Chapter 3. Experiment 1.

The effect of copra meal, palm kernel cake, cotton seed meal and sunflower meal supplements on wool growth of sheep

Abstract

Twenty-five crossbred wethers, 5 months of age and weighing on average 30.8 kg, were randomly grouped into 5 treatments: control (Ctrl), copra (Cpr), palm kernel cake (Pkc), cotton seed meal (Csm) and sunflower meal (Sfm) supplements. All sheep were offered a restricted amount of oaten chaff mixed with urea (3%) and a mineral mix (control diet) or the control diet plus one of four protein-rich supplements. The diets were formulated to be isonitrogenous. The animals were individually penned and fed once a day. There was a 10 week pre-experimental period. Feed intake, live weight change, greasy and clean wool yield and fibre diameter were measured over an experimental period of 5 weeks.

Live weight (LW) changes of wethers supplemented with Cpr, Csm, Sfm and Pkc were 11.4, -6.9, -1.7, and 11.4 g/d, respectively, which were significantly different (>0.05) from the live weight change of wethers in the Ctrl group (18.3 g/d).

Both greasy and clean wool yields of the supplemented animals were significantly higher than yields of the Ctrl group (P<0.05). The Ctrl group sheep produced only 0.23 kg greasy wool over the 35 days, whereas the greasy

wool growth for the Cpr, Csm, Sfm and Pkc groups were 0.29, 0.30, 0.26 and 0.30 kg, respectively. The growth rates of greasy and clean wool of sheep in the Cpr, Csm, Sfm and Pkc groups were respectively 16, 10, 14, 18 and 21; 17; 17; 21% higher than those of sheep in the Ctrl. Fibre diameter did not differ significantly between supplements (P>0.05).

3. 1 Introduction

In most tropical areas, ruminants have to survive and produce on mainly fibrous feedstuffs which are well known to be low in nutritive value, ie. low protein content, low digestibility, and therefore to lead to low feed intake (Bo Gohl 1975, Preston and Leng 1987). As a result, the productivity of ruminants kept in the tropics is generally low.

Feeding ruminants involves feeding both rumen micro-organisms and the host. It is necessary to provide essential nutrients to optimise rumen fermentation and, at the same time to provide bypass nutrients, in particular protein, to meet the host's requirement for a balanced array of absorbed nutrients.

To support microbial fermentation and growth in the rumen, soluble carbohydrates, molasses for instance, and non-protein-nitrogen (NPN) sources have been economically used for several decades in many countries. Ammoniation of cereal straws and the use of molasses-urea blocks in cattle feeding to improve beef and milk production are spectacular examples (Preston and Leng 1987, Leng 1991, Dolberg and Finlayson 1995). Rumen bacteria are able to convert NPN into valuable protein in their biomass. When passing into the lower digestive tract they become a source of amino acids for the host, thereby increasing the P:E ratio of the absorbed materials.

Furthermore, bypass nutrients especially bypass protein, can considerably promote the productivity of ruminants by providing amino acids, or/and other nutrients. The proportion of bypass protein required in diets depends on a number of factors, which determine the degradability of protein in the rumen such as the solubility and the structure of the protein, and protection by chemicals. Given this, different sources of protein can provide different proportions of bypass protein, and thus have different effects on the productivity of animals. Csm and Sfm are known to be good sources of protein for animal feeding. Cpr and Pkc, on the other hand, contain less protein but their quality may be high (Mc Donald *et al* 1992). Cpr and Pkc are produced in the tropics in considerable amounts. However, limited research concerning their utilisation by ruminants has been done.

Because amino acids absorbed in the small intestine are critical to wool growth (Reis 1979, Kempton 1979, Williams 1992), the rate of wool growth can be a useful indicator of the potential value of supplements as sources of bypass protein (Leng *et al* 1984). This experiment was conducted to evaluate Csm, Sfm, Pkc and Cpr as supplements by determine their effects on wool growth of sheep fed a basal diet of oaten chaff.

3.2 Method and materials

3.2.1 Animals and experimental design

Twenty-five crossbred wethers, five months old and 30.8 kg (27.4-34.3kg) from 'Kirby' research farm were individually penned in an animal house at the University of New England. The animals were fed once a day at 09.00h and drinking water was continuously available. Faeces were collected.

Period 1: *Pre-experimental.* All of the animals were fed *ad libitum* the same diet which consisted 50% oaten chaff and 50% lucerne chaff for 5 weeks. Live weight and wool growth were recorded to enable the animals to be ranked according to their production under the same conditions and thus provide information for an analysis of covariance later.

Period 2: *Introductory.* The purpose of this 5 week period was to train the animals in the treated groups to eat the supplements. The sheep were allocated to one of five live-weight categories at the end of the previous period. The animals from each rank were randomly assigned into five treatment groups: control (Ctrl), copra (Cpr), cottonseed meal (Csm), sunflower meal (Sfm) and palm kernel cake (Pkc) . All animals were given oaten chaff (Oc) *ad libitum* as basal diet and all except the control animals were given additional supplements.

Period 3: *Experimental.* All sheep were offered isonitrogenous diets (Table 3.1). Sfm (50g) was used as 'basal supplement' for all treated groups to increase the acceptability of the protein concentrates, particularly Pkc. The amounts of supplements given were calculated, according to the protein content of each supplement, to provide a total dietary nitrogen intake of 19.5g/head/day. This period lasted another five weeks.

3.2.2 Feed analysis

Feeds used in this experiment were analysed for dry matter (DM), crude protein (by Micro Kjeldahl method), acid detergent fibre (Goering and Van Soest 1970), ash (by combustion of DM at 600°C for 3h) and gross energy (Bomb calorimeter, DDS CP 500, USA).

3.2.3 *In vitro* degradation of proteins

Rumen degradation of protein to NH_3 was examined *in vitro* by a method described by Simco Stachiw and Frank Ball, Department of Animal Science, the University of New England, Australia (1995). Feeds were incubated in fresh rumen fluid obtained from sheep at 39°C. Five samples were taken at 1 hour intervals, for 5 hours and analysed for ammonia concentration on an auto-analyzer. Ammonia production was calculated based on an isonitrogenous incubation for each feed.

3.2.4 Feed intake

Feed intake of both basal diets and supplements was recorded daily.

3.2.5 Live weight

Live weight of the animals was measured at the beginning and the end of both period 1 and period 3.

