

## Chapter 3

### Effect of Different Sources of By-pass Protein on Liveweight Gain and Wool Production of Wethers Fed Fibre-based Diet

#### 3.1. Introduction

Ruminants, with the assistance of micro-organisms in the rumen, are able to use low quality fibrous diets such as roughages and produce valuable products, for example meat, milk, hair and wool. However, fibrous diets are usually deficient in protein and low in digestible nutrients. Supplementation with NPN is necessary to provide adequate ammonia to the rumen microbes but high quality protein is not necessary for the rumen microbes because most dietary protein will be degraded to NPN compounds before being used by the microbes. When animals are given high protein diets, degradation of the ingested protein by rumen microbes causes a serious wastage of nitrogen (Hogan and Weston, 1967; MacRae *et al.*, 1972).

The major sources of amino acids in the small intestine are microbial protein produced in the rumen and dietary protein that bypasses rumen fermentation (Hvelplund and Madsen, 1985; Clark *et al.*, 1992). Supplementing ruminant diets or a low protein diet with rumen bypass protein is one way to increase the availability of amino acids for the host (Zinn *et al.*, 1981; Titgemeyer *et al.*, 1989; Cecava and Parker, 1993).

A number of protein meals in Australia are considered to be useful sources of energy and bypass protein, e.g. cottonseed meal and sunflower meal (Leng, 1992). In recent years, some research has been done to determine the value of such bypass protein sources as supplements for ruminants (Mathers and Miller, 1980; Hvelplund, 1985; Hvelplund and Madsen, 1985; Zimmerman *et al.*, 1992; Cecava and Parker, 1993).

Sources of protein and energy available in the tropics include PKC, palm oil sludge, copra meal, rice bran, soybean, fish meal, rubber seed, and groundnut cake. Almost all of these sources of protein are agro-industrial by-products. These sources are less fibrous and have a higher nutrient content than crop residues (Devendra,

1989). Some research has been done to investigate the efficiency of utilization of PKC (Jelan, 1991; Devendra, 1977; Boer and Sanchez, 1989; Abdullah *et al.*, 1991; Abdullah and Hutagalung, 1988; Rahman *et al.*, 1990) and copra meal (Gulbransen *et al.*, 1990; Ehrlich *et al.*, 1990; Galgal *et al.*, 1994) as supplements in ruminant diets. These studies have demonstrated that PKC and copra meal have the potential to increase animal performance. Jalaludin (1989) showed the characteristic of PKC (Table 2). However, there do not appear to be any direct comparisons of the efficiency of utilization of palm kernel cake (PKC) and copra meal as alternatives to cottonseed meal and sunflower meal as protein supplements for inclusion in diets for sheep.

The present study was conducted to investigate the efficiency of utilization of these sources of protein (PKC, copra meal, cottonseed meal, and sunflower meal) by sheep for liveweight gain and wool production. In this study iso-nitrogenous amounts of PKC, copra meal, cotton seed meal, sunflower meal were used as alternative protein supplements for sheep given a basal diet of urea-treated oaten chaff. The basal diet was supplemented with urea with a view to ensuring that any response to supplement was not a response to rumen degradable nitrogen.

### **3.2. Materials and Methods**

#### **3.2.1. Animals and Feeding**

Twenty-five Merino wethers (approximately five months old and weighing 27 to 33 kg) were used in this experiment. They were housed individually in single pens and given lucerne and oaten chaff (50:50) during a pre-experimental period of 39 d. They were then divided randomly into five treatment groups (5 animals each group) and fed according to their treatment for 45 d to allow them to adapt to the experimental conditions. The sheep was not shorn at the end of the pre-experimental period, but was dye-banded. The sheep were shorn around the thigh to the tail and around the eyes at the end of the pre-experimental period in order to reduce the likelihood of fly strike. The formal experiment was started at the end of the adaptation period when the sheep were approximately 7 months old (31 to 38 kg). The experiment continued for 36 d. They were fed once a day with urea-treated oaten chaff (O.C.) (3 % urea ) plus a mineral mix as a basal diet and one of the protein

supplements according to treatment group. The treatments were : basal diet (control group), basal diet + 43.4 g cotton seed meal (C.S.M) + 50 g sunflower meal, basal diet + 100 g sunflower meal (S.F.M.), basal diet + 107 g P.K.C. + 50 g sunflower meal and basal diet + 75 g copra meal + 50 g sunflower meal. The sheep had continuous access to water. The pens were cleaned daily. To measure the feed intake, both supplement and basal diet residues were recorded daily.

### **3.2.2. Diet**

Each sheep was fed a fixed amount of feed. The basal diet consisted of urea-treated oaten chaff and 7 g mineral mix. The amount of oaten chaff offered was 823 g/d for the control group (unsupplemented group) and 600 g/d for supplemented groups because they also had access to concentrate. The amount of supplement offered was sufficient to provide 18.4 g N/d. Thus, because each supplement had a different crude protein content, the amount of the ration offered differed among the treatments (in order to provide the same amount of N). Supplements were given separately before the basal diet was offered. Each supplemented group was given 50 g sunflower meal mixed in the supplement. The quantity of diets and the amount of nitrogen offered to the sheep is given in Table 4.

