# Improving the nutritive value of whole-crop wheat for dairy cows

## Thesis

# IMPROVING THE NUTRITIVE VALUE OF WHOLE-CROP WHEAT FOR DAIRY COWS

BY

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Marie Jackson

## ABSTRACT

A series of experiments was carried out to investigate ways of improving the nutritive value of whole-crop wheat (WCW) for dairy cows. Experiment One examined the effects of mechanical processing and alteration of forage cutting height at harvest. Digestibility was improved when urea-treated WCW was mechanically processed at harvest with no effect on milk yield and composition. Increasing forage cutting height decreased dry matter intake (kg/day), increased condition score and reduced milk fat content. Experiment Two investigated the effects of carbohydrate supplementation to mechanically processed urea-treated WCW and included a comparison between maize silage and mechanically processed urea-treated WCW. Cows fed lactose supplemented WCW had numerically higher milk yields than those supplemented with wheat or molasses with no effect on milk component yield (kg/day). Dairy cows fed mechanically processed urea-treated WCW and maize silage performed similarly but animals fed WCW ate proportionally 0.24 more than those fed maize. Experiment Three comprised two parts; the first examining the effects of form of urea applied at ensiling at two stages of maturity. All forages harvested at 550 g DM/kg (except those receiving the urea + urease additive), fermented, indicated by their low pH (< 4.5). At 800 g DM/kg all forms of urea were released resulting in an alkaline pH. Part two of the experiment examined the effects of three stages of maturity and additive treatment to WCW. All forages were aerobically stable with additive application reducing microbial numbers. Additive application is necessary at dry matter contents of 600-700 g DM/kg to prevent microbial growth and additional urease is required at ensiling to promote rapid, extensive hydrolysis of urea and an enhanced NDF digestibility. The production of high DM urea-treated WCW without a reduction in starch digestibility is now possible through the use of mechanical processing. There is also potential to improve performance through the development of appropriate supplementation strategies.

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**Jackson, M. A., Readman, R. J., Huntington, J. A. and Sinclair, L. A.** 2004. The effects of processing at harvest and cutting height of urea-treated whole-crop wheat on performance and digestibility in dairy cows. *Animal Science* **78:** 467-476.

**Jackson M. A., Readman R. J., Huntington J. A., Sinclair L.A.** 2003. The Effect of stage of maturity and additive type on aerobic stability and chemical composition of processed whole-crop wheat. *Proceedings of the 11th International Symposium Forage Conservation.* p.152-153

**Jackson, M. A., Sinclair, L. A., R. Readman and J. A. Huntington.** 2003. The effects of processed whole-crop wheat, maize silage and supplement type to whole-crop wheat on the performance of dairy cows. *Proceedings of the British Society of Animal Science* p.117.

**Jackson, M. A., Sinclair, L. A., Readman, R. and Huntington, J.** 2002. The effect of forage grinding and cutting height of urea-treated whole-crop wheat on the milk production and diet digestibility in dairy cows. *Proceedings of the British Society of Animal Science* p. 13.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# 1.0 LITERATURE REVIEW

## 1.1. Introduction

Whole-crop cereals offer advantages to the dairy producer in a number of ways. Firstly, they require only one harvest, unlike grass silage that requires two or three (Weller, 1992). In agronomic terms, whole-crop forages are grown in the same way as crops for combine harvesting and the decision to harvest for whole-crop or grain does not have to be made until after grass silage has been made. This allows flexibility with regard to the area harvested depending on the quality and quantity of additional forage required (Leaver and Hill, 1992). A number of small grained cereals can be harvested as whole-crops, including wheat, oats, barley and maize (Corrall, 1977). Whole-crop cereals are also of increasing interest to dairy farmers due to their ability to provide a profitable forage source in areas where maize is marginal due to inadequate temperatures and/or excess rainfall (Wilkinson *et al.*, 1998). Indeed Phipps (1994) questioned the use of maize as complementary forage unless dry matters of 300-350 g DM/kg could be achieved.

The inclusion of unprocessed whole-crop wheat (WCW) into diets has been shown to increase dry matter intakes (Sutton *et al.*, 1998) but had little effect on milk yield (Abdalla *et al.*, 1999). This has been attributed to the low digestibility of the forage DM, particularly that of the starch fraction (Sutton *et al.*, 1998). Whilst anecdotal evidence exists that cracking or processing the grains of WCW at harvest increases the digestibility of the resultant forage, this has yet to be assessed, and the effects on animal performance determined.

### 1.1.1. Effect of cereal type on the nutritive value of whole-crop cereals.

Tetlow (1992) reported yields of DM for winter barley, wheat oats and triticale (Figure 1.1). The highest yield for all measured harvest dates was recorded for triticale, with the lowest observed for winter barley.

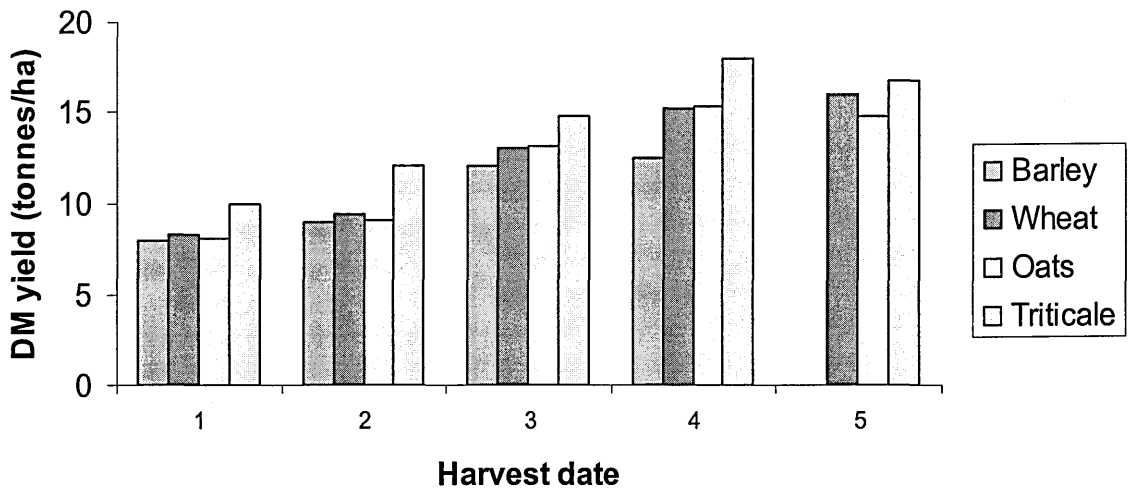


Figure 1.1 Mean DM yields of winter cereals (Tetlow, 1992)

Harvest dates were: 1 = 16 May, 2 = 23 May, 3 = 5 June, 4 = 22 June, 5 = 4 July

The results of a survey of the nutritive value of fermented whole-crop cereals is shown in Table 1.1. Wheat was reported to have a higher DM that that of barley whereas crude protein (CP) was observed to be higher for wheat than barley. The crude protein stated for winter wheat (95 g/kg DM) by Weller (1992) is within the range reported by Crovetto *et al.* (1998) but lower than the 117-124 g/kg DM recorded by Adesogan *et al.* (1998). The differences in forage crude protein levels may not, however, be a reflection of crop species. It has been shown that the level of nitrogen fertiliser applied to cereal crops can also affect nitrogen content (and hence CP content) (Selman, 1975).

Table 1.1 Chemical analyses from a survey of fermented whole-crop cereals (g/kg DM unless otherwise stated) (Weller, 1992)

| | Winter wheat | Winter barley | Triticale |
|---|---|---|---|
| DM (g/kg) | 365 (323-410) | 343 (283-380) | |
| CP | 95 (80-102) | 113 (100-125) | 96 |
| Ammonia N (% total N) | 6.9 (4.6-8.0) | 9.7 (6.4-13.0) | 15.1 |
| DOMD *in vitro* | 666 (620-697) | 671 (628-720) | 650 |

Table 1.2 shows the results of a survey of the nutritive value of urea-treated whole-crop forages carried out by Weller (1992), with the range of values recorded given in brackets. As can be seen from Table 1.2, triticale had the highest DM and winter barley the lowest. Crude protein content was similar between the three species with the value reported for urea-treated WCW somewhat lower than the 250 g/kg DM reported by Deschard *et al.* (1987) and the mean value reported for whole-crop barely being higher than the 163 g/kg DM reported by O'Kiely and Moloney (1995). It is however, likely that these differences are a result of differing levels of urea application which the forages received. Deschard *et al.* (1987) applied urea at a rate of 56.6 kg urea/t DM whereas O'Kiely and Moloney (1995) applied urea to whole-crop barley at a rate of 30 kg/t DM. The application rates reported by Weller (1992) varied between 22 to 50 kg urea/t DM and is the most likely explanation for the observed differences.

Table 1.2 Chemical analyses from a survey of alkali-preserved whole-crop cereals (g/kg DM unless otherwise stated) (Weller, 1992)

| | Winter wheat | Winter barley | Triticale |
|---|---|---|---|
| DM (g/kg) | 561 (450-830) | 432 (400-464) | 635 |
| CP | 194 (110-343) | 205 (161-249) | 219 |
| Ammonia N (% total N) | 27.7 (12.2-50.0) | 22.1 (11.1-31.3) | 13.0 |
| NDF | 456 (351-627) | 455 (540-635) | |
| Starch | 285 (245-335) | | |
| DOMD *in vitro* | 674 (544-750) | 708 (699-717) | |

Forage neutral detergent fibre (NDF) content was similar between urea-treated whole-crop wheat and barley, but the values for both species were somewhat lower that the 554 g/kg DM reported by Deschard *et al.* (1987) for winter wheat but were similar to the 486 g/kg DM reported by Sutton *et al.* (1998). Digestible organic matter of the diet (DOMD) was observed to be highest for winter wheat than winter barley.

It has been shown that there are differences between crop species in chemical composition. However, some of the recorded changes may be attributable to external factors (such as crop nitrogen application rate) and may not be a direct reflection of the effect of crop species.

### 1.1.2. Effects of variety on DM yield and forage nutritive value of WCW.

Weller *et al.* (1995) investigated the effects of ten wheat varieties on the DM yield and modified acid detergent fibre (MADF) concentration as an indicator of nutritional quality (Table 1.3 and Figure 1.2).

Table 1.3 The DM yield (t/ha) of ten winter wheat varieties at different harvest dates (Weller *et al.*, 1995)

| | Harvest date | | | | |
|---|---|---|---|---|---|
| Variety | May 14 | June 11 | July 9 | July 31 | August 14 |
| Apollo | 4.27 | 9.30 | 13.50 | 15.54 | 13.67 |
| Beaver | 4.27 | 9.58 | 15.20 | 16.14 | 16.41 |
| Fenman | 3.64 | 9.46 | 14.74 | 13.05 | 14.24 |
| Fortress | 4.79 | 8.95 | 12.85 | 15.80 | 15.27 |
| Galahad | 5.15 | 9.78 | 13.29 | 14.56 | 15.7 |
| Haven | 3.84 | 9.28 | 14.37 | 15.08 | 17.87 |
| Hornet | 4.60 | 10.61 | 14.17 | 16.38 | 17.19 |
| Norman | 4.92 | 10.61 | 15.07 | 16.50 | 13.94 |
| Riband | 3.65 | 9.53 | 12.91 | 15.58 | 15.12 |
| Slejpner | 4.27 | 8.92 | 13.90 | 16.50 | 15.59 |
| Mean | 4.35 | 9.60 | 14.00 | 15.58 | 15.50 |
| s.e.m. | 0.351 | 0.785 | 1.203 | 1.940 | 1.321 |

As can be seen from Table 1.3, the highest DM yield on 14 May was observed for the variety Norman (4.92 t DM/ha) and the lowest yield for the variety Fenman (3.64 t DM/ha). However, for crops harvested on 11 June, the highest yield was recorded for the varieties Hornet and Norman (10.61 t DM/ha) with the lowest DM yield at this harvest date for the variety Slejpner (8.92 t DM/ha). When allowed to mature further, the variety with the highest DM yield on 9 July was Beaver (15.20 t DM/ha) and the lowest yield observed for the variety Fortress (12.85 t DM/ha). The ranking of varieties changed again on 31 July

with the highest yielding varieties at this harvest date being Norman and Slejpner (16.50 t DM/ha) and the lowest Fenman (13.05 t DM/ha). At the final harvest date the highest DM yield was recorded for the Haven variety (17.987 t DM/ha) and the lowest for Apollo (13.67 t DM/ha). It can, therefore, be concluded that the effects of variety on DM yield will depend upon what stage of growth the crop is harvested at with different varieties reaching maximum DM yield at different times. There were also clear differences between varieties in MADF, as well as changes as a result of harvest date (Figure 1.2). All varieties studied had differing MADF concentrations with the highest value for material harvested on 23 July being observed for the variety Fenman and the lowest for the variety Haven. However, when allowed to mature further, the highest MADF concentration was recorded for the variety Hornet with the lowest being Haven. The pattern of change as a result of increasing crop maturity was also different between the varieties with the varieties Apollo, Beever, Fortress, Galahad, Haven and Norman each having an increased MADF concentration with increased crop maturity. Contrastingly, the other varieties in the study (Hornet and Slepjner) had lower MADF concentrations as a result of increased crop maturity. From this data it can be concluded that the yield and chemical composition of a cereal crop harvested for whole-crop can be affected by the stage of maturity of the crop and crop variety.

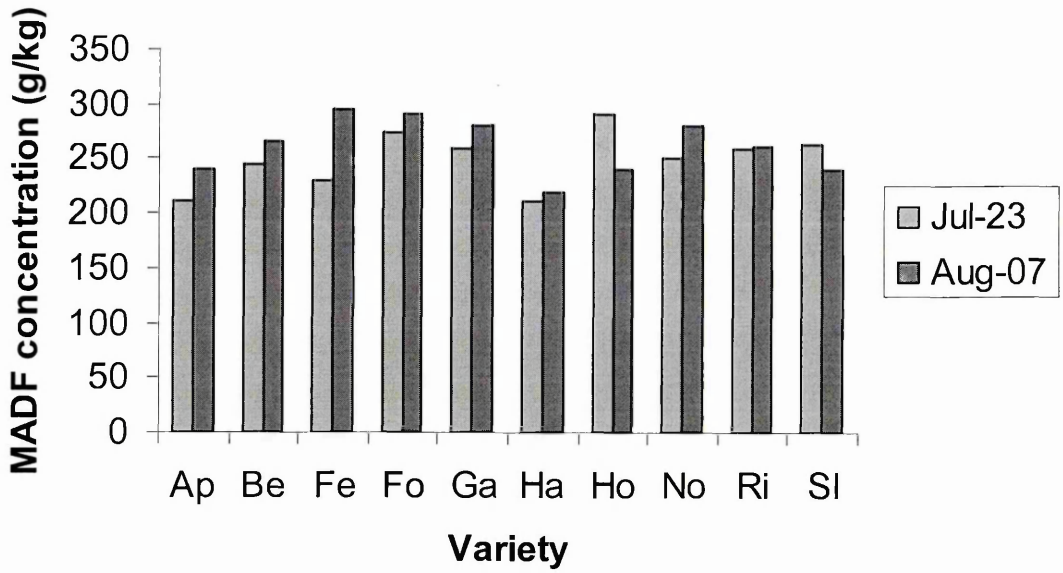


Figure 1.2 Effect of harvest date and variety on MADF concentration. (Weller *et al.*, 1995) Wheat varieties: Ap, Apollo; Be, Beever; Fe, Fenman; Fo, Fortress: Ga, Galahad: Ha' Haven; Ho, Hornet, No, Norman; Ri, Riband, Sl, Slepjner

Adesogan *et al.* (1998) also observed differences in chemical composition between the varieties studied (Table 1.4). Slepjner had the highest crude protein content and Cadenza the lowest. However, nitrogen application rate has been shown to affect crop nitrogen content (Selman, 1975) and the amount of nitrogen which the crops received was not stated by Adesogan *et al.* (1998), and may partly explain the differences in crude protein content observed between the three varieties. In addition, the work by Adesogan *et al.* (1998) was carried out over a three year period, unlike Weller *et al.* (1995) whose experimental work took place during the same growing season. The differences observed between experiments may be therefore potentially attributable to variation in growing conditions from one season to the next, rather than a direct result of variety.

Table 1.4 Effect of wheat variety on the chemical composition of WCW (g/kg DM unless otherwise stated) (Adesogan *et al.*, 1998)

| | Wheat variety | | |
|---|---|---|---|
| | Slepjner | Hussar | Cadenza |
| DM (g/kg) | 645 | 543 | 528 |
| CP | 122 | 108 | 76 |
| Ash | 70 | 71 | 94 |
| Water soluble carbohydrate | 21 | 73 | 43 |
| Starch | 237 | 260 | 231 |
| Neutral cellulase gammanase digestibility | 582 | 663 | 590 |

Water soluble carbohydrate content (WSC) was also observed to vary as a result of variety with Hussar having the highest value at 73 g/kg DM and Slepjner the lowest at 21 g/kg DM (Adesogan *et al.,* 1998). This is in contrast to the results reported by Hill and Leaver (1999b) who observed little difference in WSC content between the two varieties used (Axona and Avalon, harvested at growth stage (GS) 85 and 86 respectively (Zadoks *et al.*, 1974)). Forage starch content was reported by Adesogan *et al.* (1998) to be similar between the varieties Slepjner and Cadenza, but was higher for the variety Hussar. A difference in starch content as a result of varietal differences was also reported by Hill and Leaver (1999b) with the variety Axona having a lower starch content (248 g/kg DM) than the 262 g/kg DM reported for the variety Avalon. Adesogan *et al.* (1998) observed a higher neutral cellulase gammanase digestibility (NCGD) for the variety Hussar than the varieties Slepjner or Cadenza, potentially a result of the increased concentration of starch in the crop DM. This is similar to the results obtained by Hill and Leaver (1999b) who also reported an increased NDCD in the variety with the highest starch content. On the basis of the results of the studies presented, it appears that there are variety effects associated with the chemical composition of WCW but that other factors may also have a contributory effect on the final nutritive value.

### 1.1.3. Effects of stage of maturity on the nutritive content of wheat.

The effect of stage of maturity on crop chemical composition is shown in Table 1.5. There was an increase in crop DM as a result of increased crop maturity. This is in agreement with the findings of Ashbell *et al.* (1985) who examined wheat from the shooting stage (GS 30) to dough stage (GS 85) and Weinberg *et al.* (1991) who examined forages harvested at early milk (GS 73), milk (GS 75-77) and dough (GS 83-87). Forage NDF remained relatively constant throughout the period of the study. This is contrast to the findings of Weinberg *et al.* (1991) who observed an increase in NDF content of the whole

plant up until the dough ripening stage (GS 83-87) when NDF was observed to decrease. Filya (2003) also observed a reduction in NDF attributable to stage of maturity but the effect was observed from the flowering stage (GS 50-59). The reduction in NDF associated with increased crop maturity is a reflection of the increased forage starch content, reducing the relative proportion of NDF (Filya, 2003). However, WSC also decreased with increasing crop maturity. This decrease has also been observed by other authors (Ashbell *et al.*, 1985), and is attributable to soluble carbohydrate conversion to starch within the crop (Filya, 2003). Crop starch content also increased until cut 3 (Table 1.5) when a slight decline in starch content was observed (Tetlow and Mason, 1987). However, when the same crop was ensiled, there was no decrease in starch content as a result of increasing crop maturity observed in the resultant forage. Indeed other experiments have reported an increase in the starch content of fermented WCW with increasing crop maturity (Crovetto *et al.*, 1998).

Table 1.5 Effect of stage of maturity on the chemical composition of wheat (g/kg DM unless otherwise stated) (Tetlow and Mason, 1987)

| | Cut 1 | Cut 2 | Cut 3 | Cut 4 |
|---|---|---|---|---|
| DM (g/kg) | 328 | 420 | 498 | 641 |
| WSC | 150 | 75 | 29 | 18 |
| NDF | 607 | 508 | 588 | 589 |
| Starch | 30 | 219 | 229 | 175 |

*1.1.3.1. Effect of cutting height on the chemical composition of WCW.*

Alteration of cutting height at harvest has been shown to affect the subsequent nutritive value of the forage. Weller *et al.* (1995) demonstrated that the proportion of grain within the crop increased with increasing cutting height (Table 1.6). This increase in grain percentage was at the expense of DM yield with crops cut at a height of 10 cm having a yield of 15.7 t DM/ha, whereas those cut at 40 cm had a yield of 12.4 t DM/ha (Weller *et al.*, 1995).

Table 1.6 Effect of cutting height on dry matter yield of WCW (Weller *et al.*, 1995)

| | Harvest date August 7 | | |
|---|---|---|---|
| Cutting height (cm) | 10 | 25 | 40 |
| Mean DM yield (t/ha) | 15.7 | 14.1 | 12.4 |
| Relative yield (%) | 100 | 89.6 | 60.9 |
| Proportion of grain (%) | 48.1 | 53.5 | 60.9 |

The main effect of this increase in forage grain content was an increase in forage starch content. Sinclair *et al.* (2003), harvested wheat at 450 g DM/kg and observed an increased starch content of 60 g/kg DM by increasing the cutting height from 17.9 cm to 38.2 cm (Table 1.7). The alteration of forage cutting height also had an effect on forage fibre content with the long straw material (cutting height 17.9 cm) having a higher NDF content of 433 g/kg DM than the short straw material (cutting height 38.2 cm) at 384 g/kg DM.

Table 1.7 Effect of cutting height on the nutritional composition of fermented WCW harvested at one of two cutting heights (g/kg DM unless otherwise stated) (Sinclair *et al.*, 2003)

| | Low starch 17.9 cm | High starch 38.2 cm |
|---|---|---|
| DM (g/kg) | 446 | 477 |
| CP | 100 | 103 |
| NDF | 433 | 384 |
| Starch | 232 | 292 |

## 1.2. Preservation of WCW

Whole-crop wheat can be preserved by a number of means. These include fermentation at an immature stage of growth, either with or without an inoculant (Adogla-Bessa and Owen, 1995a) or the preservation of more mature forage through the application of sodium hydroxide (NaOH) (Tetlow, 1987) or urea (Givens *et al.*, 1993).

### 1.2.2. Fermentation

1.2.2.1. *The ensiling process.*

Although it may be harvested at a range of DM's, fermented WCW is typically harvested around 380 g DM/kg (Weller *et al.*,1995), at growth stage (GS) 71-85 (Sinclair *et al.*, 2003). It can then be preserved via fermentation with or without an inoculant. Once a crop is harvested and ensiled it continues to respire using up plant sugars and converting them to water, carbon dioxide and heat (McDonald *et al.*, 1991). Lactic acid bacteria which may have been present on the original crop, or added in the form of an inoculant, then proliferate and aid in preservation by producing lactic acid as a result of their fermentation of carbohydrates (Watson and Nash, 1960). This promotes a rapid decrease in pH (Merry *et al.*, 2000). Spoilage microorganisms such as clostridia are unable to tolerate low pH's (Rogers and Whittier, 1928) and, if silage pH remains low, their growth is limited, inhibiting proteolysis (McDonald *et al.*, 1991) essential in order to reduce the breakdown of plant proteins and hence decreasing a forages nutritional value.