3.2.6 Wool growth

Wool growth was examined by the dye-banding method (Chapman and Wheeler 1963). Dye-bands were applied at the same time as the weighings. Total fleece and dye-banded staples were harvested 4 weeks after the last dye-band was applied and separated for each period by cutting along the margins of the dye-bands. The proportion of wool staple weight grown during each period was calculated and then, by reference to the wool weight obtained at shearing, used to measure the yield of greasy wool. Wool samples (taken from the midside) were also tested for clean wool and fibre diameter at the Wool Testing Centre, Walcha, Australia.

3.2.7 Statistical analysis

3.2.8

Variances, covariances and least significant differences were computed by Minitab software (version 7.1) and used to analyse the results obtained.

Table 3.1 The composition (g airdry matter) of daily diets fed to wethers during period 3.

Groups	Ctrl	Cpr	Csm	Pkc	Sfm
1. Basal diets Oaten chaff (+3%urea)	822	600	600	600	600
2 Supplements					
Cpr	-	75	-	-	-
Csm	-	-	43.4	-	-
Pkc	-	-	-	107	-
Sfm.	-	50	50	50	100
Supp. nitrogen	-	5.4	5.4	5.4	5.4
Total nitrogen	***19.5***	***19.5***	***19.5***	***19.5***	***19.5***
Estd. DE intake (MJ)	7.03	6.47	6.11	6.89	6.17

3.3 Result

3.3.1 Feed analysis

There was no significant difference in gross energy content between feeds although the estimated DE content of the diets with supplements tended to be lower than that of the control group (Table 3.1). Pkc and Cpr were lower in crude protein than Csm and Sfm.

3.3.2 Rate of nitrogen appearance from added proteins *in vitro*

Fig.3.1 shows the rate of appearance of ammonia in vitro from similar amount of proteins from each of the supplements. The protein in Sfm was the most degradable of the supplements. Both Pkc and Cpr appeared to be more protected from rumen fermentation which suggests that the portions of ruminally resistant protein in Pkc and Cpr may have been higher than in Csm and, especially, Sfm. For oaten chaff, on the other hand, there was a net removal of ammonia during the 3 hours of incubation, indicating that uptake of ammonia by rumen organisms exceeded production during the incubation.

Table 3.2 Chemical composition of oaten chaff, copra (Cpr), cotton seed meal (Csm), palm kernel cake (Pkc) and sunflower meal (Sfm).

Feeds	GE (MJ/kg)	DM(%)	CP(%)	Ash(%)
Cpr	18.3	94.4	22.0	6.24
Csm	17.6	89.0	38.0	6.24
Pkc	19.2	92.8	15.4	3.65
Sfm	17.4	89.4	33.0	7.24
Oc	17.1	88.8	6.4	5.50

3.3.3 Feed intake

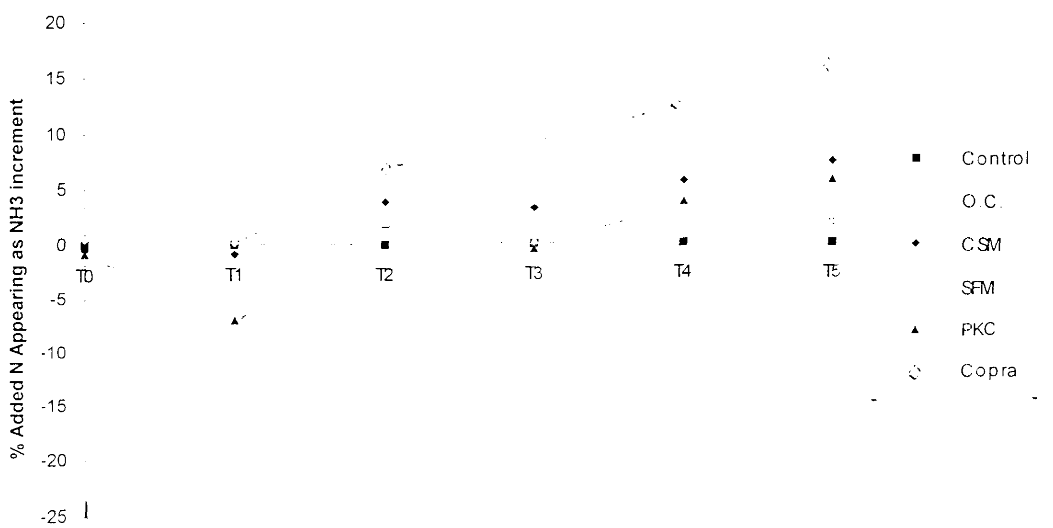
The results are shown in Table 3.3. During the *ad libitum* feeding period (period 1), there was no difference in feed intake between the animals (P>0.05). The difference in feed intake during the experimental period (period 3) was due to the restricted amounts of the basal diet and supplements given to the animals.

Table 3.3 Total feed intake by sheep during periods 1 and 3 (mean±se, g/d).

Groups	Ctrl	Cpr	Csm	Pkc	Sfm
Period 1	960[a]±71	1178[a]±107	1088[a]±137	977[a]±91	1190[a]±75
Period 3	830	732	700	764	707

[a]: Means with the same superscript in the same row do not differ significantly (P>0.05)

Fig.3.1 Percentage of added N appearing as ammonia during incubation of Oc, Cpr, Csm, Sfm and Pkc in rumen fluid *in vitro*.



3.3.4 Live weight gain

During period 1, animals in all supplemented groups had higher live weight gains than the animals fed on the basal diet. In the experimental period (period 3), on the other hand, animals in the control group showed a significantly higher live weight gain than those in the supplemented groups ($P<0.05$). During this period, control sheep gained 18.6 g/d, whereas animals in Cpr and Sfm gained only 11.5 and 11.4 g/d, and sheep in Csm and Pkc groups lost weight, 7.0 and 1.9 g/d, respectively (Table 3.4).

Table 3.4. Live-weight gain of the sheep during periods 1 and 3 (mean±se, g/d).

Groups	Ctrl	Cpr	Csm	Pkc	Sfm
Period 1	$91.6^{a}\pm19.1$	$115.3^{b}\pm3.6$	$134.7^{b}\pm11.8$	$137.9^{b}\pm4.5$	$125.8^{b}\pm5.6$
Period 3	$18.6^{a}\pm8.1$	$11.5^{b}\pm4.4$	$-7.0^{c}\pm5.3$	$-1.9^{d}\pm5.3$	$11.4^{b}\pm6.4$

a,b,c,d,: Means with different superscripts in the same row differ significantly ($P<0.05$)

3.3.5 Wool production

There were no significant differences in total fleece weight ($P>0.05$) between the groups. Greasy wool grown in period 1 appeared to show no significant differences ($P>0.05$) between the control and treated animals, although the sheep in the Pkc group tended to have higher clean wool growth rates. During period 3 significant differences ($P<0.05$) were found (Table 3.5). However, after adjusting the results by using the results from period 1 as covariates, only animals supplemented with Pkc and Sfm showed a significant difference (P<0.05) in greasy wool production (Table 3.5). There was no difference in fibre diameter between the animals in the control and supplemented groups (P>0.05).

