THE EFFECTS OF BENTONITE ON RUMEN PROTOZOAL POPULATION AND RUMEN FLUID CHARACTERISTICS OF SHEEP FED PALM KERNEL CAKE

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Summary

The effects of bentonite (B) on rumen protozoal population and rumen fluid characteristics of sheep fed palm kernel cake (PKC) were studied for a period of 21 days. Two groups, each comprising two sheep were fed either PKC or PKC + B *ad libitum*. A third group was left at pasture. Rumen fluid was sampled through a rumen cannula three times daily from all animals. Palm kernel cake contained 16% crude protein, 1% crude fat and high amounts of copper, zinc, iron and manganese. Protozoal population in the rumen fluid decreased significantly ($p < 0.05$) after the onset of feeding PKC or PKC + B. However, sheep given bentonite supplementation at 2% of the dietary dry matter, maintained higher protozoal densities (15×10^4/ml) when compared to animals fed only PKC (8×10^4/ml). With both diets, the protozoa were mainly of the small entodinia species. Animals at pasture had higher protozoal population (47×10^4/ml) with varying species of entodiniomorphids and holotrichs. Rumen fluid pH and ammonia concentration was significantly ($p < 0.05$) higher in animals at pasture compared to animals fed PKC or PKC + B. Volatile fatty acid concentration was significantly ($p < 0.05$) lower in animals fed PKC when compared to animals at pasture. There was a shift in fermentation pattern in animals fed PKC or PKC + B towards a lower acetate; and higher propionate, isovalerate and valerate. Studies *in vitro* also showed the positive effect of bentonite on protozoal numbers.
(**Key Words** : Bentonite, Protozoa, Palm Kernel Cake.)

Introduction

The palm oil industry in Malaysia produces a number of by-products including palm kernel cake (from the kernel) and palm press fibre (from the mesocarp layer) after the extraction of oil from the fruits; the empty fruit bunch, oil palm trunk and fronds. Of these by-products, palm kernel cake (PKC) has been found to be a good feed material for some ruminants. It is produced at about 60×10^3 tonnes annually and has a high nutritive value with 16% crude protein and dry matter (DM) digestibility of around 70-80% (Jalaludin et al., 1991). However, the copper (Cu) content is quite high−25 to 55 ppm (Jalaludin et al., 1991). Earlier studies showed that cattle and buffaloes fed with PKC as supplements or basal diets generally result in improved growth performance (Hutagalung and Mahyuddin, 1985; Jelan et al., 1991).

Although PKC has been used quite extensively as concentrate feed for cattle and buffalo, it has adverse effects in sheep and goats. Prolong feeding with PKC in sheep and goats resulted in problems related to Cu toxicity (Wan Mohamed et al., 1987).

Rumen protozoa have been implicated to be involved in Cu metabolism (Ivan et al., 1986, 1991). Their presence may alleviate Cu toxicity as it has been shown that protozoa can decrease both Cu solubility in the rumen and concentration in the liver of sheep fed corn silage with or without soybean meal and Cu supplement (Ivan, 1988, 1989). Ivan et al. (1986) suggested that rumen ciliates can increase production of sulphide through increase in breakdown of rumen degradable proteins. The

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sulphide then forms an insoluble complex with Cu, making it unavailable for absorption. However, ciliates do not metabolise rumen soluble proteins such as casein and, therefore, in such a case, have no effect on absorpiton of Cu (Ivan, 1989; Ivan et al., 1991).

In a later study, Ivan et al. (1992a) showed that fauna-free sheep given a corn silage-based diet supplemented with 0.5% bentonite (a finely divided montmorillonite clay) had both low Cu solubility in the rumen and Cu concentration in the liver. These effects were enhanced in faunated sheep. It seems that bentonite can lower the bioavailability of dietary Cu with or without the presence of protozoa.

