

SUPPLEMENTATION OF COCKLE SHELLS-DERIVED CALCIUM FOR MAINTANING SERUM CALCIUM LEVEL IN PREGNANT AND LACTATING JAMNAPARI AND BOER GOATS

HUSNA FASIHAH BINTI MOHD YUSOFF

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By

HUSNA FASIHAH BINTI MOHD YUSOFF

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2016

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Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfilment of the requirement of the Degree of Master of Science

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The optimum nutrition, minerals and better care of pregnant and lactating does are very important. One of the most important mineral in the diet is calcium. Calcium supplements used in this study come from cockle (Anadara granosa) shells. This is because shells are very similar to bones and in form of CaCO₃ that can provide the strong basis for the calcium potential source. Blood and feed samples were taken to evaluate the level of calcium in diet and serum calcium level in goats, respectively. The observation in this study was first looking at the feed analysis and followed by calcium serum concentration analysis. Feed analysis was used to analyze the calcium content in feed provided by the farmer. Then the research proceeds with blood analysis. Samples were collected before and after supplementation of calcium in order to identify the calcium level in goat's blood. A total of 45 goats were chosen in this study. Calcium is an essential mineral to have in the diet as it builds strong bones and sometimes we may not be aware of. Deficiency of calcium can cause reproductive problems. This is because calcium is very important for smooth muscle function. This study is very important to highlight the influence of pregnancy and lactation on the calcium concentration in goats' blood serum. Most of the feed given by the farmer contain less calcium level compared to the calcium level suggested by the previous from other other researchers. Thus, it is important for the goats to consume enough calcium in order to maintain adequate blood and bone calcium levels. Independent t-test was used to compare serum calcium concentration in mmol/L. Result were expressed as mean \pm standard error mean (SEM). A probability of p<0.05 was considered significant for the statistical test. There was a statistically significant difference between groups as determined by t-test for post-delivery stage. A test revealed that the changes between with and without supplementation were statistically significantly at p < 0.05, whereas, significant difference occurred between mid-trimester and late-gestation stages in Jamnapari goats. However from overall results from the tables shows no significant different happened among all other stages. Finally, it seems that most of the feed types were shown a lower calcium level compared with the calcium level suggested by the

other researches. As the feeds contain low concentrations of calcium, it is important to supplement the does with calcium. In this study calcium source was obtained from cockle shells. Besides that, the declining and rising of serum calcium level shown in supplemented group, contrarily it was not happening to the non-supplemented group. It seems that, the serum calcium level during pregnancy and lactating stage is really unpredictable. This situation happened might be due to several factors such as hormonal concentration (progesterone and estrogen level) along gestation and lactation period, fetus development, nutrition, excretion through waste (feces, urine and sweat) and skeletal formation and mineralization process. On the other hand, there was no significant different happened between two different breeds in calcium serum level during gestation and post-delivery. Finally, through this research and from overall observation, animal with calcium supplement maintain the normal calcium level in the body within the range and the level keep on increase appropriately for both breeds.



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DIET TAMBAHAN DARI KULIT KERANG UNTUK MENGEKALKAN TAHAP YANG SESUAI BAGI KALSIUM SERUM PADA KAMBING JAMNAPARI DAN BOER KETIKA BUNTING DAN LAKTASI