Table 3.5 Effect of supplements on the total fleece weight (FW), the greasy wool weight grown per each period (GW), the growth rate of clean wool (CW) and fibre diameter (FB) of the experimental wethers (mean±se). During period 1 all sheep were given the same diet, and during period 3 four of the groups were given protein-rich supplements. Results for period 3 are given, adjusted for results obtained during Period 1, and unadjusted.

Items	Period	Ctrl	Cpr	Csm	Sfm	Pkc
FW, kg		2.54±0.01	3.01±0.24	3.05±0.09	2.67±0.06	2.78±0.08
GW, kg	1	0.29±0.01	0.31±0.02	0.33±0.02	0.29±0.01	0.32±0.01
	3	0.23^{a}±0.02	0.29^{b}±0.02	0.30^{b}±0.02	0.26^{ab}±0.01	0.30^{b}±0.01
	Adjusted	0.24^{a}±0.01	0.29^{b}±0.01	0.28^{b}±0.01	0.28^{b}±0.01	0.29^{b}±0.01
CW, g/d	1	5.4±0.3	5.8±0.4	5.7±0.3	5.5±0.3	6.4±0.3
	3	4.7^{a}±0.5	6.0^{b}±0.3	5.5^{c}±0.3	5.6^{c}±0.4	6.7^{bc}±0.4
	Adjusted	5.1^{a}±0.3	5.9^{b}±0.2	5.6^{b}±0.2	5.8^{b}±0.2	6.0^{b}±0.2
FB, μ	1	24.0±0.8	24.3±0.4	24.3±1.1	24.5±1.2	28.1±1.3
	3	23.5±1.0	24.5±0.2	24.7±1.1	24.8±1.1	28.0±1.0
	Adjusted	24.2±0.5	25.0±0.2	25.2±0.4	25.2±0.3	25.8±1.2

a,b,c: Means with different superscripts in the same row differ significantly (P<0.05)

Table 3.6. Responses (% higher than control) of greasy and clean wool growth of sheep when given different supplements for a period of 5 weeks.

Supplements	Greasy wool	Clean wool
Cpr	21	16
Csm	17	10
Sfm	17	14
Pkc	21	18

3.4 Discussion

Animals use low digestibility roughages and fibrous by-products inefficiently and these materials are not capable of supporting efficient growth of ruminants even though they are not deficient in energy *per se* (Preston and Leng 1984). Supplementation of these diets can therefore provide better conditions for rumen fermentation and thus improve the nutrition of the host. While non-protein nitrogen supplementation is used to promote the growth of micro-organisms in the rumen, bypass protein, on the other hand, provides an additional source of absorbable amino acids in the intestine. As a result, bypass protein can improve productivity of ruminants with high genetic merit. It is well known that, in the case of *ad libitum* feeding, supplementation of low protein feeds with nitrogen, either NPN or protein N, firstly increases feed intake (Williams 1982, Preston and Leng 1987, Leng 1992). Smith (1984) reported that sheep supplemented with oats plus urea, lupins and pelleted cotton seed increased roughage intake by 27, 43 and 48% respectively. The significant effect of lucerne on live weight gain of the treated animals in period 1 of the present study was in complete agreement with the above mentioned literature. The animals in the treatment groups (except for Pkc) had a significantly higher feed and digestible energy intake and therefore had higher live weight gains than those in the control group.

In period 3, however, none of the supplemented diets supported live weight gain, and even the animals in the Csm and Sfm groups lost weight. The reason for this was firstly presumed to be an inadequate supply of digestible energy. A restricted feeding scheme was applied to make the N intakes of supplemented groups similar, and as a result the supplemented animals ingested about 10% less digestible energy than those in the control group, i.e the estimated digestible energy intake was 7.03MJ for control animals compared to 6.47, 6.11, 6.89 and 6.17MJ for animals in the Cpr, Csm, Pkc and Sfm groups, respectively. In the case of insufficient intake of digestible energy, dietary protein may be used as an energy source by animals to satisfy their energy requirements (McDonald *et al* 1992). But in this study the amount of digestible energy available from the supplements was relatively low. As mentioned earlier, this experiment aimed at testing the effects of the used supplements on wool growth rather than on overall nutrition of sheep, thus the experimental protocol did not allow to interpret their effects on other aspects. On the other hand, the amino acids in Csm and Sfm might be highly suitable for the growth of wool, and it is possible that the absorbed amino acids from these supplements were probably mostly partitioned to wool growth rather than to live weight gain.

It is well known that the major problem in wool production centres on the conversion of raw material, vegetation, normally containing a low concentration of sulphur (0.1-0.3, Williams 1982), into product containing a much higher concentration of sulphur (3-4% in wool, Reis 1979). Kempton (1979), Williams (1982) and Reis (1979) found that sulphur containing amino acids (cystine and methionine) are the most likely amino acids to limit wool growth. Kempton (1979), also stated that the availability of absorbed amino acids rather than of energy is the major factor that affects wool production. The higher rate of wool growth of all supplemented animals compared with that of the control animals, from 10 to 18%, might be brought about by the higher availability of absorbed sulfur containing amino acids. Numerous researchers have concluded that wool growth was increased when either protein supplements or readily digestible forage, lucerne for example, were added into cereal straw-based diets (see Preston and Leng 1987). Although sheep on all supplement treatments as well as in the control group had the same total N intake, the absorbed amino acids especially those of dietary origin might be different in terms of their amino acids profile and their degradability in the rumen. Even though the NPN in the control diet was perhaps efficiently converted to microbial protein, the amino acids which are critical for the growth of wool follicles, were still limiting. In contrast, proteins of the supplements, especially of Pkc and Cpr may contain more of those amino acids that are necessary for wool growth, compared with those supplied by microbial protein.

Furthermore, when comparing the rates of wool production between dietary treatments, it was found that Pkc and Cpr appeared to be more effective than Csm and Sfm. This result is supported by a tendency for a lower rate of ammonia production from Pkc and Cpr proteins *in vitro*. This means that proteins in Pkc and Cpr are more protected from rumen fermentation than those in Csm and Sfm, and thereby are likely to provide a higher proportion of escape proteins.