In view of the proposed role of protozoa in Cu metabolism and the effect of bentonite on protozoal densities, a study was conducted to determine the effects of feeding PKC and PKC plus bentonite (PKC + B) on protozoal population and rumen fluid characteristics in sheep. An *in vitro* study was also conducted with a mixed population of protozoa isolated from sheep to determine the effects of adding PKC and PKC + B on protozoal growth.

Materials and Methods

In vivo Study

The animals used were six Dorset × Marlin sheep about 3 years old, weighing 20-30 kg, each fitted with a rumen cannula and left to graze guinea grass (*Panicum maximum*) *ad libitum* in the paddock before the experiment was conducted. The animals were then divided randomly into three groups, each comprising two animals. The animals in the first two groups were placed in individual crates, while the animals in the third group were left at pasture. Each crate was fitted with a feeding trough and drinking water. The sheep in the first group were fed PKC and those in the second group were fed PKC + B *ad libitum*. The PKC + B diet contained on DM basis 2% bentonite (Fisher Scientific, Fair Lawn, New Jersey). The PKC used in this study was the solvent-extracted type. The amount of feed consumed by each animal in the first two groups was recorded daily during the experimental period.

Sampling

About 50 ml of rumen fluid was removed from each sheep fed (PKC or PKC + B) three times daily at 3 h intervals starting at 09:00 h (before fresh feed was offered) for 21 days. Similar sampling times were also followed with sheep at pasture. Each sample was strained through two layers of muslin cloth and determined for pH. A subsample of 3 ml was fixed with formaline-methylene blue for protozoa counting and identification (Ogimoto and Imai, 1981). The rest of the sample was preserved with 24% metaphosphoric acid for ammonia and volatile fatty acids (VFA) analyses. Ammonia concentration was determined by distillation and automatic titration (Vapodest, Gerhardt Instrument), while VFA were analysed by gas liquid chromatography (Shimadzu GC-14A) fitted with a flame ionisation detector and a glass column packed with 10% (w/v) PEG 600, using nitrogen gas as the carrier. Isocaproic acid was used as the internal standard.

In vitro Study

For the *in vitro* study, a mixed population of protozoa was obtained from sheep fed guinea grass. Isolation and washing procedures were carried out using the method described by William and Strachan (1984). The protozoal preparation contained about 1.5×10^6 cells per ml and 1.5 ml were innoculated into 15 ml of salt 'simplex' medium (Coleman, 1987) under anaerobic conditions. The salt medium contained 5 mg of either PKC or PKC + B, or 1.5 mg wholemeal flour (instead of grass). The protozoa, grown at 39°C, were counted in duplicates daily for four days.

Proximate and Mineral Analyses of PKC

The palm kernel cake used was analysed for its dry matter, crude protein, crude fat and ash (A.O.A.C., 1984); acid detergent fibre and neutral detergent fibre (Goering and Van Soest, 1970). The mineral contents were also analysed by atomic absorption spectrophotometry after digestion in nitric and perchloric acids (Hoffman et al., 1968). Four replicates were used in the analyses.

Statistical Analyses

Analysis of variance and Least Significance Differences (LSD) were used to comparre treatmeat Means.

Results and Discussion

The chemical composition of PKC is shown in table 1. It contained about 16% crude protein and 1% crude fat which are similar to those reported by Jalaludin et al. (1991). Besides high amount of Cu, PKC also contained large amounts of zinc (Zn), iron (Fe) and manganese (Mn), but trace amounts of cobalt and selenium. The chemical constituents of PKC might vary with different batches, but the proximate analysis seemed to be within the range of values reported earlier (Miyashige et al., 1987).

TABLE 1. CHEMICAL CONTENTS OF PALM KERNEL CAKE[1]

Proximate analysis (%)	
Dry matter	89.9
Crude protein	15.9
Crude fat	1.1
Acid detergent fibre	43.9
Neutral detergent fiber	79.7
Ash	5.1
Macro-minerals (%)	
Calcium	0.3
Phosphorous	0.6
Magnesium	0.3
Potassium	0.8
Sulphur	0.2
Micro-minerals (μg/g)	
Copper	28
Zinc	45
Iron	3,369
Manganese	200
Cobalt	trace
Selenium	trace

[1] Results reported on 100% dry matter basis, except the dry matter content.

