Effects of ammonia treatment and undegradable protein supplementation on nutrient digestion of sheep fed on wheat straw based diets

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SUMMARY
A study was conducted to investigate the effects of wheat straw ammonisation and supplementation with a rumen undegradable protein (UDP) source on nutrient digestion and nitrogen balance by lambs while diets were supplemented with kibbled carob pods as energy source. Ammonisation increased the crude protein content of wheat straw by nearly 100% and decreased the contents of neutral detergent fibre and acid detergent fibre by 7% and 1.7% respectively. Treating the straw with ammonia resulted in significant (P<0.01) increase in nitrogen (N) intake and intakes of organic matter (OM) and dry matter (DM) tended toward significance (P<0.1). The UDP source had no effect (P>0.05) on DM and OM intakes but resulted in an increase (P<0.05) of N intakes. Both, ammonization and UDP supplementation increased (P<0.01) the DM, OM and N digestibility. In conclusion, the results of this study suggest that ammonisation and UDP supplementation is a practical dietary manipulation option to improve the nutritional status of ruminants fed on roughage-based diets.

Key words: In vivo digestibility, kibbled carob pods, undegradable protein, ammoniated straw

INTRODUCTION
Ruminants fed on poor quality forages require both rumen degradable nitrogen (RDN) and undegradable dietary protein (UDP) in their diets. Dietary RDN is converted mainly to ammonia in the rumen and an adequate rumen ammonia concentration is required for optimal microbial growth and fermentation. Nevertheless, microbial protein supply may still be inadequate to meet production requirements and so supplementation with a source of UDP may be required.

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Rumen UDP or bypass protein sources resist rumen fermentation and make the amino acids available in the small intestine. Fernandez-Rivera and Klopfenstein (1989) concluded that protein is the first limiting nutrient for growing cattle grazing corn stalks and that daily gain increased as the level of supplemental UDP was increased. In addition, providing the rumen with fermentable carbohydrates as well as sources of supplemental protein that degrade slowly and maintain an optimal ruminal concentration of ammonia is also recommended to improve animal productivity (Preston and Leng 1987).

Males (1987) suggested that wheat straw utilization could be optimized by protein supplementation, and that natural protein sources were more effective than non-protein N. It has been suggested that the release of N from natural sources matches more closely the rate of ruminal fibre digestion of untreated straw than the release of N from urea. Wu et al (2005) stated that a synchronized supply of N and energy to rumen microbes should be considered to improve the efficiency of N utilization.

Microbial protein production from straw alone can not meet the protein requirements of ruminants consuming such forage. For this reason sources of undegradable protein may also be required in the diet.

Carob tree (Ceratonia siliqua), is originally from the Mediterranean region and the western part of Asia. The Mediterranean countries have traditionally grown carob as a highly profitable species for human and livestock food use. The main constituents of carob bean are carbohydrates and tannins.

The total soluble sugar content in carob cake is high (410–520 g kg\(^{-1}\) DM) making it a palatable feed and an excellent source of energy (Bravo et al., 1994). Sucrose constitutes about 69.9 % of the total soluble sugars of carob (Saura-Calixto, 1987; Karabulut et al, 2006).

Carob pods are very palatable to ruminants, but they contain appreciable amounts of tannins about 18.5 % (Saura-Calixto, 1987). These compounds are known to reduce digestibility, especially of protein, and if large amounts are fed, the digestibility of other feeds incorporated in the ration may also be reduced (Saura-Calixto and Canellas, 1982).

Improving the feeding value of straw by ammonisation is well established, but appropriate supplementation of ammoniated wheat straw for growing sheep is less completely studied. This study was conducted to investigate the effects of feeding either degradable or undegradable protein supplements with untreated and ammoniated wheat straw on the nutrient digestion and nitrogen balance in lambs. Kibbled carob pods were used as the source of supplementary energy.

**Material and Methods**

**Animals and housing**

Four Suffolk crossbred entire male lambs (average initial live weight 33.5 kg) were used in the study. Prior to the experiment, they were drenched with a broad spectrum antihelminthic against gut and lung round worms and
vaccinated by subcutaneous injection against clostridial diseases and pasteurellosis (HEPTAVAC-P Hoechst, Milton Keynes, Buckinghamshire, UK).