One emerging issue in this experiment is that the appropriate form of strategic supplementation of cereal straws might be different between wool and meat production. A trend towards higher live weight gain of sheep fed solely oaten chaff plus 3% urea during period 3 could have resulted from a higher energy intake. To feed animals having low or medium growth rates, NPN addition by optimising microbial yield from the rumen, may satisfy their requirement for CP. Thus, improving intake of basal diets to provide more digestible energy may be more cost-effective than supplementing with expensive proteins. In other words, the quality of crude protein may be less important in feeding growing animals than when feeding animals for wool production, reproduction, or in feedlots. Ryder and Stephenson (1968) stated that only 2% of ingested net energy is converted to wool. This means that protein supplementation of low protein diets is likely to give more efficient response in wool production than energy supplementation. By and large, in order to improve wool production economically, the strategy should firstly be to provide essential elements such as NPN, S, etc., to maximise rumen fermentation and microbial growth, and then the use of bypass proteins can be considered if economic.

Another interesting problem that was observed in the experiment is the preference for the different supplements exhibited by the sheep. Although 5 weeks were allowed for animals to become familiar with supplements, Pkc was not willingly consumed, whereas the other supplements were readily accepted. The reasons have not been investigated yet. However, the observation agreed with conclusion made by McDonald *et al* (1992) that 'the palatability of Pkc is poor'. Probably, the odour of Pkc affects its palatability. Preston and Leng (1987) reported that smell is the most important sense affecting feed intake of ruminants, however other sensory factors such as touch and taste may also be important for sheep in diet selection.

3.5 Conclusion

Supplementation of sheep with Cpr, Csm, Sfm and Pkc significantly promoted (P<0.05) greasy wool and clean wool production. Cpr and Pkc appeared more effective for stimulating wool production than Sfm and Csm although differences were not significant Cpr and Pkc may have had a higher content of sulfur-containing amino acids or better bypass characteristics, or both. The supplements did not affect fibre diameter significantly.

The results were interpreted as showing that all supplements were effective sources of 'escape' amino acids but the availability of escapable amino acids obtained from Pkc and Cpr might be greater than that from Csm and Sfm. In terms of wool production, Pkc and Cpr seem to be better supplements than Csm and Sfm.

Chapter 4.

The effects of urea treatment of rice straw and copra and whole cotton seed supplemented on feed intake, live weight gain of cattle fed on rice straw.

Abstract

This experiment was conducted at Quang Ngai Research Station, Vietnam from December 20, 1995 to February 14, 1996. Sixteen F1 (Local yellow x Brahman) cattle (30 months, 221 kg) were assigned to 4 treatment groups, viz: untreated rice straw (URS), treated rice straw (TRS), URS plus 1.3 kg copra (Copra) and URS plus 1 kg whole cotton seed (WCS), ie. group 1 (as control), 2, 3 and 4, respectively. Feed intakes, live weight gains, rumen degradation of supplemented proteins were measured and statistically computed.

Urea treatment of rice straw or supplementation of untreated rice straw with copra and WCS brought about significantly higher ($P<0.01$ and $P<0.05$) rice straw dry matter intakes (DMI) by cattle. DMI were 1.4, 2.41, 1.67 and 1.73kg/100kg live weight (LW) for animals in group 1, 2, 3 and 4, whereas total digestible energy intake were estimated to be 20, 35, 42 and 40MJ, respectively. Live weight gains of supplemented animals (183 g/d for copra and 235g/d for WCS) and those fed on TRS (165g/d), were significantly higher ($P<0.01$ and $P<0.05$) than cattle given only URS (-49.1 g/d).

Live weight gains of cattle in two of the supplemented groups were not predictable solely on the basis of their rates of protein degradation of the supplements *in vitro*.

4.1 Introduction.

Yellow cattle (indigenous breed) and buffalo are kept in Viet Nam by village farmers who depend on crop by-products to provide feed for their animals. Seasonal patterns and intensive use of land for crops strongly influence the availability of feeds for ruminants. Many studies have shown that during the dry and/or extreme rainy seasons when cattle and buffalo depend on very poor and limited native grasslands during the day and rice straw at night, adult animals loose weight, weaners grow at a very low rate (Nguyen Tien Von, 1992).

In Viet Nam, rice straw is freely available. The estimated yield of dry straw is more than 30 million tons annually (Nguyen Tien Von, 1991). Although rice straw has been traditionally used as feed for cattle and buffalo, it is potentially under-utilised as a source of ruminant feed. This is partly due to an increased production of rice and partly due to its low feed intake and digestibility by the animals.

Although farmers view rice straw as a good source of feed for their cattle and buffalo, it supports only low performance of animals, especially when production of milk and meat is the goal rather than draught power. Thus, the development for making better use of rice straw and finding into an optimum feeding of cattle and buffalo in Vietnam, is an important goal for research.

Possible strategies include chemical treatment to increase the nutritive value of rice straw or developing ways of supplementing the animal. There are many studies that confirm the advantages of urea treatment (Jackson 1977[a], 1977[b]; Doyle, Devendra and Pearce 1986) and of strategic supplementation of rice straw-based diets with rich-protein feeds (see Preston and Leng, 1987) as a means of improving production of cattle. However, within the Vietnamese farming context, and elsewhere in developing countries, it is necessary to determine which method is most economic and most easily taken up by a large number of farmers.

As Preston and Leng (1987) have emphasised, feeding ruminants involves feeding both rumen microbes and the host animal, but this may mean that feeding ruminants with rich-protein feeds is not necessarily better, from the point of view of cost or practicality, than feeding them with non-protein-nitrogen compounds. Therefore, this experiment was carried out to enable comparison of two different strategies for feeding cattle on rice straw based diets. These included treating rice straw with urea and supplementation of rice straw based diets with copra or cotton seed.

4.2 Methodology

4.2.1 Experimental design

Sixteen male cross-bred cattle (Yellow x Brahman), 30 months and weighing on average 221 kg were selected from a breed herd at Quang Ngai Research Station, Vietnam. The cattle were housed in a shed with a concrete floor and provided with group feed troughs. From 13 to 17h each day, the animals were allowed out to be on adjacent areas for movement purpose. The 16 cattle were allocated at random from live weight strata into 4 treatment groups receiving, respectively untreated rice straw, treated rice straw, untreated rice straw plus copra or untreated rice straw plus whole cotton seed. After a one-week adaptation period, feed intake and live weight change were recorded. The trial lasted for 56 days (from December 20, 1995 to February 14, 1996).

