UNIVERSITI PUTRA MALAYSIA

THE EFFECTS OF MOLYBDENUM AND / OR SULFUR SUPPLEMENTATION ON THE MINERAL STATUS OF PLASMA AND LIVER OF SHEEP FED PALM KERNEL CAKE

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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

1999

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By

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Thesis Submitted in Fulfilment of the Requirements for the
Degree of Master of Science in the
Faculty of Agriculture
Universiti Putra Malaysia

May 1999

ACKNOWLEDGEMENTS

I would like to express my appreciation and sincere gratitude to Associate Professor Dr. Abdul Razak Alimon for his help and guidance during my course of study. I would also like to thank my co-supervisors Dr. Mohd Hair-Bejo, and Dr. Wan Zahari Mohamed (MARDI), for their valuable guidance and advice throughout this study. I am indebted to Professor Dr. Michael Ivan, for his patience and valuable guidance in planning and designing the feeding trial of my experiment. My thanks go to Professor Dr. Li, De Fa, deputy dean of College of Animal Science, China Agricultural University, and Mr. Oh Beng Tat for encouraging me to pursue my Master of Science degree at UPM.

My appreciation goes to Dr. Jothi M. Panandam, for her rewarding advice on data analysis of this research.

Appreciation is also extended to Miss Yusnita Maizul binti Yasin, Mr. Lan Gan Qiu, and Mr. Siddiq Abdalla for their tremendous assistance during the feeding trial.

The technical assistance of Mr. Ibrahim bin Mohsin, Mr. Bakari bin Abd. Rahman, and Mr. Saparin bin Demin is gratefully acknowledged.

I would like to thank the government of Malaysia (through IRPA, Project No. 51186) for funding this research program and my graduate research assistantship.

Lastly, but not least, my deepest gratitude goes to my parents, my brother and sister and their families for their support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Atomic Absorption
AAS	Atomic Absorption Spectrophotometer
ADF	Acid Detergent Fibre
ADP	Adenosine Diphosphate
AMP	Adenosine Monophosphate
ATP	Adenosine Triphosphate
CF	Crude Fibre
CP	Crude Protein
DFI	Daily Feed Intake
DM	Dry Matter
DMD	Dry Matter Digestibility
DVS	Department of Veterinary Services, Ministry of Agriculture, Malaysia.
ME	Metabolic Energy
MT	Metallothionein
OM	Organic Matter %
OPF	Oil Palm Fronds
OPT	Oil Palm Trunk
PKC	Palm Kernel Cake
POME	Palm Oil Mill Effluent
POS	Palm Oil Sludge
PPF	Palm Press Fibre
SD	Standard Deviation
SEM	Standard Error of Mean
TTM	Tetrathiomolybdate
UPM	Universiti Putra Malaysia

Abstract of the thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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May 1999

Chairman: Associate Professor Abdul Razak Alimon, Ph.D

Faculty : Agriculture

Although palm kernel cake (PKC) has been widely used as a feed for large ruminant, its high copper (Cu) content is potentially poisonous to sheep and possibly goats. It is well known that sheep are extremely intolerant of Cu toxicity. Sheep fed high levels of PKC, showed high levels of Cu in the liver, which caused jaundice and liver damage. Some studies showed that molybdenum (Mo) and/or sulfur (S) could form complexes with Cu to make Cu unavailable, hence reducing the accumulation in the liver. However, the optimum amount of S and Mo need to be added to PKC has not been established.

In the present study, from 80 six months to one-year-old Malin cross sheep , 72 were selected and allocated randomly into 9 groups with 8 animals each. The animals were offered PKC pellet (96-97%) supplemented with different levels of S and Mo, hence, Diet A (Mo, 13ppm), Diet B (Mo, 26ppm), Diet C (52ppm), Diet D (Mo, 103ppm), Diet E (S, 4400ppm, Mo, 13ppm), Diet F (S, 4400ppm, Mo, 26ppm), Diet G (S, 4400ppm,Mo, 52ppm), Diet H (S, 4400ppm, Mo, 103ppm), Diet I (S, 4400ppm).