*1.2.2.2. Effects of stage of maturity on the chemical composition of fermented WCW.*

Fermented WCW is typically harvested at an immature stage of growth at DM's around 380 g DM/kg (Weller *et al.*, 1995) but has been harvested at a range of DM's including 197 g DM/kg (Crovetto *et al.*, 1998), 346-392 g DM/kg (Filya, 2003) and 335-637g DM/kg (Adesogan *et al.*, 1998). This can lead to differences in the chemical composition as the crop matures. Table 1.8 summarises the results obtained by a number of authors over a range of crop DM's.

Table 1.8 Effect of stage of maturity at harvest on the chemical composition of fermented WCW (g/kg DM unless otherwise stated)

| | Crovetto *et al.* (1998) | | | | Adesogan *et al.* (1998) | | |
|---|---|---|---|---|---|---|---|
| Stage of maturity | 40-49 | 64-65 | 70-79 | 80-89 | 73 | 76 | 92 |
| DM (g/kg) | 197 | 224 | 290 | 360 | 335 | 492 | 637 |
| CP | 127 | 98 | 83 | 79 | 124 | 119 | 117 |
| OM | 931 | 944 | 938 | 944 | 920 | 927 | 940 |
| NDF | 575 | 594 | 594 | 487 | | | |
| Acid detergent fibre | 349 | 346 | 350 | 310 | 310 | 285 | 302 |
| Starch | 23 | 26 | 27 | 188 | 116 | 215 | 243 |
| pH | 3.6 | 3.6 | 3.6 | 3.8 | 3.9 | 4.1 | 5.7 |

Both DM and starch increased with increasing crop maturity. Differing effects of stage of maturity on NDF content have been observed, with some authors reporting an increase in forage NDF (Crovetto *et al.,* 1998; Hill and Leaver, 1999a), whereas others such as Adogla-Bessa and Owen (1995a) reported a decrease in forage NDF. However, this decrease is likely to be a result of the increased amounts of starch present in the forage, rather than a decrease in the fibre content (Arieli and Adin, 1994). A similar pattern of change to that reported by Adogla-Bessa and Owen (1995a), namely a decrease in forage NDF with increasing crop maturity was also observed by Adesogan *et al.* (1998) and Sinclair *et al.* (2003).

*1.2.2.3. Effect of additive application on the chemical composition of WCW.*

Previous work (Weinberg *et al.*, 1993b; Williams *et al.*, 1995) has determined that enzyme and inoculant additives can be used successfully to preserve WCW. However, most work has been carried out at low DM's (e.g. 357 g DM/kg Weinberg *et al.,* 1993b, or 208 g DM/kg Williams *et al.*, 1995), when maximum DM yield had not been achieved (Bolsen *et al.,* 1983). It was suggested by Adogla-Bessa and Owen (1995a) that cellulase and hemicellulase enzymes could be used to hydrolyse the polysaccharides within WCW, hence increasing the amounts of simple sugars available for fermentation. The effects of these enzymes on the nutritive value of WCW are shown in Table 1.9.

Table 1.9 Effect of application of cellulase-hemicellulase enzymes on the chemical composition of WCW forage (g/kg DM unless otherwise stated) (Adogla-Bessa and Owen, 1995a)

| | Enzyme application rate | | | | |
|---|---|---|---|---|---|
| | Untreated | Low | Medium | High | s.e.d. |
| Total nitrogen | 14.0 | 14.9 | 14.5 | 14.6 | 0.42 |
| NDF | 528 | 537 | 507 | 503 | 13.9 |
| Acid detergent fibre | 320 | 309 | 302 | 305 | 11.6 |
| pH | 4.8 | 4.5 | 4.6 | 4.5 | 0.08 |
| Cellulose | 280 | 270 | 260 | 260 | 9.1 |

The application of cellulase-hemicellulase enzymes decreased forage NDF and acid detergent fibre (ADF) content, but had little effect on total nitrogen (N) or cellulose content. Additive application did, however, improve silage fermentation, as indicated by the lower pH observed for the enzyme treated material. When WCW treated with cellulase-hemicellulase enzymes was fed to steers no effect on dry matter intake (DMI), diet digestibility or liveweight gain was observed between the treated and untreated forages (Adogla-Bessa and Owen, 1995b). In a subsequent experiment Adogla-Bessa *et al.* (1999) found similar effects, namely a decrease in forage NDF and ADF content. However, ammonia-N was also observed to decrease with increasing rate of additive application which, when linked to the decrease in pH observed confirms that the application of a cellulase-hemicellulase additive to WCW improves silage fermentation.

It was suggested by Weinberg *et al.* (1999) that the application of *Lactobacillus buchneri* may improve the aerobic stability of cereal silages by producing volatile fatty acids which have been observed to inhibit the growth of fungi and yeasts. Weinberg *et al.* (1999) observed an improved aerobic stability (indicated by decreased pH and yeast counts (5.6 *vs.* 6.6 $\log_{10}$ colony forming units/g fresh material respectively)) for material treated with *L. buchneri* compared to material receiving no additive. This was attributed to be a result of the higher acetic acid concentrations of these silages (28 *vs.* 16 g/kg DM, $P < 0.05$) which has been observed to inhibit fungi (Moon, 1983). A higher level of silage acetic acid as a result of *L. buchneri* application was also observed by Weinberg *et al.* (2002),

with means of 17 *vs.* 10 g/kg DM of acetic acid for treated and untreated material respectively ($P < 0.05$). The effects of stage of maturity and the addition of lactic acid bacteria (*Streptococcus faecium)* on forage composition reported by Weinberg *et al.* (1993a) are presented in Table 1.10.

Table 1.10 Effect of inoculation of WCW forage with *Streptococcus faecium* on chemical composition of forages (g/kg DM unless otherwise stated) (Weinberg *et al.*, 1993a)

| Growth Stage | 60-69 | | 70-79 | | 80-89 | |
|---|---|---|---|---|---|---|
| Treatment | Control | Inoc | Control | Inoc | Control | Inoc |
| WSC | - | 60 | 62 | 91 | 35 | 40 |
| NDF | 644 | 561 | 472 | 518 | 605 | 500 |
| ADF | 436 | 373 | 293 | 309 | 322 | 306 |
| pH | 5.0 | 3.8 | 3.9 | 3.8 | 4.0 | 4.0 |

As can be seen in Table 1.10, the effects of inoculation resulted in a decrease in pH at the flowering (GS 60-69), stage but less effect was observed at the milk and dough stages of maturity (GS 70-79 and 80-89). Both NDF and ADF contents were observed to decrease with additive application at the flowering and dough stages at maturity but an increase in NDF was observed at the milk stage for inoculated material compared to material receiving no additive. It can, therefore, be concluded that the application of an additive to fermented WCW may result in improved silage fermentation, as well improving the stability of the forage when the clamp is opened and exposed to air.

### 1.2.3. Sodium hydroxide treated WCW.

The effects of sodium hydroxide (NaOH) on straw and grain has well been established. Ørskov (1977) reported an increase in DM degradation (from 4.9 % to 79.8 % of DM) when wheat grain was sprayed with 50 g NaOH/kg DM. Cereal straws have also been treated with NaOH with Moss *et al.* (1990) reporting an increase in the digestibility of OM (0.400 *vs.* 0.634 kg/kg, $P < 0.01$) and crude fibre (0.428 *vs.* 0.734 kg/kg, $P < 0.001$) as a result of the addition of 45 kg NaOH/t DM to wheat straw.

*1.2.3.1. Effect of NaOH application on the chemical composition of WCW.*

The major effect of NaOH on forage chemical composition is on the fibre content. A decrease in NDF content of WCW (from 528 to 508 g/kg DM) as a result of NaOH application was observed by Tetlow and Mason (1987). Although investigating the effects on wheat straw and not WCW, Moss *et al.* (1990) also found a decrease in straw fibre content as well as a reduction in cellulose and hemicellulose (Table 1.11). This is likely to be as a result of the effect of NaOH on plant cell walls with the application of alkali to forages having been shown to solubilise plant cell walls and hence decrease forage/straw fibre content (Van Soest *et al.*, 1984).

Table 1.11 Chemical composition of wheat, barley and oat untreated straws and straws treated with 45 kg/t DM NaOH (g/kg DM unless otherwise stated) (Moss *et al.*, 1990)

| | Control | NaOH | Significance |
|---|---|---|---|
| DM | 841 | 762 | * |
| CP | 44 | 39 | |
| CF | 429 | 416 | |
| NDF | 799 | 681 | *** |
| ADF | 523 | 511 | |
| Cellulose | 407 | 398 | |
| Hemicellulose | 276 | 171 | *** |

*1.2.3.2. Effects of NaOH application on the digestibility of WCW.*

The addition of NaOH to WCW (applied at 50 g/kg DM) was found to increase *in vitro* organic matter digestibility (Table 1.12, Tetlow and Mason, 1987). Similar increases in forage and straw digestibility as a result of the application of NaOH have been observed by a number of authors (Wanapat *et al.*, 1985; Moss *et al.*, 1990; Ng'ambi and Campling, 1991). Increases in digestibility have also been reported *in vivo* with wheat straw and grain treated with NaOH being observed to have a higher NDF digestibility (0.689 *vs.* 0.626 kg/kg) than ensiled WCW when fed to heifers (Leaver and Hill, 1995). Sutton *et al.* (2001), also observed an increase in the NDF digestibility of WCW treated with 50 kg caustic soda/t DM when measured in lactating dairy cows with values of 0.628 *vs.* 0.593 kg/kg for treated and untreated material respectively ($P < 0.05$). Although NaOH has been

shown to enhance the digestibility of WCW, due to the hazards presented by its application as an additive, a safer alternative alkali source was desired (Tetlow, 1992).

Tetlow (1992) compared the effects of urea application and NaOH application on the *in vitro* and *in vivo* digestibility of WCW (Figure 1.3). The application of NaOH resulted in a higher OM digestibility *in vitro* at all application rates.

Table 1.12 Effect of NaOH application (50 g/kg DM) to WCW on organic matter digestibility *in vitro* (Tetlow and Mason, 1987)

| | | Organic matter digestibility (g/kg organic matter) | |
|---|---|---|---|
| Harvest date | NaOH -/+ | Fresh crop | Silage |
| 6 July | - | 602 | 476 |
| 6 July | + | 700 | 648 |
| 6 August | - | 620 | 530 |
| 6 August | + | 704 | 720 |
| 20 August | - | 572 | 490 |
| 20 August | + | 733 | 762 |
| 29 August | - | 556 | 478 |
| 29 August | + | 799 | 752 |

However, this situation was reversed *in vivo,* with urea-treated forages having a higher OM digestibility than NaOH treated forages (Figure 1.3). On the basis of this data Tetlow (1992) concluded that urea was the more appropriate preservation agent for mature/high DM WCW.

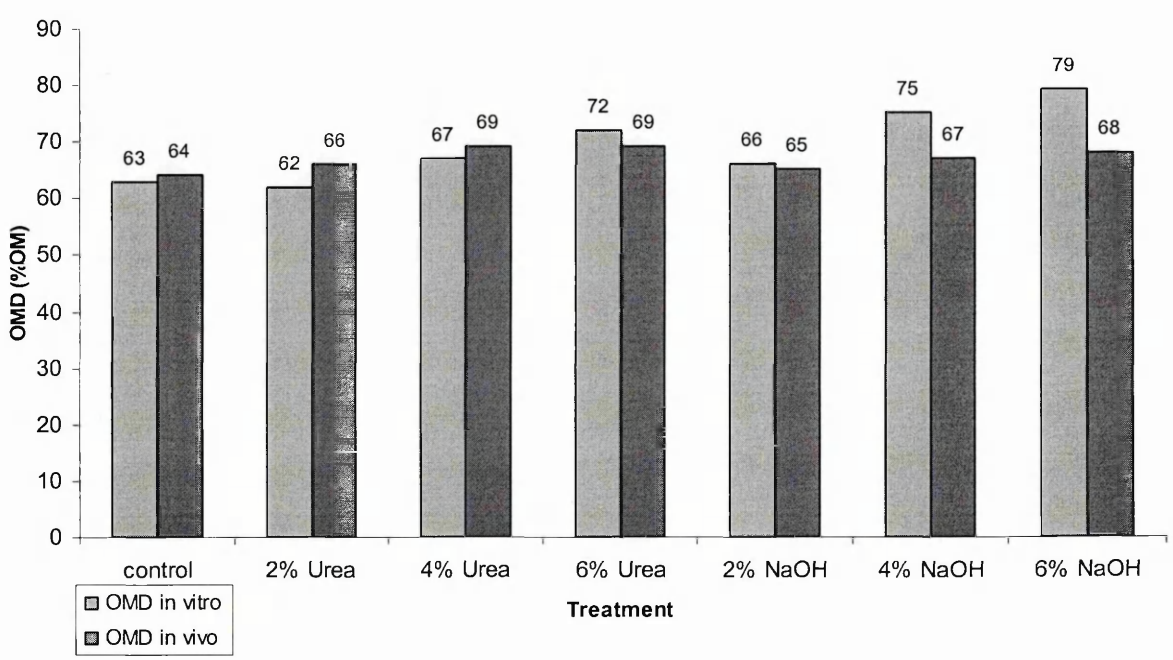


Figure 1.3 Effects of NaOH and urea treatment on the *in vitro* and *in vivo* organic matter digestibility (OMD) of WCW (Tetlow, 1992).

### 1.2.4. Urea-treated WCW.

Urea is applied to mature whole-crop cereals to assist in the preservation of the crop and to reduce losses at feed out (Hill and Leaver, 1999a). The preservation of WCW with urea is reliant upon the conversion of urea into ammonia, with the liberated ammonia being the preservative agent (Givens *et al.*, 1993). Ammonia has also been shown to inhibit fungi and yeast growth in silage (Yu *et al.*, 1975; Sahnoune *et al.*, 1991).

#### *1.2.4.1. Effect of urea treatment on the chemical composition of WCW.*

The application of urea to a forage results in a number of changes to its chemical composition. These changes are illustrated in Table 1.13.

Table 1.13 Effect of urea treatment on the chemical composition of WCW (g/kg DM unless otherwise stated) (Hill and Leaver, 2002).

| | Treatment | |
|---|---|---|
| | untreated | urea-treated (40 kg/t DM) |
| DM (g/kg) | 669 | 689 |
| Total Nitrogen | 13.8 | 33.0 |
| Ammonia N (g/kg total N) | 20 | 386 |
| NDF | 486 | 476 |
| ADF | 282 | 286 |
| Starch | 214 | 237 |
| pH | 6.16 | 8.86 |

The major differences between urea and untreated WCW were in the total nitrogen content, with the application of urea resulting in an increase in the forage N content (from 13.8 to 33.0 g/kg DM). The total N values obtained by Hill and Leaver (2002) for urea-treated forage (treated with 40 kg urea/t DM) are lower than those reported by Sutton *et al.* (1997) (40.6 g/kg DM for material treated with 40 kg urea/t DM) but higher that that observed by Phipps *et al.* (1995) (26.1 g/kg DM for material treated with 40 kg urea/t DM). Ammonia N was also increased by the application of urea, at 386 g/kg total N (Hill and Leaver, 2002). This is similar to the values obtained by Leaver and Hill (1995), (314 g/kg total N) and to those recorded by Phipps *et al.* (1995) (356 g/kg DM) but substantially higher than

those of Sutton *et al.* (1997) of 150 g/kg DM. Forage pH was also higher for the urea-treated forage, which is the case for urea-treated WCW (Phipps *et al.*, 1995; Leaver and Hill, 1995; Hameleers, 1998), indicating the alkaline nature of the additive. A decrease in NDF was found as a result of urea treatment and an increase in ADF, thought to be a result of solubilisation of hemicellulose due to urea treatment (Van Soest *et al.*, 1984).

Work has been carried out by a number of authors (Hill and Leaver, 1991; Sutton *et al.*, 1997) to examine the level of urea needed to preserve WCW. Results reported by Sutton *et al.* (1997) and Hill and Leaver (2002) from applying urea at two different levels, 20 kg/t DM and 40 kg/t DM on the nutritional composition of WCW are presented in Table 1.14.

Table 1.14 Effect of level of urea application at ensiling on the chemical composition of urea-treated WCW (g/kg DM unless otherwise stated).

| | Sutton *et al.* (1997) | | Hill and Leaver (2002) | |
|---|---|---|---|---|
| Level of urea application | 20 kg/t DM | 40 kg/t DM | 20 kg/t DM | 40 kg/t DM |
| DM (g/kg) | 757 | 761 | 662 | 689 |
| CP | 220 | 254 | 186 | 206 |
| Ammonia-N (g/kg total N) | 105 | 150 | 349 | 386 |
| Ash | 63 | 57 | 55 | 52 |
| WSC | 17 | 24 | 23 | 24 |
| NDF | 556 | 552 | 482 | 476 |
| ADF | 336 | 343 | 287 | 286 |
| Starch | 168 | 184 | 236 | 237 |
| pH | 7.3 | 8.6 | 8.7 | 8.9 |
| Lactic acid | 9 | 2 | 2 | 0 |
| Acetic acid | 8 | 16 | 3 | 3 |

The major effect of urea application rate on forage composition was on CP and ammonia N, both being higher as a result of increased urea application. The higher urea application rate was also shown to affect pH, with forages treated with 40 kg urea/t DM having a more alkaline pH than forage treated with 20 kg urea/t DM. Additionally, Hill and Leaver (1991) reported reduced DM losses for WCW treated with 40 kg urea/t DM compared to that treated with 20 kg urea/t DM. It was concluded by Sutton *et al.* (1997) that the higher urea

application rate (40 kg urea/t DM) was necessary to ensure even application of urea within the clamp.

In order that urea can hydrolyse into ammonia, a source of urease needs to be present in the forage (Van der Merwe, 1977). Coxworth (1978) investigated the effects of urea treatment on the feeding value of straw and reported that the addition of urea plus a urease source resulted in a significant increase in digestibility (*in vitro*), but the addition of urea without a urease source resulted in little improvement (Table 1.15). It was concluded by Coxworth (1978) that wheat straw did not contain sufficient urease to hydrolyse urea into ammonia. Coxworth (1978) also reported a greater increase in digestibility for straw treated with urea and a source of urease compared to straw receiving aqueous ammonia.

Table 1.15 Effect of urea, with or without added jack bean meal as a source of urease enzyme, on the *in vitro* digestibility of Neepawa wheat straw (Coxworth, 1978)

| Treatment | | | Digestible organic matter (%) |
|---|---|---|---|
| Urea % | Jack bean meal % | Aqueous ammonia % | |
| 0 | 0 | 0 | 31 |
| 5 | 0 | 0 | 33 |
| 5 | 5 | 0 | 39 |
| 0 | 0 | 0 | 34 |
| 5 | 0 | 0 | 38 |
| 5 | 5 | 0 | 40 |
| 5 | 2 | 0 | 41 |
| 0 | 0 | 3 | 39 |

The presence of water is also necessary for the conversion of urea to ammonia (Blakeley and Zerner, 1984). The lower availability of water at high DM's may result in urea remaining unhydrolysed in the forage when fed (Tetlow, 1992). This creates the potential problem of ammonia toxicity at feed out caused by the rapid hydrolysis of urea in the rumen (West, 1992), particularly if there has been an uneven distribution of the additive in the clamp.

Urease can be supplied from two sources, the forage itself or an external additive applied to the forage (Sahnoune *et al.*, 1991). An external enzyme can either be applied as a pure culture or a material that contains high amounts of urease such as ground dehulled soya bean meal (Sahnoune *et al.*, 1991). It was concluded by Sahnoune *et al.* (1991) that it was useful to add urease when urea treating dry forages due to their low water content.

## 1.3. Effect of whole-crop wheat on performance.

Dairy cow performance is affected by a number of factors including physiological factors such as stage of lactation (Thomas and Rook, 1983), genetic potential (Oldham and Sutton, 1979) as well as nutritional factors such as energy and protein intake (Thomas and Rook, 1983).

### 1.3.1. Effects of fermented WCW on animal performance.

*1.3.1.1. Effects of inclusion rate of fermented WCW on intake and performance.*

When fermented WCW was included into dairy cow rations containing grass silage, there was an increase in DM intake (from 16.3 to 18.0 kg DM/day, $P$ <0.001) (Leaver and Hill, 1995). Smaller increases in intake as a result of fermented WCW inclusion into grass silage based diets (inclusion rate 33:67 fermented WCW: grass silage, DM basis) were also observed by Phipps *et al.* (1995) with the inclusion of fermented WCW resulting in a higher forage DM intake (10.6 kg DM/day) compared to intakes observed for sole grass silage diets (9.3 kg DM/day). Similar results were also observed by Hameleers (1998) when fermented WCW was included into grass silage based rations (40 % fermented WCW to 60 % grass silage). It was concluded by Leaver and Hill, (1995) that WCW has a greater intake potential than grass silage.

Leaver and Hill (1995) reported no effect on milk yield or milk constituent yield between animals fed sole grass silage diets or diets containing fermented WCW despite the higher intakes observed for these forages, in agreement with the findings of Phipps *et al.* (1995). Live weight change was also observed to be unaffected by fermented WCW inclusion (Phipps *et al.*, 1995; Leaver and Hill, 1995). The lack of a response to the higher intakes of fermented WCW in the form of milk yield, was attributed by Phipps *et al.* (1995), to be due to the low metabolisable energy (ME) of the forage (9.9 MJ/kg DM) compared to that of the grass silage it replaced (10.9 MJ/kg DM). Hence the benefit of the increase in intake was reduced due to the lower ME of the fermented WCW. Phipps *et al.* (1995) went further to state that the inclusion of fermented WCW into diets based on high energy density well fermented grass silage would be unlikely to result in an increase in milk production.

*1.3.1.2. Effect of stage of maturity of fermented WCW on intake and performance.*

Differing effects have been observed on DM intake as a result of forage maturity. Adogla-Bessa and Owen (1995b) observed no effect of stage of maturity on DM intake when WCW was fed to steers. Contrastingly, Bolsen and Berger (1976), reported that there was a significant effect on forage DM intake when WCW was fed to lambs, with wheat harvested at the milk stage of maturity (GS 70-79, 326 g DM/kg) having a lower dry matter intake (490 g/day) than forage harvested at the dough stage of maturity (653 g/day, GS 83-87, 320 g DM/kg). No statistical difference was observed by Sutton *et al.* (2002) on DM intake when fermented WCW harvested at two different stages of maturity (301 g DM/kg and 511 g DM/kg) was fed to lactating cows. However, in the second year of the study, a difference was observed with low DM WCW (321 g DM/kg) having a total DM intake of 20.1 kg/day compared to 21.7 kg/day for the high DM material (496 g DM/kg; $P < 0.01$). In common with the first year of the study, no effect was observed on milk yield and composition (Sutton *et al.*, 2002).