The sheep were housed in individual pens (120 x 120 cm) with slatted wooden floors in a well ventilated animal house. Wool was clipped around the tail to facilitate the fitting of bags for subsequent faecal collection.

The experiment was a 4 x 4 randomised block design such that the four animals were fed each of the four diets over four experimental periods. The diets consisted of untreated (UWS) or ammoniated (AWS) wheat straw based diets. Both straw-based diets were supplemented with kibbled carob pods and either groundnut/cottonseed (GNC/CSC) cake or protomix (PM). The GNC/CSC cake was a 50:50 mixture (fresh weight basis) of groundnut cake and cottonseed cake, whereas Protomix (Feed specialities, Redditch, Worcestershire, U.K) is a groundnut and cottonseed cake mixture treated in such a way that its UDP content is over 50% of the protein content. The concentrate mixture of all the four diets included 12 g/d of a mineral/vitamin premix (Downland Molassed Sheep Mineral, Central wool Growers, U.K). The ingredients of the experimental diets and amounts of each ingredient in the offered diets are shown in Table 1.

The feed was offered daily in two equal meals (0900 and 1600 h). Feeding of the concentrate mixture was followed by feeding of the straw. Clean, fresh water was available continuously throughout the experiment.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>UWS</th>
<th>AWS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GNC/CSC</td>
<td>Protomix</td>
</tr>
<tr>
<td>Wheat straw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated straw</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Ammoniated straw</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KCP</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>CSC</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>GNC</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Protomix</td>
<td>-</td>
<td>200</td>
</tr>
<tr>
<td>Trace minerals</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

UWS: Untreated wheat straw, AWS: Ammoniated wheat straw, KCP: Kibbled carob pods, CSC: Cottonseed cake, GNC: Groundnut cake.

**Measurements**

Each experimental period consisted of a 14 day acclimatisation phase followed by a 7-day collection phase in which complete collections of refusals and faeces were carried out. A 100 g sample of feed (both of concentrate and straw) was taken during collection periods, bulked, and a subsample (100 g)
was kept for analysis. Total refusals were collected, weighed daily and bulked within sheep over the collection period. The bulked refusals were sampled for analysis. Faeces were collected in zipped canvas bags attached to webbing harnesses that were fitted to the lambs. Daily faecal outputs of individual sheep were weighed and a sample (10% aliquot) taken. Faecal samples were stored at -20°C and bulked within individual animals at the end of the experimental period. Bulked samples were stored at -20°C for subsequent analysis.

Samples of feeds, refusals and faeces were dried at 60°C over 48 h to determine the actual moisture and to facilitate the grounding. The samples were ground through a 1-mm screen and stored in air-tight glass jars at room temperature pending analysis.

Daily urine outputs from individual sheep were collected over 20 ml of 50% v/v sulphuric acid (final pH of the urine < 2) and a proportion (10%) of the daily amounts was sampled, later bulked and stored at 5°C pending N analysis.

**Experimental design**

The experiment was a 4 x 4 randomised block design such that the four animals were fed each of the four diets. The diets consisted of untreated (UWS) or ammoniated (AWS) wheat straw. Both straw based diets were supplemented with kibbled carob pods and either groundnut/cottonseed cake (GC) or protomix (PM). The amounts of the ingredients offered (g fresh weight/head/day) are shown in Table 1.

Supplemental proteins were a 50:50 w/w mixture of groundnut cake and cottonseed cake, or protomix (which is a groundnut cake/cottonseed cake mixture treated in such a way as to increase its undegradable protein content to over 50% of the protein (Feed specialities, Redditch, Worcestershire, U.K).

**Laboratory analyses**

Samples of refused feeds and faeces were analysed for dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents. DM content was determined by drying the samples to a constant weight at 60°C for 48 h. The residual moisture content was determined by drying at 102°C for 24 h. OM content was determined by ignition in a muffle furnace at 550°C overnight. The total nitrogen content was determined using a Kjeldahl technique and CP was calculated by multiplying N content by 6.25 (AOAC, 1980). NDF and ADF contents were determined by the methods of Goering and Van Soest (1970). Hemicellulose was estimated as the difference between NDF and ADF.