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Pengambilan nutrisi, mineral serta penjagaan yang optimum adalah sangat penting bagi kambing yang sedang bunting dan diperingkat laktasi. Kalsium adalah salah satu contoh mineral penting yang patut ada di dalam diet. Kulit kerang (Anadara granosa) telah di gunakan di dalam kajian ini sebagai sumber kalsium di dalam diet tambahan. Ini adalah kerana keadaan cengkerang adalah sama dengan tulang dan kalsium karbonat dapat menjadi penyumbang utama sumber kalsium. Sampel darah dan makanan diambil bagi mengkaji tahap kalsium. Pemerhatian yang pertama di dalam kajian ini adalah analisis makanan. Analisis makanan dijalankan bagi menganalisa kandungan kalsium di dalam makanan yang diberi oleh penternak. Kemudian disusuli dengan analisis darah. Sampel diambil sebelum dan selepas kalsium diet tambahan diberi untuk mengetahui tahap kalsium di dalam darah kambing. Sejumlah 45 ekor kambing dipilih di dalam kajian ini. Kalsium penting di dalam diet bagi membentuk tulang yang kuat dan ianya seringkali tidak dititikberatkan. Kekurangan kalsium akan menyebabkan masalah reproduksi. Ini adalah kerana kalsium adalah penting bagi fungsi otot licin. Kajian ini adalah amat penting bagi menekankan kaitan di antara kebuntingan dan laktasi kepada kepekatan kalsium di dalam serum. Hampir kesemua makanan yang diberi oleh penternak tidak mengandungi kalsium yang mencukupi jika dibandingkan dengan kandungan kalsium yang telah diusulkan di dalam kajian-kajian pengkaji lain. Maka, ianya adalah sangat perlu untuk menyediakan kalsium yang mencukupi bagi mengekalkan tahap kalsium di dalam darah dan tulang haiwan. Ujian t bagi min tak bersandar digunakan bagi membandingkan kepekatan kalsium serum di dalam unit mmol/L. Keputusan dilampirkan di dalam min ± min ralat piawai. Kebarangkalian bagi p < 0.05 ditakrif sebagai signifikan bagi ujian statistik. Terdapat perbandingan signifikan yang berlaku di antara kumpulan yang ditentukan dari ujian t bagi fasa selepas pelahiran. Kajian menyatakan bahawa perubahan antara diet tambahan dan tanpa diet tambahan adalah signifikan secara statistic apabila p < 0.05. Manakala, perubahan yang signifikan berlaku antara fasa bunting pertengahan dan bunting berat bagi kambing Jamnapari. Sungguhpun begitu, keputusan keseluruhan dari jadual-jadual lain menunjukkan tiada perubahan signifikan yang berlaku pada fasa yang lain. Akhirnya, didapati kebanyakkan jenis makanan adalah kurang kandungan kalsium jika dibandingkan dengan jumlah yang telah diusul oleh kebanyakan pengkaji. Oleh kerana makanan haiwan mengandungi kepekatan kalsium rendah, diet makanan adalah penting bagi menyediakan kalsium kepada kambing. Sumber kalsium di dalam kajian ini adalah dari kulit kerang. Selain dari itu, penurunan dan penaikan kalsium serum berlaku pada kumpulan yang diberi diet tambahan, manakala keadaan sedemikian tidak berlaku pada kumpulan yang tidak diberi diet tambahan. Keadaan menerangkan bahawa tahap kalsium serum bagi fasa bunting dan laktasi adalah tidak boleh dijangka. Situasi ini berlaku adalah mungkin disebabkan oleh beberapa faktor seperti kepekatan hormon (progesteron and estrogen), nutrisi, perkumuhan (tinja, urin, peluh) dan proses pembentukan dan pemineralan rangka. Selain dari itu, tiada perubahan signifikan berlaku bagi kalsium serum semasa tempoh bunting dan selepas pelahiran yang berlaku bagi dua baka yang berbeza. Akhir sekali, melalui keseluruhan pemerhatian dari kajian ini didapati haiwan yang diberi diet tambahan mengekalkan tahap kalsium di antara julat normal dan ianya meningkat secara bersesuaian bagi kedua-dua baka.

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I Certify that the Thesis Examination Committee has met on 21 June 2016 to conduct the final examination of Husna Fasihah Binti Mohd Yusoff on her thesis entitled "Supplementation of Cockle Shells-Derived Calcium Maintains Appropriate Serum Calcium Level in Pregnant and Lactating Jamnapari and Boer Goats" in accordance with the Universities and University College act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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	R		

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LIST OF ABBREVIATIONS

HCL Hydrochloric Acid

Gram

- DM Dry Matter
- CP Crude Protein
- Kg Kilogram
- g
- mg Milligram
- L Litre

6

- Mmol Millimole
- DVS Department of Veterinary Services



CHAPTER 1

INTRODUCTION

Goat's feed can be divided into two types; roughage and concentrate. Roughage is high in fiber. It comes from plants and can be divided into two types which are grasses and browse (leaves, twigs and shoots). Hay and straw are roughages that have been cut and cured, and making it ideal for storage. While, concentrate are complementary to roughages and usually refer to grain. It is commonly low in fiber but high in energy or protein (Cheryl, 2010). These two types of feed are very essential in a goat's diet. In fact, the optimum nutrition and better care of pregnant does are very important to achieve. If the deficiency in any of the necessary nutrients such as mineral likes calcium, it will affect the growth of the foetus. Fetuses put at 80% of their growth during the last few weeks of pregnancy. In four season countries, does are usually supplied with specially formulated and balanced goat pellets, goat mash, cracked corn or barley (Barbara, 2005). Some common feeds in other countries are corn, oats, barley and soybean (Cheryl, 2010). In summer, the goats will browse their own feed on pasture or on weed. While during the winter season, if the does live in an area where foraging is a possibility year round, the farmers keep their goats grazing or provide hay (Yvonne, 2012).