The findings of Sutton *et al.* (2002) are in agreement with those of Phipps *et al.* (1998) who recorded no effect on DM intake or milk yield as a result of feeding fermented WCW at differing stages of maturity (343 and 518 g DM/kg) with no difference also being observed as a result of forage maturity on milk composition (Phipps *et al.*, 1998). Sinclair *et al.* (2003) reported similar results for material harvested at 296 and 371 g DM/kg.

Contrastingly, Arieli and Adin (1994) reported a decrease in milk yield as a result of increasing crop maturity (36 kg/day *vs.* 32.8 kg/day; $P < 0.001$) for forages harvested at 301 and 379 g DM/kg. Milk fat content was reported to increase with increasing crop maturity (2.45 % *vs.* 2.79 % $P < 0.001$) whereas milk protein yield decreased (1.06 kg/day *vs.* 0.97 kg/day respectively, $P < 0.001$). It was concluded that the harvesting of fermented WCW at such different crop DM's was unlikely to be of any practical nutritional significance, although benefit may be obtained in forage DM yield by allowing the crop to reach a greater stage of maturity (Sutton *et al.*, 2002).

### 1.3.2. Urea-treated WCW.

*1.3.2.1. Effect of inclusion rate of urea-treated WCW on intake and performance.*

When compared to sole grass silage diets, the introduction of urea-treated WCW into diets based on grass silage has been observed to increase forage DM intake by Sutton *et al.* (1997). Intakes were observed to increase from 9.26 kg DM/day to 10.29 kg DM/day when urea-treated WCW was introduced into grass silage based diets (in the ratio 2:1, 2 parts grass silage to 1 part WCW, DM basis) (Sutton *et al.*, 1997). Similar trends were also reported by Hameleers, (1998), Sutton *et al.* (1998) and Phipps *et al.* (1995).

Despite the observed increases in DM intake, no effect was observed on milk yield (Sutton *et al.*, 1997). This is in agreement with other studies (Phipps *et al.*, 1995; Hameleers, 1998). Urea-treated WCW was also reported to have no effect on milk constituent yield

(Phipps *et al.*, 1995; Hameleers, 1998). However, an increase in milk protein yield as a result of urea-treated WCW inclusion was observed by Sutton *et al.* (1997). It was stated by Sutton *et al.* (1997) that this supported the findings of Phipps *et al.* (1995), namely that cows perform better on forage mixtures rather than sole grass silage diets.

The exact reason behind the inconsistencies between DM intake and milk and milk constituent yield with regard to urea-treated WCW remains, at present, contested. It has been suggested by Leaver and Hill (1992), that the poor utilization of whole-crop cereals may be attributable to the high intake of ammonia and the metabolic costs of excreting this substance. However the low digestibility of urea-treated WCW has also been cited as the reason behind the poor performance of the forage (Sutton *et al.*, 1998). The effects of inclusion rate on DM intake and animal performance are summarised in Table 1.16.

An increase in forage DM intake from 10.3 kg DM/day to 10.7 kg DM/day when the inclusion rate was increased from 2:1 to 1:2 was recorded by Sutton *et al.* (1997) (for material treated with 40 kg urea/t DM). There was, however, a decrease in intake when urea-treated WCW was offered as a sole forage. This decrease may be explained by the lack of an additional forage source in the diet as it has been determined that when an additional forage is introduced into a ration, intakes increased (Leaver and Hill 1995; Hameleers, 1998).

Table 1.16 Effect of inclusion rate of urea-treated WCW on dairy cow intake and performance

| | Sutton *et al.* (1997) | | | Sutton *et al.* (1998) | | Abdalla *et al.* (1999) | |
|---|---|---|---|---|---|---|---|
| Treatment† | 2:1 40 | 1:2 40 | WCW40 | 2:1 40 | 1:2 40 | 2:1 40 | 1:2 40 |
| Forage intake (kg/day) | 10.3 | 10.7 | 10.1 | 11.5 | 11.7 | 11.5 | 12.4 |
| Milk yield (kg/day) | 20.5 | 21.6 | 19.9 | 26.2 | 25.8 | 21.2 | 22.2 |
| Fat (g/kg) | 46.9 | 43.6 | 44.7 | 42.6 | 45.0 | 43.7 | 44.1 |
| Protein (g/kg) | 32.3 | 32.7 | 32.5 | 28.0 | 29.2 | 29.0 | 29.5 |
| Lactose (g/kg) | 46.4 | 46.2 | 45.7 | 47.7 | 47.2 | 48.0 | 47.2 |
| Fat yield (g/day) | 958 | 939 | 886 | 1113 | 1139 | 927 | 974 |
| Protein yield (g/day) | 660 | 704 | 644 | 733 | 742 | 614 | 654 |
| Lactose yield (g/day) | 951 | 997 | 909 | 1248 | 1223 | 1018 | 1047 |

†2:1 20 = grass silage mixed 2:1 (DM basis) with WCW treated with 20 kg urea/t DM. 2:1 40 = grass silage mixed 2:1 (DM basis) with WCW treated with 40 kg urea/t DM. WCW 40 = WCW treated with 40 kg urea/t DM fed as sole forage.

Increasing the rate of WCW inclusion was observed to result in an increase in milk fat and milk protein concentration (Abdalla *et al.*, 1999). However, there was no effect on milk yield (Table 1.16). Similar effects of performance were observed by Sutton *et al.* (1998). By contrast, Sutton *et al.* (1997) observed a decrease in milk fat concentration when the ratio of grass silage to WCW treated with 20 and 40 kg urea /t DM was increased from 2:1 to 1:2 DM, but a slight increase in milk fat levels when WCW was fed as the sole forage (Table 1.16). However milk protein content increased when WCW inclusion rate was decreased from 2:1 to 1:2 but fell slightly when WCW was offered as a sole forage (Sutton *et al.*, 1997; Table 1.16). It can be concluded that there may be benefits to increasing the inclusion rate of WCW but that there are disadvantages in performance if urea-treated WCW is fed as the sole forage.

*1.3.2.2. Effects of level of urea application to WCW on intake and performance.*

There have been a number of experiments carried out to investigate the effects of differing levels of urea application to WCW on intake and performance in dairy cows (Table 1.17). Sutton *et al.* (1997) fed WCW preserved with either 20 kg urea/t DM or 40 kg urea/t DM

and observed no significant effect on either forage or total DM intakes. This is in agreement with Sutton *et al.* (1998) and Abdalla *et al.* (1999). No effect on milk yield and composition as a result of level of urea application was also recorded (Sutton *et al.*, 1997). In subsequent work an effect on milk protein yield was observed, which was greater for cows fed WCW treated with 40 kg urea/t DM than that achieved from cows fed WCW treated with 20 kg urea/t DM (Sutton *et al.*, 1998; Abdalla *et al.*, 1999). There was also an increase in milk fat content when forage was treated with a higher amount of urea (20 *vs.* 40 kg urea/t DM) (Sutton *et al.*, 1998; Abdalla *et al.*, 1999).

Table 1.17 Effect of rate of urea application on intake, milk yield and composition

| | Sutton *et al.* (1997) | | Sutton *et al.* (1998) | | Abdalla *et al.* (1999) | |
|---|---|---|---|---|---|---|
| Treatment† | 2:1 20 | 2:1 40 | 1:2 20 | 1:2 40 | 1:2 20 | 1:2 40 |
| Forage DMI (kg/day) | 10.43 | 10.29 | 12.13 | 11.70 | 12.36 | 12.41 |
| Total DMI (kg/day) | 16.40 | 16.24 | 18.10 | 17.64 | 18.26 | 18.32 |
| Milk yield (kg/day) | 21.2 | 21.6 | 26.2 | 25.8 | 22.1 | 22.2 |
| Fat (g/kg) | 44.3 | 43.6 | 42.6 | 45.0 | 42.1 | 44.1 |
| Protein (g/kg) | 32.7 | 32.7 | 28.2 | 29.2 | 29.3 | 29.5 |
| Lactose (g/kg) | 46.1 | 46.2 | 47.5 | 47.2 | 46.9 | 47.2 |
| Fat yield (g/day) | 935 | 939 | 1119 | 1139 | 925 | 974 |
| Protein yield (g/day) | 692 | 704 | 735 | 742 | 646 | 654 |
| Lactose yield (g/day) | 977 | 977 | 1242 | 1223 | 1038 | 1047 |

† 2:1 20 = grass silage mixed 2:1 (DM basis) with WCW treated with 20 kg urea/t DM.
2:1 40 = grass silage mixed 2:1 (DM basis) with WCW treated with 40 kg urea/t DM.

*1.3.2.3. Comparison between fermented WCW and urea-treated WCW on intake and performance.*

No significant difference in DMI in dairy cows fed either fermented or urea-treated WCW was observed by Phipps *et al.* (1995), although both forages increased intakes above that recorded for cows fed grass silage alone (Table 1.18). This is in agreement with the findings of Leaver and Hill (1995). However, Hameleers (1998) reported a significant ($P$ <0.01) increase in intakes between animals fed urea-treated WCW and fermented WCW (Table 1.18). Forage DM intakes for urea-treated WCW were higher (at 13.1 kg DM/day) than those recorded for animals fed fermented WCW (12.2 kg DM/day).

Table 1.18 Effect of inclusion of either fermented WCW or urea-treated WCW on intake and milk production

| | Phipps *et al.* (1995) | | | Hameleers, (1998) | | |
|---|---|---|---|---|---|---|
| | GS† | FW | UW | GS‡ | FW | UW |
| Forage DMI (kg/day) | 9.3 | 10.6 | 10.2 | 10.6 | 12.2 | 13.1 |
| Milk yield (kg/day) | 23.0 | 24.2 | 24.0 | 27.4 | 27.1 | 26.9 |
| Milk composition (g/kg) | | | | | | |
| Fat | 41.7 | 41.7 | 42.1 | 48.9 | 49.0 | 48.1 |
| Protein | 29.9 | 30.8 | 30.8 | 34.1 | 34.0 | 34.3 |
| Lactose | 41.1 | 43.9 | 44.7 | 45.8 | 45.8 | 45.4 |

† GS = sole forage, FW = fermented WCW mixed grass silage (330 g/kg DM fermented WCW:670 g/kg DM grass silage, UW = urea-treated WCW (40 kg/t DM) mixed with grass silage (330 g/kg DM urea-treated WCW:670 g/kg DM grass silage).
‡ GS = sole grass silage, FW = fermented WCW mixed with grass silage (400 g/kg DM fermented WCW:800 g/kg DM grass silage, UW = urea-treated WCW (40 kg/t DM) mixed with grass silage (400 g/kg DM urea-treated WCW:800 g/kg DM grass silage).

However, both forages failed to promote a production response compared with grass silage, with milk yield and composition being similar across all treatments (Hameleers, 1998). This is in agreement with the results of Leaver and Hill (1995) but in contrast to that reported by Phipps *et al.* (1995) who observed an increase in milk protein yield as a result of feeding either fermented or urea-treated WCW compared with sole grass silage diets. This is potentially a result of the increased energy intakes associated with the inclusion of urea-treated WCW (243 MJ/day) compared to fermented WCW (229 MJ/day) coupled with the increases in N intakes (625 g/day *vs.* 528 g/day), as it has been shown that both energy and protein intake have effects on milk protein content (Oldham and Sutton, 1979).

The lack of a response in milk yield as a result of feeding fermented WCW was attributed by Phipps *et al.* (1995) to be a result of the low ME of the forage (9.9 MJ/kg DM) compared to that of the grass silage (10.9 MJ/kg DM). It was concluded by Phipps *et al.* (1995) that it was unlikely that an improvement in milk production would be obtained when fermented WCW was included into diet based on good quality grass silage.

## 1.4. Digestion of urea-treated WCW in the rumen

Whole-crop wheat as a forage is different to traditional forms of silage (e.g. grass silage) as it contains both grains and straw, both of which in the case of urea-treated WCW are mature and not readily digested (Abdalla *et al.*, 1999). The application of urea in its self also has effects on the rumen environment as it increases the non protein nitrogen supply to the rumen which is readily available (Abdalla *et al.*, 1999). Increasing levels of starch that is associated with an increase in crop maturity, have been shown to result in a decrease in rumen pH (Ørskov, 1976) with potential negative effects on rumen fibre digestion (Ørskov, 1982).

The effect of inclusion of WCW treated with both 20 and 40 kg urea/t DM on rumen metabolites was investigated by Abdalla *et al.* (1999). Total volatile fatty acid (VFA) concentrations were unaffected by treatment, but acetic acid increased with increasing WCW inclusion, with propionic acid decreasing (Table 1.19). This would not be expected to occur when coupled with the increased amount of starch being digested in the rumen (Table 1.19), as propionate would be expected to increase (Overton *et al.*, 1995). However, Sutton *et al.* (2001) reported no effect in rumen VFA concentrations in animals fed WCW diets, despite varying starch digestibilities.

Table 1.19 Effect of WCW inclusion on daily mean total VFA (mmol/l) and molar proportions of individual VFA in the rumen (mmol/mmol) (Abdalla *et al.*, 1999)

| | Treatments † | | | | | Significance | |
|---|---|---|---|---|---|---|---|
| | GS | 2:1 40 | 1:2 20 | 1:2 40 | s.e. | Treat | Linear |
| Total VFA concentration | 107 | 113 | 110 | 111 | 4.0 | | |
| Molar proportions | | | | | | | |
| Acetic acid (Ac) | 638 | 647 | 657 | 659 | 3.0 | ** | ** |
| Propionic acid (Pr) | 223 | 205 | 193 | 197 | 2.9 | * | ** |
| Iso-butyric acid | 8 | 8 | 7 | 7 | 0.1 | ** | ** |
| n-butyric acid (Bu) | 102 | 113 | 119 | 113 | 3.4 | | |
| (Ac + Bu)/Pr | 3.32 | 3.73 | 4.02 | 93.93 | 0.072 | ** | ** |

† GS = grass silage, 2:14= 2:1 GS to WCW (DM basis) treated with 40 kg urea/t DM, 1:2 20 = 1:2 GS: WCW (DM basis) treated with 20 kg urea/t DM, 1:2 40 = 1:2 GS to WCW (DM basis) treated with 40 kg urea/t DM.

Rumen pH was also observed to take longer to recover post feeding with increasing WCW inclusion, potentially a result of the increased amounts of starch digested in the rumen, resulting in a drop in pH (Ørskov, 1976). Peak rumen ammonia concentrations were observed to be highest in animals receiving 1:2 40 WCW (grass silage:WCW treated with 40 kg urea/t DM) and lowest in animals receiving grass silage. It was suggested by Abdalla *et al.* (1999) that this was likely to be a reflection of the lower organic matter digestibility observed (Table 1.20), resulting in a lower capacity of the rumen microorganisms to utilize the non-protein nitrogen supplied by the diet.

Abdalla *et al.* (1999) reported that the amount of DM apparently digested within the rumen was lower in diets containing WCW. This was attributed to the higher DM flow at the duodenum observed for WCW diets. Digestibility of NDF and ADF in the rumen decreased when WCW was included into diets based on grass silage but, as the rate of WCW inclusion increased, no significant effect was observed on either NDF or ADF digestibility. Starch flow to the duodenum (kg/day) was observed to increase with increasing WCW inclusion, with the amount being digested in the rumen decreasing with increasing inclusion rate (Table 1.20).

The primary reason behind the increase in DM outflow and decrease in digestibility was ascribed to the passage of whole grains to the duodenum (Abdalla *et al.,* 1999). However, N flow to the duodenum was also observed to increase with increasing WCW inclusion, with amounts of N being lost in the rumen decreasing with increasing WCW inclusion. It was concluded by Abdalla *et al.* (1999) that the increase in forage intake associated with WCW, in conjunction with the decrease in rumen dry matter digestibility, suggests that the inclusion of urea-treated WCW within a ration increases the rate of passage of undigested food from the rumen.

Table 1.20 Effect of the inclusion of urea-treated WCW on the apparent digestibility (kg/kg) in the rumen of dry matter, organic matter and starch (Abdalla *et al.*, 1999 )

| | Treatments † | | | | |
|---|---|---|---|---|---|
| | GS | 2:1 40 | 1 :2 20 | 1:20 40 | s.e. |
| DM | 0.374 | 0.264 | 0.248 | 0.227 | 0.0297 |
| OM | 0.494 | 0.372 | 0.345 | 0.321 | 0.0308 |
| NDF | 0.639 | 0.542 | 0.533 | 0.515 | 0.0181 |
| ADF | 0.662 | 0.567 | 0.563 | 0.561 | 0.0145 |
| Total N | -0.019 | -0.099 | -0.097 | -0.126 | 0.0387 |
| Starch | 0.706 | 0.431 | 0.502 | 0.426 | 0.0914 |

† GS = grass silage, 2:14= 2:1 GS to WCW (DM basis) treated with 40 kg urea/t DM, 1:2 20 = 1:2 GS: WCW (DM basis) treated with 20 kg urea/t DM, 1:2 40 = 1:2 GS to WCW (DM basis) treated with 40 kg urea/t DM.

## 1.5. Whole tract digestibility of WCW.

The digestibility of feed depends on a number of factors including the feed intake of the animal (Forbes, 1986). It has been shown that as feed intake increases, there is an increase in rumen outflow rate (Colucci *et al.*, 1982) and a corresponding decrease in diet digestibility (Tyrrell and Moe, 1975). Hence when investigating the digestibility of a feed, the type of animal (i.e. ovine or bovine) as well as feeding rate need to be considered as both have an effect on digestibility. The composition of the total diet can also affect digestibility. Diets high in readily fermentable carbohydrate have a lower fibre digestibility (Chamberlain and Choung, 1995). This is attributable to the effect of the rapid breakdown of carbohydrate promoting a depression in rumen pH which has a negative effect on the metabolism of fibre digesting microorganisms within the rumen (Ørskov, 1982). In forages, stage of growth also has an effect with the digestibility of fibre decreasing with increasing crop maturity (Crovetto *et al.*, 1998) owing to the increasing levels of lignified material present in the forage (Tamminga and Van Vuuren, 1988).

### 1.5.1. Digestibility of fermented WCW.

Wheat as a cereal crop undergoes various chemical changes as it matures (Crovetto *et al.*, 1998). It is therefore logical to assume that the stage of maturity of a wheat crop intended for WCW production is likely to affect the nutritive value of the subsequent forage.

*1.5.1.1. Effects of stage of maturity on the digestibility of fermented WCW.*

Lignin is indigestible (Beever, 1993), and hence material which contains a high amount of lignified cell walls such as straw, (Lindberg *et al.*, 1984) has a lower digestibility than less lignfied material such as grass, (Chesson and Murison, 1989). A reduction in DM digestibility attributable to an increase in stage of maturity was reported by Sutton *et al.* (2002). The digestibility of DM was observed to be significantly lower in fermented WCW harvested at the medium dough growth stage than that harvested at the early soft dough stage (Table 1.21). A decrease was also observed in NDF digestibility, a finding consistent with those of Crovetto *et al.* (1998) who also reported a reduced OM digestibility when crop stage of maturity was increased (Table 1.22). The decrease in NDF digestibility observed with increasing crop maturity has been attributed by Tamminga and Van Vuren (1988) as reflecting the increasing lignification of the crop. This increase in the level of lignification was attributed by Cherney and Marten (1982) to be a major factor in the decrease in digestibility observed with increasing crop maturity and would certainly explain the results obtained by Crovetto *et al.* (1998) and Sutton *et al.* (2002).

Table 1.21 Effect of stage of maturity of fermented WCW on diet digestibility (kg/kg) in dairy cows (Sutton *et al.*, 2002)

| | Low DM† | High DM |
|---|---|---|
| DM | $0.680^{a}$ | $0.657^{c}$ |
| OM | $0.702^{a}$ | $0.679^{b}$ |
| NDF | $0.568^{a}$ | $0.516^{c}$ |
| Starch | $0.964^{a}$ | $0.966^{a}$ |

Means with different superscripts differ significantly at $P < 0.05$.
† Low DM = fermented WCW harvested at the early soft dough stage, High DM = fermented WCW harvested at the medium dough stage

Table 1.22 Effect of stage of maturity on the digestibility (%) of fermented WCW when fed to sheep

| | Crovetto *et al.* (1998) | | | | |
|---|---|---|---|---|---|
| Growth stage | 40-49 | 64-65 | 70-79 | 80-89 | S.E.M. |
| DM | 73.4 | 67.1 | 59.2 | 59.6 | 0.76 |
| OM | 75.6 | 68.9 | 61.4 | 62.0 | 0.68 |
| NDF | 71.8 | 65.5 | 52.9 | 34.5 | 1.68 |
| ADF | 72.6 | 67.7 | 53.0 | 34.5 | 1.35 |
| Crude Fibre | 75.9 | 68.5 | 52.5 | 29.9 | 3.77 |

### 1.5.2. Digestibility of urea-treated WCW.

It has been well established that the application of alkali to forages and/or cereals results in an increase in digestibility (Tetlow and Mason, 1987). The action of ammonia was suggested by Horn *et al.* (1989) to act on the plant cell walls, facilitating the access of bacteria to the polysaccharides contained within, increasing DM digestibility (Fondevila *et al.*, 1994; Al-Masri and Guenther, 1999) and hence DM intake (Fondevila *et al.*, 1994). Mason *et al.* (1988), using ammonia treated straw (anhydrous ammonia applied at 35 kg $NH_3$/t straw DM), reported that between 8–9 % of the cell walls of straw were solubilised through ammonia treatment with forage cell wall content decreasing from 854 to 786 g/kg DM. There was a corresponding increase of 91 g/kg in the digestibility of forage OM (determined *in vitro*). The findings of Mason *et al.*, (1988) are in agreement with Deschard *et al.* (1988) (Table 1.18) who reported that the digestibility of OM, cellulose and organic matter digestibility (OMD, (*in vitro*)) was observed to be higher for urea-treated forage than forage receiving no additive when the forages were fed to steers.

Table 1.23 Apparent digestibility (g/kg) of WCW receiving either no additive or urea fed ad-libitum to steers (Deschard *et al.*, 1988).

| | Treatment | |
|---|---|---|
| | Control | 55.6 kg urea/t DM |
| Organic matter | 647 | 663 |
| Cellulose | 462 | 660 |
| OMD (*in vitro*) | 594 | 678 |

When roughage which is low in nitrogen (such as straw), is made more digestible through the application of alkali based treatments, the nitrogen requirements of the rumen microorganisms increase (Ørskov, 1979). In the case of urea-treated WCW this is provided through the ammonia absorbed by the forage (Ørskov, 1983).

*1.5.2.1. Effects of level of urea application on the digestibility of WCW.*

The effects of applying either 20 or 40 kg urea/t DM to WCW have been determined by Sutton *et al.* (1998) and Leaver and Hill (1995) and are summarised in Table 1.24. Digestibility of DM and OM increased with increasing urea application ($P < 0.05$), and non significant increases were observed for NDF, and starch digestibility (Sutton *et al.*, 1998).