4.2.2 Treatment of rice straw with urea

Rice straw was put in a brick pit in stages. Straw was added to create a layer (about 20 cm thick). The layer was well pressed (by jumping) and then sprayed with a 4% (w/w) solution of urea (1 litre per 1 kg rice straw). The process was repeated layer by layer until the pit was filled with straw. The pit it was then well covered by nylon sheet to exclude the atmosphere and to retain ammonia evolved by bacterial degradation of urea. After 3 weeks, treated rice straw was ready to be taken to feed animals.

4.2.3 Feeding scheme

Based on the crude protein contents of feeds, diets were calculated as shown in Table 4.1. The animals were fed *ad libitum* on rice straw, both treated and untreated. Supplements were offered to the animals once per day at 08.00h.

4.2.4 Feed intake measurement

Dry matter (DM) intake of each group of cattle was determined by measuring the feeds offered and refused daily.

4.2.5 Feed analysis

Feeds used in this experiment were analysed for dry matter (DM), crude protein (by Micro Kjeldahl method), acid detergent fibre (Goering and Van Soest 1970), ash (by combustion of DM at 600°C for 3h) and gross energy (by Ballistic Bomb calorimeter, Gallenkamp).

4.2.6 In vitro degradation of proteins

Rumen degradation of protein to NH_3 was examined *in vitro* by method described by Simeo Stachiw and Frank Ball, Dept of Animal science, the University of New England, Australia (1995). Feeds were incubated in fresh rumen fluid (39°C) obtained from cattle. Samples were taken at 1 hour interval, for 5 hours to analyse for ammonia on the auto-analyzer. Ammonia production was calculated based on isonitrogenous level for each feed.

4.2.7 Growth of animal

The cattle were weighed on the first and last days of the experiment and live weight change was calculated.

4.2.8 Data analysis

The data were computed on Excel for Windows software (Version 5.0) for statistical parameters such as variance, covariance, least square deviations and least significant differences.

Table 4.1 The composition of the diets and amounts fed to cattle during the experiment.

Feeds	Group 1	Group 2	Group 3	Group 4
Untreated RS (kg)	4.5	-	4.5	4.5
Nitrogen (g)	*24.5*	-	*24.5*	*24.5*
Treated RS (kg)	-	9.0	-	-
Nitrogen (g)	-	*63.7*		
Copra (kg)	-	-	1.3	-
Nitrogen (g)	-	-	*35.8*	-
Cotton seed (kg)	-	-		1
Nitrogen (g)	-	-		*36.3*
Total Nitrogen (g)	***24.5***	***63.7***	***60.3***	***60.8***

4.3 Experimental results

4.3.1 Feed analysis

Feeds used in the experiment were analysed for their main components, and results are shown in Table 4.2

4.3.2 Feed intake

Average feed intakes of cattle in the experiment are presented in Table 4.3. Treatment of rice straw by urea ensiling brought about a significant increase in rice straw intake by cattle. Animals in Group 2 fed solely on urea treated rice straw had a significantly higher intake ($P<0.01$) than animals fed untreated rice straw as well as those supplemented with copra or whole cotton seed.

Although there were no differences in rice straw intakes by supplemented animals across groups 3 and 4, intakes were significantly higher ($P<0.05$) than those of animals given untreated rice straw.

Mean straw dry matter intakes (DMI) of cattle in this experiment were 1.4; 2.41; 1.67 and 1.73 kg/100kg LW, for animals in Groups 1, 2, 3, 4 respectively.

Table 4.2 Chemical composition of feeds (%DM)

Feeds	DM	CP	GE (MJ/kg)	Fibre	Ash
Rice Straw	88.4	3.4	14.4	31.7	14.1
Treated Rice Straw	60.8	6.8	NA	NA	12.2
Cotton Seed Whole	92.5	22.7	14.5	24	4.8
Copra Meal*	91.0	17.2	11.6	14.5	6.3

*: Processed by villagers

Table 4.3 Average total airdry matter intake of rice straw and supplements (kg/h/d: Mean±SE) by cattle during a 56 day growth trial.

Feeds	Group1	Group2	Group3	Group4
Rice straw	3.5 ± 0.02^{a}	$9.\pm0.06^{b}$	4.3 ± 0.45^{c}	4.5 ± 0.03^{c}
Copra	-	-	1.3	-
Cotton seed whole	-	-	-	1
Straw DMI/100kg LW	1.[illegible] ^{a}	2.41^{b}	1.67^{c}	1.73^{c}
Straw DMI/head	*3.[illegible]*	*5.4*	*3.8*	*3.9*
Total DMI/head	***3.[illegible]***	***5.4***	***5.0***	***4.8***

a,b,c,: Means with different superscripts in the same row differ significantly, where: ($P<0.05$)

4.3.3 Live weight gains

As can be seen from Table 4.4, cattle in the control group were not able to maintain their live weight (average LW gain was -49.1 g/day) while those in the 3 other groups gained live weight. Differences in LW gain between animals fed on untreated rice straw and those fed on treated rice straw and/or protein supplemented animals were significant ($P<0.05$ to $P<0.001$). There was also a significant difference ($P<0.05$) in live weight gain between cattle supplemented with copra and those supplemented with whole cotton seed, whereas copra supplement gave the same effect as the urea-treated rice straw diet.

Table 4.4 Effects of supplementation with copra (Group 3) and whole cotton seed (Group 4), and of urea-treatment (Group 2) or non-treatment of rice straw on live weight of cattle during 56 day growth trial.

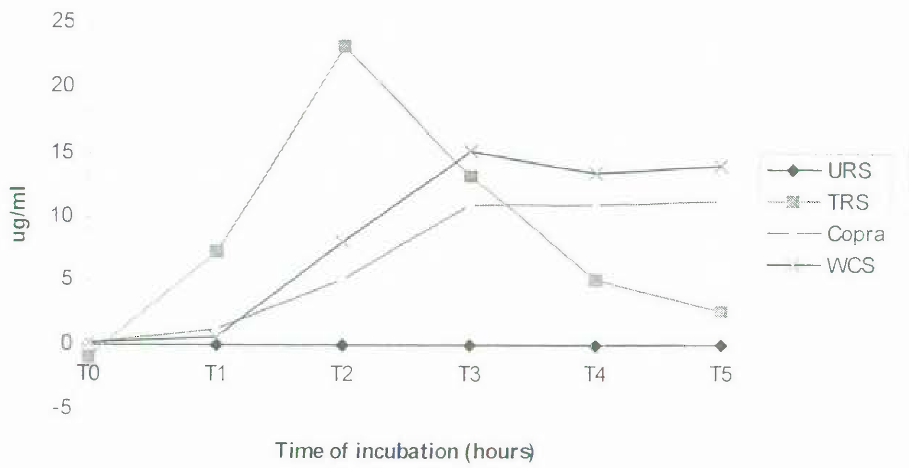
	Values	Group 1	Group 2	Group 3	Group4
Initial LW (kg)	M	221	221	221	220
	SE	1.65	1.49	1.79	0.85
Final LW (kg)	M	218^{a}	230^{b}	232^{b}	235^{b}
	SE	1.08	1.47	1.04	0.81
LW gain (g/d)	M	-49.1^{a}	165.2^{b}	183^{b}	276.79^{c}
	SE	13.4	11.2	18.4	13.2

a,b,c: Means with different superscripts in the same row differ significantly, where: ($P < 0.05$)