There are different types of goat's feed in certain part of Asia such as in Thailand and Indonesia. Leucaena is preferred in these two countries as a supplement to complete the diet. In fact, it is a plant that favoured by goats in parts of the tropics. Goats also been supplemented with another type of common feed, for example, in Thailand they were fed with cereal crop residues which increased the dry matter intakes and digestibility of the basal diet. In the previous research, it has been reported that the total dry matter intake of goats given Leucaena was higher than those given soybean meals (Aung, 2007). Leucaena leaf seemed to have higher calcium content than that was observed in grasses fed to the animals. Body weight of goats in the Leucaena consumption group tended to be higher than that of control animals (Maki et al., 2004). In Indonesia, the roughage used can be divided into four groups: Legumes (Sesbania sesban, Leucaena and Kancu leaf), grass (king grass and native grass), leaves (jackfruit, banana, and mahogany) and agriculture waste products (Corn stover and dry cassava tuber skin) (Kustantinah et al., 2005). Even though leucaena is widely used as a supplement that rich in protein and calcium (Maki et al., 2004) around the Asia, unfortunately research made by Colin (1963), Jabbar et al. (1996) and Peter (1994) are against the statement by reporting that leucaena contains anti-nutritional factors in the leaves and harmful used in livestock feeding. The anti-nutritional for leucaena is mimosine (Colin, 1963; Jabbar et al., 1996; Peter, 1994). Concern has arisen after the importance of this plant to the animals as a supplement increased. In addition, there are bad effects on the consumption of the mimosine to the ruminant. Research made by the report of the Philippine council for agricultural and resources, research and the united states national academy of sciences (1997) reported that the main symptoms of toxicity in ruminants



are poor growth, loss of hair and wool, swollen and raw coronets above the hooves, lameness, mouth and esophagus lesion, and depresses the serum thyroxin level. Reduction in pregnancy production due to leucaena feeding has also been noted (Jones *et al.*, 1989).

Contrarily, there are different varieties of feed commonly used in Malaysia for ruminants. Examples of the feeds are Napier grass, silage, soy waste, and concentrate. Napier is widely used in Malaysia as a main source of roughage. It has high cellulosic fiber content and is, therefore, an excellent cheap feedstock. Some of it does not need nitrogenous fertilizer to grow fast and it gives an advantage to the farmer to cut the cost. In addition, nitrogen fertilizer is not good for the environment as it requires the greatest amount of fossil fuel energy to produce chemicals and by avoiding their use, greenhouse gas emissions are also can be avoided. The advantage of using Napier is, it can propagate easily; it has the soft stem that is easy to cut; it has deep roots; so is fairly drought resistant; the tender young leaves are excellent animal feed and it grows very fast compared to other type of grass (Roland, 2012).

Even though, there are lots of advantages of using Napier in a goat's diet. Unfortunately, it contains low calcium content and high oxalate level. Thus, with the increase of Napier silage intake, there was a significant increase of oxalate too, with subsequent increase of the calcium excretion through feces (Das *et al.*, 2010). Hsieh-Weinchang *et al.* (2004) reported a higher oxalate in feces by feeding high amount of Napier grass as well. Dodson (1959) found a portion of oxalates is degraded in rumen with the probable formation of carbonate and bicarbonate. Moreover, the soluble part of oxalate also bind with available calcium in the rumen and excreted in feces. This is the probable cause of calcium deficiency occur in pregnant does (Das, *et al.*, 2010).

There are few reproductive problems that happen due to deficiency of calcium. One of the problems is the uterine prolapse. Uterine prolapse occurs within 12-18 hours after kidding or causes a difficult delivery (George and Paula, 2011). The study made by Pugh and Baird (2012) found that hypocalcaemia can contribute to flaccid that predisposed to uterine prolapse. If the deficiency of calcium is suspected, the female should be given a calcium solution as oxytocin is indicated to aid uterine contraction. Other than that, hypocalcaemia can also overlap with the clinical sign of pregnancy toxaemia as these two diseases often seen as concurrent. Animals will recumbent and paralyzed due to this problem. This happens because little to no calcium is available to release acetylcholine at the neuromuscular junction and influence muscle contractibility (Pugh and Baird, 2012). The other problem caused due to hypocalcaemia is dystocia (Jill *et al.*, 2008).