The feeding trial lasted 6 months. The animals were slaughtered in two groups, i.e., at the end of 3 months and 6 months of the feeding trial respectively.

When the animals were slaughtered, their livers were removed, prepared and were analyzed for mineral concentration.

The blood was collected from the jugular vein of each sheep in heparinized vacuum tubes and centrifuged to obtain plasma once every 2 months throughout the trial. The plasma was prepared for the analyses of the mineral content and this was carried out by Atomic Absorption Spectrophotometer (AAS).

The results showed that Mo when combined with S and Mo alone in high levels were more efficient in reducing the liver Cu levels in sheep than Mo in low levels or S. However, except for Ca, other elements analyzed also showed different variations among treatments and the initial values.

The plasma minerals showed some differences from that of liver minerals. Again, except for Ca, other elements presented some changes over time.

The causes of the above may be complicated, however, the major reason considered is the dietary treatments. It is evident in this study that Mo and / or S supplementation have prevented the sheep from chronic Cu toxicity as caused by PKC, and the animals survived between 3-6 months of feeding trial. When those treatments were compared, all Mo + S groups seem to be more efficient in lowering the liver Cu levels.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains.

KESAN PENAMBAHAN MOLIBDENUM DAN SULFUR KE ATAS STATUS MINERAL DALAM PLASMA DAN HEPAR BIRI-BIRI YANG DIBERI MAKAN HAMPAS ISIRUNG KELAPA SAWIT

Oleh

LI JUAN

Mei 1999

Pengerusi: Profesor Madya Dr. Abdul Razak Alimon, Ph.D.

Fakulti: Pertanian

Sungguhpun PKC diguna secara meluas sebagai bahan makanan ruminan besar, kandungan kuprum (Cu) nya yang tinggi berpotensi untuk meracuni biri-biri dan mungkin juga kambing. Biri-biri yang diberi makanan yang mengandungi PKC aras tinggi menyebabkan aras Cu yang tinggi di dalam hati dan ini menyebabkan 'jaundice' serta kerosakan hati. Sesetengah kajian menunjukkan bahawa molibdenum (Mo) dan / atau sulfur (S) dapat membentuk kompleks dengan Cu dan seterusnya mengurangkan pengumpulan di dalam hati. Walau bagaimanapun, amaun optimum S dan Mo yang perlu ditambah kepada PKC tidak diketahui.

Dalam kajian ini, daripada 80 ekor biri-biri kacukan Malin yang berumur enam bulan hingga satu tahun, 72 ekor telah dipilih dan dibahagi secara rawak kepada 9 kumpulan dengan 8 ekor bagi setiap kumpulan. Biri-biri ini diberi makan pelet PKC (96-97%) yang ditambah dengan S dan Mo pada aras yang berbeza, iaitu, Diet A (Mo, 13ppm), Diet B (Mo, 26ppm), Diet C (52ppm), Diet D (Mo, 103ppm), Diet E (S, 4400ppm, Mo, 13ppm), Diet F (S, 4400ppm, Mo, 26ppm), Diet G (S, 4400ppm,Mo, 52ppm), Diet H (S, 4400ppm, Mo, 103ppm), Diet I (S, 4400ppm).

Kajian pemberian makanan berlangsung selama 6 bulan. Haiwan ini disembelih dalam 2 kumpulan pada 3 bulan dan 6 bulan, manakala yang lainnya disemblelih pada penghujung percubaan.

Apabila haiwan disembelih, hati diasingkan, dan dianalisa untuk kandungan mineral.

Semasa percubaan darah diambil dari salur jaguler setiap ekor biri-biri menggunakan tiub vakum berheparin dan diempar untuk mendapatkan plasma. Plasma diproses untuk menentukan kandungan mineral menggunakan Atomic Absorption Spectrophotometer(AAS).

Hasil kajian menunjukkan kombinasi Mo dan S serta Mo sahaja pada aras yang tinggi adalah lebih berkesan untuk mengurangkan aras Cu hati pada biri-biri berbanding dengan Mo beraras rendah atau S. Walau bagaimanapun, kecuali Ca elemen-elemen lain yang dikaji, menunjukkan varasi yang berlainan di antara perlakuan dan nilai asal.