Figure 1 shows the daily DM intake by animals fed PKC or PKC + B. Intake seemed to vary from day to day; the reason is not known. There was no measurement on grass intake of animals at pasture.

Table 2 shows the mean feed intake during the experimental period. Animals fed PKC + B tended ($p < 0.07$) to consume higher amount of feed when compared to animals fed PKC. This may be due to elevated bacterial activity in the rumen and, consequently, improved digestibility of PKC + B diet. Ivan et al. (1992b) showed a 10% higher duodenal flow of bacterial nitrogen in faunated sheep fed a bentonite-supplemented diet.

Figure 2 shows the average daily protozoal counts for three weeks for the three groups of animals. The mean numbers of protozoal cells for the whole sampling period are shown in table 2. Protozoal population in the rumen fluid dropped immediately after the consumption of PKC in the first two groups of animals. The predominant species in these animals after 21 days of consuming PKC or PKC + B were the small entodinia. The larger holotrichs were seldom detected. The protozoal population in sheep at pasture consisted of various species of

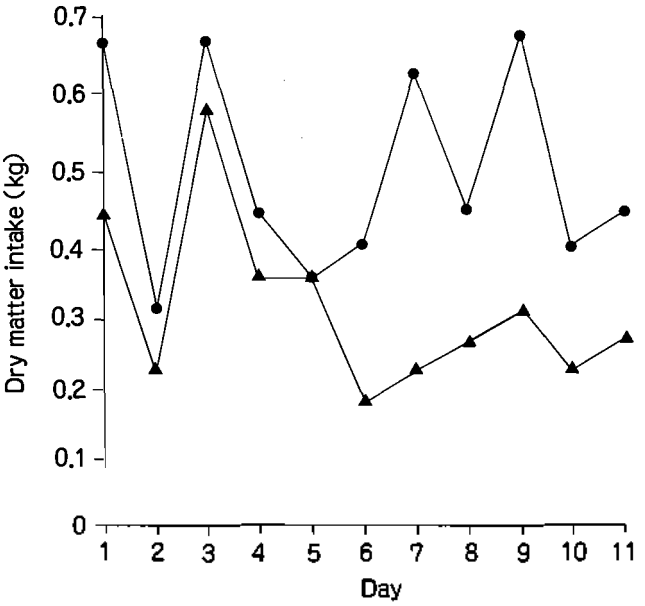

Figure 1. Average daily dry matter intake during the last 11 days of the experimental period for sheep fed PKC (▲) or PKC + B (●).

TABLE 2. FEED INTAKE, PROTOZOAL COUNTS AND CHARACTERISTICS OF RUMEN FLUID OF EXPERIMENTAL SHEEP

Parameters	Diet		
	Grass (Mean ± S.E.M.)	PKC (Mean ± S.E.M.)	PKC + B (Mean ± S.E.M.)
Feed intake			
(% body weight)	–	1.50 ± 0.15^{a}	1.75 ± 0.15^{a}
($g/W^{0.75}$)	–	32.0 ± 3.1^{a}	40.0 ± 3.0^{a}
Protozoa (cells per ml)	47×10^{4a}	8×10^{4b}	15×10^{4c}
pH	6.98 ± 0.49^{a}	6.30 ± 0.25^{b}	6.02 ± 0.39^{c}
Ammonia (mg N per 1)	166 ± 57^{a}	131 ± 46^{b}	107 ± 49^{b}
Total VFA (mM)	96.2 ± 6.9^{a}	74.8 ± 5.5^{b}	86.8 ± 6.0^{a}
Molar %:			
Acetic	66.1 ± 0.6^{a}	54.4 ± 2.7^{b}	52.5 ± 1.6^{b}
Propionic	19.6 ± 0.5^{a}	26.4 ± 1.3^{b}	27.9 ± 1.6^{b}
Butyric	11.2 ± 0.6^{a}	11.6 ± 1.6^{a}	10.9 ± 0.6^{a}
Isovaleric	1.6 ± 0.3^{a}	3.7 ± 0.8^{b}	5.4 ± 0.7^{b}
Valeric	1.1 ± 0.1^{a}	4.4 ± 0.8^{b}	4.3 ± 0.5^{b}

[a, b, c] Means within the same row with different superscipts are statistically different ($p < 0.05$).