4.3.4 Rate of degradation of feed proteins *in vitro*

Rate of degradation of crude protein of untreated rice straw, treated rice straw, copra and whole cotton seed were determined by measuring rates of net ammonia production from added proteins *in vitro* (Fig.4.1). Ammonia production from urea-treated rice straw was greater than from copra and whole cotton seed. Protein in copra appeared to be more protected from rumen digestion than whole cotton seed protein.

Fig. 4.1 Net rate of ammonia production when urea-treated rice straw, copra and whole cotton seed were incubated in rumen fluid *in vitro*.



4.4 Discussion

Rice straw is generally considered to be of low nutritional value feed due to both its low digestibility and poor nutrient composition, and its low intake by ruminants. A summary of some major nutritional disadvantages of rice straw is as follows:

1) low protein content, (normally less than 4%),

2) high silica and low phosphorus contents

3) low digestibility

4) low feed intake.

Analysis in the experiment showed that rice straw has a low crude protein content (3.4% dry matter and a higher ash content (14.1 %) in comparison with other cereal straws, for example maize stover 8.5%, sorghum stover 3.9% (Khajarern and Khajarern, 1985). However, the rice straw used in this study had a lower ash content (14.1%) compared with those (18% average) reported by Doyle, Devendra and Pearce (1986). As with other cereal straws, rice straw contains a large pool of fibre which can be degraded as an energy source for ruminants by rumen micro-organisms.

Both copra and whole cotton seeds in this analysis had a lower crude protein content compared with that found in these feeds in our first experiment in Australia.

According to Doyle *et al* (1986), on the basis of live weight, intakes of buffalo and cattle have ranged from 1.2 to 2.7 kg/100 kg live weight, intakes of sheep and goats have ranged from 1.0 to 2.7 kg/100 kg live weight. However, when compared on the basis of metabolic weight, which assumes that $LW^{0.75}$ is the appropriate scaler for comparison between species, then large ruminants consumed between 46 and 105 g/kg $LW^{0.75}$ (mean 74) compared to only 25 to 60 g/kg $LW^{0.75}$ (mean 46) by small ruminants.

DMI of cattle fed on urea treated rice straw were similar to those obtained by Wanapat *et al* (1984), Sriwattanasombat and Wanapat (1985), Perdok *et al* (1982), which ranged from 1.6 to 2.3 kg/100kg LW, but less than those achieved by Saadullah *et al* (1983) and Ibrahim *et al* (1984) which varied from 2.9 to 3.1 kg/100kg LW with a maximum of 4.0 kg/100 kg LW (Jaiswal *et al*, 1983). In this experiment the animals in Group 2 were fed *ad libitum* with treated rice straw and a significantly higher feed intake was obtained in this group compared with group 1 (URS). The mechanisms involved have been an increase in solubilisation of some of cell wall components or disruption of lignin and cell wall carbohydrate complexes (Jackson, 1977a Sundstøl *et al*, 1979, Sundstøl, 1984). Moreover, rice straw given to animals in the group 2 was ensiled in wet condition (ie. rice straw: water is 1:1, w/w).

thus the soaking process itself contributed partly to increased intake. The main effect of wetting rice straw is likely to be improved palatability due to reduced dustiness of feed (Doyle *et al* 1986).

Effects of urea treatment on straw intake have been discussed by a number of scientists (Jackson, 1977[d]; Owen *et al* 1984, Orskov *et al* 1981, Preston and Leng, 1987, Perdok *et al*, 1982, etc). Consensus agreement was that urea treatment can soften cell wall structures, reduce silica content, increase crude protein and, as a result, increase digestibility and, at the same time, decrease its residence time in the rumen

Straw intakes of animals supplemented with whole cotton seed and copra were also higher than for animals in the control group. This suggests that whole cotton seed and copra may have provided a supplementary source of nitrogen for microbial growth in the rumen. Moreover, as proteins contained in straw are bound in structural carbohydrates in cell walls, they are not readily exploited by micro-organisms. It is reasonably assumed that the increased population of micro-organisms enhanced the rumen degradation of rice straw material, and as a result, reduced residence time and increased turn-over rate of rice straw in the rumen. Increased available volume and throughput, lastly permitted animals to increase their intake.

A clear reason for liveweight loss of animals fed solely on untreated rice straw was their lower intake compared with the intakes of animals in group 2, 3 and 4. It was also clear that treatment with urea increased remarkably MEI by the cattle in group 2 compared with those in control group. As calculated, gross energy intake of animals in Group 1 was only 43.2, compared to 78.1 MJ/d for animals in Group 2, and assumed digestibility was 45 % for both groups, ME was only 19.4 MJ, compared to an estimated 35.7 MJ of those in Group 2.

According to Kearl (1982) the maintenance requirement of cattle fed on rice straw is 26.8 MJ metabolizable energy for a 200 kg animal. it is clear that animals in Group 1 would have to consume an additional 7 MJ of ME in order to maintain a constant weight.

Live weight gain of cattle supplemented with whole cotton seed was significantly higher than that of animals supplemented with copra (276 vs 183 g/h/d). This result indicates that cotton seed protein may have a higher nutritional value for cattle than copra protein, although their net nitrogen intakes were similar. Many papers have suggested 2 main aspects of the role of protein supplements in fibre-rich diets: 1) to provide rumen microbes with more readily useable nitrogen source, 2) to provide the host animal with amino acids which can be absorbed from the small intestine.