### **3.2.3. Feed analysis**

The chemical composition of the feeds given to the sheep is shown in Table 3. The dry matter content of the feed was determined on ground samples (1 mm sieve) by drying in an oven at 70 °C for 48 h or until weight was constant. Crude protein (N x 6.25) was determined using the Kjeldahl method. Ash content was measured by heating samples in a furnace at 600 °C for 6 h. The energy content of the feeds were determined using a bomb calorimeter (Atomic calorific processor, CP500).

### 3.3. Measurements

#### 3.3.1. Feed intake

Sheep were fed daily during the pre-experimental period and the experimental period. Daily feed intake was calculated by subtraction of the amount of the feed residues from the amount of feed offered. Dry matter intake ( $\text{g/kg } W^{0.75}$ ) was calculated based on the dry matter content of the diet and the most recent estimate of liveweight.

Table 3. The composition of feed ingredients

<u>Feeds</u>	<u>DM (%)</u>	<u>CP (%)</u>	<u>Ash (%)</u>	<u>GE (MJ/kg)</u>
O.C.	88.8	6.4	5.5	17.1
C.S.M.	89.0	38.0	6.24	17.6
S.F.M.	89.4	33.0	7.24	17.4
Copra meal	94.4	22.0	6.24	18.3
P.K.C.	92.8	15.4	3.65	19.2

#### 3.3.2. Liveweight gain

Animals were weighed at the beginning and end of both the pre-experimental period and the experimental period. The sheep were weighed in the morning before any feed was offered. Liveweight gain ( $\text{g/d}$ ) was calculated for the pre-experimental period and the experimental period. Daily liveweight gain of sheep in the pre-experimental period was used as a covariate when analysing the daily liveweight gain data obtained in experimental period.

#### 3.3.3. Wool dye-banding

A mixture of 0.8 g Durafer Black in 100 ml of distilled water mixed with 2.5 ml  $\text{H}_2\text{O}_2$  (0.08 % concentration) was used as a dye-banding solution (Chapman and Wheeler, 1963). The dye bands hopefully were placed at the base of wool staples on

the skin and were approximately 10 cm long. Dye bands were applied at the beginning and end of both the pre-experimental period and the experimental period. The application of dye-bands to the staples was done using syringes, with a 20 gauge needle attached, instead of with a pipette as suggested by Chapman and Wheeler (1963).

Table 4. Feed, crude protein and total N offered to sheep during the experimental period of 36 days

Treatment group	Quantity fed (g/h/d)	Dry matter (g/h/d)	Crude protein (g/h/d)	Total nitrogen (g/h/d)
Group A				
O.C. + Urea (3 %)	823	708.9	45.4 + 24.7 g	
Mineral mix.	7		urea	18.6
Group B				
O.C. + Urea (3 %)	600	516.8	33.1 + 18 g urea	
Mineral mix.	7			
SFM.	50	44.7	14.8	
CSM.	43.4	38.6	14.7	18.3
Group C				
O.C. + Urea (3 %)	600	516.8	33.1 + 18 g urea	
Mineral mix.	7			
SFM.	100	89.4	29.5	18.4
Group D				
O.C. + Urea (3 %)	600	516.8	33.1 + 18 g urea	
Mineral mix.	7			
SFM.	50	44.7	14.8	
PKC.	107	99.3	15.3	18.4
Group E				
O.C. + Urea (3 %)	600	516.8	33.1 + 18 g urea	
Mineral mix.	7			
SFM.	50	44.7	14.8	
Copra meal	75	70.8	15.6	18.4

### 3.3.4. Feed conversion efficiency

Feed conversion efficiency (FCE) into liveweight during the experimental period was calculated as :

$$\text{FCE} = \frac{\text{liveweight gain (g/d)}}{\text{dry matter intake (100 g DM consumed/d)}}$$

This was expressed as liveweight gain (in g) per 100 g dry matter consumed.

### 3.3.5. Wool measurements

Wool samples were obtained from each animal at the end of the experimental period. Wool was cut into two parts, the first grown in the pre-experimental period and the second grown in the experimental period. Greasy wool weight, clean wool weight and mean fibre diameter were measured. Mean fibre diameter and clean wool weight were analysed at the Chiswick wool testing centre, NSW.

### 3.4. Statistical analysis

Data were analysed with analysis of variance of complete randomized design using the Minitab program release 8.2. Data from the pre-experimental period was used as a covariate in the analyses of the data from the experimental period. When the covariate was significant the data was adjusted. The differences among the treatments were tested using Duncan's New Multiple Range Test (Steel and Torrie, 1981).