S.E.M. Standard error of mean.

entodiniomorphids and holotrichs. The adverse effect of PKC on protozoa has been reported in cattle (Abdullah and Hutagalung, 1988) fed a PKC-based diet, but the reason is not known. Some dietary factors may reduce or eliminate ruminal protozoa. The presence of high amounts of Cu, Zn, Fe and Mn may contribute to the defaunation effect as excessive dietary supplementation of Cu and zinc can reduce the protozoal population (see William and Coleman, 1992). Bentonite could alleviate the defaunation effect of PKC, but the protozoal population was still very much lower than that of animals fed grass. Reparts on the effect of bentonite supplementation on protozoal density is rather contradictory. Fenn and Leng (1990) observed an increase in protozoal density in sheep fed roughage diet supplemented with 15-16 g bentonite/day, whereas Ivan et al. (1992a) did not find any increase in protozoal numbers in sheep fed a corn-silage-based diet supplemented with bentonite.

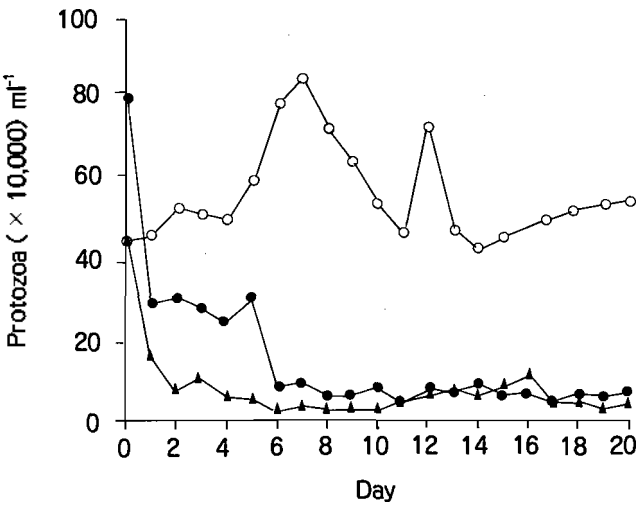

Figure 2. The mean daily protozoal numbers per ml of rumen fluid of sheep fed PKC (▲), PKC + B (●) or at pasture (○).

The pH of rumen fluid was within the physiological range (table 2), but animals fed PKC + B showed the lowest value. Lower rumen fluid pH was also observed in sheep fed corn-silage supplemented with bentonite (Ivan et al., 1992a). Rumen ammonia was higher in animals fed PKC than in animals fed PKC + B. Bentonite had been reported to absorb ammonia in the rumen (Jacques et al., 1986). However, the difference was not significant between the two diets because of the large variations in daily ammonia contents. Total VFA for animals fed grass and PKC + B were almost similar, but the concentration was significantly ($p < 0.05$) reduced in animals fed PKC. The high VFA concentration in animals fed PKC + B reflects a more active fermentation activity when compared to animals fed PKC. Molar proportions of VFA of animals at pasture were comparable to those of other animals fed roughage reported by Abdullah et al. (1991). However, animals fed PKC and PKC + B showed a reduction in acetate, but with a significant ($p < 0.05$) increase in propionic acid. Only a small amount (about 1 %) of isobutyric was detected in the rumen fluid of all animals. Butyric acid production in animals fed PKC and PKC + B was comparable to animals at pasture, but was lower when compared to animals fed concentrate-based diet (Jackson et al., 1971). The reduction in protozoal population may result in lower butyrate production as protozoa are also butyrate producers and significant reduction in butyrate production had been observed in defaunated lambs (Eadie and Gill, 1971). The fermentation pattern of low levels of acetate and butyrate and high level of propionate on PKC-based diet had been observed in cattle (Hamali and Shukri, 1993). The fermentation activity in animals fed PKC produced higher amounts of isovaleric and valeric acids compared to animals at pasture. Similar increase in isovaleric acid had been reported earlier in cattle fed PKC-based diet (Abdullah and Hutagalung, 1988). The increase in isovaleric acid could be an advantage to the animal as earlier reports showed that the use of isoacids in the diet improved microbial protein synthesis and cellulose digestion (Russell and Sniffen, 1984; Gorosito et al., 1985).