An increase in digestibility as a result of increased urea application rates was also observed by Leaver and Hill (1995). When WCW was fed to heifers, digestibility of all measured components, with the exception of starch, were observed to increase with increasing levels of urea application (Leaver and Hill, 1995).

Table 1.24 Effect of level of urea application on diet digestibility (kg/kg)

| | Sutton *et al.* (2002) | | Leaver and Hill (1995) | |
|---|---|---|---|---|
| | 20 kg urea/t DM | 40 kg urea/t DM | 20 kg urea/t DM | 40 kg urea/t DM |
| DM | 0.627 | 0.645 | | |
| OM | 0.639 | 0.659 | 0.655 | 0.661 |
| NDF | 0.579 | 0.591 | 0.638 | 0.643 |
| ADF | 0.573 | 0.554 | 0.516 | 0.520 |
| Starch | 0.805 | 0.900 | 0.898 | 0.888 |

(Sutton *et al.*, 1998) WCW mixed 1:2 DM basis with grass silage
(Leaver and Hill, 1995) WCW was mixed 33:67 oven DM basis with grass silage

*1.5.2.2. Effects of inclusion rate of WCW on digestibility.*

A number of authors have examined the effects of rate of inclusion of WCW on diet digestibility (Table 1.25). No significant difference was observed by Sutton *et al.* (1997) on NDF or ADF digestibility as a result of increasing WCW inclusion (Table 1.25), but there was a decrease in DM and OM digestibility, as well as starch. Significant reductions

in digestibility of DM, OM, NDF, ADF and starch as a result of increasing WCW inclusion have also been observed by others (Sutton *et al.*, 1998; Abdalla *et al.*, 1999).

The decrease in DM digestibility as a result of increasing WCW inclusion is a reflection of the higher lignification of the wheat crop compared to that of the grass silage which the WCW is replacing, resulting in a decrease in the digestibility of the total diet. This lignification also affects OM digestibility and fibre digestibility.

The lower starch digestibility associated with increasing inclusion rate is a result of the increasing amount of starch loss in the faeces observed (0.22 kg/day on the 2:1 mixtures and 0.39 kg/day on the 1:2 mixtures). This is likely to be as a result of increasing amounts of grain passing through the digestive tract undigested, as observed by Abdalla *et al.* (1999).

Table 1.25 Effect of increasing WCW inclusion on whole tract digestibility (kg/kg)

| | Sutton *et al.* (1997) | | | Abdalla *et al.* (1999) | | Sutton *et al.* (1998) | |
|---|---|---|---|---|---|---|---|
| | 2:1 40 | 1:2 40 | WCW-40 | 2:1 40 | 1:2 40 | 2:1 40 | 1:2 40 |
| DM | 0.67 | 0.64 | 0.61 | | | 0.67 | 0.65 |
| OM | 0.70 | 0.66 | 0.63 | 0.96 | 0.66 | 0.69 | 0.66 |
| NDF | 0.62 | 0.59 | 0.64 | 0.61 | 0.59 | 0.61 | 0.59 |
| ADF | 0.60 | 0.54 | 0.64 | 0.59 | 0.56 | 0.59 | 0.55 |
| Starch | 0.90 | 0.87 | 0.75 | 0.91 | 0.90 | 0.91 | 0.90 |

2:1 40 = grass silage mixed 2:1 with WCW treated with 40 kg urea/t DM.
1:2 40 = grass silage mixed 1:2 (DM basis) with WCW treated with 40 kg urea/t DM.
WCW-40 = WCW treated with 40 kg urea/t DM fed as sole forage.

Leaver and Hill (1992) reported that ME intakes and requirements were imbalanced such that there was 49 to 84 MJ ME/day which were unaccounted for. It was suggested by Leaver and Hill (1992) that there was a poor efficiency of ME utilisation within WCW diets, which was potentially the result of the energy required by the animal to excrete the extra ammonia ingested as a result of the urea treatment of the forage. In a subsequent experiment (Leaver and Hill, 1995) a reduction in the levels of unaccountable energy

(compared to that reported in the first experiment) was observed and was attributed by the authors to be due to the higher inclusion rates in the earlier experiment. Other workers have calculated the energy balance for urea-treated WCW diets for lactating dairy cows (Sutton *et al.*, 1998) and this is summarised in Table 1.26.

Table 1.26 Effect of urea-treated WCW on the energy balance (MJ/d) of dairy cows (Sutton *et al.*, 1998)

| | Treatments† | | | | | |
|---|---|---|---|---|---|---|
| | GS | 2:1 40 | 1:2 20 | 1:2 40 | s.e. | Significance |
| Intake | 300 | 331 | 339 | 329 | 6.3 | * |
| Faeces | 88 | 110 | 128 | 120 | 1.9 | *** |
| Methane | 21 | 23 | 23 | 23 | 0.4 | |
| Urine | 10 | 10 | 9 | 9 | 0.4 | |
| Milk | 76 | 81 | 81 | 82 | 1.4 | |
| Heat | 111 | 117 | 110 | 110 | 2.8 | |
| Retained | -6 | -11 | -12 | -15 | 5.4 | |
| Digestible energy | 212 | 220 | 211 | 209 | 5.0 | |
| Metabolisable energy | 181 | 187 | 179 | 177 | 4.7 | |

† GS= grass silage, 2:1 GS:WCW-2 = 2:1 ratio GS:WCW (DM basis) treated with 20 kg urea/t DM, 1:2 GS:WCW-2 =1:2 GS:WCW (DM basis) treated with 20 kg urea/t DM, 1:2 GS:WCW-4 = 1:2 GS:WCW (DM basis ) treated with 40 kg urea/t DM.

The results obtained indicated that there was no decrease in efficiency of ME utilisation in animals fed urea-treated WCW diets as methane production, heat and urine losses were similar across treatments. This contradicts the suggestion by Hill and Leaver (1999b) that the major reason behind the lack of a milk production response was a result of the decreased efficiency of urea-treated WCW diets. Sutton *et al.* (1998) concluded that future work should concentrate on improving the digestibility of the starch component of urea-treated WCW.

*1.5.2.3. Effects of species on digestibility*

It has been observed that the digestibility when measured in sheep differs to that when measured in cattle. In an experiment evaluating unprocessed whole-crop wheat Sutton *et al.* (2002) observed a number of differences between digestibility values obtained through dairy cows and those recorded for sheep (Table 1.27).

Table 1.27 Effect of animal species on the digestibility (kg/kg) of WCW harvested at differing stage of maturity and preserved with different additives (Sutton *et al.*, 2002).

| | Low DM WCW | | High DM WCW | | High DM/inoculant treated WCW | | Urea-treated WCW | |
|---|---|---|---|---|---|---|---|---|
| | Cows | Sheep | Cows | Sheep | Cows | Sheep | Cows | Sheep |
| DM | 0.680 | 0.689 | 0.657 | 0.670 | 0.666 | 0.660 | 0.676 | 0.713 |
| OM | 0.702 | 0.706 | 0.679 | 0.690 | 0.688 | 0.679 | 0.695 | 0.737 |
| NDF | 0.568 | 0.661 | 0.516 | 0.664 | 0.533 | 0.653 | 0.597 | 0.768 |
| Starch | 0.964 | 0.980 | 0.966 | 1.000 | 0.967 | 1.000 | 0.926 | 1.000 |

Digestibility of all measured components was higher in sheep than lactating cattle. Part of this difference could be attributed to the intake of the animals affecting rumen outflow rates with rumen outflow rates for lactating cattle of 0.08/h and sheep fed at maintenance is 0.02/h. (Agricultural and Food Research Council (AFRC), 1995). It has been established that intake has an effect on rumen outflow rates and that outflow increases with increased intakes (Colucci *et al.*, 1982). The longer rumen retention time associated with sheep fed at maintenance may be a cause for the increased digestibility values recorded. Indeed Colucci *et al.* (1982) noted that longer rumen retention times would allow for a longer rumen digestion and more time for mastication and rumination.

The digestibility of the starch component of urea-treated WCW when recorded in lactating dairy cattle by Sutton *et al.* (2002) was 0.926 kg/kg, whereas when fed to sheep, the digestibility of the starch increased to 1.000 kg/kg. This could be due to the physiological differences between the two species as it has been reported that the recticulo-omasal orifice which digesta must pass to enter the lower gut is smaller in sheep than cattle (Ørskov, 1979), with the result that whole grains can not easily pass through into the lower ovine digestive tract (Ørskov, 1979). The whole grain is then regurgitated and cracked open during rumination (Ørskov, 1979).

## 1.6. Methods of improving the digestibility of urea-treated WCW.

The lack of a production response when WCW is incorporated into a diet has been attributed to the low digestibility of the forage, particularly that of the starch fraction (Sutton *et al.*, 1995). Improving the digestibility of WCW could potentially be achieved by a number of means including the supplementation of the diet to achieve an improved diet utilisation (Sutton *et al.*, 2001) or by chemical treatment (e.g. NaOH, Tetlow and Mason, 1987) or through physical processing of the forage to alter its digestibility (Campling, 1991),

### 1.6.1. Supplementation of WCW diets.

A number of authors (Hill and Leaver, 1990; Castlejon and Leaver, 1994; Sutton *et al.*, 2001) have investigated different ways/methods of supplementing WCW diets to improve diet digestibility and animal performance. Supplementation may take the form of providing additional protein (Oldham, 1984), or additional energy sources (Obara *et al.*, 1991; Murphy, 1999; Sutton *et al.*, 2001).

#### *1.6.1.1. Protein*

Dry matter digestibility was found to increase, on average by 0.01 units for every unit change in ration CP content for rations containing at least 16 % CP (Oldham, 1980). Increasing the level of dietary protein has also been observed to result in increased DM intakes as a result of improved DM digestibility (Oldham and Smith, 1982). Crude protein supplementation may also have additional benefits as it has been shown that at high levels of feed intake, ration CP needs to be higher to meet rumen requirements for nitrogen due to decreasing protein degradation (Oldham and Smith, 1982) as a result of the increase in rumen outflow rate (Crampton *et al.*, 1980).

Milk yield was observed to be similar (21.3 *vs.* 21.9 kg/day) when urea-treated WCW diets were supplemented with 6 kg/day of 208 g CP/kg DM concentrate *vs.* 6 kg/day 276 g CP/kg DM concentrate (Hill and Leaver, 1999b). An increase was however, observed in milk protein content from 31.8 to 32.4 g/kg ($P$ <0.01) when additional CP was included into WCW diets (Hill and Leaver, 1999b). The effects of type of protein supplement on performance and digestibility have also been determined (Table 1.28). Supplementation of urea-treated WCW with additional protein in the form of fishmeal resulted in a significantly higher milk yield than that achieved through supplementation in the form of a combination of soya-bean meal, molassed sugar beet pulp and maize gluten (Hill and Leaver, 1999b). No difference was observed on milk yield or composition as a result of increasing the level of protein supplement from 4 kg/day of low protein concentrate to 4 kg/day of high protein concentrate (based on a mixture of soya-bean meal, molasses sugar beet pulp and maize gluten and not containing fishmeal). However, increasing the level of protein supplementation increased the digestibility of all measured parameters (apart from NDF where supplementation decreased digestibility) with the highest digestibilities being observed for diets supplemented with protein in the form of fishmeal (Hill and Leaver, 1999b).

Table 1.28 Apparent digestibility of urea-treated WCW (g/kg) in dairy cows (Hill and Leaver, 1999b)

| | Treatment † | | | |
|---|---|---|---|---|
| | LP | HP | HP+F | s.e.d. |
| DM | 745 | 767 | 780 | 6.73* |
| OM | 757 | 772 | 790 | 5.62** |
| NDF | 564 | 534 | 538 | 8.51* |
| ADF | 527 | 519 | 557 | 7.66** |
| Starch | 934 | 948 | 966 | 2.01*** |

† LP = 4 kg/day of 166 g CP/ kg DM concentrate, HP = 4 kg/day of 333 g CP/ kg DM concentrate, HP+F = 4 kg/day of 329 g CP/kg DM of concentrate incorporating fishmeal.

It can be concluded from these results that additional protein supply increased diet digestibility but had a small effect on milk production. However, the experiment utilised urea-treated WCW as a sole forage, not mixed with grass silage as is more commonly

reported (Leaver and Hill, 1995; Sutton *et al.,* 1998). As such the effects reported do not reflect common practice, and there may be variations in the responses observed if the effects of protein supplementation to WCW mixed with grass silage were evaluated.

*1.6.1.2. Energy*

It has been suggested that the addition of a sugar source to silage based diets, through the provision of a readily degradable energy source, can improve the capture of ammonia produced as a result of the rumen degradation of the diet by rumen microorganisms (Obara *et al.*, 1991). Indeed Sinclair *et al.* (1993;1995) noted that maximal microbial fixation of N occurred when the production of ammonia from the breakdown of dietary N sources was synchronised with the energy release from dietary carbohydrates.

Sucrose supplementation of grass silage diets has been shown to result in a decreased rumen ammonia levels (7.07 *vs.* 12.57 mmol/l for sucrose supplemented diets compared to control diets) (Khalili and Huhtanen, 1991). Sugar supplementation was also shown to increase microbial N synthesis (Rooke *et al.,* 1987). Indeed it has been observed that sucrose supplements to grass silage improves N balance when compared to starch or cellulose supplementation (2.49 g/day *vs.* 2.03 g/day and 1.56 g/day for silage diets supplemented with 15 % sucrose, 15 % starch and 15 % cellulose respectively (Syrjala, 1972).

With specific reference to urea-treated WCW, it was noted by Abdalla *et al.* (1999) that there was potential to improve microbial protein synthesis through the supplementation of diets with readily available carbohydrates. Urea-treated WCW contains a high amount of ammonia nitrogen (17-20 % ammonium nitrogen as a percentage of total nitrogen) which is rapidly released within the rumen giving rise to increased levels of ammonia (Anderson, 1967). Sugar sources such as molasses are rapidly fermented in the rumen (Czerkawski and

Breckenridge, 1969) and provide the rumen microorganisms with a rapidly available supply of energy so that they may capture the ammonia produced as a result of urea breakdown in the rumen (Murphy, 1999).

Molasses is a good source of rumen fermentable energy (Murphy, 1999) and contains approximately 50 % soluble sugar (sucrose, fructose and glucose) (Karalazos and Swan, 1976). It is produced as a by-product of the production of sugar (Karalazos and Swan, 1976). The majority of the carbohydrates contained within molasses are rapidly fermented in the rumen, thus supplying energy to the rumen microorganisms (Murphy, 1999). This leads to a more effective utilisation of the rapidly available nitrogen contained within silages, resulting in increased microbial protein synthesis (Sinclair *et al.,* 1993; Richardson *et al.,* 2003). As microbial protein is a good source of amino acids for milk synthesis, milk protein levels in molasses supplemented diets may be expected to increase. Significant increases in milk yield have been achieved through the addition of molasses to dairy cow diets. Murphy (1999) observed a significant increase in milk yield, protein yield and concentrations and total DMI with increasing concentration of molasses in grass silage based diets (Table 1.29) although lower milk fat levels were observed for animals receiving molasses (Murphy, 1999).

Table 1.29 Effect of the inclusion of molasses into grass silage based diets on the performance of dairy cows (Murphy, 1999)

| | Treatment† | | | | | |
|---|---|---|---|---|---|---|
| | 0M | 50M | 100M | 150M | SED | Effect |
| Milk yield (kg/day) | 22.1 | 23.2 | 23.3 | 23.7 | 0.27 | IL (P <0.001) |
| Fat yield (g/day) | 834 | 860 | 859 | 846 | 19 | |
| Protein (g/day) yield | 668 | 712 | 715 | 739 | 8 | IL (P <0.001) |
| Total DMI (kg/day) | 14.8 | 16.6 | 17.4 | 18.2 | 0.34 | IL (P <0.001) |

† 0M = 0g molasses/1000g grass silage, 50M = 50g molasses/950g grass silage 100M = 100g molasses/900g grass silage, 150M= 150g molasses/850g grass silage, I = dietary inclusion of molasses (0M *v.*. mean of 50M, 100M and 150 M. L = linear effect of molasses inclusion.

The inclusion of cane molasses into grass silage based diets has been shown to significantly increase ($P$ <0.05) the digestibility of DM and OM (Table 1.30; Givens *et al.*, 1992). An improvement in nitrogen balance was also observed, attributed to a reduction in rumen ammonia losses (Givens *et al.*, 1992).

Table 1.30 Effect of the inclusion of molasses into grass silage based rations on diet digestibility measured in sheep (Givens *et al.*, 1992)

| | Molasses inclusion rate (g/kg TDM†) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 38.5 | 75.9 | 105.1 | 151.2 | SED |
| Digestibility (kg/kg) | | | | | | |
| DM | 0.81 | 0.80 | 0.82 | 0.81 | 0.83 | 0.011 |
| OM | 0.83 | 0.82 | 0.83 | 0.83 | 0.85 | 0.010 |
| NDF | 0.86 | 0.84 | 0.85 | 0.86 | 0.87 | - |
| Nitrogen | | | | | | |
| N Loss as proportion in intake | | | | | | |
| Faeces | 0.23 | 0.23 | 0.22 | 0.22 | 0.23 | |
| Urine | 0.84 | 0.78 | 0.74 | 0.63 | 0.64 | |
| N Balance (g N/day) | -1.49 | -0.39 | 0.94 | 3.39 | 2.25 | |
| Nitrogen retention g kg N intake | -68.1 | -17.5 | 40.5 | 144.7 | 100.5 | |

† Toluene DM

Decreased rumen ammonia levels have been reported in steers fed diets supplemented with molasses compared to those supplemented with barley (5.63 mg dl-1 *vs.* 9.26 mg dl-1 for steers receiving diets supplemented with molasses and barley respectively, $P < 0.001$; (Moloney *et al.*, 1994)). As a result of the decrease rumen ammonia levels, blood urea levels were also found to decrease (4.24 mmol l-1 *vs.* 5.56 mmol l-1 for steers receiving diets supplemented with molasses and barley respectively (Moloney *et al.*, 1994)).

The effect of feeding molasses mixed with the forage or concentrates (at a rate of 2 kg/day) and the effect of caustic treatment on WCW are presented in Table 1.31. The addition of molasses to the diet resulted in a reduction in NDF digestibility when compared to the control forage. All forms of supplementation reduced starch digestibility (Sutton *et al.*, 2001).

Table 1.31 Effect of supplementation of WCW diets on the digestibility (kg/kg) of WCW (Sutton *et al.*, 2001)

| | Control | Caustic | Molasses/ forage | Molasses/ concentrates | s.e. | Significance |
|---|---|---|---|---|---|---|
| DM | 0.673 | 0.680 | 0.665 | 0.667 | 0.0064 | |
| OM | 0.701 | 0.701 | 0.687 | 0.690 | 0.0058 | |
| NDF | 0.593 | 0.628 | 0.564 | 0.557 | 0.0085 | ** |
| Starch | 0.909 | 0.862 | 0.880 | 0.899 | 0.0039 | *** |

It was observed that the mean daily ammonia level in the rumen remained unaffected by molasses supplementation, possibly explaining the lack of a production response (Sutton *et al.*, 2001). Supplementation with molasses resulted in a lower milk protein content than the control treatment, an effect that was attributed by Sutton *et al.* (2001) to be a result of a reduction in starch intake, as a direct consequence of increasing sugar intake. Molasses supplementation of WCW treated with 40 kg urea/t DM resulted in no effect on milk yield or milk protein yield (Table 1.32).

Table 1.32 Effect of supplementation of WCW with molasses or caustic soda on milk production (Sutton *et al.*, 2001).

| | Control | Caustic | Molasses/ forage | Molasses/ concentrates | s.e. | Significance |
|---|---|---|---|---|---|---|
| Experiment 2 | | | | | | |
| Milk yield (kg/day) | 26.3 | 27.6 | 26.5 | 27.6 | 0.39 | |
| Milk fat (g/kg) | 41.6 | 42.4 | 43.7 | 41.9 | 0.60 | |
| Milk Protein (g/kg) | 31.6 | 31.7 | 30.8 | 30.2 | 0.38 | |
| Fat yield (g/day) | 1093 | 1164 | 1161 | 1155 | 13.3 | * |
| Protein yield (g/day) | 828 | 867 | 815 | 828 | 12.3 | |
| Experiment 3 | | | | | | |
| Milk yield (kg/day) | 24.3 | 25.6 | 25.4 | 24.9 | 0.32 | |
| Milk fat (g/kg) | 48.3 | 46.5 | 46.9 | 46.3 | 0.64 | |
| Milk Protein (g/kg) | 34.5 | 34.4 | 33.3 | 33.6 | 0.21 | * |
| Fat yield (g/day) | 1165 | 1189 | 1188 | 1141 | 27.4 | |
| Protein yield (g/day) | 832 | 879 | 843 | 827 | 11.4 | |

In contrast, in an experiment investigating the effects of molassed sugar beet pulp (MSBP) supplementation to WCW treated with 40.6 kg urea/t DM, an increase in the *in vivo* digestibility of WCW when supplemented with 1.8 kg of MSBP/day was observed (Castlejon and Leaver, 1994). There were also increases in NDF, ADF and starch

digestibility (Table 1.33). However, the study had additional supplementation in the form of the increased fibre that the sugar beet pulp provided (356 g/kg DM). It has been suggested that by-product feeds which are high in NDF may be a useful tool to help regulate low rumen pH (Younker *et al.*, 1998), as can be seen when diets contain a large amount of readily available carbohydrate (Durand, 1989). The changes in digestibility seen may, therefore be a reflection of the MSBP potential ability to maintain rumen pH and hence increase fibre digestibility rather than an effect of molasses.

Table 1.33 The *in vivo* digestibility (g/kg) of WCW and of the total diet by dairy heifers (Castlejon and Leaver, 1994).

| | Treatment | | | | |
|---|---|---|---|---|---|
| | W | WB | WBF | s.e.d. | Significance |
| WCW | | | | | |
| DOMD | 654 | 651 | 640 | 5.3 | * |
| NDF | 609 | 682 | 673 | 22.0 | ** |
| ADF | 561 | 592 | 622 | 15.1 | ** |
| Starch | 852 | 856 | 829 | 7.0 | ** |
| Total diet | | | | | |
| DOMD | 654 | 675 | 665 | 6.2 | * |

W = no supplement, WB = supplement of 2.0 kg/day molassed sugar beet pulp, WBF = supplement of 1.8 kg/day molassed sugar beet pulp plus 0.2 kg/day fishmeal.