### 3.5. Results

#### 3.5.1. Pre-experimental period

##### 3.5.1.1. Feed intake

Intake of feed which was offered *ad libitum* during the pre-experimental period was increased every day until a total amount of 1200 g/h/d of the oaten chaff and lucerne mix (50:50) was offered. There was no difference in average feed intake ( $P>0.05$ ) among the groups in this period. However, the average intake of sheep in Group 3 (the group later supplemented with sunflower) tended to be less than the other groups. The feed intakes of Groups 1 - 5 during the pre-experimental period are given in Figure 3:

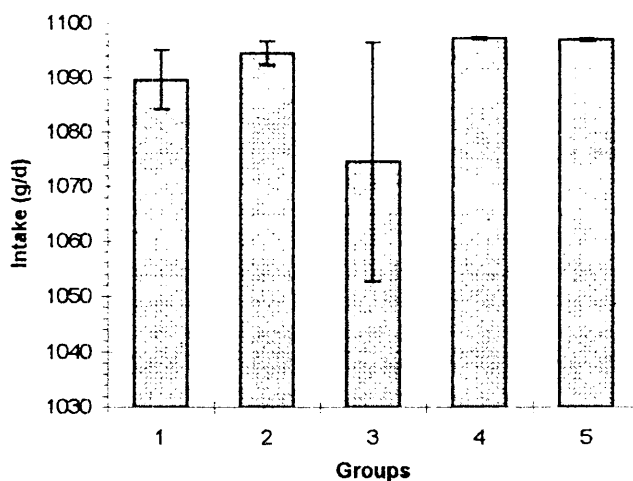


Figure 3. Average daily feed consumption (as fed) by the group of sheep during the pre-experimental period.

##### 3.5.1.2. Liveweight gain

All sheep gained weight at an average rate of 118 g/d during the pre-experimental period (Figure 4). There were significant differences in daily liveweight gain ( $P<0.01$ ) among the groups. Sheep in Group 1 had the lowest live weight gain ( $89 \pm 18.7$  g/h/d) and which was significantly different from sheep in Group 2, 3 and 4,

but it was not differ significantly from Group 5. Liveweight gain of sheep in Group 5 ( $112 \pm 3.5$  g/h/d) was statistically not different ( $P>0.05$ ) from the other groups.

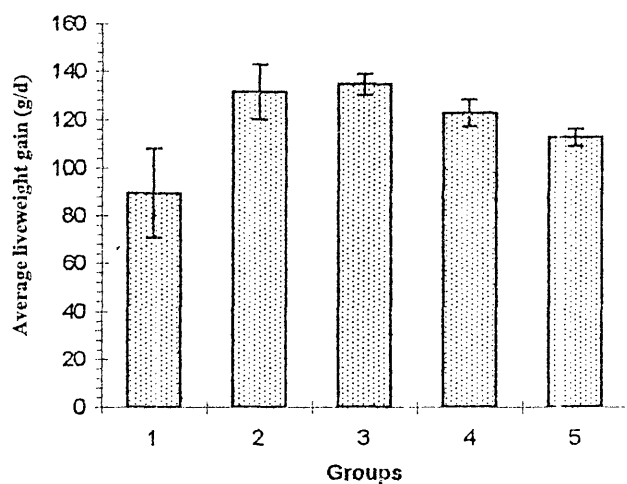


Figure 4. Average daily liveweight gain of sheep given an oaten chaff and lucerne mix during the pre-experimental period.

### 3.5.1.3. Greasy wool production during the pre-experimental period

There was no significant difference in greasy wool production ( $P>0.05$ ) among the groups during the pre-experimental period which had an average production 7.9 g/d (Figure 5).

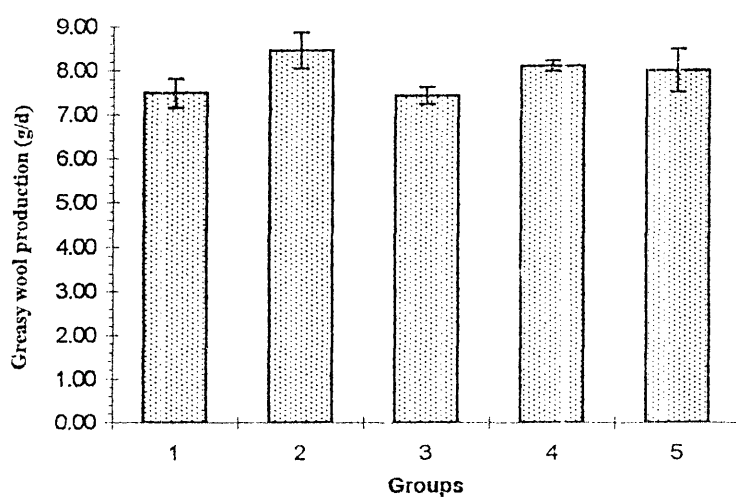


Figure 5. Average daily greasy wool production of sheep given an oaten chaff and lucerne mix during the pre-experimental period.



### 3.5.1.4. Percentage of clean wool production during the pre-experimental period

Clean wool production differed ( $F < 0.05$ ) between the sheep in group 2 ( $66 \pm 2.3\%$ ) and group 4 ( $77 \pm 2.9\%$ ) during the pre-experimental period (Figure 6).