Kandungan mineral plasma didapati berlainan dibandingkan dengan kandungan mineral hati. Kecuali Ca, elemen-elemen lain menunjukkan beberapa perbezaan dan ini bergantung kepada masa.

Keputusan kajian di atas mungkin agak kompleks. Faktor utama mungkin disebabkan perawatan pemakanan. Hasil kajian ini menunjukkan dengan jelas bahawa penambahan Mo dan / atau S dapat mengelakkan biri-biri daripada keracunan Cu yang kronik, sebagaimana yang disebabkan oleh PKC. Ternakan didapati dapat bertahan 3 – 6 bulan selepas tarikh ujian. Apabila kesemua rawatan dibandingkan, semua kumpulan Mo + S dapat mengurangkan aras Cu hati dengan lebih berkesan.

CHAPTER I

INTRODUCTION

The poor growth of the ruminant industry in Malaysia is said to be mainly attributed to the high cost of feed and poor availability of feed. Malaysia produces large quantities of agro-industrial by-products and crop residues. Extensive use of these by-products and residues could alleviate this situation markedly.

As one of these by-products, palm kernel cake (PKC) comes from palm oil industry, and the production is approximately 1 million tons annually. As a matter of fact, PKC contains reasonable amount of protein (CP 16-18%), energy (ME 9.5-10 MJ/ kg), and fiber (CF 13-16%) to meet ruminants requirements. It has been successfully included in the diets of large animals, such as cattle, buffaloes and even deer. However, because of high level of copper (Cu 20-50 ppm) in PKC, the utilization of this by-products to small ruminants should be with much caution, since the ovine, especially sheep, is extremely susceptible to Cu toxicity when compared with bovines.

To reduce the Cu toxicity in sheep, most methods involved zinc (Zn), molybdenum (Mo), sulfur (S), calcium (Ca), iron (Fe), and cadmium (Cd). These elements usually interact with Cu affecting its absorption and metabolism in ruminants. The most significant interaction and intensively studied among these elements are those between Cu and S, as well as between Cu, S and Mo. These interactions result in formation of insoluble and biologically unavailable copper sulfide (CuS) or cupric thiomolybdates, respectively. Such compounds are not absorbed from the intestinal tract and are excreted in feces. Moreover, the dietary ratio of calcium (Ca) to phosphorus (P) is between 0.3:1 - 1:2 in PKC, and this imbalance needs to be corrected as it may enhance the bioavailability of dietary Cu, as well as other macroelements, particularly Ca.

Supplements of S plus Mo were used to prevent Cu toxicity in sheep in many studies. However, the optimal level of S and / or Mo added is still not established. Therefore, 5 levels of Mo, 2 levels of S, and some of their combinations were designed in the present study, so as to investigate:

i. whether Mo alone function in reducing Cu toxicity of PKC in sheep;

ii. whether S alone function in reducing Cu toxicity of PKC in sheep;

iii. which treatments are effective;

iv. which treatment is the best (optimal levels of Mo and / or S supplementation).

CHAPTER II

LITERATURE REVIEW

Status of Ruminant Industry in Malaysia

Malaysia is self sufficient in non-ruminant products such as pork, poultry meat and eggs since early 1980s and will continue to be so in future. Importation of beef, mutton and milk will be continued. These ruminant products are still lower than the level of self sufficiency (Mahyuddin, 1993; DVS, 1998). This situation can be presented in Table 1.

Table 1: Production and Consumption of Animal Products (1996)

Items	Local Production	Total Consumption	%Self Sufficiency	Per Capita Consumption
Beef (mt)	14,915	84,335	17.7	5.1
Mutton (mt)	529	14,306	3.7	0.9
Pork (mt)	242,772	174,405	139.2	10.5
Poultry (mt)	584,400	486,590	120.1	29.2
Eggs (mil. Nos.)	6,132	5,521	111.1	331.6
Milk (mil. L)	32.17	638.3	5.0	38.3

DVS (1998) Livestock Statistics.

It is projected that Malaysia will be 20% self-sufficient in beef, 10% in mutton, and 10% in dairy product by the end of this century. Although the government is making great effort to improve the ruminant industry, availability of breeding stocks and feeds remains two major and serious constraints.