Lactose is a by-product from the production of skimmed milk powder and consists of glucose and galactose (McDonald *et al.*, 1995). Lactose was reported by Hussain and Miller (1999) to reduce protozoal numbers in the rumen, thus increasing bacterial numbers and, potentially increasing microbial protein yield. Similarly, Chamberlain *et al.* (1993) reported that feeding lactose resulted in a greater yield of microbial protein than cereal starch (89 g/day as a result of feeding lactose compared to 74 g/day when maize starch was fed). It has also been reported that feeding lactose results in a decrease in propionic acid from 241 mmol/mol, when the basal diet was supplemented with maize starch to 225 mmol/mol, when lactose was the supplement source (Chamberlain *et al.*, 1993). This effect has the potential to decrease insulin production and hence may have effects on the partitioning of nutrients (Bines and Hart, 1986). The inclusion of lactose (fed in

conjunction with a protected fat source) into dairy cow diets also led to a significant increase in forage DM intake from 20.5 kg DM/day to 21.8 kg DM/day (Allison and Garnsworthy, 1997).

### 1.6.2. Effects of processing grains on their nutritional value.

It has been demonstrated that whole cereal grains incubated within the rumen for several days are not digested, and it was assumed by Ørskov *et al.* (1978) that the grains needed to be cracked/damaged to allow the rumen microorganisms access. Indeed it was suggested by Sutton *et al.* (1997) that future work should focus on ways to improve the low digestibility of the grain component of WCW. Therefore it could be desirable to incorporate a form of processing when feeding mature, urea-treated WCW to cattle.

Grain processing can affect the rate and extent of digestion, digestibility and voluntary feed intake of forage (Campling, 1991). There are two types of grain processing; physical and chemical. Physical processing breaks the exterior tissues of the grain, thus allowing the access of rumen microorganisms and digestive enzymes (Campling, 1991). Chemical processing of crops through treatment with alkalis (sodium hydroxide or ammonia), has a similar effect to that of physical processing, namely facilitating easier access for the rumen microorganisms and digestive enzymes (Campling, 1991).

#### *1.6.2.1. Chemical processing*

Chemical processing has long been used as a means to improve the digestibility of grain. As mentioned earlier, Ørskov *et al.* (1978), stated that a degree of processing of grain was necessary to ensure efficient use of grains by cattle due to anatomical differences between themselves and ovines. The addition of NaOH to grain has been shown to increase the digestibility of the grains (see section 1.2.3.). It has also been applied with success to

WCW and has enhanced the digestibility of this forage (see section 1.2.3.2.). However, its nature as an additive means that it is unpleasant to handle and so other additives have been sought. Urea has been used also successfully to improve digestibility (see Tables 1.20, 1.23, and 1.24).

*1.6.2.2. Physical processing*

The physical processing of grain has been shown by Theurer (1986) to result in an increase in starch digestion in cattle. This improvement was hypothesised to be a result of increased ruminal fermentation of processed starch, and an increased digestion of starch in the small intestine (Theurer, 1986) and it was also suggested that processing enhanced energy and nitrogen efficiency through the minimization of starch fermentation and microbial synthesis in the hind gut (Ørskov *et al.*, 1970).

Overton *et al.* (1995) concluded that an increase in starch digestion would usually lead to an increase in the proportion of propionic acid produced in the rumen. It has been found that the fermentation of starch by bacteria produces a higher proportion of propionic acid (35 to 45 mol/100 mol VFA) than bacterial fermentation of cellulose or hemicellulose (15 to 20 mol/100 mol (Ørskov, 1986)). This idea is supported by Dhiman *et al.* (2000) who observed an increase in rumen propionate concentrations in dairy cows when processed maize silage was fed. Propionic acid formed as a result of the digestion of grain diets may also cause digestive problems in lactating animals (Ørskov, 1986). An increased level of propionate production results in an increase in insulin production (Ørskov, 1986). While insulin may not, as suggested by Reynolds *et al.* (1997) be the sole cause behind low milk fat, there is a shift in nutrient partitioning towards body fat deposition, accompanied by a depression in milk fat. However, recently it has been suggested that ruminally produced trans-10, cis-12 conjugated linoleic acid may have a greater influence on milk fat content (Bauman *et al.*, 2001).

Physical processing maize silage at harvest has been observed to reduce the number of intact grains present in the forage (Johnson *et al.*, 2002a) with an average of 13.5 % of total kernels being intact compared to an average of 0.4 % intact kernels for processed material. Processing was observed to increase total tract starch digestibility of maize silage ($P$ <0.01) when compared to unprocessed forage (Johnson *et al.*, 2002b). Dhiman *et al.* (2000) also observed this and reported an increased digestibility of starch recorded in dairy cows fed processed maize silage compared to unprocessed maize silage (87.4 % *vs.* 84.3 % $P$=0.09). Ruminal digestion of maize starch was observed by Johnson *et al.* (2003) to be unaffected by processing but there was a tendency ($P$=0.09) for animals receiving processed maize silage to have a higher postruminal digestion of starch. As not all of the starch contained within a feedstuff is digested in the rumen (Nocek and Tamminga, 1991), it is, therefore, logical that excess starch will flow through into the intestine and will be metabolised in the hind gut (Nocek and Tamminga, 1991), thus potentially explaining the increased postruminal digestion of starch observed by Johnson *et al.* (2003). Processing also had an effect on NDF digestibility with cows fed processed maize silage tending ($P$ <0.10) to have lower total tract digestibilities (Johnson *et al.*, 2003). This could potentially be a result of the increased starch digestibility resulting in a lower rumen pH and hence inhibiting fibre degrading microorganisms (Ørskov, 1982). Indeed it was reported by Johnson *et al.* (2003) that cows fed processed maize silage had a lower rumen pH 2hr post feeding that those fed unprocessed maize silage.

Recently, a processor designed specifically for small grained cereals has been developed, with the objective of cracking the grains at harvest. Although there is anecdotal evidence that cracking, or processing of the grains improves nutrient availability in urea-treated whole-crop cereals, there is little experimental evidence available.

## 1.7 Conclusions

The inclusion of urea-treated WCW in dairy cows diets has been shown to result in increases in DMI with little effect on milk yield and composition. The low digestibility of the starch component of urea-treated WCW has been stated to be a potential cause of the lack of a production response when this forage was incorporated into rations. However all studies to date have investigated the effects of the incorporation of unprocessed urea-treated WCW on intake, digestibility and performance. Therefore the effects, or lack of effect, reported may be a reflection of the unprocessed nature of the forage, rather than a direct result of feeding urea-treated WCW. Anecdotal evidence exists that the processing of WCW at harvest improves digestibility but this has not yet been determined experimentally.

### 1.7.1. Experimental objectives

The objectives of the present study were to determine the effects of processed or unprocessed urea-treated WCW at harvest on the performance and diet digestibility in dairy cows. Secondly, different dietary supplements to processed urea-treated WCW were evaluated as a potential means to increase performance of dairy cows. The effects of alternative means of preservation of high DM WCW were also investigated.

# 2.0. GENERAL MATERIALS AND METHODS

Feed and faecal samples from Experiments 1, 2 and 3a were analysed by NRM Laboratories Ltd, Berkshire UK. Samples from Experiment 3b were analysed at Harper Adams University College using the same methods or modified as stated.

## 2.1.1. Dry matter

Dry matter in Experiments 1, 2 and 3a was determined by drying in a force-draught oven at 90°C until a constant weight was obtained. The grass and maize silage dry matter was then corrected for volatile losses according to the equation:

Corrected DM (CDM) (g/kg) = 0.99 [Oven DM (ODM)] + 18.2 (AFRC, 1993)

Dry matter analysis for Experiment 3b was carried out according to the method of MAFF (1986). The dry matter of sub samples of all feeds was determined by drying a known weight of sample in an oven at 100°C to constant weight. Faecal samples were dried to a constant weight at 60°C. Dry matter content was calculated according to the following formula:

$$\text{Dry matter (g/kg)} = \frac{\text{weight of dry sample (g)}}{\text{weight of original sample (g)}} \times 1000$$

Unless otherwise stated, all subsequent analyses were carried out on dried material, ground to pass through a 0.1 mm sieve using a Cyclotec 1024 mill (FOSS UK, Warrington, UK).

### 2.1.2. Organic matter

A known weight of dried ground sample (approximately 2 g) was weighed into a crucible and ashed overnight at 500$^0$C in a muffle furnace (Gallenkamp Muffle furnace Size 3 GAFSE 620, Gallenkamp, Loughborough, UK). Ashed samples were placed in a desiccator and cooled to room temperature before being re-weighed.

$$\text{Ash (g/kg DM)} = \frac{\text{weight of ash (g)}}{\text{weight of original sample (g)}} \times 1000$$

Organic matter was calculated as 1000-ash (g/kg) (AOAC, 2000)

### 2.1.3. Nitrogen

*2.1.3.1. Total Nitrogen*

Nitrogen was determined by Kjeldahl digestion with an automated Kjeldahl procedure using a Tecator 1035 autoanalyser (Foss UK Ltd, Warrington, UK). The sample was weighed into a Whatman No. 1 filter paper (Whatman plc, Maidstone, UK) and added to a digestion tube, 2 kjeltab catalyst tablets (C + K Thompson and Capper Limited, Runcorn, Cheshire, UK) were added to the tube along with 16 ml of 98% (w/v) low nitrogen, sulphuric acid. The samples were then digested at 400°C on a heating block for 45 minutes. Samples were allowed to cool slightly and then quenched with 75 ml of distilled water. Nitrogen content was then estimated via back titration using HCL (0.2 M) as the titrant and crude protein calculated as N x 6.25.

*2.1.3.2. Ammonia nitrogen*

Ammonium nitrogen for Experiments 1, 2 and 3a was extracted from forages according to the procedures of MAFF (1986) by placing 20 g of fresh forage into a shaker bottle and adding 100 ml of distilled water. The samples were then shaken for 1 hour before being filtered through a 150 mm Whatman No. 1 filter paper (Whatman plc, Maidstone, UK). The ammonium nitrogen content of the sample was then determined using a colorimetric method by reaction with alkaline hypochlorite and phenol to produce indophenol blue (EPA, 1984).

The ammonia nitrogen content of the forages was determined for Experiment 3b according to the procedures of MAFF (1986) by placing approximately 10 g of forage in a glass shaker bottle and adding 100 ml of distilled water. The bottle was then shaken for 1 hour (using a HS501 digital shaker, Kika labourtechnik, Germany). The resultant liquor was then filtered through a Whatman No 1 filter paper (Whatman plc, Maidstone, UK). Exactly 5 ml of the filtrate was then placed in a digestion tube and 6 ml of magnesium oxide (17 g ignited magnesium oxide to 100 ml distilled water) was added. The resulting solution was distilled into a solution of 4 % boric acid and titrated with 0.02M HCL using a Tecator 1030 (Foss UK, Warrington, UK). The nitrogen content of the sample was then calculated as:

$$\text{Ammonium-nitrogen (g/kg DM)} = \frac{7 \times T \times (120-(wt \times DM))}{DM \times vol}$$

Where T = titration reading

DM = dry matter of the sample (g/kg)

wt = grams of silage (kg)

vol = volume of filtrate

### 2.1.4. Forage pH

Approximately 10 g of fresh forage was placed in a glass shaker bottle and 100 ml of distilled water added. The sample was shaken for 1 hour on a shaker (HS501 digital, Kika labourtechnik, Germany). The resultant liquid was filtered through a filter paper (Whatman No. 1, Whatman plc, Maidstone, UK) and the pH of the liquid measured using a pH probe (Russell RL150). The probe was recalibrated every 10 samples using two pH solutions (colour key buffer solution yellow, pH 7 (product number 192403F) and colour key solution red, pH 4 (product number 192393U), VWR International Ltd, Poole, UK).

### 2.1.5. Neutral detergent fibre

Neutral detergent fibre (NDF) was determined according to Van Soest *et al.* (1991). Approximately 0.5 g of ground sample was accurately weighed into a previously dried and weighed crucible (50 ml borosilicate glass, sinter porosity No. 1, Soham Scientific, Cambridge, UK). The crucible was placed into the Fibretec apparatus (Tecator Fibretec 1020 Hot Extractor, Foss UK Ltd, Warrington, UK) and 25 ml of cold neutral detergent fibre (NDF) solution was added (93 g disodium ethylene tetra-acetate dihydrate (EDTA), 34 g di-sodium tetraborate decahydrate, 150 g sodium lauryl sulphate, 50 ml of 2-ethoxyethanol mixed with 22.8 g of anhydrous di-sodium hydrogen phosphate made up to 5 litres and pH adjusted at pH 7) along with 0.5ml of octanol. The samples were boiled for 30 minutes after which the heat was turned off and 2 ml of α-amylase solution (2 g α-amylase E.C. 3.2.1.1 from *Bacillus sybtilis* was dissolved in 90 ml distilled water, filtered and then 10 ml 2-ethoxyethanol added. The resultant solution was stored at 4°C) added with an additional 25 ml of cold NDF solution. The sample was again heated to boiling and digested for a further 30 minutes before being washed and filtered under vacuum 3 times with 20 ml of hot (80°C) de-ionised water and once with of 20 ml acetone. Samples were

removed from the Fibretec apparatus and dried overnight at 100°C, allowed to cool in a desiccator and weighed. Samples were placed in a muffle furnace (Gallenkamp Muffle furnace Size 3 GAFSE 620, Gallenkamp, Loughborough, UK) and ashed at 550°C for 4 hours, cooled in a desiccator and re-weighed. NDF content (g/kg DM) was calculated as:

$$\text{NDF (g/kg DM)} = \frac{\text{residue weight (g)} - \text{ash content (g)}}{\text{sample weight (g DM)}} \times 1000$$

### 2.1.6. Neutral cellulase plus gamanase digestibility

Exactly 0.5 g of dried ground sample was weighed into a crucible (50 ml borosilicate glass, sinter porosity No. 1, (Soham Scientific, Cambridge, UK). Any oil present in the samples was removed by washing with 3 x 25 ml of petroleum spirit. Surplus solvent was removed from the sample using gentle vacuum and neutral detergent solution (50 ml) was added (93 g disodium ethylene tetra-acetate dihydrate (EDTA), 34 g di-sodium tetraborate decahydrate, 150 g sodium dodecyl sulphate, 50 ml of 2-ethoxyethanol mixed with 22.8 g of anhydrous di-sodium hydrogen phosphate made up to 5 litres and pH adjusted at pH 7). The sample was then mixed thoroughly and heated to boiling and maintained under reflux for 30 minutes. At this point 25 ml of cold neutral detergent solution and 2 ml of amylase was added (2 g $\alpha$-amylase E.C. 3.2.1.1 from *Bacillus sybtilis* was dissolved in 90 ml distilled water, filtered and then 10 ml 2-ethoxyethanol added. The resultant solution was stored at 4°C). The sample was then reheated to boiling and maintained under reflux for a further 30 minutes before being filtered again under vacuum and the residue washed three times with 20 ml of hot (80°C) distilled water. When filtration was completed, a suba seal was placed onto the bottom of the crucible and 25 ml of hot distilled water (80°C) added followed by 2 ml of amylase solution. The sample was then mixed and allowed to stand for 15 minutes, the cap removed and 30 ml of buffered cellulase/gamanase solution added

(buffered cellulase solution consisted of 20 g cellulase (VWR International Ltd, Poole, UK) and 0.1 g of chloramephenicol to which 1 litre of buffer solution (1.36 g sodium acetate dissolved in 500 ml distilled water and 0.6 ml of glacial acetic acid, the resultant solution diluted to 1 litre, and adjusted to pH 4.8). The resultant solution was shaken and incubated at $40^0$C for at least 1 hour before use). Buffered cellulase solution and Gammanase (Gammanase Novozyme, Netherlands) were mixed together in the proportion 9:1)). The sample was shaken and incubated at 40°C for 24 hours, shaking twice daily. The cap and supa-seal were then removed and any solid washed back into the filter tube. The enzyme solution was removed by vacuum and undigested fibre washed with hot distilled water, with a final rinse consisting of 20 ml acetone. The resultant residue was dried at 100°C. The sample was cooled in a desiccator and reweighed before being ashed at 550°C for 4 hours. The sample was allowed to cool and re-weighed. The loss in organic matter (indigestible organic matter) was calculated as a percentage of the dry matter of the original 0.5 g of sample. The percentage of total ash and dry matter in separate sub-samples was determined and calculated on a dry matter basis and NCGD determined according to the equation given below. The organic matter in the sample digested by the neutral detergent solution and cellulase plus gammanase is denoted by the abbreviation 'NCGD'.

NCGD (g/kg DM) = 1000 – (indigestible organic matter (g/kg) + total ash (g/kg)).

### 2.1.7. Acid detergent fibre

Acid detergent fibre (ADF) was determined according to the procedures of Goering and Van Soest (1970). Approximately 1 g of dried ground sample was transferred into a previously weighed crucible (borocilicate glass, porosity No. 2, Soham Scientific, Ely, UK). The crucibles were fitted into the Fibretec apparatus (Tecator Fibretec 1020 Hot

Extractor, Foss UK Ltd, Warrington, UK). To each sample 100 ml of ADF reagent (10 g of cetyltrimethylammonium bromide (CTAB) in 1 litre of 0.5 M sulphuric acid) was added. The sample was then boiled for 60 minutes, filtered under gentle vacuum and the residue washed three times with 50 ml of hot (80°C) distilled water and once with acetone (20 ml). The crucibles were removed from the Fibertec apparatus and dried in an oven overnight at 102°C overnight. The crucibles were then allowed to cool in a desiccator and re-weighed. ADF content (g/kg DM) was calculated as:

$$\text{ADF (g/kg DM)} = \frac{\text{residue weight (g)} - \text{ash content (g)}}{\text{sample weight (gDM)}} \times 1000$$

### 2.1.8. Starch

Starch was determined for Experiments 1, 2 and 3a according to the procedures of MAFF (1982). Starch content of the feeds was determined through two separate processes. The sample was treated with warm diluted hydrochloric acid, clarified and filtered and the optical resolution of the sample determined. The sample was then extracted with 40% ethanol and filtered. The resulting filtrate was acidified using hydrochloric acid, clarified and re-filtered and its optical rotation determined.

Starch was determined in Experiment 3b according to the procedure of Rasmussen and Henry (1990). Samples containing free glucose were pre-treated with warm aqueous ethanol according to the procedures of Henry (1985). Approximately 0.05 g of sample was weighed into a culture tube (16 mm x 100 mm) fitted with a screw cap and PTFE-faced rubber seal, and 3 ml of ethanol (AnalaR) was added to the sample and the sample mixed on a vortex mixer (Fisherbrand whirlimixer). The sample was then heated in a water bath at 80°C for 10 minutes. The extract was removed by centrifuging at 2000 *g* for 5 minutes

and pipetting off the supernatant. A further 3 ml of ethanol was then added and the sample re-mixed and centrifuged and the procedure repeated.

After extraction of free glucose, 2.5 ml of acetate buffer (148 ml of 0.2 M acetic acid added to 352 ml of 0.2 M sodium acetate, diluted to 1 litre, adjusted to pH 5.0 with the addition of either sodium hydroxide or hydrochloric acid) was added along with 20 $\mu$l Teramyl ($\alpha$-Amylase EC 3.2.1.1 Sigma Aldrich, A-3403, 5000,000 units/ml). The sample was then incubated for 30 minutes in a boiling water bath and was mixed on a vortex mixer three times during this period. The sample was then removed from the water bath and allowed to cool to room temperature. Exactly 10 $\mu$l of amyloglucosidase (EC 3.2.1.3 from *Aspergillus niger,* Sigma Aldrich, A-9913, 3 times concentration) was added to each sample, and the sample incubated for 16 hours at 60°C. The sample was then centrifuged at 2200 g for 15 minutes and 0.5 ml of the supernatant added to 10 ml of distilled water. From this 0.2 ml of the diluted supernatant was transferred to a small test tube (15 x 85 mm) and 5 ml of glucose oxidase reagent (24.8 g disodium hydrogen orthophosphate (AR grade), 12.4 g sodium dihydrogen orthophosphate (AR grade), 4.0 g benzoic acid (GPR grade, dispersed in a small volume of ethanol), 0.2 g 4-amino-antipyrine (Sigma Aldrich, A-4382) and 3.0 g p-hydroxybenzoic acid (Sigma Aldrich, H-5376) in 1800 ml distilled water in a 2 litre volumetric flask. To this 40 mg glucose oxidase (EC 1.1.3.4 from *Aspergillus niger* (Sigma Aldrich, G2133 Type VII)) and 10 mg peroxidase (EC 1.11.1.7, from horse radish (Sigma Aldrich, P8375 Type VI)) were added and the solution diluted to 2 litres with distilled water and stored at 4°C in a dark bottle) was added to the sample. Triplicate 0.2 ml of glucose standard (100 $\mu$g glucose per ml (prepared from AR glucose after drying under vacuum) was also transferred into three empty tubes for the determination of the glucose standard. The samples, standards, method blank (two empty tubes introduced at the beginning of the test and treated the same as the samples) and glucose blank (three tubes containing 0.2 ml of distilled water, introduced into the procedure after the 16 hour

incubation step) were incubated in a water bath for 15 minutes at 40°C. Samples were then removed and allowed to stand at room temperature for 60 minutes. The absorbance of the sample was then read at 505 nm (10 mm cuvette) on a Beckman DU640 Spectrophotometer (Beckman Coulter (UK) Ltd, High Wycombe, Buckinghamshire, UK). The absorbance of the glucose blank was read against distilled water, with the method blanks, glucose standards and samples being read against the glucose blanks. The starch concentration in the original sample was calculated according to the equation;

$$\text{Starch in original sample (g/kg)} = \frac{0.4555 \times A_s}{W \times A_g} \times 100$$

Where W = sample weight (g), $A_s$ = net absorbance of sample and $A_g$ = net absorbance of glucose standard.

### 2.1.9. Water soluble carbohydrate

Water soluble carbohydrate was determined according to the procedures of Thomas (1977). Exactly 0.2 g of dried sample was added to a shaker bottle and 200 ml of distilled water added. The sample was then shaken for 60 minutes (using a HS501 digital shaker, Kika labourtechnik, Germany), and filtered through a Whatman No. 1 filter paper (Whatman plc, Maidstone, UK). The filtrate was retained and 2 ml of the extract pipetted into a test tube and left to stand for 10 minutes. Whilst still in the ice, 10 ml of anthrone reagent (760 ml nitrogen-free sulphuric acid (98% *w/v* $H_2SO_4$) was added slowly to 330 ml of water and cooled rapidly, 1 g of thiourea and 1 g of anthrone added and the solution stirred until dissolved. The reagent was stored at 4°C and discarded after three days)) was slowly added and the contents of the test tube gently mixed. The tube was then loosely stoppered and the samples placed in a boiling water bath for 20 minutes. The samples were

removed from the water bath and rapidly cooled by placing in ice and water before reading the absorbance of the samples at 620 nm using a Beckman DU640 spectrophotometer within 30 minutes of the samples being removed from the water bath.