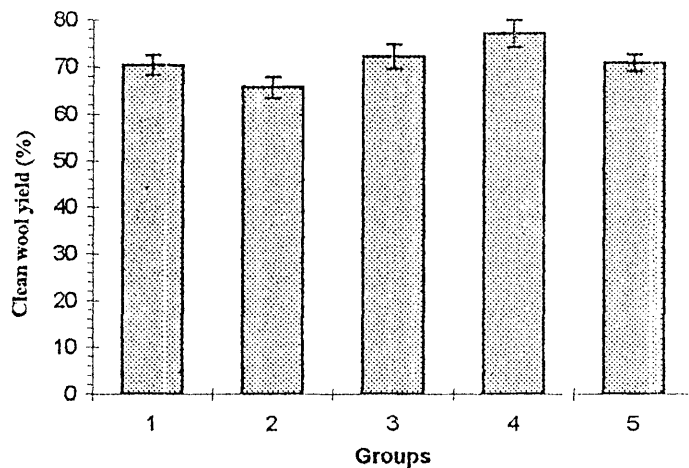


Figure 6. Percentage of clean wool production of sheep given an oaten chaff and lucerne mix during the pre-experimental period.

### 3.5.1.5. Clean wool production during the pre-experimental period

There was no difference in clean wool production ( $P > 0.05$ ) among the groups with the production of  $5 \pm 0.3$ ,  $6 \pm 0.3$ ,  $5 \pm 0.3$ ,  $6 \pm 0.2$  and  $6 \pm 0.4$  g/d for Group 1, 2, 3, 4 and 5 respectively for 39 d during the pre-experimental period (Figure 7).

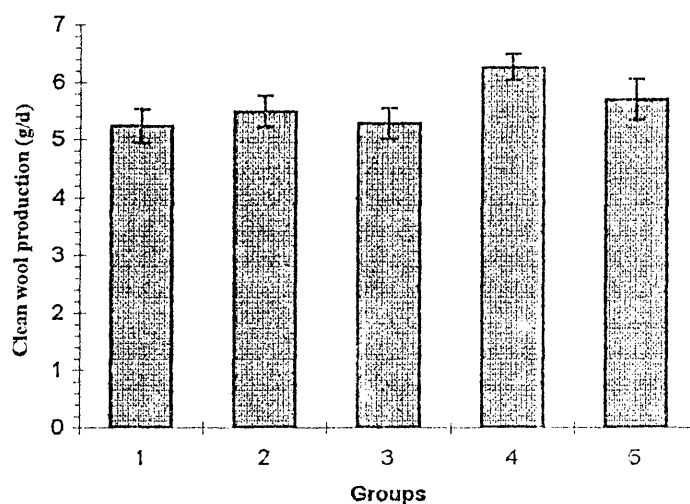


Figure 7. Average daily clean wool production of sheep given an oaten chaff and lucerne mix during the pre-experimental period.

### 3.5.2. Experimental period

#### 3.5.2.1. Feed intake

Differences in intake of feed in this experiment could not be evaluated as an effect of the different supplements, because they were given in a restricted amount in order to supply the same amount of nitrogen in each diet. The sheep supplemented with cottonseed meal, sunflower meal or copra meal consumed all the feed offered, but the sheep supplemented with PKC and the unsupplemented sheep refused a small amount of the feed offered (Table 5).

Table 5. Average feed intake and percentage of feed consumed by the sheep during the experimental period

Treatments	Feed offered per day (g/h)	Average feed consumed/day (g/h)	Percentage of feed consumed* (%)
Control	823	812 ± 6.9	98.7
C.S.M.	693	693 ± 0.0	100
S.F.M.	700	700 ± 0.0	100
P.K.C.	757	750 ± 6.8	99
Copra meal	725	724 ± 0.6	99.8

\* Feed consumed as a percentage of the feed offered.

#### 3.5.2.2. Dry matter intake

The differences in dry matter intake were due to the different amounts of feed offered to the sheep (Table 6).

Table 6. Average dry matter intake by sheep in the groups

Treatments	Average dry matter intake per day (g/ kg W <sup>0.75</sup> /d)
Control	50 ± 1.0
C.S.M.	43 ± 0.9
S.F.M.	44 ± 0.5
P.K.C.	50 ± 0.5
Copra meal	45 ± 0.9

### 3.5.2.3. Predicted digestible energy

Intake of digestible energy was calculated using digestible energy values for feedstuffs from International Feedstuffs Institute (1982) (Table 7).

Table 7. Predicted digestible energy intake by sheep in the groups

Treatments	Predicted digestible energy (MJ per d)
Control	5.97 ± 0.04
C.S.M.	7.10 ± 0.26
S.F.M.	8.15 ± 0.002
P.K.C.	7.90 ± 0.35
Copra meal	7.21 ± 0.01

### 3.5.2.4. Liveweight gain

There was no significant effect of diet ( $P > 0.05$ ) on liveweight gains of sheep in the experiment (Figure 8). Supplementation of the basal diet with cotton seed meal or with sunflower meal during the experimental period resulted in a loss in weight whereas sheep in the other groups gained weight. The average liveweight changes of control group, cottonseed meal, sunflower meal, PKC and copra meal group were 11 ± 6.6, 16 ± 5.7, 9 ± 4.8, 3 ± 5.4, and 2 ± 4.3 g/h/d for 36 d respectively (Figure 8).