The ruminant industry is still in the hands of small holder farmers. The farmers keep small numbers of animals under the extensive system of farming involving grazing by road shoulders, rice bunds, waste lands, as well as the plantations of rubber and oil palm. Over the last 30 years, Malaysia has been importing exotic breeds to upgrade the indigenous animals. As a result, there has been a steady increase in sheep population (Chee and Jalil, 1994). But little progress has been made in the management and nutrition of these animals. Other ruminant population such as cattle has been increasing at rate of less than 5% per annum (p.a.), while the goats and buffaloes are decreasing at the rate of 3-5% p.a. The poor growth of the ruminant industry has been attributed mainly to the unavailability of good quality feeds at reasonable cost (Alimon, 1993).

Feed Situation in Malaysia

Feed is one of the important components in any animal industry; it makes up a large proportion of the cost in animal production. About 60 to 70% of the total cost of production is spent on the feeding livestock. The cost of feeding is even more when the efficiency of animals are poor.

Malaysia does not produce sufficient raw ingredients for animal feeds, although some feedstuffs are available, e.g., tapioca chips, rice bran, copra meal, and soybean meal. The amounts produced are small and contributed to less than 10% of the total feed required (Rajion, *et al.*, 1993). Therefore, the livestock industry, especially the poultry and swine sectors are forced to rely on imported feedstuffs. Even though the cost is very high, for example, the expenditure in 1992 was RM820 million, the non-ruminant industry is viable because of increased efficiency in management and high feed conversion efficiencies of pigs and poultry (Alimon, 1993). However, the ruminant industry can not be supported by imported feedstuffs owing to their low feed conversion efficiencies. Therefore, this sector need to be boosted by some local available feedstuffs. Malaysia is an agricultural country and produces large quantities of agro-industrial by-products and crop residues, such as rice straw, pineapple waste, sago waste, spent brewers grain and tofu waste, and various palm oil by-products. If these by-products and residues could be used to meet the feed requirements of the animals, the feed shortage in Malaysia would be alleviated.

It is an established fact that ruminants can use a wide variety of agricultural by-products and crop residues which otherwise are wasted and create pollution problems. This is because of their forestomachs that are capable to digest high fibrous materials as compared to monogastric animals. However, at present, these by-products and residues are not fully utilized in Malaysia, due to several reasons such as poor understanding of the nutritive value of these materials, logistics of collection and transportation and more important, lack of techniques to use them safely and efficiently (Alimon, 1993).

Oil Palm By-products as Feed

As a major agro-industry in Malaysia, palm oil industry yields large quantity of by-products while it produces oil. Many of these by-products can be used as feed for ruminants and these include the following:

Palm Press Fiber (PPF), obtained after the epicarp of the oil palm fruit is pressed to remove its oil. It constitutes the largest part of the fruit that is discarded after oil extraction. Owing to its poor feeding value, the use for feeding ruminant is limited. It is mainly used for mushroom growing and most of the time burned as fuel to generate heat energy in the process of extracting oil (Alimon, 1993). Studies by Jalaludin *et al.*

(1991) showed that after treated with alkali, for example NaOH, PPF can be included in ruminant diets successfully.

Palm Kernel Cake (PKC), obtained after the shell is cracked and the kernel is pressed to remove its oil. This is an important by-product and is a useful feed for ruminants because of its reasonable high level of crude protein (16-18%), crude fiber (16%), as well as metabolizable energy (10 MJ/kg) contents. In Malaysia PKC has been successfully used in diets of cattle, buffaloes and small ruminants (Jalaludin, 1995).

Palm Oil Mill Effluent (POME) also named palm oil sludge (POS). This material is obtained after partial drying of the solids that is reclaimed from POME. It can be included as sources of protein and energy up to 15% in poultry and 30% in cattle. The growth of bull consuming 100% POME was significantly retarded due to the poor skeletal growth and poor bone mineralization attributed to the lower digestibility of Ca and Mg in POME-fed animals (Yusoff *et al*., 1989)

Oil Palm Fronds (OPF), in several studies by researchers in Malaysian Agricultural Research & Development Institute (MARDI) (Abu. Hassan and Azizan, 1992) and in Universiti Putra Malaysia (UPM) (Dahlan, 1992a,b), it has been indicated that OPF can be a suitable source of roughage for large and small ruminants.

