Bogor)
Prof., Dr. drh., Pudji Srinto, M.Kes. (Universitas Airlangga, Surabaya)
Dr. drh. I Nengah Kerta Besung, M.Si. (Universitas Udayana, Bali)
Prof. Dr. drh. Siti Isrina Oktavia Salasia (Universitas Gadjah Mada, Yogyakarta)
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Greetings From The Dean

Assalamu’alaikum warohmatullahi wabarokatuh.
Good Morning.

I take this opportunity to welcome our honorable:
Rector of Universitas Gadjah Mada (Prof. Ir. Panut Mulyono, M.Eng. D.Eng)
Vice President of Foreign Affairs, Yamaguchi University (Prof. Fusanori Miura)

I would love to welcome our distinguished keynote speakers:
Dean of The United Graduate School of Veterinary Medicine, Yamaguchi University
Dean of The United Graduate School of Kagoshima University
Dean of The United Graduate School of Tottori University
Prof. Byeong Chun Lee, College of Veterinary Medicine, Seoul National University
Head of Indonesian Association Faculty of Veterinary Medicine
All Deans of Faculty Veterinary Medicines in Indonesia
as well as dear participants.

Distinguished delegates, Ladies, and Gentlemen,

Faculty of Veterinary Medicine, Gadjah Mada University is proud to become the host of the 2nd International Symposium in Veterinary Sciences. This symposium is an annual meeting that initiated, firstly, in Bogor in 2017 by a group of veterinary faculty representatives from Indonesia and Japan as initiators. The theme of this symposium is "Strengthening the regional veterinary education and research for the future excellent veterinary graduates".

As the Dean of Veterinary School, Universitas Gadjah Mada and on behalf of the Organizing Committee, I am very pleased indeed to welcome all of you to Yogyakarta. For years, Yogyakarta has experienced being a student and cultural-rich city leading to the destination for higher-learning study from all over the country and as the second tourist destination in Indonesia, as well as being endowed with a handful of beauty and a wide range of cultures. In addition, the city is generally safe to visit, therefore I do hope you may have some other chance to visit Borobudur and Prambanan temples, a part of the world heritages as awarded by UNESCO. This seminar will allow you the opportunity to experience some of its richness.

In this symposium we invite deans and faculty members from 15 universities as follows: Yamaguchi University, Kagoshima University, Tottori University, Seoul National University, Gadjah Mada University, Bogor Agricultural University, Syah Kuala University, Airlangga University, Udayana University, Hasanuddin University, Nusa Cendana University, Brawijaya University, Wijaya Kusuma University, Nusa Tenggara Barat University and Padjajaran University.

Recent advances in veterinary research and education would be discussed in this symposium. Every faculty representative would present their leading activities in research and its contribution to educational improvements in term of standardization system. During
the discussion, we could do an institutional capacity mapping as a fundamental base for future collaborative works and synergies.

Last but not least, the role of these networks will not be maximized if they are not accompanied by strong mutual commitments from each institution. With this, it is hope every individual will enable to contribute actively and progressively with good outcomes for strengthening the veterinary education and research for the future excellent veterinary graduates.

We sincerely hope that from this symposium will give a positive impact for every institution. We also hope to develop more mutual collaboration in the future. Thank you, have a nice and fruitful discussion and God Bless You.

Wassalamu'alaikum warahmatullahi wabarakatuh

Dean of Faculty of Veterinary Medicine UGM

Prof. Dr. Siti Isrina Oktavia Salasia, DVM
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Study of Blood Calcium and Phosphorus Level in Sumatran Reticulated Python (Malayopython reticulatus)
Slamet Raharjo, Soedarmanto Indarjulianto, Yanuartono, Ong Rui Yi
STUDY OF BLOOD CALCIUM AND PHOSPHORUS LEVEL IN SUMATRAIN RETICULATED PYTHON (*Malayopython reticulatus*)

Slamet Raharjo	extsuperscript{1*}, Soedarmanto Indarjulianto	extsuperscript{1}, Yanuartono	extsuperscript{1}, Ong Rui Yi	extsuperscript{1}

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Introduction

Indonesia was one of the world’s top biodiversity rich countries. It was home to many species of non-venomous snakes including reticulated python snake (*Malayopython reticulatus*) (Reynold, et al., 2014; Low et al., 2016). *Malayopython reticulatus* has large, muscular build, iridescence and patterned skin for which it’s named. The back typically had a series of irregular diamond shapes which were flanked by smaller markings with light centers (Auliya, 2002; de Lang and Vogel, 2006).