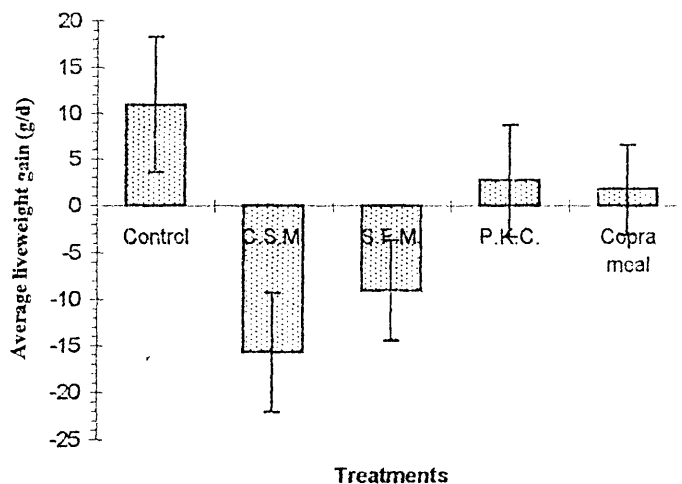


Figure 8. Average daily liveweight gains of sheep given diets containing different protein-rich supplements during the experimental period.

### 3.5.2.5. Feed conversion efficiency

The control sheep fed with urea-treated oaten chaff tended to have higher feed conversion efficiency liveweight (2 g/100 g DMI) than those supplemented with P.K.C. (0.4 g/100 g DMI) and copra meal (0.3 g/100 g DMI) (Figure 9).

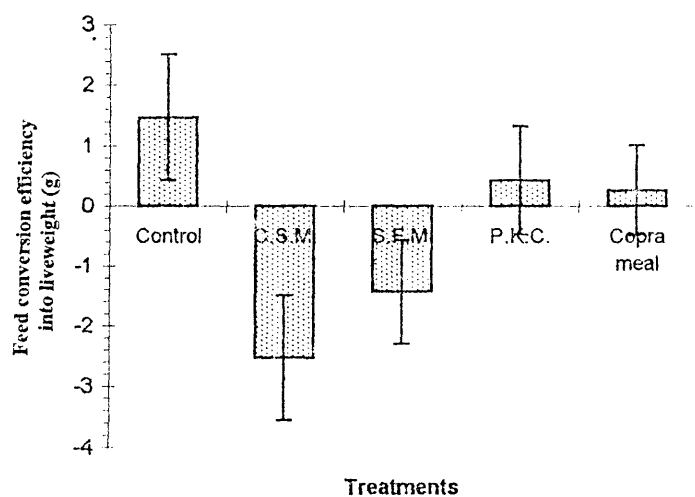


Figure 9. Feed conversion efficiency into liveweight of sheep given diets containing different protein-rich supplements during the experimental period.

### 3.5.2.6. Greasy wool production during the experimental period

There was no significant effect of diet ( $P>0.05$ ) on greasy wool production of sheep in the experiment. The sheep given urea-treated oaten chaff without any supplement produced  $7 \pm 0.6$  g/d greasy wool production and average greasy wool production of the sheep given supplements was  $8 \pm 0.4$  g/d. There were no statistical differences in greasy wool production among the sheep in the groups given the diets containing protein-rich concentration (Figure 10).

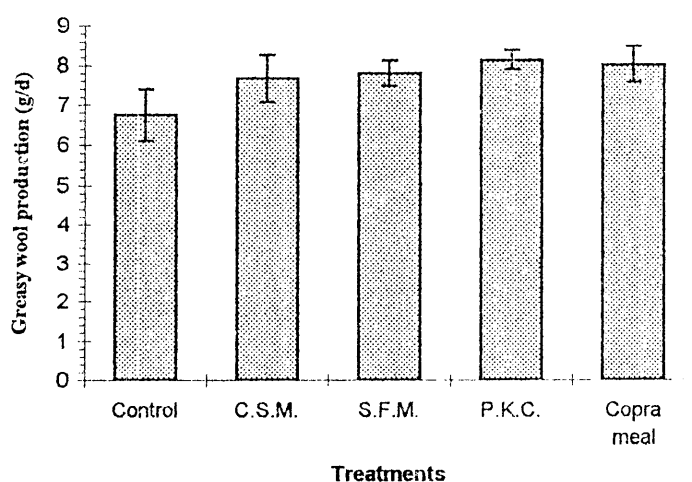


Figure 10. Average daily greasy wool production of sheep given diets with different protein-rich supplement during the experimental period.

### 3.5.2.7. Percentage of clean wool production during the experimental period

There were no significant differences in the clean wool production ( $P>0.05$ ) among the treatments during the experimental period. The average percentage clean wool production was  $72 \pm 2.3$  % (Figure 11).