Water soluble carbohydrate content of the sample was calculated through the preparation of a standard graph by reading the absorbance of glucose standard solutions (0, 0.04, 0.08, 0.12, 0.16 and 0.20 mg/l of glucose) prepared according to the method described above. The absorbance of the samples and blank determinations were compared with that achieved from the standards and the difference between the two values multiplied by 500.

$$\text{WSC (g/kg)} = 500 \times (0.0111 + (0.184 \times \text{absorbance of sample}))$$

$$\text{WSC corrected for weight} = \text{WSC (g/kg)} \times \frac{0.2}{\text{sample weight}}$$

**2.2.0. Volatile fatty acids**

Volatile fatty acids were determined according to the method of Jones and Kay (1976) after extraction from the silage in water through the use of gas chromatography using Flame Ionisation Detection by reference to internal standards (provalic acid, AnalaR grade, Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK) and standard acids (acetic, propionic, isobutyric, butyric, AnalaR grade Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK).

### 2.2.1. Lactic acid

Lactic acid was determined following extraction from the silage with the aid of water (Bergmeyer, 1974). The lactic acid was oxidised in the presence of NAD by the enzyme L-lactate dehydrogenase and D-lacate dehydrogenase to form pyruvic acid. The amount of NADH formed was proportional to the concentration of lactic acid in the original sample and was measured at an absorbance of 340 nm.

### 2.2.2. Oil

Oil was determined for Experiments 1, 2 and 3a according to the procedure of MAFF (1982). Oil content of the samples was determined through extraction with the aid of light petroleum. The resultant residue was treated by heating with hydrochloric acid and the mixture cooled, filtered and extracted again with hydrochloric acid. The solvent was then removed by distillation and the residue dried and weighed.

The oil content of feeds on Experiment 3b was determined according to the procedures of (MAFF, 1986). Approximately 2 to 3 g of feed was accurately weighed into a previously weighed cellulose extraction thimble (Whatman plc, Maidstone, UK). The thimble was then plugged with defatted cotton wool and placed in the extraction unit (Tecator Soxtec 1043, Foss UK, Warrington, UK). Approximately 25 ml of pet ether (30-40 °C, AnalaR, VWR International Ltd, Poole, UK) was added to each preweighed extraction cup, and placed into the Soxtec apparatus, the sample was lowered into the ether. The solvent was then heated to boiling point (40°C) and the sample refluxed for 30 minutes. The solvent was then allowed to evaporate and the extraction cups were then removed from the apparatus and allowed to cool in a fume cupboard before being re-weighed. Ether extract (EE) content of the sample was calculated using the following formula:

$$EE\ (g/kg\ DM) = \frac{(\text{Extraction cup + fat weight (g)}) - (\text{Extraction cup weight (g)})}{(\text{Thimble + sample weight (g)}) - (\text{Thimble weight (g)})} \times 1000$$

### 2.2.3. Metabolisable energy (ME)

Metabolisable energy (ME) was estimated according to AFRC (1995) for grass silages, maize silage and dairy concentrates from NCDG using the following equations;

Grass silage ME

Digestible organic matter in the DM as g/kg of oven DM ($DOMD_o$)

$$DOMD_0\ (g/kg) = [OMD] \times (1000 - \text{total ash})/1000$$

Digestible organic matter including volatiles (DOMCc) (g/kg)

$$DOMDc\ (g/kg) = 1000 - \{(1000 - [DOMDo]) \times [ODM]/[CDM]\}$$

where OMD = oven DM and CDM = corrected DM

$$ME\ (MJ/kg\ corrected\ DM) = 0.016\ [DOMD_C]$$

Maize ME

$$ME\ (MJ/kg\ DM) = 3.62 + 0.0100[NCD]$$

Concentrate ME

$$ME\ (MJ/kg\ DM) = 0.0140[NCGD] + 0.025\ [EE]$$

### 2.2.4. Blood metabolites

Blood samples were taken via venepuncture from the tail vein into tubes containing potassium oxylate and sodium fluoride for the determination of urea, total protein and albumin and into tubes containing lithium heparin for samples taken for the determination of β-hydroxybutyrate, glucose and non-esterified fatty acids (NEFA).

Samples were centrifuged immediately at 1118 g for 5 minutes using a Sanyo Centaur 2 centrifuge, (Sanyo Watford UK, Watford, Herts, UK). The resultant plasma was pipetted into 1.5 ml micro-centrifuge tubes before being frozen at –80°C. Prior to analysis the plasma samples were allowed to thaw slowly at room temperature. The plasma samples were analysed using a Bayer Technicon RA 1000 autoanalyser (Bayer plc, Newbury, Berkshire, UK), blood urea was analysed using a Bayer Diagnostics test kit, (kit catalogue number T01-1823-56), glucose using a Bayer Diagnostics test kit (kit catalogue number T01-1833-56), β-hydroxybutyrate using a Randox laboratories kit (kit catalogue number RB 1008) and NEFA using a Wako chemicals test kit (catalogue number 994-75409). Albumin and total protein were analysed using Randox laboratories kits (kit catalogue number AB 361 and TP 245 respectively).

### 2.2.4. Milk analysis

Milk samples were preserved via the addition of 2 Lactabs mark II per 30 ml of milk (Thompson and Capper Limited, Hardwick Road, Astmoor, Runcorn, Cheshire, UK) and stored at $4^0$C prior to analysis and analysed using a Dairy Lab 2 spectrophotometer (Foss UK Ltd, Warrington, UK) for fat, protein and lactose. The spectrophotometer was calibrated using standards containing known concentrations of fat ranging from 247 to 629

g fat/kg and 305 to 366 g protein/kg (QM Ltd, Bury, Lancashire, UK). Samples were analysed weekly and never stored for longer than 2 weeks.

# 3.0. EXPERIMENT 1 EFFECT OF FORAGE PROCESSING AND CUTTING HEIGHT OF UREA-TREATED WHOLE-CROP WHEAT ON THE MILK PRODUCTION AND DIET DIGESTIBILITY IN DAIRY COWS.

## 3.1. Introduction

Previous work has shown that the inclusion of urea-treated whole-crop wheat (WCW) into dairy cow rations resulted in a significant increase in DM intake (Leaver and Hill, 1995; Phipps *et al.,* 1995; Sutton *et al.,* 1997). However, this increase in intake was not translated into an increase in milk or milk component yield. This was attributed by Abdalla *et al.* (1999) to poor ruminal digestibility of the starch fraction of the forage resulting in the passage of intact grains through the digestive tract. Attempts to improve the utilization of urea-treated WCW through dietary supplementation with readily available carbohydrate source, caustic treatment prior to feeding (Sutton *et al.*, 2001) or the inclusion of feed enzymes (Sutton *et al.*, 2002), have generally been unsuccessful and Abdalla *et al.* (1999) concluded that some form of physical processing was required. Recently, a processor fitted within the forage harvester and specifically designed for small-grained cereals has been developed. Whilst there is anecdotal evidence that this processor improves whole tract digestibility, and may subsequently enhance animal performance, no work has been published in this area.

Weller *et al.* (1995) demonstrated that by increasing the cutting height (i.e. leaving a longer stubble length), the proportion of grain within the forage was increased from 0.48 for material harvested at a height of 10 cm, to 0.61 when harvested at 40 cm, with a corresponding decrease in the modified acid detergent fibre content from 250 to 195 g/kg DM. Increasing cutting height was not, however, shown to significantly alter the metabolisable energy content of WCW when harvested at 450 g DM/kg (Sinclair *et al.*, 2003), although it was suggested that the effects of cutting height may be greater in forages that are harvested at a more mature stage.

The objectives of this experiment were to determine the effect of processing and cutting height at harvest of urea-treated WCW on intake, milk production, diet digestibility and the blood metabolite profile in lactating dairy cows.

## 3.2. Materials and Methods

### 3.2.1. Forage production

A commercial crop of winter wheat (*cv.* Equinox) was grown according to standard agronomic practice. The seed was dressed with fludioxnil, and the crop was sown on 19 October 1999, with a target seed rate of 150 kg/ha. The crop received 155 kg N/ha applied as follows; 45 kg N/ha on 17 March 2000 at growth stage (GS) 22 (tillering, main shoot and 2 tillers, Zadoks *et al.*, 1974) and 110 kg N/ha applied on 1 May 2000 at GS 32 (stem elongation, second node detectable). An aphicide apray (cypermethrin) and manganese were applied on 17 March 2000 at GS 22. The fungicide programme comprised of two sprays: kresoximmethyl and fluquinconazole + prochloraz was applied on 2 May 2000 at GS 32, followed by azoxystrobin and epoxiconazole + fenpropimorph applied on 30 May 2000 at GS 51 (inflorescence emergence, first spikelet of inflorescence just visible). Weed control consisted of isoproturon + diflufenican applied on 17 March 2000 at GS 22 followed by mecoprop-p and bromoxynil applied on 2 May 2000 at GS 32. The crop also received a growth regulator (chlormequat) at GS 32.

To ensure that a representative area of the field was harvested for each of the four forage treatments (LU, long straw, unprocessed, LP, long straw processed, SU, short straw unprocessed, SP, short straw processed), the field was divided into 12 plots, which in turn were arranged in three randomised blocks with treatments randomly allocated within each block. Crop DM yield was determined by manually cutting one quadrat (0.72 $m^2$) per plot to ground level on the day prior to harvest and harvestable yield calculated for each cutting height. The samples were oven dried at 100°C and the yield at each of the cutting heights calculated. The crop was harvested on 14 August 2000 at GS 87 (hard dough) using a self propelled forage harvester fitted with a combine header and a grain processor (Claas

Jaguar 800 series, Class, Bury St. Edmunds, UK). The processor was mounted directly after the cutting cylinder and consisted of two serrated steel contra-rotating rollers with their axis of rotation parallel to the cutter head. Each roller (circumference of 616 mm) contained saw tooth serrations (125 per circumference) with one rotated at 5000 revolutions per minute (rpm) and the other at 3100 rpm and with a gap of approximately 0.5 mm between the two rollers. For the unprocessed forages the processor was disengaged. Mean crop height prior to harvest (mean +/- s.d.) was 66.6 cm (2.02) cm and was cut to leave one of two cutting heights: long straw: (stubble height of 17.8 (5.13) cm) or short straw (stubble height of 37.3 (4.13) cm), and either unprocessed or processed at harvest. The four forages were ensiled in separate concrete walled, roofed clamps with a urea + urease additive ('Home 'n' Dry', Volac, Royston, UK; a mixture of feed grade urea and soya beans) applied by hand to provide approximately 20 kg urea per tonne of forage DM. After filling, all clamps were rolled well and double sheeted. As an indicator of the efficacy of the processor, the number of whole and undamaged grains in 100 g DM of fresh forage (4 samples per forage) was determined by visual examination. The grass silage which was fed in conjunction with the WCW forages was harvested on 30 May 2000 from a predominantly perennial ryegrass sward, wilted for 24 hours, received no additive and was ensiled in a concrete walled clamp.

### 3.2.2. Animals and experimental procedure

Forty-four Holstein-Friesian dairy cows (8 primaparous and 36 multiparous) were used in a 14-week continuous design study. Prior to the start of the experiment animals were fed a complete diet consisting of grass and maize silages (1:2 on a DM basis) with 7.5 kg/cow/day of concentrates. An additional 2.5 kg/cow/day of proprietary concentrates were fed through out of parlour feeders. Animals commenced the study at approximately week nine of lactation with week eight used as a covariate period. The cows were blocked and

randomly allocated to one of the four treatments (LU, LP, SU and SP as described earlier) according to parity (prima or multi), milk yield (mean 30.9 (4.41) kg) and body condition score (mean 2.7 (0.48)). Animals were housed in cubicles fitted with rubber mattresses and were bedded with sawdust twice weekly, with loafing areas scraped twice daily. All animals were offered water *ad-libitum*.

The WCW forages were mixed 2:1 on a DM basis with grass silage, with the ratio between the WCW and grass silage being maintained by oven drying forage samples taken twice weekly and adjusting the diets appropriately. The forage component of the ration was fed through individual electronic feed bins (Insentec, Marknesse, Holland) which measure intake electronically by means of weigh cells located within the feed bins and collar transponders worn by the cows. The concentrate component of the ration consisted of 2 kg/cow/day of rapeseed meal that was mixed with the forage component of the diet, plus 8.5 kg/cow/day of a standard dairy concentrate (Table 3.1), fed through out of parlour feeders.

Diets were formulated to satisfy the metabolisable energy and protein requirements for a milk yield of 30 kg/day (AFRC, 1995). Animals were fed the forage mix once daily at 08.00h at a rate of 1.05 of the previous calculated daily intake with refusals being collected twice weekly. Intake data was downloaded daily into a spreadsheet and dry matter intake calculated. Cows were milked twice daily at approximately 06.00 h and 17.00 h. Milk yield was recorded at each milking and milk samples taken for the determination of milk composition weekly at two consecutive milkings, on a Monday afternoon and Tuesday morning. Animals were weighed and condition scored (Lowman *et al.*, 1976) weekly after the Wednesday afternoon milking by the same person throughout the experiment.

Table 3.1 Ingredient composition of the concentrate

| | kg/t fresh weight |
|---|---|
| Wheat | 265 |
| Rapeseed meal | 100 |
| Sugarbeet pulp | 181 |
| Palm kernal extract | 75 |
| Maize | 31 |
| Soyabean meal | 125 |
| Sunflower meal | 69 |
| Molasses | 77 |
| Vegetable oil | 23 |
| Megalac† | 30 |
| Minerals and vitamins | 24 |

† Calcium salts of palm oil (Volac, Royston, UK).

During weeks 3, 8 and 13 of the experiment, blood samples were taken from 24 multiparous cows (six cows per treatment) at 11:00 h via venepuncture from the tail vein. Samples were collected into vacutainers containing either lithium heparin (for samples used to determine urea, total protein and albumin) or potassium oxylate and sodium fluorite (for samples used to determine $\beta$-hydroxybutyrate, glucose and non-esterified fatty acids (NEFA)). Following collection, samples were centrifuged immediately at 1120g for 5 minutes, the plasma collected and frozen at $-80^0$C prior to analysis.

Apparent digestibility was determined in 20 multiparous cows, (five cows per treatment) when animals were in week 13 of the experiment. Chromic oxide ($Cr_2O_3$, GPR grade, VWR International Ltd, Poole, UK) was used as an indigestible marker and was mixed with the dairy concentrate to provide an intake of 42.7 g DM of chromic oxide per cow per day. The marker was fed for 14 days with faecal grab samples taken from the rectum taken twice daily at 09:00 h and 18:00 h over the last seven days. To determine diurnal variation of marker excretion, faecal samples were also taken over the last 48 h of the sampling period at 03:00, 06:00, 09:00, 12:00, 15:00, 18:00 and 21:00 h. The diurnal variation data was then used to correct the mean daily chromium concentration for time of sampling. Samples were stored at $-20^0$C prior to subsequent analysis.

### 3.2.3. Chemical analysis

Feed samples were taken twice weekly and bulked every four weeks and a subsample analysed for acid detergent fibre (ADF), ammonium nitrogen, pH, crude protein, neutral detergent fibre (NDF), starch, oil, water soluble carbohydrates (WSC), digestible organic matter in the dry matter (DOMD), and volatile fatty acids according to the methods described in Chapter 2.

Faecal samples were analysed for chromium content according to the method of Siddons *et al.* (1985) using a Smith-Hjete 1000 atomic absorption spectrophotometer (Thermo Jarrell Ash Corporation, Massachusetts, USA). Approximately 0.5 g of sample was ashed at 500°C overnight (16 h) and was placed in a 250 ml conical flask. To this 6 ml of digestion acid (250 ml orthophosphoric acid (880 g/l), 50 ml manganese sulphate (100 g/l), 250 ml concentrated sulphuric acid) was added to the ashed sample along with 3 mls of potassium bromide solution (45 g/l). The sample was then heated until the solution turned a deep purple colour and then allowed to cool and 100 ml of distilled water added. The chromium content of the samples was then determined and read against a standard curve. A range of standards were made (from 0 to 50µl) from a standard solution of chromium (Spectrosol, VWR International Ltd, Poole, UK) which contained 1000 ppm of chromium. Standards were made up to 100 ml with deionised water and subjected to the same analysis as the faecal samples. Faecal starch and NDF content was determined according to methods described in Chapter 2.

Milk samples were analysed weekly for milk fat, protein and lactose content whilst blood samples were analysed for urea, albumin, total protein, NEFA, β-hydroxybutyrate and glucose as described in Chapter 2.

### 3.2.4. Statistical analysis

The performance and digestibility data was analysed by analysis of variance as a 2 x 2 factorial design with milk production or liveweight in the week prior to the experiment being used, where appropriate, as a co-variate. The three treatment degrees of freedom were split into main effects of processing (P), straw height (H) and the interaction between processing and straw height (P x H). Blood metabolite data were analysed using analysis of variance and repeated measures analysis of variance where appropriate, with processing and cutting height together with interactions forming the "between animal" stratum and sample time with associated interactions with processing and cutting height forming the "within animal by time" stratum. All analysis was conducted using Genstat version 5 (VSN Int. Ltd, Oxford, UK) with means and standard error of the difference (s.e.d) presented. Significance is denoted in tables as NS for $P > 0.05$, * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$.

## 3.3. Results

### 3.3.1. Crop yields

Harvestable yield was higher ($P$ <0.01) for the long straw forages at 18.2 t DM/ha compared with 14.9 t DM/ha for the short straw forages (s.e.d. 0.94).

### 3.3.2. Forage analysis

Dry matter content was similar across all four whole-crop wheat forages with an average value of 696 g DM/kg, whilst the grass silage had a lower value of 232 g DM/kg (Table 2.2). Crude protein was lowest in SP at 134 g/kg DM and highest in LP at 146 g/kg DM with treatments SU and LP having intermediate values. Forage NDF was higher for the long compared with the short straw forages (422 *vs.* 337 g/kg DM for L *vs.* S respectively). By contrast, starch content was higher for the short compared to the long straw forages (419 *vs.* 356 g/kg DM respectively). The grass silage had an estimated ME content of 10.9 MJ/kg DM and a pH of 3.7. The number of whole grains in 100 g DM of forage was considerably higher ($P$ <0.001) in the unprocessed forages with values of 874, 145, 960 and 178 grains/100 g DM for treatments LU, LP, SU and SP respectively (s.e.d. 70.3). The concentrate had an estimated ME of 13.4 MJ/kg DM and a crude protein content of 200 g/kg DM. The rapeseed meal had a crude protein content of 345 g/kg DM.

Table 3.2 Chemical composition (g/kg DM unless otherwise stated) of the concentrates, grass silage, and whole-crop wheat that was either unprocessed (U) or processed (P) at harvest and cut at one of two heights to produce a forage that contained either long (L) or short (S) straw.

| | Grass Silage | LU | LP | SU | SP | Dairy concentrate | Rapeseed meal |
|---|---|---|---|---|---|---|---|
| Dry Matter (g/kg) | 232 | 713 | 653 | 707 | 709 | 871 | 878 |
| CP | 120 | 137 | 146 | 140 | 134 | 200 | 345 |
| Ammonia-N (g/kg total N) | 100 | 170 | 230 | 170 | 200 | nd | nd |
| OM | 912 | 958 | 952 | 956 | 954 | 913 | 922 |
| DOMD | 681 | 722 | 667 | 754 | 743 | nd | nd |
| Metabolisable energy (MJ/kg DM) | 10.9 | nd | nd | nd | nd | 13.4 | nd |
| WSC | 18.1 | 16.8 | 14.8 | 16.3 | 19.8 | 117.7 | 52.9 |
| NDF | 609 | 425 | 419 | 331 | 342 | 238 | 341 |
| ADF | 360 | 264 | 251 | 197 | 199 | nd | 247 |
| Oil | 34.4 | 17.6 | 19.2 | 17.7 | 19.3 | 69.9 | 356 |
| Starch | 23 | 369 | 342 | 417 | 420 | 223 | 8.7 |
| pH | 3.7 | 7.6 | 7.5 | 7.4 | 7.9 | nd | nd |
| Lactic acid | 23.5 | nd | nd | nd | nd | nd | nd |
| Acetic acid | <1.0 | nd | nd | nd | nd | nd | nd |
| Butyric acid | <1.0 | nd | nd | nd | nd | nd | nd |

Grass silage DM on a volatile corrected basis
nd = not determined

### 3.3.3. Milk production, composition, liveweight and condition score

Data was excluded for cow 41 (treatment SP) due to inconsistent use of the out of parlour concentrate feeders, cow 45 (treatment LU) due to mastitis and cow 202 (treatment SP) due to ill health that was not related to dietary treatment. Milk production, milk composition, liveweight and condition score are presented in Table 3.3. and Figures 3.1, 3.2 and 3.3.

There was no significant effect of treatment on milk yield which averaged 30.1 kg/day across all four treatments and is illustrated in Figure 3.1. There was an effect ($P$ <0.05) of cutting height on milk fat content with animals receiving the short straw forages (S) having a lower milk fat content than animals receiving the long straw (L) forages (mean values of 37 g/kg and 42 g/kg respectively). This effect was evident after week 3 of the experiment and remained so for the remainder of the experimental period (Figure 3.2). There was also

an effect of cutting height on milk fat yield with cows fed the short straw forages having a lower yield ($P$ <0.01) than animals receiving the long straw forages (1.12 kg/day *vs.* 1.26 kg/day respectively).

Table 3.3 Average milk yield, composition, liveweight change and condition score of cows fed whole-crop wheat that was either unprocessed (U) or processed (P) at harvest and cut at one of two heights to produce a forage that contained either long (L) or short (S) straw.

| | Diets | | | | | Sign. of main effects | | |
|---|---|---|---|---|---|---|---|---|
| | LU | LP | SU | SP | s.e.d. | P | H | PxH |
| Milk yield (kg/d) | 30.8 | 29.9 | 30.0 | 29.8 | 0.82 | NS | NS | NS |
| Fat (g/kg) | 41.8 | 41.9 | 38.4 | 35.6 | 2.50 | NS | * | NS |
| Protein (g/kg) | 34.4 | 34.6 | 33.3 | 33.0 | 1.11 | NS | 0.10 | NS |
| Lactose (g/kg) | 46.1 | 45.4 | 46.4 | 46.2 | 0.59 | NS | NS | NS |
| Fat yield (kg/d) | 1.28 | 1.24 | 1.16 | 1.07 | 0.071 | NS | ** | NS |
| Protein yield (kg/d) | 1.05 | 1.02 | 1.01 | 0.99 | 0.034 | NS | NS | NS |
| Lactose yield (kg/d) | 1.42 | 1.34 | 1.40 | 1.40 | 0.062 | NS | NS | NS |
| Daily live weight change (kg) | 0.33 | 0.44 | 0.39 | 0.44 | 0.131 | NS | NS | NS |
| Condition score | 2.59 | 2.61 | 2.83 | 2.76 | 0.108 | NS | * | NS |

P = main effect of processing, H = main effect of cutting height and P x H interaction between processing and cutting height.