In recent years there had been a number of confusing changes in the scientific name of this species (Kaiser et al., 2013). The formerly name was *Python reticulatus* (Shine et al., 1998). Fossey (2004) giving a re-classification genus name into Broghammerus, so the species name change to *Broghammerus reticulatus* (Rawlings, et al., 2008). A new change in 2011 turn the genus name Broghammerus back to *Python reticulatus* (Relox, et al., 2011) and the latest changes was turn to *Malayopython reticulatus* widely accepted (Reynolds, et al., 2014; Barker et al., 2015). Now, *Malayopython reticulatus* classified under the Kingdom of Animalia, Phylum of Chordata, subphylum Vertebrata, class Reptilia, order Squamata, family Pythonidae, genus Malayopython and species Malayopython reticulatus (Das, 2012; Uetz and Hosek, 2016).

In Indonesia, reticulated pythons (*Malayopython reticulatus*) (Figure 1) were quite commonly reared as pets especially the Sumatra and Java island locality (Raharjo, 2008). As pet, the reticulated pythons suffered many medical problems due to improper husbandry and diets. So, vets need physiological data and blood figure including blood calcium and phosphorus level for diagnostic reference.

![Figure 1. Malayopython reticulatus (Barlett and Bartlett, 1998)](image_url)

Calcium (Ca) was the most abundant chemical (mineral) element in the animal body. It was an essential constituent of the skeleton and teeth. In addition, calcium was a cation in intracellular and extracellular fluid, making it an integral component of living cells and tissue fluids (Meyer and Donnelly, 2012). *Malayopython reticulatus* had the blood calcium level 23.5 mg/ dl, ranged 8.6 – 78 mg/ dl (Mader and Divers, 2013). Lack of calcium causes retarded growth and deformed bones in the young, while prolonged and extreme deficiency of calcium causes rickets (Kaplan, 2014).
Phosphorus was a major intracellular anion, and most intracellular phosphorus was organic. Most inorganic phosphate was located extracellular (Mayer and Donnelly, 2012). Phosphorus was derived mainly from the diet, especially from meat. Phosphorus involved in bone and teeth constituent, and plays an important role in storage, release and transfer of energy and acid-base metabolism. Vitamin D was stimulated by hypophosphataemia, and must be hydroxylated in the liver and in the kidney to produce the active form of vitamin D. Vitamin D acts to increase intestinal resorption of phosphorus and calcium. Abnormalities in phosphorus levels were often complex problems and interdependent with other parameters (Mayer and Donnelly, 2012). *Malayopython reticulatus*’s blood phosphorus level was 3.9 mg/dl, ranged 1.9 – 7.2 mg/dl (Mader and Divers, 2013).

For reptiles, the normal calcium to phosphorus ratio was approximately 2:1 (Stahl, 2006). In general, the calcium to phosphorus ratio in snakes was approximately 1.5-2:1. Snakes with renal impairment frequently have inverse calcium to phosphorus ratio (Mitchell and Tully, 2009). The blood calcium and phosphorus level in some snakes shown in Table 1.