Oil Palm Trunk (OPT), obtained when the trees are chopped down at the end of their production, usually about 20-25 years. OPT has been chopped and chipped and treated with NaOH and ensiled. It appears that OPT can be used as a source of fiber for ruminants though not at high levels (Oshio, *et al*., 1991). However, processing OPT demands high energy and can be costly.

Figure 1 shows the extraction rates of products and by products from the oil palm.

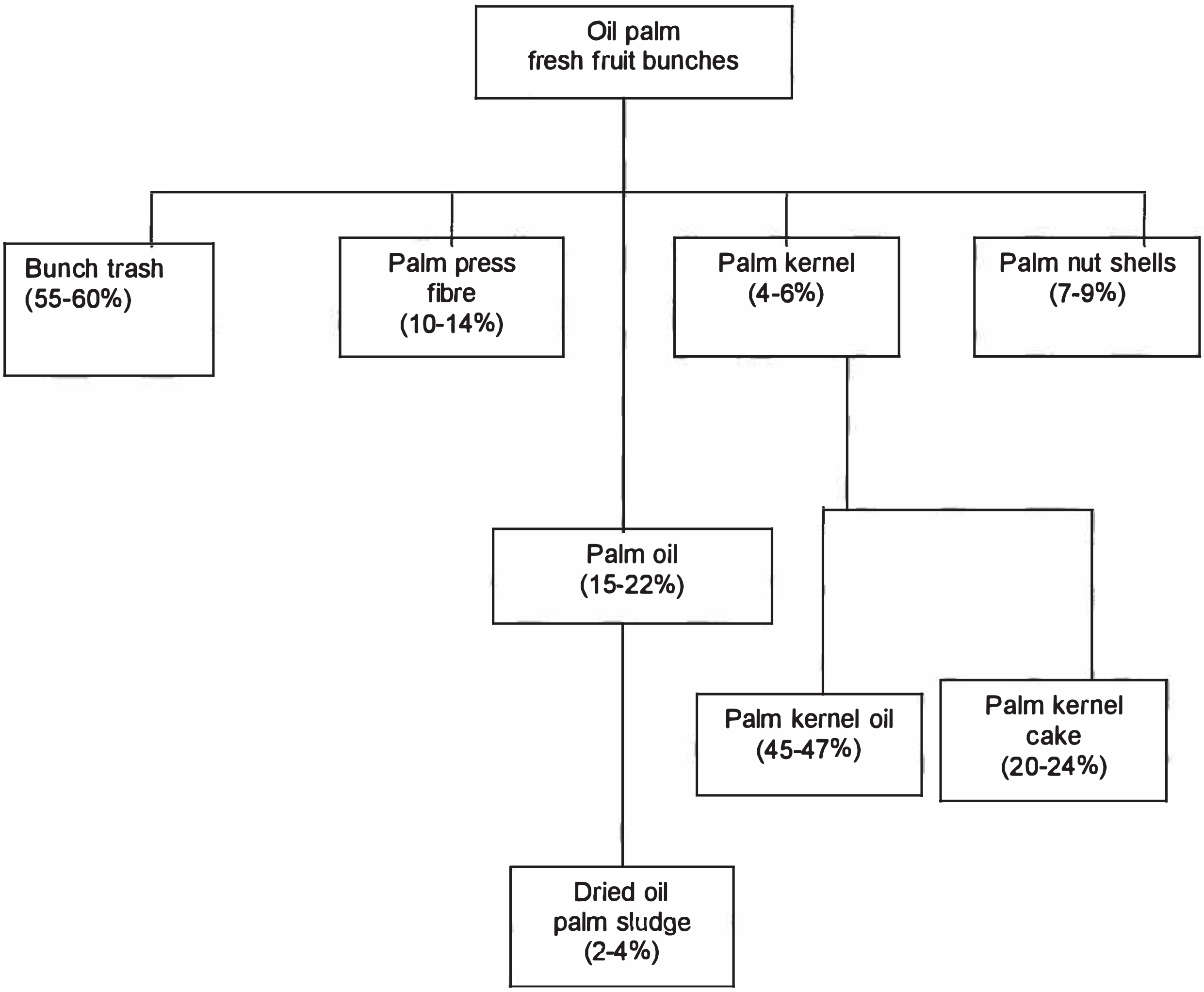

Figure 1: Extraction Rates of Products and By-products from the Oil Palm (Hutagalung, 1981)