The actual feed intakes and overall apparent digestibility of DM, OM and nitrogen for each diet was then calculated, and the overall apparent nitrogen balance was estimated.
Statistical analysis
Data were analysed assuming a completely randomised design with the aid of the GENSTAT package.

RESULTS AND DISCUSSION

Chemical composition
The composition of feedstuffs used in the experiment is shown in Table 2. Ammonisation increased the CP content of the straw by 100%, and decreased the contents of NDF and ADF by 7% and 1.7%, respectively.

In kibbled carob pods, the content of ADF was greater than that of NDF, resulting in an estimation of hemicellulose content that is negative. One reason for this may be that the acidic condition for ADF determination might have caused some kind of polymerisation of sugars with the fibre fraction resulting in an artificial increase in apparent ADF content (Ibrahim and Pearce, 1982). Among the protein supplements it was noted that GNC was the highest in CP contents followed by the Protomix and then lastly the CSC. The low N content of carob (43 g kg⁻¹ DM) is comparable with that reported by Albanell et al (1993) and Bravo et al (1994).

Table 2. Dry matter (g/kg fresh weight) and chemical composition (g/kg DM) of the feed ingredients

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>DM</th>
<th>OM</th>
<th>NDF</th>
<th>ADF</th>
<th>HC</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>UWS</td>
<td>880</td>
<td>948</td>
<td>798</td>
<td>555</td>
<td>243</td>
<td>58</td>
</tr>
<tr>
<td>AWS</td>
<td>878</td>
<td>942</td>
<td>741</td>
<td>545</td>
<td>196</td>
<td>115</td>
</tr>
<tr>
<td>KCP</td>
<td>877</td>
<td>765</td>
<td>203</td>
<td>241</td>
<td>-</td>
<td>43</td>
</tr>
<tr>
<td>CSC</td>
<td>887</td>
<td>933</td>
<td>356</td>
<td>255</td>
<td>101</td>
<td>408</td>
</tr>
<tr>
<td>GNC</td>
<td>886</td>
<td>935</td>
<td>335</td>
<td>122</td>
<td>213</td>
<td>578</td>
</tr>
<tr>
<td>Protomix</td>
<td>892</td>
<td>932</td>
<td>533</td>
<td>196</td>
<td>336</td>
<td>533</td>
</tr>
</tbody>
</table>

UWS: Untreated wheat straw, AWS: Ammoniated wheat straw, KCP: Kibbled carob pods, CSC: Cottonseed cake, GNC: Groundnut cake; DM, dry matter; OM, organic matter.

In vivo digestibility
The main effects of straw ammonisation and protein supplement on feed intake and feed digestibility is presented in Table 3. There was no interaction effects (P>0.05) of ammonisation × protein supplement on feed intake or feed digestibility. Treating the straw with ammonia tended to increase (P > 0.05) OM and DM intakes. Not surprisingly, the increase in N intake due to ammonisation was highly significant (P < 0.01). Similarly, the type of protein fed had no effect on DM and OM intakes, but N intake was increased (P < 0.05) by the UDP source. In this study straw was fed at fixed amounts (400 g/head/d). Nevertheless, Ammonisation as well as the UDP supplement (Protomix) increased the digestibility of DM, OM even though N digestibility was not affected (P > 0.05) (Table 3). This is in contrast with the results reported by Salisbury et al (2004) who suggest that in lambs fed low-quality forage there
was no difference in apparent total-tract digestion or N balance (percentage of N intake) between lambs fed supplements that had the same CP but differed in the proportion of UIP and DIP. The increased intake of AWS compared with that of UWS may be at least partly attributed to its greater overall apparent digestibility. The increased digestibility would be also associated with a faster transit time through the gut, enabling the animal to increase its intake. Feeding the protein in relatively undegradable form also increased the overall apparent digestibility of the diets. This would suggest that in the course of processing the groundnut and cotton to render the protein fractions less degradable in the rumen, a secondary effect was to increase the overall digestibility of these two feeds. This may have been brought about by breaking down crystalline and other relatively resistant structures in the feed. This would then facilitate the access by digestive enzymes.

The fact that there was no interaction between straw treatment and protein degradability, suggests that the severity of processing in the manufacture of proteins is sufficiently mild that enough degradable protein remains in the supplement to ensure that, in this case, the supply of degradable protein was not limiting. The surplus protein is then more efficiently absorbed by feeding it in an undegradable form.