**Table 1. The Blood Calcium and Phosphorus Level in Some Snakes**

<table>
<thead>
<tr>
<th>Snakes</th>
<th>Calcium level (mg/dL)</th>
<th>Phosphorus level (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Python (<em>Python curtus</em>)</td>
<td>14.7 (13.5-16.2)</td>
<td>2.3 (1.6-2.8)</td>
</tr>
<tr>
<td>South Asian Python (<em>Python molurus spp</em>)</td>
<td>19 (9.9-34)</td>
<td>2.7 (1.7-3.9)</td>
</tr>
<tr>
<td>Ball Python (<em>Python regius</em>)</td>
<td>15.3 (10.8-22.2)</td>
<td>2.5 (1.8-4)</td>
</tr>
<tr>
<td>Green Tree Python (<em>Morelia viridis</em>)</td>
<td>23.4 (10.3-80.1)</td>
<td>2 (1.3-2.7)</td>
</tr>
<tr>
<td>Jungle Carpet Python (<em>Moleria spilota cheynei</em>)</td>
<td>14.4 (12.8-16.5)</td>
<td>3.1 (2.8-3.3)</td>
</tr>
<tr>
<td>Reticulated Python (<em>Malayopython reticulatus</em>)</td>
<td>23.5 (8.6-78)</td>
<td>3.9 (1.9-7.8)</td>
</tr>
</tbody>
</table>

(Mader and Divers, 2013)

Calcium and phosphorus were essential macrominerals that needed by pythons for body metabolism and for constructing new skin in the shedding cycle of snake. Blood calcium and phosphorus level on *Malayopython reticulatus* Sumatran locality have no data that can be used as a guideline. Thus, research about blood calcium and phosphorus level in Sumatran *Malayopython reticulatus* need to be carried out for further diagnostic reference.

The aim of the research was to study the blood calcium and phosphorus level in reticulated python (*Malayopython reticulatus*) of Sumatran locality. The results of this research to provide a better understanding about blood calcium and phosphorus level of Sumatran reticulated python (*Malayopython reticulatus*) for diagnostic purpose related to calcium and phosphorus.

**Materials and Methods**

Eleven adult healthy reticulated python of Sumatran island locality pet snakes consist of 4 snakes from Medan of Northern Sumatra, 3 snakes from Palembang of Middle Sumatra and 4 snakes from Lampung of Southern Sumatra were used in this research. As much as 1 mL blood samples collected with 3 ml disposable syringe from ventral coccygeal vein and then slowly deliver the blood into a sterile eppendorf tube with no anticoagulant. The blood samples then process for collecting the sera. For separating serum from blood, firstly incubate the eppendorf in an upright position at room temperature for 30 – 45 minutes to allow clotting. Next, centrifuge the eppendorf for 5 minutes with speed of 3000 rpm without break. Carefully aspirate the supernatant, which was the serum, at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. Use a clean
pipette for each tube. Finally, inspect serum for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble matter. If the sera need storing, it can be stored at temperature of -20°C. Sera collected then sent to Laboratory of ParaHita in JogjaKarta, Indonesia for calcium and phosphorus level analyses. Analyses of calcium and phosphorus level in sera using Cobas® 6000c 501 equipment (Roche Diagnostic Ltd., Switzerland). The blood calcium and phosphorus level count in mg/dl.

Results and Discussion

The results of blood calcium and phosphorus level in 11 blood samples from Sumatran reticulated python were shown in Table 2. All snakes used in this research were in healthy condition based on physical examination.

Table 2. The Blood Calcium and Phosphorus Level in Reticulated Python (Malayopython reticulatus) Sumatran locality

<table>
<thead>
<tr>
<th>No</th>
<th>Calcium Level (mg/dl)</th>
<th>Phosphorus Level (mg/dl)</th>
<th>Standard*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.8</td>
<td>5.28</td>
<td>Ca: 8.6-78</td>
</tr>
<tr>
<td>2</td>
<td>16.4</td>
<td>8.47</td>
<td>P: 1.9-7.8</td>
</tr>
<tr>
<td>3</td>
<td>15.9</td>
<td>5.94</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16.3</td>
<td>6.03</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>16.2</td>
<td>6.33</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>16.6</td>
<td>6.30</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>16.0</td>
<td>5.91</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16.4</td>
<td>8.25</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>16.7</td>
<td>8.21</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>17.4</td>
<td>5.26</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>17.2</td>
<td>5.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>16.26</td>
<td>6.54</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>13.8 – 17.4</td>
<td>5.26 – 8.47</td>
</tr>
</tbody>
</table>

*Based on Mader and Divers, (2013)

Based on standard level by Mader and Divers (2013), the normal calcium level range was 8.6-78 mg/dl; while the normal phosphorus level range was 1.9-7.8 mg/dl. Base on Table 2, for snake 1, 3, 4, 5, 6, and 7, the calcium and phosphorus level were in the normal range. As for snake 2, 8, and 9, the phosphorus level was higher than normal range, which was 8.47, 8.25 and 8.21 respectively. This might due to excessive dietary phosphorus, excessive vitamin D3 or having renal disease. For the average value calcium level shows value of 16.26, which falls in the normal range, average of phosphorus level was 6.54, which also falls in the normal range.