The real value of the ingested protein for the host depends on the extent to which it is degraded by rumen micro-organisms since this affects the ratio of ruminally degradable N supplied (1) to the amount of 'escape' amino acids (2). The higher live weight gain of animals supplemented with whole cotton seed suggests that its protein might be less degraded in the rumen than protein in copra. In an attempt to test the above hypothesis. rates of degradation of diet proteins, expressed as rates of ammonia production, were examined *in vitro*. Results showed that crude protein in cotton seed produced a higher rate of ammonia production than that in copra. This result implies that proteins in copra are more resistant to rumen fermentation than those in cotton seed and that the former are therefore more valuable as sources of escape protein for ruminant nutrition. Obtained live weight gains did not support this conclusion. however. A reasonable explanation might be proteins in whole cotton seed, in comparison to proteins in copra, tend to pass to the omasum without thoroughly mixing with ruminal contents. It is a fact that intake protein that is resistant to fermentation passing down to the lower digestive tract consists of two fractions: (1) protein that resists microbial attack in the rumen and (2) protein that is more readily degraded in the rumen but for some reasons evades attack in the rumen and passes to the omasum. Result obtained from *in vitro* measurement may only describe the first fraction. Therefore further studies should conduct *in vivo* measurements in order to include both fractions (Chen and Gomes 1992).

Furthermore, does protein in whole cotton seed have a more appropriate amino acid spectrum and/or a higher efficiency in digestion and absorption in the small intestine than those in copra? These aspects also need to be intensively investigated. Nevertheless, live weight gain results indicated that supplementation of whole cotton seed in the rice straw diet brought about a higher growth rate of cattle in comparison with copra supplementation.

Interestingly, animals in group 2 were given only treated rice straw but had almost the same live weight gain of those supplemented with copra. The reason for that was a significantly higher rice straw intake and therefore higher energy intake by animals fed on treated rice straw. On the other hand, in the treated rice straw diet, urea was a source of non-protein nitrogen which was very important for growth of rumen micro-organisms. As a result, rumen digestibility was probably increased by an increased population of rumen organisms. The availability of absorbable protein in the small intestine was also higher due to the higher availability of nitrogen from urea for synthesis into microbial protein.

During this trial, all animals were put on natural grazing area for 4 h/d. The role of grazing intake has been discussed by many scientists (Preston and Leng 1984; Devendra 1983; Mogan *et al* 1983, etc.). Although the quantity of ingested forage may not always be high, animals have an opportunity to selectively obtain specific nutrients, vitamins, nitrogen, minerals and readily available energy. The grazing intake of cattle in this trial was unknown. However, from observing the animals, it seemed to be very low in both quantity and quality. Actually, adjacent areas were almost clear ground where animals could only do 'excersise'. Nevertheless, effects of grazing intake on nutrition of all cattle were assumed to be the same.

4.5 Conclusion

Cattle fed solely on rice straw lost weight and a major reason for this was a low voluntary intake and thus a low intake of digestible energy. Treating rice straw with urea (ensiling) or supplementing with copra or whole cotton seed, resulted in a significant increase in live weight gain of cattle. The former appeared to be a more practical means of making better use of rice straw because: 1) it gave a reasonable effect on the growth of animal, i.e. the same that given by copra supplement and 2) it must be more feasible in practice than the latter because urea is highly available and relatively inexpensive.

In a small farming system context, however, there are many considerations that affect whether or not farmers will adopt new management practices, economic and sustainable practices for instance. The considerations will be important in further studies aimed at the optimising utilisation of rice straw as a ruminant feed in tropical countries. Supplementation of rice straw with concentrates, green fodders, or other cheap and locally available products are further possibilities that deserve to be evaluated by scientists.

Chapter 5

General Discussion

5.1 Production responses to feeding urea-treated straw or to protein supplementation.

Both urea-treatment and protein supplementation of oaten chaff or rice straw can improve the performance of ruminants. Increases in intake brought about by ammoniation or protein supplements have been recorded in the majority of experiments, as discussed in section 3.3. Improvements in intake have generally resulted in improvements in the growth and performance of animals. In experiment 2, the statistically higher live weight gains of animals receiving urea-treated rice straw or protein-rich supplements were believed to be the result of higher EI intakes in the animals, compared to those in the control group.

Interestingly, in experiment 2, urea treatment showed a significantly higher effect on rice straw intake compared to supplementation with Cpr and whole cotton seed ($P<0.01$). It is more important that although urea-treatment gave a lesser effect on live weight gain compared to whole cotton seed ($P<0.05$), it gave, however, the same result that brought about by 1.3kg of Cpr supplemented to untreated rice straw. Studies which compared treated and untreated straws fed with different amounts of energy concentrates, have shown that the effects of ammoniation on roughage digestibility may be reduced, or completely eliminated when the concentrate is more than 50% of the diets (Horton 1978, Garrett *et al* 1979, Streeter *et al* 1983). However, it is different in cases of small amounts of bypass protein supplemented. Lawlor and O'Shea (1979) showed that protein supplements may be more effective in augmenting straw intake than energy source (barley grain). Saadullah *et al* (1983) and Wanapat *et al* (1984) reported that with low or medium levels of protein supplements, for example 1kg/d for cattle, responses of animals in straw intake and growth were both positive. On the other hand, Horton and Holmes (1976) observed that 1.2kg of a barley-alfalfa supplement depressed *ad libitum* consumption of barley straw and that increasing levels continued to depress straw intake, although total organic matter intake increased gradually. Recently Dolberg and Finlayson (1995) cited research results obtained in China which showed a tendency that the higher levels of cotton seed cake given to cattle (ranged from 0.25 to 4 kg/d), the lower treated straw (NH_3 and urea treatments) intakes were observed. Unfortunately, in experiment 2, effects of a combination between treatment and protein supplement have not been examined. It is therefore necessary to detect this interesting point locally in the future in order to have a better feeding strategy.

In experiment 1, however, supplementation of the lambs with various supplements did not support growth of the animals. This was firstly because of the inadequate supply of energy imposed by restricted feeding of basal diet. It is well known that the major effect of bypass protein supplementation on the growth of ruminants fed fibre-rich diets is an increase in feed intake (Egan 1965, Kempton et al 1979, Hennessy 1984). The main source of energy for ruminants is VFAs derived from rumen fermentation of carbohydrates (McDonald *et al* 1978). In the experiment 1, to achieve isonitrogenous rations all supplemented sheep were offered only 600 g oaten chaff, which was less than that offered to control animals (822g). Thus, VFA production in the rumen of supplemented animals should be correspondingly lower than control sheep. Furthermore, in the intestine of the supplemented animals, amino acids might be more available for absorption compared to the control ones, and as a result, the ratio of protein to energy absorbed from the gut might be higher in supplemented animals. The ratio of calories to protein in the diet as well as in the gut is important. Most animals tend to eat to satisfy energy requirements. In this case, caloric intake of supplemented animals was insufficient therefore absorbed amino acids may be partly diverted to supplying energy.