Table 3. Effects of ammonisation and protein sources on the mean feed intakes (g/head/d) and apparent digestibility (g/kg) of dry matter (DM), organic matter (OM) and nitrogen (N) of each diet

<table>
<thead>
<tr>
<th>Ammonisation</th>
<th>Protein source</th>
<th>Item</th>
<th>UWS</th>
<th>AWS</th>
<th>s.e.d</th>
<th>Signif.</th>
<th>GNC/CSC</th>
<th>Protomix</th>
<th>s.e.d</th>
<th>Signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Feed intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DM</td>
<td>895</td>
<td>910</td>
<td>9.9</td>
<td>NS</td>
<td>900</td>
<td>905</td>
<td>9.9</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OM</td>
<td>856</td>
<td>869</td>
<td>9.2</td>
<td>NS</td>
<td>858</td>
<td>867</td>
<td>9.2</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>21</td>
<td>23</td>
<td>0.72</td>
<td>***</td>
<td>22</td>
<td>23</td>
<td>0.72</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apparent digestibility as g/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DM</td>
<td>564</td>
<td>627</td>
<td>7.6</td>
<td>***</td>
<td>588</td>
<td>603</td>
<td>7.6</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OM</td>
<td>581</td>
<td>621</td>
<td>8.0</td>
<td>***</td>
<td>590</td>
<td>612</td>
<td>8.0</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>532</td>
<td>605</td>
<td>30.6</td>
<td>***</td>
<td>560</td>
<td>577</td>
<td>30.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

UWS: Untreated wheat straw, AWS: Ammoniated wheat straw, GNC/CSC: Groundnut and cottonseed cake. Significance: NS: not significance, * P < 0.05, *** P<0.001.

Dietary nitrogen partitioning and nitrogen efficiency

There was no interaction effects (P>0.05) of ammonisation × protein supplement on N partitioning or N efficiency. There were no significant differences between treatments in terms of the amount of N excreted in either the faeces or urine (Table 4). The amount of N excreted as either metabolic faecal N or endogenous urinary N would have been approximately the same in all cases. However, despite increases in N intake brought about by treating the straw and the protein supplements, no increase in exogenous N excretion was
observed. This would explain the increase in N digestibility already reported. This increase in overall apparent digestibility with increased N intakes resulted in an increase (P<0.05) in the amount of nitrogen retained for the ammoniated straw. The increase in N intake achieved by the UDP source tended to increase N retention, but this effect was not significant. Since the endogenous losses of N are relatively constant, the efficiency of N retention increases as supply of N increases. This is because the requirement for N to replace endogenous losses constitutes an increasingly smaller proportion of N consumed. This explains why the efficiency of N retention tended to increase with treatment of both straw and protein supplement. This observed tendency was not significant, although, in the case of straw treatment, it tended towards significance (P<0.1).

Table 4. Effects of ammoniation and protein source on N intake partitioning of dietary nitrogen (g/d) and nitrogen efficiency

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ammoniation</th>
<th>Protein source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UWS</td>
<td>AWS</td>
</tr>
<tr>
<td>N intake g/d</td>
<td>21.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Faecal N g/d</td>
<td>9.8</td>
<td>9.9</td>
</tr>
<tr>
<td>Urinary N g/d</td>
<td>7.1</td>
<td>7.2</td>
</tr>
<tr>
<td>Retained N g/d</td>
<td>4.1</td>
<td>6.4</td>
</tr>
<tr>
<td>N efficiency</td>
<td>198</td>
<td>269</td>
</tr>
</tbody>
</table>

N efficiency: calculated as g N retention/kg N consumed, Significance: NS: not significance, * P < 0.05, *** P < 0.001, UWS: Untreated wheat straw, AWS: Ammoniated wheat straw, KCP: Kibbled carob pods, CSC: Cottonseed cake, GNC: Groundnut cake

CONCLUSIONS

Results of this study showed that the intake and digestibility of straw-based rations supplemented with carob is increased by ammoniation of straw and the protein supplement used is rendered relatively undegradable. These dietary manipulations seem to enhance straw digestibility by altering the physical composition of the feed and thereby optimising rumen function.

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