Based on Table 2, the average calcium level of Sumatran Malayopython reticulatus was 16.26 mg/dl, lower than Mader and Divers (2013) that showed 23.5 mg/dl but still in normal range. The average phosphorus level of Sumatran Malayopython reticulatus was 6.54 mg/dl, higher compare to Mader and Divers (2013), that showed 3.9 mg/dl but still in normal range.

The calcium to phosphorus ratio base on Stahl (2006) for reptiles was approximately 2:1 as for snake base on Mitchell and Tully (2009) was 1.5-2:1. Based on Table 2, the calcium to phosphorus ratio for Sumatran Malayopython reticulatus was 2.486:1, which was approximate 2.5:1, shows slightly higher value compare to Mitchell and Tully (2009). This might due to difference in food consumption rate in tropical region and other region, sex of
snakes, and age of the snakes. The type or amount of food will also affect the calcium and phosphorus value for snake. For example, chicken-based food and rodent-based food contain different calcium and phosphorus value. For sex, the female snake calcium and phosphorus value might differ to male due to egg production. The calcium and phosphorus level for juvenile and adult snakes will be different depend on age.

Calcium has the function for constituent of the skeleton and teeth. There are three organs involved in supplying calcium to blood and removing it from blood when necessary, which are the small intestine, bone and kidney. The small intestine is the site where dietary calcium is absorbed; the bone serves as a vast reservoir of calcium; and kidney involved in calcium homeostasis. When blood calcium level decreases, this stimulates parathyroid hormone (PTH) to be released from parathyroid gland. PTH will act on bone for releasing of calcium into blood serum, and act on kidney to increase uptake of calcium. Kidney will release active vitamin D, to stimulate the intestine, for increase uptake of calcium; on the other hand, when the blood calcium level elevates, this stimulate the thyroid gland to release calcitomin hormone, which will act on bones for calcium deposition, and the intestine and kidney, for reduce uptake of calcium. As for phosphorus, the function involved in bone and teeth constituent, and plays an important role in storage, release and transfer of energy and acid-base metabolism (Mayer and Donnelly, 2012). For phosphorus homeostasis, when Phosphorus level in blood serum increases, this will stimulate PTH to be releases from parathyroid gland. PTH will act on kidney to decrease uptake of phosphorus. Besides, PTh will decrease active vitamin D synthesis, thus causes decrease of Phosphorus uptake in the intestine, and vice versa.

Ecdysis or shedding is known as periodically renew of the outer covering of the skin. Ecdysis was a natural process in the snake that is regulated by the thyroid gland. Once the ecdysis process is started, it generally takes approximately 14 days to complete. Hormones secreted by the thyroid glands play an important role in ecdysis. Parathyroid glands produce parathormone, or also known as parathyroid hormone, which helps regulate levels of calcium and phosphate in the blood. Regulation of calcium was also important for homeostasis (Mitchell and Tully, 2009). Calcium level increases during shedding period, as high level of estrogen will inhibit parathyroid hormone secretion from parathyroid gland, and thus cases elevation of calcium level in serum. It has been thought that scales are storage sites of calcium, and that calcium is put in and mobilized for some physiological use from the scales (Sasayama, 1999; Mayer and Donnelly, 2012).

Conclusion

Based on the research’s results and discussion, it could be concluded that reticulated python (Malayopython reticulatus) Sumatra island locality had calcium level range 13.8-17.4 mg/dl, with average of 16.26 mg/dl and phosphorus level range from 5.26-8.47 mg/dl, with average of 6.54 mg/dl. It needs further research with more samples, different ages and geographic variation of Malayopython reticulatus Sumatran locality.

References


Keywords: Malayopython reticulatus, Sumatra island, blood, calcium, phosphorus
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