Palm Kernel Cake

Among oil palm by-products, palm kernel cake (PKC) is the only feedstuff, which is produced in relatively large quantities in Malaysia. The production of PKC is around 1 million tonnes annually, valued at RM 260 million (Jalaludin *et al*., 1991). However, this by-product is mainly exported to Netherlands and Germany. If PKC could be fully used as the feed for domestic livestock, especially for ruminants under an intensive system, the ruminant industry in Malaysia would be improved dramatically.

Chemical Composition of PKC

As mentioned earlier, PKC contains sufficient level of crude protein and energy to be used as feed for ruminant. However, the chemical composition of PKC is variable due to different processing methods. Its crude protein content ranges form 7.7% to18.7% in a study by Jalaludin *et al*. (1991). The shell content varies from 5.5% to 27.8%. A high level of shell reducing PKC degradation by 18-20 units was shown in the same study. The studies by Sattapukdee and Jalaludin (1991) have also shown that the digestibility of PKC was high, with dry matter digestibility (DMD) of 70%. It was suggested that PKC could be an excellent ruminant feed (Jalaludin, *et al*., 1991). Information concerning the intestinal digestibility of rumen escape protein in PKC is scarce. The average digestibility (82%), as presented by Van Straalen and Tamminga (1990), is based on two widely differing observations (Hindle, *et al*., 1995). Therefore, the ruminal protein degradability of PKC, is still not ascertained.

PKC has a high mineral content, especially copper (20-50 ppm), iron (800-6,000ppm), and phosphorus (4000-6000 ppm). The copper content of PKC is potentially toxic to sheep and possibly goats if given in large quantities (Hair-Bejo and Alimon, 1992a,b). However, incidences of copper toxicity are rare in other ruminants when fed similar levels of PKC. Ovines are much more susceptible to copper toxicity than the bovines. The high level of iron in PKC may cause deformed legs in lambs (Hidiroglou, *et al.*, 1978) and decrease productivity in dairy cows (Coup and Campbell, 1964).

Moreover, the calcium and phosphorus levels in PKC are not in the proper proportion for ruminants (0.3:1 – 1:2), but this can be corrected with calcium supplements.

PKC as Animal Feed

PKC is highly palatable to all ruminant species such as cattle, buffaloes, goats, sheep and even deer, and has been categorized as a protein feed. It's CP and ME are in the same level, as required by those large ruminants. Progress has been made in using this by-product in ration of cattle (Camoens, 1978; Dalzell, 1978) and pigs (Hutagalung, 1981). The inclusion levels in poultry and pig rations were not high (usually 10-15%) because of it's high crude fiber content (Jalaludin, 1995). With proper mineral and vitamin supplementation it can be fed at levels up to 100% in feedlot beef cattle. Growth rates of up to 1.1kg/day with Droughtmaster cattle have been recorded (Alimon, 1993).

Studies have also shown that PKC when combined with small quantity of feed additives (e.g. minerals and vitamins) produced gains as much as 0.7-1.0 kg/head/day in cattle (Hutagalung, 1985).

Ganabathi (1983) has also reported PKC in feeds of dairy cows and bull calves of crossbred dairy cattle. Feeder cattle finished in the feedlot are generally younger than grass fed cattle because of the higher weight gain. Thus, the meat when slaughtered is generally tender (Mahyuddin, 1993).

PKC is also widely used in feeding sheep under intensive and semi-intensive farm systems. Supplementation at rate of 200-400 g/head/day appear sufficient in promoting daily gains ranging between 120-150 g (Wan Mohamed *et al*., 1988). Davis *et al*. (1995) reported that Dorsimal lambs could be fed a grass free diet of 83% PKC and 17% fishmeal supplemented with 20 g/kg siromin (copper free) from weaning to early maturity giving a good growth rate suitable for prime lamb production.