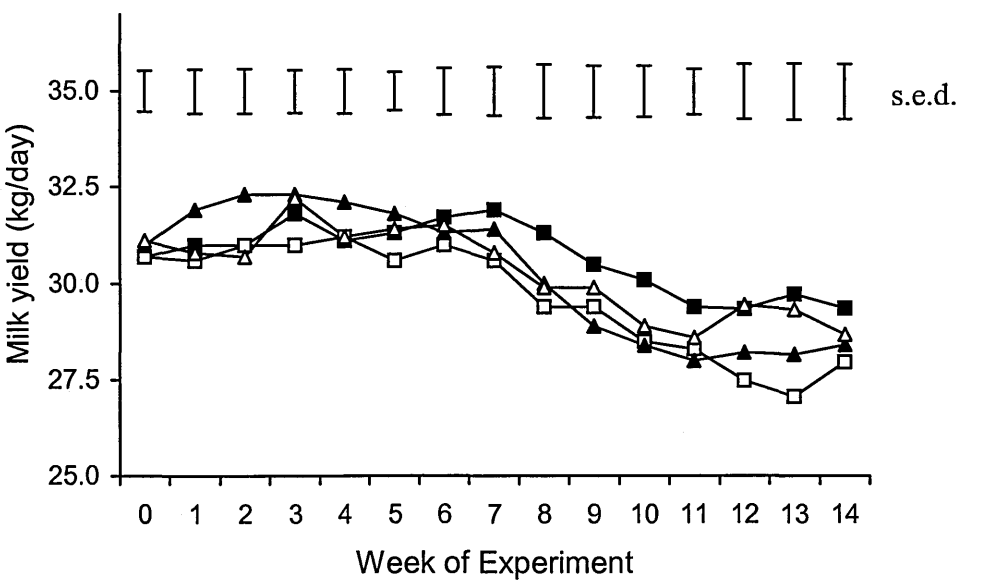


Figure 3.1 Milk yield (kg/day) in cows fed treatments of urea-treated whole-crop wheat that were either long straw and unprocessed (■), long straw and processed (□), short straw and unprocessed (▲) or short straw and processed (△).

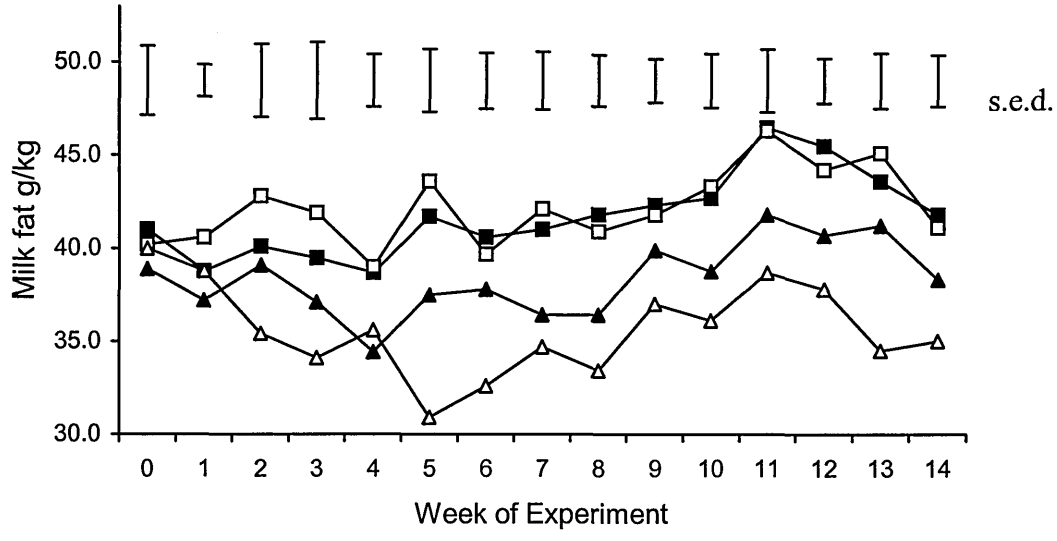


Figure 3.2. Milk fat (g/kg) in cows fed treatments of urea-treated whole-crop wheat that were either long straw and unprocessed (■), long straw and processed (□), short straw and unprocessed (▲) or short straw and processed (△).

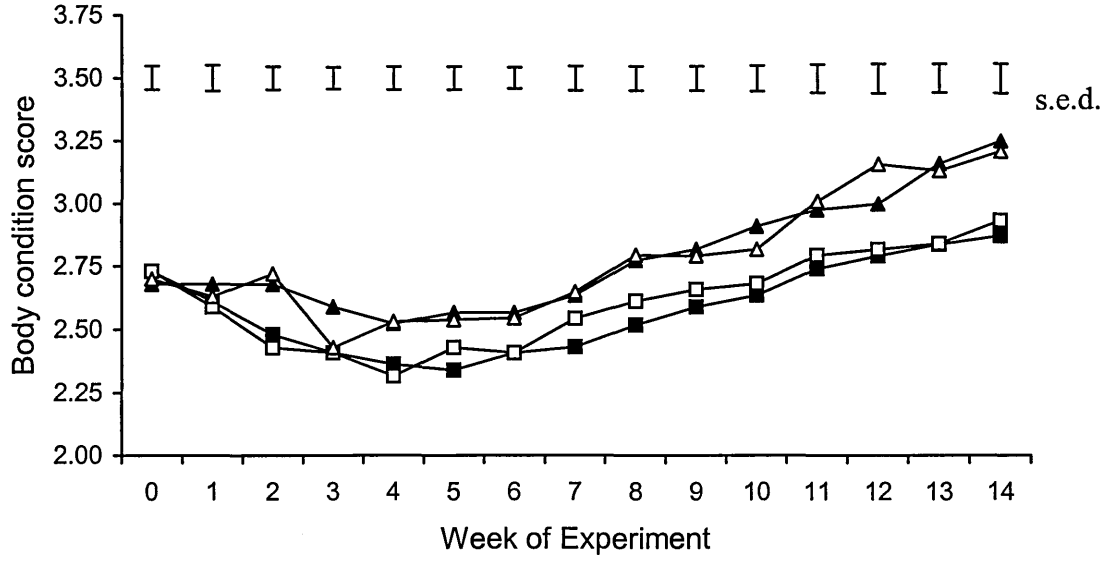


Figure 3.3 Body condition score in cows fed treatments of urea-treated whole-crop wheat that were either long straw and unprocessed (■), long straw and processed (□), short straw and unprocessed (▲) or short straw and processed (△).

Dietary treatment had no significant effect on live weight change; however, cutting height had an effect on average body condition score ($P$ <0.05), with animals receiving the short straw forages having a higher body condition score of 2.8 *vs.* 2.6 for animals fed the long

straw forages. This difference became evident after week 2 of the experiment (Figure 3.3) and continued to increase throughout the remainder of the experimental period.

### 3.3.4. Dry matter intake

Concentrate, forage and total dry matter intakes (DMI) are presented in Table 3.4. Concentrate intake was similar across all experimental treatments with a mean total intake of 8.89 kg DM/day. By contrast, forage intake was lower in cows fed the processed forages (mean value of 13.7 *vs.* 12.5 kg DM/day for the main effects of U *vs.* P respectively; $P < 0.05$), whilst animals receiving the short straw forages tended to have a lower intake ($P = 0.08$), than those receiving the long straw (L) forages. The effect of processing on forage intake was also reflected in total DM intake, which was lower in cows receiving the processed than the unprocessed forages ($P < 0.05$). There was also a tendency ($P = 0.10$) for animals receiving the short straw whole-crop forages to have a lower total DM intake than those receiving the long straw forages.

Table 3.4 Average intake (kg DM/day) of dietary components of cows fed urea-treated whole-crop wheat that was either unprocessed (U) or processed (P) at harvest and cut at one of two heights to produce a forage that contained either long (L) or short (S) straw.

| | Diets | | | | | Sign. of main effects | | |
|---|---|---|---|---|---|---|---|---|
| | LU | LP | SU | SP | s.e.d. | P | H | PxH |
| †OPF concentrates | 7.23 | 7.10 | 7.03 | 7.08 | 0.094 | NS | NS | NS |
| ‡ Total concentrates | 9.12 | 8.72 | 8.84 | 8.86 | 0.192 | NS | NS | NS |
| Grass silage | 4.80 | 4.33 | 4.33 | 3.92 | 0.268 | * | * | NS |
| WCW | 9.28 | 8.64 | 9.00 | 8.11 | 0.570 | * | NS | NS |
| Total forage | 14.09 | 12.97 | 13.32 | 12.03 | 0.834 | * | 0.08 | NS |
| Total DMI | 23.21 | 21.65 | 22.16 | 20.83 | 0.983 | * | 0.10 | NS |

P = main effect of processing, H = main effect of cutting height and P x H interaction between processing and cutting height.
†OPF = out of parlour concentrates
‡ Total concentrate = out of parlour concentrates plus rapeseed meal

### 3.3.5. Blood metabolites

Neither plasma urea nor NEFA levels were significantly affected by treatment, with an average value across treatments of 5.89 mmol/l and 0.283 μmol/l respectively (Table 3.5). There was, however, a tendency ($P = 0.08$) for plasma urea levels to be higher in cows fed the processed compared to the unprocessed forages (Table 3.5).

Table 3.5 Average plasma metabolites in cows fed whole-crop wheat that was either unprocessed (U) or processed (P) at harvest and cut at one of two heights to produce a forage that contained either long (L) or short (S) straw.

| | Diets | | | | | Sign. of main effects | | |
|---|---|---|---|---|---|---|---|---|
| | LU | LP | SU | SP | s.e.d. | P | H | PxH |
| Urea (mmol/l) | 5.78 | 6.02 | 5.52 | 6.23 | 0.359 | 0.08 | NS | NS |
| Glucose (mmol/l) | 3.36 | 3.32 | 3.50 | 3.53 | 0.093 | NS | * | NS |
| BHB (mmol/l) | 0.90 | 0.97 | 0.82 | 0.67 | 0.083 | NS | ** | 0.09 |
| NEFA (μmol/l) | 0.282 | 0.274 | 0.294 | 0.283 | 0.0097 | NS | NS | NS |
| Albumin (g/l) | 34.62 | 33.57 | 33.09 | 33.04 | 0.762 | NS | NS | NS |
| Total protein (g/l) | 74.2 | 74.2 | 75.8 | 75.2 | 3.41 | NS | NS | NS |

P = main effect of processing, H = main effect of cutting height and P x H interaction between processing and cutting height.

There was an effect of cutting height on plasma glucose levels; animals receiving the short straw forages had higher levels than those receiving the long straw forages (3.52 *vs.* 3.34 mmol/l for the main effects of S *vs.* L respectively; $P < 0.05$). However, this effect may be attributable to the differences observed during week 3 of the experiment (Figure 3.4). Cows receiving the short straw forages also had lower average plasma BHB levels than animals receiving the long straw forages ($P < 0.01$), although most of this effect could be attributed to differences occurring during week eight of the experiment (Figure 3.5). Animals receiving LP had a higher BHB content than those receiving any of the other three treatments (mean value 1.26 mmol/l, $P < 0.05$, s.e.d. 0.139). There was no effect of dietary treatment on either plasma albumin or total protein of treatment, with an average value across treatments of 33.58 g/l and 74.9 g/l respectively.

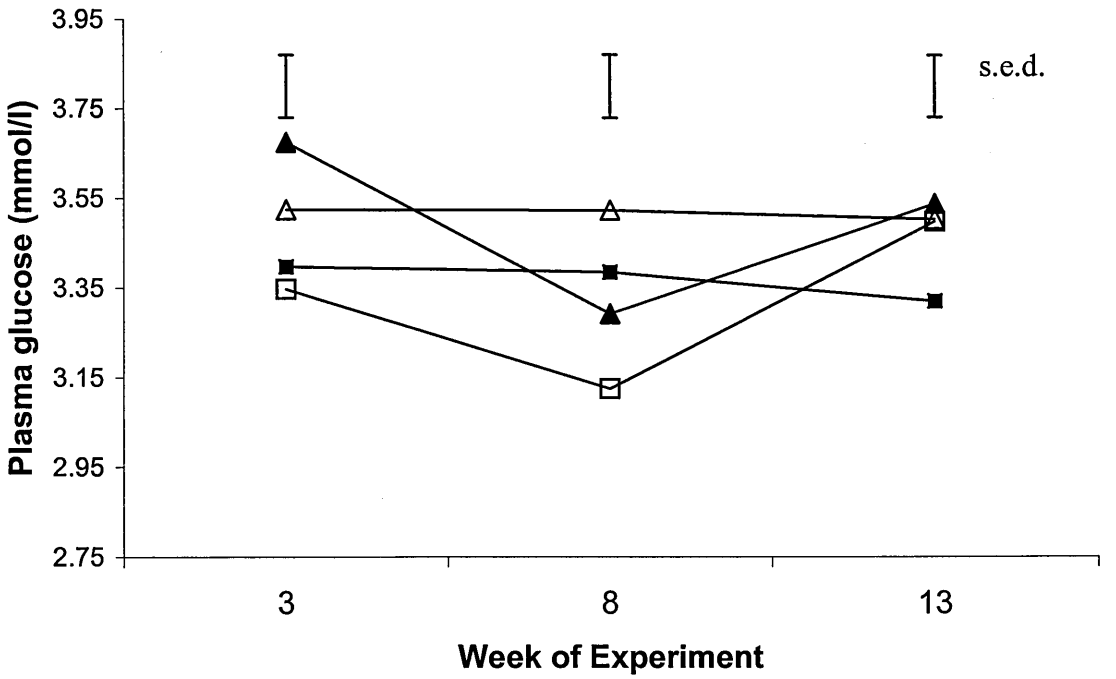


Figure 3.4. Plasma glucose (mmol/l) in cows fed treatments of urea-treated whole-crop wheat that were either long straw and unprocessed (■), long straw and processed (□), short straw and unprocessed (▲) or short straw and processed (△).

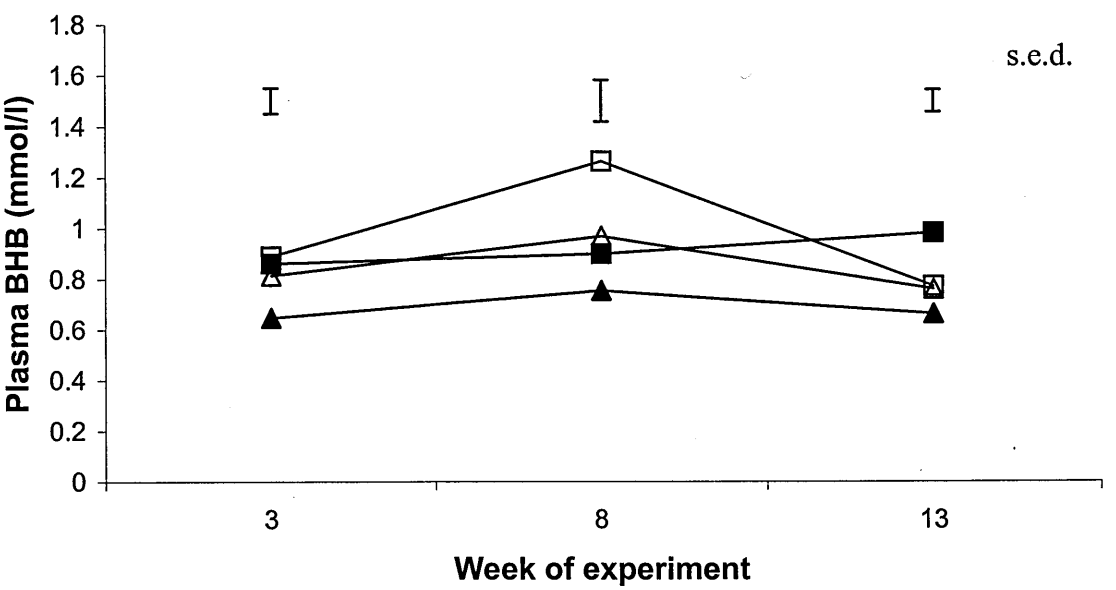


Figure 3.5. Plasma BHB (mmol/l) in cows fed treatments of urea-treated whole-crop wheat that were either long straw and unprocessed (■), long straw and processed (□), short straw and unprocessed (▲) or short straw and processed (△).

### 3.3.6. Apparent digestibility

There was no significant effect of dietary treatment on organic matter (OM) intake, with an average value across treatments of 20.99 kg/day (Table 3.6). There was an interaction between processing and cutting height on faecal OM output; OM output was increased ($P$ <0.05) due to processing in cows fed the long straw WCW (LU *vs.* LP) but decreased in cows fed the short straw WCW (SU *vs.* SP). There was, however, no significant effect of dietary treatment on OM digestibility. Intake of NDF was higher in animals receiving the long compared with the short straw forages (average intake of 8.92 kg/day *vs.* 7.88 kg/day for L *vs.* S respectively; $P$ <0.05), although there was no significant difference between treatments on NDF digestibility. Starch intakes were not significantly affected by treatment with an average value of 4.98 kg/day. Processing at harvest altered starch output with cows receiving processed forages having lower faecal outputs than those receiving the unprocessed forages (0.17 *vs.* 0.63 kg/day respectively; $P$ <0.001), whilst animals receiving the short straw forages had higher faecal starch outputs than those receiving the long straw forages (0.49 *vs.* 0.31 kg/day respectively; $P$ <0.05). Starch apparent digestibility was higher in cows fed the processed compared with the unprocessed forages (0.96 *vs.* 0.88 kg/kg respectively; $P$ <0.001) and was higher in cows fed the long compared with the short forages (0.94 *vs.* 0.91 respectively; $P$ <0.05), although most of this effect could be attributed to the higher digestibility of starch in cows fed LU.

Table 3.6 Intake, faecal output (kg DM/day) and apparent digestibility (kg/kg) of organic matter, fibre and starch of cows fed whole-crop wheat that was either unprocessed (U) or processed (P) at harvest and cut at one of two heights to produce a forage that contained either long (L) or short (S) straw.

| | Diets | | | | | Significance of main effects | | |
|---|---|---|---|---|---|---|---|---|
| | LU | LP | SU | SP | s.e.d. | P | H | PxH |
| Organic matter | | | | | | | | |
| Intake | 21.6 | 21.3 | 21.6 | 19.5 | 1.24 | NS | NS | NS |
| Faecal output | 7.73 | 7.88 | 8.67 | 6.99 | 0.532 | 0.07 | NS | * |
| Digestibility | 0.64 | 0.63 | 0.60 | 0.64 | 0.033 | NS | NS | NS |
| NDF | | | | | | | | |
| Intake | 9.02 | 8.8 | 8.27 | 7.49 | 0.590 | NS | * | NS |
| Faecal output | 3.65 | 3.86 | 3.82 | 3.45 | 0.255 | NS | NS | NS |
| Digestibility | 0.59 | 0.56 | 0.54 | 0.53 | 0.046 | NS | NS | NS |
| Starch | | | | | | | | |
| Intake | 4.78 | 4.79 | 5.48 | 4.87 | 0.291 | NS | 0.08 | NS |
| Faecal output | 0.46 | 0.16 | 0.80 | 0.18 | 0.103 | *** | * | 0.05 |
| Digestibility | 0.90 | 0.97 | 0.86 | 0.96 | 0.017 | *** | 0.05 | NS |

P = main effect of processing, H = main effect of cutting height and P x H interaction between processing and cutting height.

## 3.4. Discussion

### 3.4.1. Forage analysis

The DM content of the whole-crop forages was similar between treatments, averaging 696 g DM/kg. This value is higher than reported in a number of other studies that have examined urea-treated WCW (Leaver and Hill, 1995; Sutton *et al.*, 2001) but lower than the 761 g DM/kg reported by Abdalla *et al.* (1999) or the 813 g DM/kg used in the work of Sutton *et al.* (1998). The average starch levels in the long straw WCW of 356 g/kg DM are also comparable to the 293-328 g/kg DM reported for urea-treated WCW by Sutton *et al.* (2001) and the 306 g/kg DM used by Sutton *et al.* (2002). Increasing the stubble height at harvest increased the starch concentration proportionally by 0.20, a value similar to the 0.25 increase in fermented WCW reported by Sinclair *et al.* (2003). By contrast, altering cutting height reduced forage NDF levels by approximately 0.20, a value considerably higher than the 0.11 reported in the less mature forage in previous work (Sinclair *et al.*, 2003). Forage ADF concentration followed similar patterns to that observed for NDF, being lower in the short straw forages, although values were somewhat lower than those observed by Sinclair *et al.* (2003). Mean ammonia nitrogen levels were similar between treatments with a mean value of approximately 190 g/kg total N. This value is greater than the 105 and 150 g/kg total N achieved by Sutton *et al.* (1997) for material preserved with 20 and 40 kg urea/t DM respectively but similar to the 183 g/kg total N observed by Sutton *et al.* (1998) for material preserved with 20 kg urea/t DM. Crude protein levels were consistent between treatments, at approximately 140 g/kg DM. Previous studies have applied feed-grade urea at either 20 or 40 kg urea/t DM forage, which has resulted in larger but inconsistent increases in forage crude protein levels. For example, the application of urea at the rate of 20 kg/t DM resulted in a WCW crude protein content of 220 g/kg DM (Sutton *et al.*, 1997), whereas in other work the application of urea at 40 kg/t DM resulted

in a crude protein level of only 171 g/kg DM (Sutton *et al.*, 1998). The mean forage pH of 7.6 is somewhat higher than the 7.3 and 6.7 reported by Sutton *et al.* (1997) for material treated with 20 kg urea/t DM but lower than the pH of 8.2 achieved by Abdalla *et al.* (1996), also for material preserved with 20 kg urea/t DM.

### 3.4.2. Digestibility and intake

Processing of whole-crop wheat at harvest was hypothesised to increase the digestibility of the forage, particularly that of the starch component. The current results clearly demonstrate that processing reduced the number of whole grains in the forage (mean value of 917 grains/100g DM for unprocessed forages compared to a mean value of 162 grains/100g DM for processed forages) and improved the whole tract digestibility of starch, although organic matter and fibre digestibility were not significantly affected. Assuming that the digestibility of starch for all the dietary components other than the WCW was constant at 0.97 kg/kg (Sutton *et al.*, 1998), then the apparent digestibility of the starch component in the whole-crop forages can be estimated at 0.87, 0.97, 0.80, and 0.96 kg/kg for forages LU, LP, SU and SP respectively (s.e.d. 0.026; $P$ <0.001). The mean value for the unprocessed WCW treatment of 0.83 kg/kg is higher than the 0.63-0.81 kg/kg reported by Sutton *et al.* (1998), although higher values have been reported elsewhere (Sutton *et al.*, 2002). Despite this, processing of the forage at harvest increased the whole tract digestibility of starch to almost 100 %, a value comparable to that when measured in sheep (Sutton *et al.*, 2002). Increasing cutting height at harvest has also been suggested as a means of increasing the energy value of WCW (Weller *et al.*, 1995). However, the effects of altering the cutting height on organic matter, starch or fibre digestibility in fermented WCW when determined in sheep were small and non-significant (Sinclair *et al.*, 2003). In the current experiment there was also no significant effect of cutting height on

whole tract digestibility, although there was a tendency for fibre digestibility to be higher in the long straw treatments.