Thus, it is important for the goats to consume enough calcium in order to maintain adequate blood and bone calcium levels. If calcium level drops below normal, calcium will be mobilized from the bone and passed into the blood in order to maintain blood calcium levels (Linda *et al.*, 2004). A calcium supplement is very important to the goats in order to avoid these problems from happening. The common calcium

supplement used by the local feed industry in Malaysia is derived from limestone (Raghavan, 2000). Calcium supplementation is still practiced in this sector, as the calcium absorption process can be easily accomplished by the addition of calcium carbonate (feed grade limestone). The example of calcium supplement sells for livestock consumption is by Jolly German Livestock Supplies-Dolomite (Limestone) by The Jolly German, Pete Lien and sons in depositing mined in northern Colorado and southern Wyoming and much more company who sell calcium supplement for livestock consumption. Malaysia also produces calcium limestone powder for animals' consumption in local industries. The example of the company is in Pulai calcium carbonate industries Sdn Bhd. It was incorporated in 1995 with modest start in Ipoh, Malaysia. From the entire example above, it shows really wide production of calcium supplement produce by using limestone source. In addition, Department of Veterinary Services (2009) suggest that calcium level for pregnant goats should be around 2 grams/ day for each head.

A calcium supplement is usually produced via CaCO₃ sources that are from limestone, which is obtained through mining and quarrying limestone hills. Yet, this study also able to exploit the vast availability of waste resources in Malaysia, which is cockle shell. Cockle shell had been chosen in this study as the source of calcium supplement to the pregnant does. As shells are very similar to bones and predominantly aragonite form of CaCO₃ that can provide the strong basis for the calcium potential source (Hemabarathy et al., 2014), this study was conducted to analyze the calcium level after supplementation with this cockle shell powder. The shells represent a huge portion of waste products after the consumption of mussels. This was happening as it is widely consumed in South East Asian countries in various delicacies. The research is environmentally friendly and could be scaled up for industrial production, as it permitted a greener synthesis of naturally available material and utilizing the waste shells that are the by-product of one of the important seafood industry in Malaysia (Kamba et al., 2013). Research by Awang-Hazmi et al. (2007), Islam et al. (2012) and Zuki et al. (2004) had found that potential use of the cockle shell based CaCO₃ powder as a source of biominerals for bone tissue applications. Thus, it shows CaCO₃ from cockle shell is very safe for animal consumption. A study by Awang-Hazmi et al. (2007) and Zuki et al. (2004) found that overall mineral composition of cockle shells of Peninsular Malaysia are CaCO₃ 98.7%; Mg 0.05%; Na 0.9%; P 0.02%; others 0.2%. And CaCO3 97.6%-98.7%; Mg 0.2%-0.52%; Na 0.72%-0.96%; P 0.02%- 0.05%; the rest was identified as another, respectively. Those studies proved there is high CaCO₃ contain in cockle shells and can make full use of it.

Hypothesis

Through this research, animal with calcium supplement maintain their normal calcium level in the body within the range and the level keep on increase appropriately for both breeds.

1.1 Significant of Study

This study is very significant to highlight the effect of pregnancy and lactation on the calcium concentration in goat's blood serum with the calcium supplement in two breed of goats, Jamnapari and Boer. The findings benefit the ruminant industry in Malaysia. As farmers will be more aware of the importance of providing sufficient calcium to their animals during pregnancy and post-delivery as the calcium level will be more fluctuate and imbalance during these stages.

1.2 Problem Statement

Most of the studies carried out so far were related to calcium concentration in blood serum during pregnancy and lactation period were only held in foreign countries. In addition, there are limited numbers of pregnant goats present in about the same age, weight and body score make the research more challenging. Besides that, the cockle shells powder productions are very hard to produce manually by using 90µm siever.

1.3 Research Objectives

The general aim of this research was to evaluate the effects of oral supplementation of cockle shell-derived $CaCO_3$ on calcium level in the blood between pregnant and lactating goat.

The specific objectives are:

- i. To analyze and determine the calcium level in goat's diet.
- ii. To determine the serum calcium level during pregnancy until post-delivery (with and without supplementation).
- iii. To determine the serum calcium level during pregnancy until post-delivery between two breeds of goats.

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APPENDICES

Appendix A

Production of Supplement (Cockle Shell)







Cockle Shell

Break into small pieces

Heating







Grinding

6

Sieving

Heating



Packaging

Appendix B

Blood Sampling and Analysis







Blood sampling

Centrifuge

Serum separation



Loading serum for reading

C





Transfer into tube

Result

Appendix C

Feed Analysis



Digest

6

Digest

Filter







Reading

Dilute

Dilution



Result

BIODATA OF STUDENT

Husna Fasihah binti Mohd Yusoff was born on 26th January 1990 at General Hospital Parit Buntar. She was raised in Perak Darul Ridzuan, and studied at Sekolah Kebangsaan Selama Jalan Menteri and Sekolah Menengah Kebangsaan Dato' Haji Hussein. After finishing her school, she furthered her study at the Perak Matriculation College and then entered the Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin, Terengganu, Malaysia to do the Bachelor of Animal Production and Health. She was accepted to the Master of Veterinary Science Program at Faculty of Veterinary Medicine in Universiti Putra Malaysia.