The improvement of the growth rate of lambs could be achieved by providing PKC as a supplement in the diet at the level of 1.35% body weight (Ginting *et al*., 1987). Another study by Settapukdee and Jalaludin indicated that lambs fed wholly on PKC showed good performance, gaining by as much as 150 g/day (Jalaludin, *et al*., 1991).

PKC has also been fed to deer as a supplement although little work has been done to evaluate PKC for deer in the long term (Alimon, 1993).

Toxicity Effects of PKC

PKC must be used with caution since an excess of 30% in diet can cause a ten-fold increased in blood glucose in the sheep (Zainur, 1991), whilst higher levels can cause death, as a result of Cu poisoning (Hair-Bejo and Alimon, 1992ab; 1995; Hair-Bejo, *et al*., 1995a).

Although there is a dearth of information on the use of PKC for animal feed, very little information is available on its nutritive value and factors affecting its utilization by ruminants, it is well known that symptoms of copper-toxicity can develop as early as 4 to 6 weeks after feeding high levels of PKC (90%) in sheep (Alimon and Hair-Bejo, 1995). When animals were fed solely with PKC, there is a negative balance of –21.5% for Mn, -20% for Fe and –52.9% for Zn (Wong and Moh Salleh, 1989).

It has been established that sheep do not tolerate high levels of PKC in the diet and show symptoms of copper toxicity that coincide with those described by Dick *et al.* (1975). Generally, sheep fed high level of PKC showed elevated Cu concentrations in the liver, kidneys and blood. When sheep died due to Cu toxicity, normally the main clinical signs are generalized jaundice and haemoglobinuria. The kidneys are firm, enlarged and reddened or darkened. Histologically, the hepatocytes are swollen, vacuolated and necrotized, particularly at the periacinar zone (Hair-Bejo and Alimon, 1992a, b). Bremner (1980) has suggested that the greater susceptibility of sheep and calves to chronic Cu poisoning could be related to their inability to accumulate large amount of Cu as monomeric metallothionein in their livers. The increased concentration of Cu in the liver of cattle, goats and sheep with increased Cu intake is well known (Underwood, 1977). Liver and other internal organs are widely consumed by the Malaysian population and might pose a health hazard, but the effect of high Cu intake by dairy cows or goats on the Cu accumulation in milk is little known. Thus, the effects of high concentrations of Cu in PKC on accumulation of Cu in the internal organs and milk must be investigated.

Copper Toxicity

Copper (Cu) is both an essential and a toxic element (Underwood, 1977). A number of metalloenzymes containing Cu were identified in the cells and tissues, including ascorbic acid oxidase, monoamine oxidase, benzalamine oxidase, cytochrom oxidase, ceruloplasmin (ferroxidase), and erythrocuprein or superoxide dismutase (SOD). The concentrations and activities of many of these enzymes were then related to the specific functional and structural disorders that develop in the Cu-deficient animals. On the other hand, the excessive Cu may cause toxicity in the animal. The sudden ingestion of large amounts of Cu may produce acute toxicity. However, this situation is relatively uncommon. The occurrence of chronic Cu toxicity has been recognized as a major hazard in the intensive rearing of sheep, but much less in cattle and monogastric animals. Nevertheless, Shand and Lewis (1957) reported that young calves are almost as sensitive as sheep to Cu toxicity.

Copper Absorption, Storage and Excretion

Cu can be absorbed in all segments of gastrointestinal tract. Although sites in the upper section of the small intestine appear to play the major role of Cu absorption in the majority of the animals, considerable net absorption of Cu was found taking place in the large intestine of the sheep (Grace 1975). Homeostasis is effected by controlling the rate of absorption, which in turn is regulated by the intestinal mucosa and its excretion (Davis and Mertz, 1987). The absorbability or dietary Cu differ between foodstuff, and the efficiency of Cu absorbability usually ranges between 32-60% (Wan Aahari, personal communication).