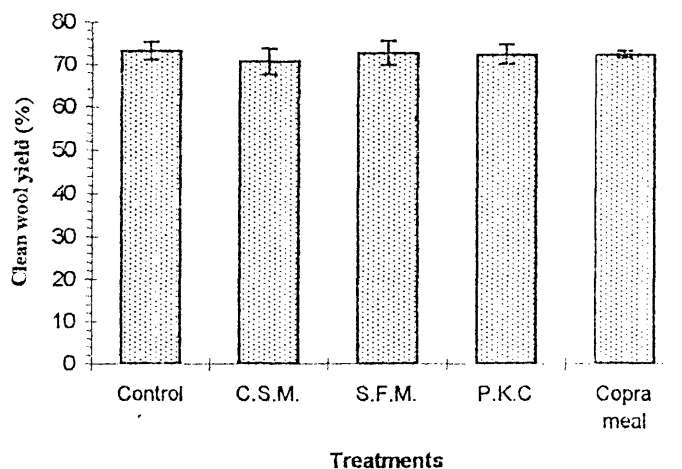


Figure 11. Percentage clean wool production of sheep given diets containing different protein-rich supplements during the experimental period.

### 3.5.2.8. Clean wool production during the experimental period

Clean wool production did not differ ( $P > 0.05$ ) between the groups fed different diets during the experimental period but tended to be higher in sheep given protein-rich supplement. The average clean wool production was  $6 \pm 0.36$  g/d for 36 d during the experimental period (Figure 12).

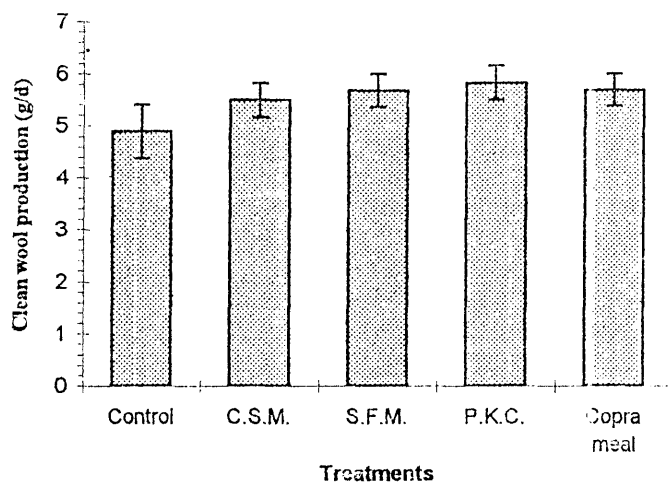


Figure 12. Average daily clean wool production of sheep given diets containing different protein-rich supplements during the experimental period.

### 3.5.2.9. Mean fibre diameter ( $\mu\text{m}$ ) of wool grown during the experimental period

Diet had no effect on wool mean fibre diameter ( $P>0.05$ ) but mean fibre diameter tended to be higher in sheep given a protein-rich supplements. The mean fibre diameter was  $25 \pm 0.88 \mu\text{m}$  (Figure 13).

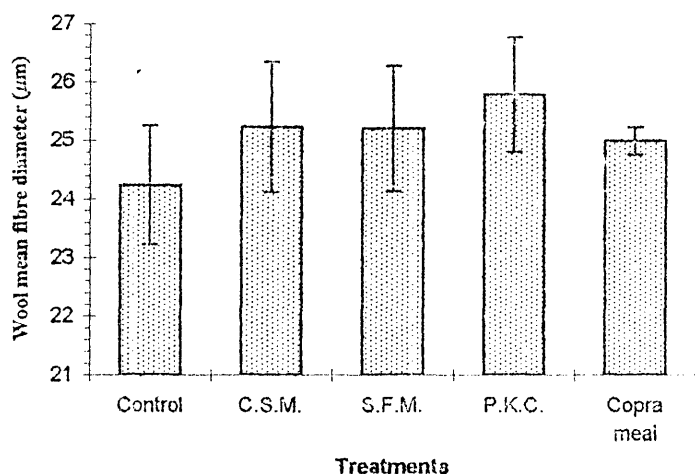


Figure 13. Wool mean fibre diameter of sheep given diets containing a protein-rich supplements during the experimental period.

## 3.6. Discussion

### 3.6.1. Pre-experimental period

The sheep in Group 3 tended to consume less than the other groups. This was probably because the sheep in this group had lower initial liveweight than the other groups. Intake per  $w^{0.75}$  of this group also tended to be lower than group 2, 4 and 5, but seem to be higher than group 1.

The low correlation between liveweight gain and feed intake indicated there were differences in efficiency of utilization of feed between groups. The sheep in group one were less efficient because they ate more but gained less liveweight. On the other hand, the sheep in Group 3 had the highest liveweight gain. Therefore sheep in Group 3 were the most efficient in converting feed into meat and wool, whereas animals in group one were the least efficient.

The difference of percentage of clean wool production between sheep in group 2 and in group 4 was due to the influence of individual sheep in both groups, i.e. 1

sheep in group 2 had very low clean wool production (7.2 % less than the average) and 2 sheep in group 4 had very high wool production (8.3 % higher than average). This shows there was considerable variability between animal in wool growth of animals fed the same diet.