Protozoal counts in 'simplex' medium containing either wholemeal flour, PKC or PKC + B are shown in figure 3. Protozoal numbers decreased gradually over the four-day incubation period for all the three substrates used. The survival rate of protozoa *in vitro* declined with longer incubation period. Decrease in protozoal numbers with longer incubation period had been reported by Wallace and Newbold (1991) in their studies on rumen ciliate protozoa using the Rusitec. In the present study, protozoal numbers in medium containing wholemeal flour were significantly ($p < 0.05$) higher than those containing PKC + B or PKC. The addition of bentonite seemed to improve protozoal growth as the numbers in the medium containing PKC + B were higher than those containing PKC. The mechanism involved is not clearly understood, but bentonite may absorb substances present in PKC that are toxic to the protozoa. On the other hand, Wallace and Newbold (1991) reported that the addition of 2 g bentonite/day to fermentation vessels (Rusitec) with rumen fluid from sheep and a mixture of forage and concentrate as food depressed protozoal growth, and that bentonite prevented motility of protozoa. Nevertheless, the

results from the *in vitro* study confirmed the observation obtained *in vivo*, where PKC depressed protozoal growth and bentonite reduced this effect.

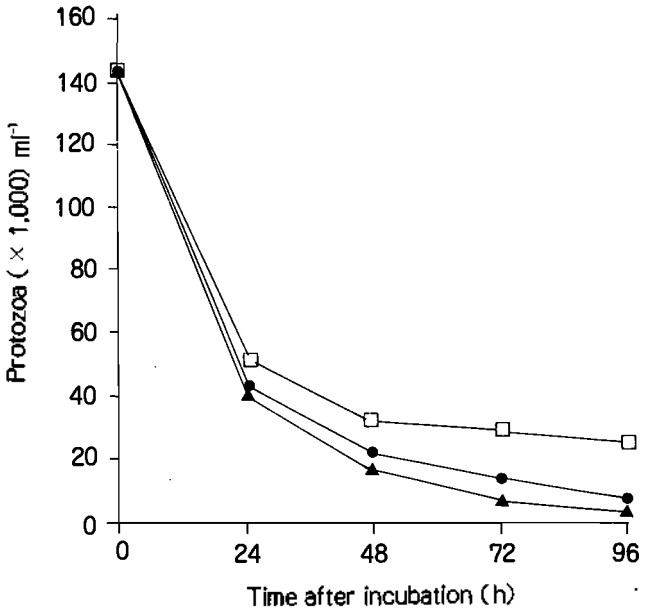

Figure 3. The mean daily protozoal numbers per ml in 'simplex' medium containing wholemeal flour (□), PKC (▲) or PKC + B (•).

Besides protozoa, the rumen bacteria and fungi were also affected by PKC. The number of cellulolytic bacteria was significantly ($p < 0.05$) reduced in buffalo fed 5 kg of PKC (Hussain et al., 1993). The same phenomenon was observed in the rumen fungi, where PKC greatly reduced fungal population in animals fed or supplemented with PKC (Y. W. Ho, personal communication).

Further studies on the effects of the large amounts of Cu, Fe, Mg and Mn in PKC on protozoa and other microbial population have to be conducted. The information would be useful in developing strategies for maintaining a normal microbial population in the rumen of animals fed PKC.

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