Although in restricted feeding of basal diets, supplemented sheep in experiment 1 lost weight, the supplements did support wool growth. The positive effect of protein supplements on wool growth may be explained by a better ratio of protein to energy absorbed in the intestine, as above discussion. More importantly, duodenal amino acid profile derived from supplements may be more suitable for wool production rather than for weight gain. Findings from experiments done by Kempton (1979), Reis (1979) and Williams (1982) indicated that sulphur containing amino acids, cystine and methionine, are the most limiting amino acids for wool production. The more that ingested protein is protected from degradation in the rumen, the more sulphur containing amino acids are available for intestinal absorption, leading to a higher rate of wool growth. Rate of wool growth has been used as a method for determining the degradability of food proteins (Leng *et al* 1984), but the method may also detect differences in the amounts of sulfur-containing acids in the profiles of different sources of protein.

5.2 Feeding values of the supplements used

Csm and Pkc appear to be more effective than Sfm and Csm in supporting wool growth. NRC (1985) concluded that Csm is a larger source of bypass protein than Sfm and soybean meal. Compared to CSM, however, PKC tended to be even more effective in promoting wool production. A reasonable explanation is that PKC may contains more ruminally protected sulphur containing amino acids per unit of crude protein than CSM does, because according to Van Straalen and Tamminga (1990), the rumen degradabilities of protein in PKC and CSM were almost the same, i.e. 0.42 and 0.43 respectively.

In both experiments, rates of ammonia production from supplemented proteins were measured *in vitro*. The results suggested that rumen degradability was highest for Sfm followed by Csm and Pkc, and the lowest was Cpr. The tendency was the same in both experiments and this suggests that Cpr must contain the highest bypass-protein content, followed by Pkc, Csm and Sfm, respectively. However, wool growth of Cpr supplemented sheep in experiment 1 and cattle in experiment 2 did not support this implication. As discussed already in Chapter 4, a fact that protein which is resistant to fermentation in the rumen which passes down to the duodenum may consist of two fractions: (1) protein that resists rumen microbial attack and (2) protein that is more readily degraded in the rumen but which for some reasons can escape rumen degradation and passes to omasum without any microbial attack. Ammonia production rate *in vitro* may only indicate the first fraction. Thus, it is necessary to develop a technique for measurements of both fractions by *in vivo*.

In experiment 1, sun flower meal appeared to be most preferred of the four supplements by sheep. In contrast, Pkc had the lowest acceptability, and even after a five-week training period sheep did not accept Pkc readily. This observation agreed with McDonald *et al* (1978) who indicated that the palatability of Pkc is poor. Reasons for that have not been determined in the experiments. However, it might be due to its unfamiliar smell or unknown secondary compounds. According to Preston and Leng (1987), smell is the most important sense affecting feed intake of ruminants.

5.3 Considerations of practical methods

The main objective of the two experiments was to investigate ways to make better use of cereal straws as ruminant feeds. The methods investigated were either treatment with urea or supplementation with protein-rich concentrates. Although both techniques have shown promising results, several other alternatives may need to be concerned and tested. Whichever method can be used, the economic and sustainable issues should be involved in feeding strategy. Furthermore, techniques must be simple and relatively low inputs required to ensure an wide-uptake by a large numbers of farmers.

In Asia, apart from rice straw which is widely abundant, many other crop residues are still underutilised such as maize stalk, sugar cane top and bagasse, straw of legume crops (mung bean or soybean for example). These sources of ruminant feed are also highly available and they are low in nutritional value as well. Thus, it is necessary to develop suitable techniques to fully exploit these non-conventional sources to feed ruminants (Doyle *et al* 1986, Preston and Leng 1987).

The first priority for Asian countries may be to feed ruminants with urea-treated rice straw plus various sources of green fodders. Many studies have been undertaken in Asia (Devendra 1982, Doyle *et al* 1986). However, which proportion of treated rice straw in diets relative to green fodders is the best, still remains a question. On the other hand, as human population pressure increases and the area devoted to food crop production is extended the use of land for pasture becomes very limited. The approach of using grass or green fodders in Asian farming systems must be different from developed countries where conventional feeding standards exist. Cut-and-care grasses from every possible places, i.e. road sides, under tree plantations, between-rows in certain crops, or grazing on the field after harvest is traditional ways of feeding animals in many Asian countries. Other possibilities could be encouraged. Using multipurpose trees/plants in the integrated farms is a good example. Leucaena (*Leucaena leucocephala*), gliricidia (*Gliricidia sepium*), erythrina (*Erythrina glauca*) can be grown to provide 'live' fences, fuel, fertiliser and a source of supplements for feeding ruminants. Leaves of many fruit trees/crops like jack-fruit, banana and fresh crop residues such as sweet potato vines, vegetables can also be considered a good supplements to ruminants fed on rice straw or other fibre-rich based diets.

An option for ruminant feeding may be a combination of treated straw, green fodders and protein supplements. This may be more suitable to integrated farming systems where small farmers can find diverse sources of feedstuffs to feed their small herd of livestock. In case of feeding genotypes with a high production potential (crossbreds or imported ruminants for instance), another strategy might be supplementation with high energy concentrates solely or in combination with protein supplements.

Any feeding strategy needs to achieve: (1) an optimum development of rumen ecosystem and (2) a balance of nutrients absorbed from the intestine. For the former, treatment straws with urea or feeding ruminants with urea-molasses block may satisfy fermentable nitrogen and other nutrient requirement of rumen micro-organisms. However, minerals, in particular S, Co and P, needed to be added into diets to ensure an efficient level for microbial development. For the latter, a range of options can be used. Supplementation of rice straw/fibrous crop residues with bypass nutrients (protein, LCFAs, starch) will provide a better profile of absorbable nutrients in the gut driving a higher absorbed P:E ratio.

Another important issue needs to be discussed is the application of new technology in feeding ruminants. In the context of integrated farming systems it is ideal to optimise the use of local feed resources rather than to maximise animal production by using expensive feeds. This means that urea treatment and using urea-molasses blocks appear to be an important means of a better utilisation of rice straw or other fibrous by-products in the developing countries where protein supplements have been found relatively expensive and readily high competitive between people and monogastric animals. Supplementation with bypass nutrients is, however, necessary in certain circumstances such as milk production, reproduction or growing animals. In these cases, urea treatment or urea-molasses blocks alone can not satisfy a high requirement of animals for nutrients. However, supplements must be used economically and sustainably.

Finally, disease/parasitic and climatic constraints must be considered in order to have an optimum feeding strategy. Interaction between climate, diseases, parasites and nutrition is an important field for study. In a complex of many factors involved in ruminant production, whether or not supplementation can bring benefit to producers is questionable and needs to be investigated.