### 3.6.2. Experimental period

Sheep given the diet of urea-treated oaten chaff (control group) had the highest feed intake among the treatments. This is because they were given more oaten chaff in order to have the same amount of nitrogen content in the diet. Therefore, the differences of total feed intake were due to the different amount given and could not be ascribed to the different sources of protein. The sheep supplemented with sunflower meal consumed 100 % of the ration offered and sheep on the other treatments had feed intake almost 100 % of the ration offered (Table 5). This is may be because the *ad libitum* intake (about 1090 g/d) achieved during the pre-experimental period exceeded the amount given in the experimental period. So, the rumen capacity was not a limiting factor when the treatment diets were consumed and the satiety signal may not have been sent to the nervous system to stop eating. However, intake may also have been enhanced by the amount of rumen bypass protein in the diets. Tan and Bryant, (1991) and Kempton *et al.* (1977) stated that the protein status of a ruminant is the first-limiting factor to feed intake in ruminants fed low protein diets.

The sheep supplemented with PKC had a higher dry matter intake because PKC contains lower nitrogen than the other supplements.

The sheep supplemented with cottonseed meal and with sunflower meal tended to lose liveweight but did not differ from other groups. Bird and Dicko (1987) found that sheep fed with basal diets (oaten chaff) + urea had higher liveweight gains (g) than sheep fed with basal diet plus urea supplemented with 100 g/d cottonseed meal. The loss of liveweight of sheep supplemented with cottonseed meal might also be due to an over protection of protein during the processing causing protein to be also protected from enzymatic digestion in the small intestine.

The pattern of amino acids supplied to the intestine for intestinal absorption may also affect the responses of ruminants to bypass protein (Huber and Kung, 1981). In this experiment, 18.4 g nitrogen/d offered to the sheep may be more than adequate



for maintenance, but the lack of availability of energy may have been a limiting factor. Thus, the explanation for the lost of weight in the sheep supplemented with CSM and SFM may be due to excessive amino acid supply or NAN entering the intestine which was more than adequate for maintenance, so the surplus was deaminated and contributed to the synthesis of urea. Therefore, the sheep fed these diets may have had insufficient energy for growth due to the use of energy for anabolism of urea in the liver.

The efficiency of feed conversion into meat in this experiment tended to be higher in sheep fed with urea-treated oaten chaff than in sheep supplemented with protein-rich supplements. This indicated that, in this experiment, nitrogen availability for microbial protein synthesis in the rumen was more important than a source of by-pass protein. Micro-organisms in the rumen use ammonia for synthesis of amino acids which are then available for the host animal. This may also be due to the better balance of absorbed nutrients in this group compared with supplemented group.

Greasy wool production, clean wool production and mean fibre diameter tended to be higher in the sheep supplemented with protein-rich supplements than in those given urea-treated oaten chaff alone. Coombe (1985) found that the wool growth rate of sheep supplemented with rapeseed meal or sunflower meal was about 2 times higher than that of sheep supplemented with urea-starch providing the same nitrogen intake. This indicates that dietary by-pass protein influences wool production (Coombe, 1992). More specifically, the wool production responses in this experiment may have been due to the intake of additional sulphur amino acids from the supplements. These amino acids are known to increase wool growth (Downes *et al.*, 1970). Extra amino acids absorbed in the intestine are needed to increase wool growth (Leng *et al.*, 1989). Two sulfur-containing amino acids, methionine and cysteine are generally limiting for wool growth (Williams, 1991). Cysteine is the first limiting amino acid for wool growth in sheep (Staples *et al.*, 1993).

### 3.7. Conclusions

The differences of feed intake and dry matter intake of sheep was due to the restricted amount of feed offered in order to have the same nitrogen intake in all treatments. There was no effect of iso-nitrogenous amount of supplements on

liveweight change among the treatments which indicated that the efficiencies of utilization of nitrogen by sheep in the different treatment groups did not differ. Greasy wool production and mean fibre diameter were however influenced by the availability of dietary protein in the intestine. Therefore, the sheep supplemented with a protein-rich supplement tended to produce more wool with a broader mean fibre diameter than unsupplemented sheep, but all four protein-rich supplement appeared to be similar in their effects when compared at the same level of nitrogen intake.

## Chapter 4

### Estimation of intake of cottonseed meal and copra meal using lithium chloride as a marker in penned sheep

#### 4.1. Introduction

The accuracy of measurement of feed intake, and particularly of supplements, in grazing or penned animals is usually limited by factors such as the consumption of feed by wild animals in the grazing system, or the loss of feed residues due to the animals eating habits in pens. The loss of feed due to the consumption of feed by the wild animals may cause an inaccuracy in evaluating feed intake of supplements by the grazing animals. The loss of feed in the pen causing by the eating behaviour of animals may also bring about a problem in estimating intake of supplements by animals.

The problem in estimating intake of supplements by grazing or penned animals has encouraged scientists to find an appropriate technology to provide accurate estimates of feed intake measurement. Many experiments have been done using different chemical agents such as tritiated water (Nolan *et al.*, 1976),  $\text{Cr}_2\text{O}_3$  (Lobato and Pearce, 1978), and lithium chloride (Suharyono *et al.*, 1991; Suharyono, 1992) to measure feed intake. The use of tritiated water and  $\text{Cr}_2\text{O}_3$  have shown a high variation in estimated feed intake by grazing sheep. Lithium chloride has been successfully estimate intake of pellet in penned sheep (Suharyono *et al.*, 1991).

Lithium chloride has also been used to create feed aversion in ruminants (Burritt and Provenza, 1989) because it is a known emetic agent (Ralphs and Cheney, 1993). Gastrointestinal illness occur in sheep when they are given 150 mg LiCl/kg liveweight (Burritt and Provenza, 1989). However, Suharyono (1992) found that these problems can be avoided by using lithium chloride below 50 mg/kg liveweight per day. Suharyono *et al.* (1991) found that LiCl gave a good indication of the intake of pellets by individual sheep, and concluded that LiCl is a good marker for estimation of intake of supplement.

In this experiment, lithium chloride was used as a marker to estimate intake of cottonseed meal and copra meal. The aim of this study was to test the accuracy of the

lithium chloride technique in estimating intake of supplements (cottonseed meal and copra meal).

## **4.2. Materials and Methods**

### **4.2.1. Animals and diet**

Four sheep supplemented with copra meal and four sheep supplemented with cottonseed meal (some of the same sheep as used in the first experiment) were used in this experiment. Both of the supplements, 75 g copra meal + 50 g SFM (total 125 g) and 43 g cottonseed meal + 50 g SFM (total 93 g), were mixed with 800 mg LiCl per sheep (average liveweight of 35 kg), to give the equivalent of 23 mg LiCl/kg liveweight. The lithium chloride (800 mg) was dissolved in 2 ml of water and mixed with the respective supplement in a plastic bag by shaking. This supplement was then fed to the animals.

### **4.2.2. Blood samples collection and lithium chloride analysis**

One blood sample from each sheep was taken from the jugular vein using a 10 ml vacutainer containing heparin 24 h later. They were collected in ammonium heparin tubes. The blood samples were centrifuged at 3000 g for 10 min. Supernatant was decanted and stored at -20 °C. After thawing the blood plasma was diluted with water within the ratio 1 : 5 before it being analysed. Standard solutions of lithium chloride were prepared, i.e. 1, 3, 7, and 10  $\mu\text{g/ml}$ . Analysis of the lithium chloride content in the diluted plasma was done triplicates using an Atomic Absorption Spectrometer (A.A.S.) (Perkin Elmer, Model 360).

Estimation of feed intake by individual sheep was calculated according to Suharyono (1992). The actual feed intake was calculated by subtraction of the supplement residues from the supplement offered.

The variation of estimated feed intake among the sheep was calculated using the Microsoft Excel program version 5.

### 4.3. Results

All sheep supplemented with cottonseed meal ate all of their diet. In the group of sheep supplemented with copra meal, 1 sheep consumed only 47 g of the 125 g of the supplement offered, but the all others ate all of the supplement offered. Visually there was no negative effect seen as the result of the presence of LiCl in the supplement.

Coefficient of variation of estimated intake of copra meal ranged between 2.5 - 23 % with the average of 8.7 % (Table 8). Variability of estimated intake of cottonseed meal ranged from 0.6 to 8.8 % with the average of 2.9 % (Table 9).

Table 8. Estimation of intake of copra meal using Lithium chloride as a marker

Sheep No.	Plasma Lithium (mmol/L)	Liveweight (kg)	Lithium X Liveweight	Individual proportion	Actual intake (g)	Estimated intake (g)	Variation of estimation (%)
7	0.05	36.2	1.85	0.19	47	75	37
9	0.08	32.1	2.47	0.25	125	113	11
11	0.08	35.4	2.76	0.28	125	115	9
24	0.08	33.7	2.73	0.28	125	119	5
Total			9.81		422		

Table 9. Estimation of intake of cottonseed meal using Lithium Chloride as a marker

Sheep No.	Plasma Lithium (mmol/L)	Liveweight (kg)	Lithium X Liveweight	Individual proportion	Actual intake (g)	Estimated intake (g)	Coeff. of Variation (%)
2	0.1	37.1	3.71	0.27	93	95.1	1.12
14	0.10	31.9	3.06	0.22	93	91.3	0.92
20	0.12	36.6	4.28	0.31	93	111	8.82
21	0.08	34.8	2.71	0.20	93	74.2	0.64
Total			13.8		372		

#### 4.4. Discussion

The sheep that did not all of its copra meal showed the highest error in estimated intake. In this case, copra meal consumed by this sheep (47 g) may have contained higher LiCl than that in the residue, possibly because LiCl was not uniformly distributed through the supplement. There was no feed aversion due to the presence of lithium chloride in the diet because the level of LiCl was less than a tolerance level of 50 mg/kg liveweight/d as suggested by Suharyono (1992).

All the cottonseed meal was consumed by the sheep. The average coefficient of variation of estimated intake of cottonseed meal was lower than 10 %. This is in agreement with the studies of Suharyono *et al.* (1991) indicated that the coefficient of variation of estimated intake was lower than 10 % when all the supplements given were consumed. Suharyono (1992) found that liveweight and plasma Li concentration were the factors that influenced the estimation of pellet intake by sheep.

#### 4.5. Conclusions

Lithium chloride is very useful for estimating intake of supplement. The use of lithium chloride as a marker gives a relatively small error in estimation of intake of supplement. The uniformity of coating of the feed with lithium chloride influenced the estimation of feed intake when the animal consumes relative small amount of the labelled feed given.