Processing at harvest reduced both forage and total DM intake ($P$ <0.05), whilst increasing cutting height tended to decrease forage DM intake ($P$ =0.08). These effects were additive and resulted in a difference in forage DM intake between cows fed the long, unprocessed and the short, processed WCW of over 2 kg DM/day. The observation that processing at harvest reduced forage DM intake is in contrast to that reported for mechanical processing of maize silage, which from a review of the literature, was shown to increase DM intake in dairy cows by an average of 3 % (Johnson *et al.*, 1999). However, certain studies reported no significant effect (Johnson *et al.*, 2003), whilst others observed a reduction in DM intake (Johnson *et al.*, 2002). The supplementation of ruminant diets with additional starch has also been shown to reduce total DM intake (Reynolds *et al.*, 1997), an effect that has been considered to be mediated by an increased ruminal production and absorption of propionate and subsequent metabolic and endocrine responses (Langhans, 1999). Similarly, the replacement of whole grains with ground and pelleted grains in cattle diets was associated with a reduced DM intake (Ørskov *et al.*, 1978). Although ruminal propionate levels were not measured in the experiment, processing of the grains at harvest and increasing the dietary starch concentration through decreasing straw length, would both be expected to result in an increased ruminal availability of starch which may lead to increased production of propionate (Theurer, 1986), and could explain the effects on intake observed here.

### 3.4.3. Animal performance and blood metabolites

There was little effect of processing at harvest of WCW on milk production, with an average yield of 29.9 and 30.4 kg/day for cows fed the processed or unprocessed forages

respectively. In an examination of the effects of mechanical processing of maize silage, milk yield was increased due to processing by a modest 0.9 %, although the response ranged from –10 to +10 % (Johnson *et al.*, 1999). Mechanical processing of maize silage has also been reported to have a variable effect on milk composition. Some studies have reported an increase in milk protein content (Johnson *et al.*, 1999) whilst others have reported no significant effect on milk fat or protein levels (Johnson *et al.*, 2002b) or a negative effect on milk protein concentrations (Johnson *et al.*, 2003). Despite the increased starch digestibility due to processing in the current experiment, there was no significant effect of processing on milk fat or protein levels, although there was a tendency for milk fat levels to be the lowest in cows fed the short, processed material. The reduction in milk fat levels and increased body condition score in cows fed the short straw treatments is in agreement with the observation that a depression in milk fat concentration is generally associated with an increase in body lipid deposition (Reynolds *et al.*, 1997). This effect is generally associated with, and partly mediated by, an elevation in peripheral insulin concentration, although more recent evidence suggests a greater influence of ruminally produced *trans*-10, *cis*-12 18:2 on milk fat concentrations (Bauman *et al.*, 2001). The lack of a response in live weight change to cutting height over the experimental period is in accordance with Sinclair *et al.* (2003) who found that live weight change did not differ between animals receiving high starch (short straw) or low starch (long straw) fermented WCW. In the absence of a measured value for the ME value of processed WCW, and the inconsistency in the results of previous attempts to calculate energy values (Leaver and Hill, 1995), it would be misleading to calculate a complete ME balance from the results reported here. However, milk energy outputs, calculated according to Tyrrell and Reid (1965) for treatments LU, LP, SU and SP were 100, 97, 93 and 89 MJ/day and indicate a reduction in milk energy output due to cutting height but that processing had little effect. This observation, in combination with the significant decrease in forage DM intake for cows fed the diets LP and SP, represents an improvement in the efficiency of forage use

for milk production due to processing. By contrast, N efficiency was similar between treatments at 0.269, 0.262, 0.268 and 0.276 kg milk N output/kg N intake for treatments LU, LP, SU and SP respectively. These values are somewhat higher than those reported by Hameleers (1998) who calculated a nitrogen efficiency of 0.229 (kg milk N output/kg N intake) for unprocessed urea-treated whole-crop wheat mixed with grass silage in the ratio of 2:3 WCW:grass silage (DM basis).

It was stated by Sutton *et al.* (1998) that the inclusion of urea-treated WCW into diets bought with it environmental problems as a result of the increased levels of N excreted. It was concluded by the authors that the high loss of N gave weight to the argument against using urea as a forage preservative. The N efficiencies in the work of Sutton *et al.* (1998) were between 0.236 and 0.238 kg milk N output/kg N intake for unprocessed urea-treated WCW (Sutton *et al.*, 1998). In a more recent experiment, efficiencies were reported of 0.253 g milk N output/g N intake for unprocessed urea-treated WCW (Sutton *et al.*, 2002). These values are substantially lower than those observed in the current work. However, in the work of Sutton *et al.* (2002), and Sutton *et al.* (1998), all the experimental diets received the same level of concentrate with an unchanged chemical composition. Hence the conclusions by the authors that the use of urea as a forage preservative brought with it significant environmental issues with regard to the excretion of N may be unsound, when the dietary treatments themselves were not balanced to reflect the additional N supply from the urea-treated WCW.

Decreasing cutting height at harvest was shown to alter the energy status of the dairy cows, with animals receiving the short straw forages (S) having higher plasma glucose levels and lower BHB concentrations than those receiving the long straw forages (L). It is well established that plasma glucose and BHB concentrations are inversely related (Ward *et al.*, 1995), and the increased glucogenic:ketogenic tissue supply in cows fed the short straw

treatments in the current experiment supports the observation of the decreased milk fat in animals fed treatments SU and SP. The lower plasma BHB levels in cows fed the short straw treatments is also in agreement with that reported previously with WCW when harvested at a less mature stage (Sinclair *et al.*, 2003). The tendency ($P$ =0.08) for animals fed the processed forages to have lower plasma urea levels however, is in contrast to that reported for milk urea levels as a result of processing maize silage (Johnson *et al.*, 2002b).

The lack of an effect of treatment on blood NEFA observed in the current experiment is in contrast to that reported by Sinclair *et al.* (2003) who observed a decrease in NEFA when short straw compared with long straw fermented WCW was fed. It has however been shown that if the diet contains a high proportion of cereal-based concentrates that plasma NEFA concentrations do not increase when energy intake is reduced (Topps and Thompson, 1984). Neither albumin nor total protein was affected by treatment with mean values of 33.6 and 74.9 g/l. These values are higher than the values obtained by Castlejon and Leaver (1994) of 27.9 g/l albumin and 56.6 g/l of total protein for animals receiving WCW as the sole forage, but lower than those observed by Hill and Leaver (1999b), with albumin values of 37.0 g/l and total protein of 73.6 g/l when urea-treated WCW was fed as a sole forage.

## 3.5. Conclusions

Processing of whole-crop wheat at harvest had no significant effect on milk yield, composition or body condition score. However, processing increased the apparent digestibility of starch and resulted in a reduction in forage DM intake and therefore offers the possibility of improved efficiency of forage use for milk production. The utilisation of a forage processor at harvest also offers the potential to harvest whole-crop cereals at higher DM contents without reducing starch digestibility. Increasing the cutting height at harvest decreased milk fat content and yield but increased body condition score, indicating an alteration in nutrient partitioning towards tissue lipid deposition. Attempts to increase the DM intake of processed WCW to levels similar to that for unprocessed forage may improve performance and warrants further investigation.

# 4.0 EXPERIMENT 2 EFFECTS OF PROCESSED UREA-TREATED WHOLE-CROP WHEAT, MAIZE SILAGE AND SUPPLEMENT TYPE TO WHOLE-CROP WHEAT ON THE PERFORMANCE OF DAIRY COWS.

## 4.1. Introduction

In Experiment 1 (Chapter 3) it was found that processing urea-treated whole-crop wheat at harvest significantly increased apparent whole tract starch digestibility. However, there was no significant effect of processing on milk or milk constituent yield. It has been suggested that the provision of a sugar source may enhance the performance of animals fed urea-treated whole-crop wheat through increased capture of ammonia in the rumen (Obara and Dellow, 1993). Urea-treated whole-crop wheat contains a high content of ammonium nitrogen (17-20 % ammonium nitrogen as percentage of total nitrogen), which is rapidly released in the rumen, giving rise to increased ruminal ammonia levels (Anderson, 1967). The rapid release of a sugar source may enhance the capture of this ammonia and may result in an increased rumen microbial protein synthesis (Abdalla *et al.,* 1999).

Lactose, a by-product from the production of skimmed milk powder, has been reported to reduce ruminal protozoa levels, thus potentially increasing microbial protein yield (Hussain and Miller, 1999). Chamberlain *et al.* (1993) found that feeding lactose to sheep resulted in a greater yield of microbial protein than that obtained from cereal starch (89 g/day microbial protein achieved from lactose supplemented diets compared to 74 g/day microbial protein obtained from starch supplemented diets). Lactose has also been reported as having a positive effect on dry matter intake in dairy cows (Allison and Garnsworthy, 1997). An increase in DMI was also observed by DeFrain *et al.* (2004) when lactose was used as a supplement to diets based predominately on corn silage. An alternative sugar source to lactose is molasses, a by-product of sugar production (Karalazos and Swan, 1976). Molasses is easily fermented in the rumen, supplying rapidly available energy to

rumen micro organisms (Murphy, 1999). This may also lead to a greater utilisation of the available nitrogen (in the form of ammonia nitrogen), and increase ruminal microbial protein synthesis (Murphy, 1999). Molasses has also been reported to result in a higher dry matter intake and milk yield when included in dairy cow rations (Murphy, 1999).

Earlier comparisons between unprocessed urea-treated WCW and maize silage have shown that maize silage resulted in a greater intake and milk yield (Phipps *et al.,* 1995). However, few studies have compared maize silage with processed, urea-treated WCW fed to dairy cows.

The objectives of the current experiment were, therefore, to investigate the effects of supplement type to processed urea-treated whole-crop wheat and to compare processed urea-treated WCW with maize silage, on the intake and production of dairy cows.

## 4.2. Materials and Methods

### 4.2.1. Crop production

*4.2.1.1. Whole-crop wheat*

A commercial crop of winter wheat (*cv* Equinox) was grown on a sandy loam soil following a three-year grass ley. The crop was sown on 26 November 2000 using fludioxinil treated seed at a target seed rate of 175 kg/ha. Soil analysis for phosphate and potash indicated P and K indices of 4 and 1 respectively and 160 kg/ha of $K_2O$ was applied as muriate of potash on 18 February 2001. Nitrogen was applied at the rate of 136 kg N/ha as a split dressing: 50 kg N/ha on 18 February 2001 (GS 21, tillering, main shoot and 1 tiller) and 86 kg N/ha on 18 April 2001 (GS 30/31, stem elongation, 1$^{st}$ node detectable). An early application of growth regulator (trinexapac ethyl) was applied on 20 March 2001 to encourage root development and tillering.

Disease control consisted of a two spray fungicide programme; kresoxim methyl + fenpropimorph applied on 5 May 2001 (GS 31), and kresoxim methyl + fenpropimorph and fluquinconazole + prochloraz applied on 30 May 2001 (GS 39, stem elongation, flag leaf ligule visable). Weed control consisted of isoproturon + diflufenican applied on 20 March 2001 to control annual meadow grass and annual broad leafed weeds, followed by fluroxypyr and metasulfron urea applied on 20 May 2001 to control wild oats. The crop was sprayed with cypermethrin against aphids on 20 March 2001, and received routine manganese sprays.

The crop was monitored twice weekly for DM content by cutting two adjacent rows (at a height of 14.5 cm) from 5 areas marked at random across the field. These samples were then dried overnight at 100°C. Immediately prior to harvest quadrat samples (1 from the

area between each tramline, 14 in total, each quadrat of area 0.72 $m^2$) were taken for the determination of total above ground DM yield, and grain yield.

The crop was harvested on 17 August 2001 using a self propelled forage harvester fitted with a forage mill (Claas Jaguar 900 series, Claas, Bury St. Edmunds, UK). The crop had a mean pre-harvest height of 70.3 cm (2.48) and was harvested at an average stubble height of 32.0 cm (1.13 cm). The crop was ensiled in a concrete walled, roofed clamp, with a urea + urease additive, ('Home 'n' Dry', Volac, Royston, UK; a mixture of feed grade urea and soya beans) applied by a fertiliser spreader at ensiling to provide approximately 20 kg urea per tonne of forage DM. After filling, the clamp was rolled well, double sheeted and weighed down with rubber tyres.

*4.2.1.2. Maize silage*

The maize variety (*cv* Nancis) was grown on a sandy loam soil following winter wheat. The crop was drilled on 25 May 2001 at a seed rate of 110,000 seeds/ha at 12 cm spacing and 76 cm row width. On 6 June 2001 the crop was sprayed with Atrazine and was harvested on 15 October 2001 using a self propelled forage harvester fitted with a corn cracker, (Claas Jaguar 890, Class, Bury St. Edmunds, UK) and ensiled with no additive, in a concrete walled, roofed clamp. After filling the clamp was rolled well, sheeted and weighed down with rubber tyres.

*4.2.1.3. Grass silage*

The grass silage fed in conjunction with the maize and WCW was made from a predominately perennial ryegrass sward. It was cut and left to wilt for approximately 36 hours and ensiled on 23 May 2001 using a trailed forage harvester (John Deere 3625, John Deere Ltd, Nottingham, UK) in a concrete walled roofed clamp. After filling the clamp was rolled well, sheeted and weighed down with rubber tyres.

### 4.2.2. Animals

Forty-four Holstein-Friesian dairy cows (36 multi-parous, 8 prima-parous), averaging 35 days (9.59) into lactation were used. Prior to commencing the experimental treatments, cows received a complete diet containing a forage mixture (kg/kg DM basis; processed and urea-treated WCW 0.24, maize silage 0.24, grass silage 0.18, bean silage 0.06 and moist sugar beet pulp 0.28) and 9.2 kg/day of concentrates, with an additional 2 kg/day of a standard concentrate fed through out of parlour feeders. Before commencing the experiment, milk yield, composition, liveweight and condition score were recorded, and animals were blocked according to parity (prima parous or multiparous), calving date, milk yield, condition score, milk composition and liveweight and randomly allocated to one of four dietary treatments. Animals were housed in cubicles that were bedded twice weekly and limed once weekly. Loafing areas were scraped using automatic scrapers and animals had continuous access to water. The experiment ran for 15 weeks.

### 4.2.3. Diets

There were four dietary treatments; Maize: maize silage and 2 kg/day rolled wheat, W-WCW: processed WCW and 2 kg/day rolled wheat, L-WCW: processed WCW and 0.7 kg/day Suga-lac (710 g lactose/kg DM, Trouw Nutrition, Cheshire, UK) and 1.3 kg/day rolled wheat and M-WCW: processed WCW and 2.4 kg/day molasses. The allocation of concentrate is presented in Table 4.1. The test forages were mixed 2:1 (DM basis) with grass silage, with the ratio being maintained by oven drying forage samples twice weekly. In addition, 2 kg/cow/day rapeseed meal was fed mixed in the forage component of the ration. The forage component of the ration was fed through individual electronic feed bins (Insentec, Marknesse, Holland). All cows also received 6.5 kg/day of a standard dairy concentrate (Table 4.2) that was fed through out of parlour feeders. The Maize diet was

supplemented with feed-grade urea, (26 g/kg maize DM) to balance the ration for crude protein content.

Table 4.1 Concentrate allocation (kg/day) to dairy cows fed maize silage supplemented with wheat or whole-crop wheat (WCW) supplemented with wheat (W), lactose (L) or molasses (M).

| | Diets | | | |
|---|---|---|---|---|
| | Maize | W-WCW | L-WCW | M-WCW |
| Rolled wheat | 2.0 | 2.0 | 1.3 | - |
| Suga-Lac | - | - | 0.7 | - |
| Cane molasses | - | - | - | 2.3 |

Diets were formulated to satisfy the metabolisable energy and protein requirements according to AFRC (1995). Animals were fed the forage mix once daily at 08:00 h at a rate of 1.05 of the previous calculated daily intake, with refusals being collected three times weekly. Intake data for all cows was downloaded daily into a spreadsheet.

Table 4.2. Ingredient composition of the concentrate

| | kg/t fresh weight |
|---|---|
| Unmolassed sugarbeet pulp | 220 |
| Rapeseed meal | 230 |
| Palm kernel extract | 140 |
| Sunflower meal | 120 |
| Soyabean meal | 120 |
| Molasses | 80 |
| Megalac† | 42 |
| Palm oil | 20 |
| Vitamins and minerals | 28 |
| Di-calcium phosphate | 7 |
| Salt | 5 |

† Calcium salts of palm oil (Volac, Royston, UK).

Cows were milked three times daily at approximately 06.30, 12.30 and 20.30 h. Milk yield was recorded at each milking and samples taken for the determination of milk composition weekly at three consecutive milkings on Mondays and Tuesdays. Animals were weighed and condition scored (Lowman *et al.*, 1976) weekly following the Wednesday afternoon milking.

After cows had been on the experiment for 3, 8 and 13 weeks, blood samples were taken from 24 multiparous cows (six per treatment) at 11:00 h via venepuncture from the tail vein. Samples were collected into vacutainers containing either lithium heparin (for samples used to determine urea, total protein and albumin) or potassium oxylate and sodium fluoride (for samples used to determine BHB, glucose and NEFA). Following collection, samples were centrifuged at 1120g for 5 minutes, the plasma collected and frozen at $-80^0$C prior to analysis.

### 4.2.4. Chemical analysis

Feed samples were bulked every four weeks and a subsample sent to NRM laboratories (Bracknell, UK) for analysis using the methods described in Chapter 2. Milk samples were analysed as described in Chapter 2. Blood samples were analysed for urea, albumin, total protein, $\beta$-hydroxybutyrate, glucose and NEFA as described in Chapter 2.

### 4.2.5. Statistical analysis

The results were analysed using a randomised block design with initial liveweight and milk yield used as co-variates where appropriate. One cow (4217:M-WCW) was removed from the experiment for reasons unrelated to dietary treatment, and her data was excluded from the statistical analysis. Two cows were also removed from the data due to recurrent mastitis (645:W-WCW and 166:L-WCW). The data from the remaining 41 cows were analysed by analysis of variance using Genstat 5 (VSN Int. Ltd, Oxford, UK). Significance is denoted in tables as NS for $P>0.05$, * for $P<0.05$, ** for $P<0.01$ and *** for $P<0.001$.

## 4.3. Results

### 4.3.1. Crop yield

The wheat crop had a grain yield of 10.04 t/ha (1.78) and a total above ground DM yield of 17.30 t DM/ha (2.41). Harvestable yield was 11.63 t DM/ha (2.00).

### 4.3.1. Feed analysis

Within the three forages the WCW had the highest DM and crude protein content (Table 4.3). Similarly, the WCW had the highest starch content, which was proportionally 0.12 more than the maize silage. By contrast, the maize silage had the lowest ammonia-N content and the lowest pH of the three forages. The grass silage was recorded as having the highest NDF and WSC content of the forages with the lowest NDF content being in the WCW which also had the lowest WSC content of the three forages.

The rapeseed meal had a crude protein content of 361 g/kg DM and a starch content of 8 g/kg DM (Table 4.4). The wheat and lactose supplements had similar crude protein contents but the wheat had a higher fibre (NDF and ADF) content than the lactose. Starch content was also higher for the wheat compared to the lactose. The molasses had the lowest crude protein content.

Table 4.3. Chemical composition (g/kg DM unless otherwise stated) of the forages and concentrate

| | Grass silage | Whole-crop wheat | Maize silage | Concentrate |
|---|---|---|---|---|
| Dry matter (g/kg) | 356† | 823 | 310 | 892 |
| CP | 122 | 143 | 76 | 249 |
| Ammonia-N (g/kg N) | 86 | 165 | 33 | nd‡ |
| OM | 927 | 967 | 961 | 886 |
| ME (MJ/kg DM) | 11.5 | nd | 11.5 | 13.5 |
| WSC | 98 | 7 | 16 | 116 |
| NDF | 509 | 410 | 442 | 286 |
| ADF | nd | 237 | nd | nd |
| Starch | nd | 350 | 308 | 62 |
| pH | 4.5 | 8.0 | 4.2 | nd |
| Volatile fatty acids | | | | |
| Lactic | 73 | nd | 82 | nd |
| Acetic | 41 | nd | 26 | nd |
| Propionic | 0 | nd | 0 | nd |
| Butyric | 10 | nd | 1 | nd |
| Ethanol | 20 | 0 | 0 | nd |

†Grass and maize silage DM on a volatile corrected basis
‡nd = not determined

Table 4.4. Chemical composition (g/kg DM) of the additional concentrates

| | Rapeseed meal | Wheat | Lactose | Molasses |
|---|---|---|---|---|
| Dry matter (g/kg) | 872 | 851 | 929 | nd |
| CP | 361 | 123 | 122 | 67 |
| OM | 925 | 983 | 911 | nd |
| NDF | 333 | 176 | 2 | nd |
| ADF | 237 | 38 | 15 | nd |
| Starch (g/kg DM) | 8 | 706 | 4 | nd |

nd = not determined

### 4.3.2. Milk production, composition, liveweight and condition score

Average milk yield was not significantly different between dietary treatments, with an average yield of 34.3 kg/day (Table 4.5). Within supplement types to WCW, animals fed lactose had higher milk yields than those receiving wheat or molasses. This tendency for an enhanced milk yield in lactose supplemented cows was observed from week 4 of the experiment and remained evident for the remainder of the experimental period (Figure 4.1). Feeding molasses as a supplement to WCW resulted in a higher milk protein content

($P$ <0.01) than feeding lactose. This difference was observed from week 2 of the experiment and remained evident for the remainder of the experimental period (Figure 4.2). Cows fed WCW supplemented with molasses also had numerically higher milk fat levels. However, there was no significant difference between supplement sources on milk protein or fat yield (kg/day). No significant difference was observed in milk fat or protein content or yield in cows fed Maize or W-WCW. Liveweight change averaged 0.39 kg/day and was not significantly different between treatments. Condition score was also unaffected by dietary treatment.

Table 4.5. Average milk yield, composition, live weight change and condition score of cows fed maize silage supplemented with wheat or whole-crop wheat (WCW) supplemented with wheat (W), lactose (L) or molasses (M).

| | Diets | | | | | Significance |
|---|---|---|---|---|---|---|
| | Maize | W-WCW | L-WCW | M-WCW | s.e.d. | |
| Milk yield kg/day | 34.0 | 34.4 | 35.6 | 33.1 | 1.21 | NS |
| Fat g/kg | 37.7 | 34.3 | 34.3 | 38.4 | 2.20 | NS |
| Protein g/kg | 31.2 | 32.5 | 31.5 | 33.3 | 0.61 | ** |
| Lactose g/kg | 45.5 | 45.6 | 46.3 | 46.3 | 0.53 | NS |
| Fat yield kg/day | 1.31 | 1.18 | 1.22 | 1.27 | 0.075 | NS |
| Protein yield kg/day | 1.07 | 1.13 | 1.11 | 1.10 | 0.040 | NS |
| Lactose yield kg/day | 1.54 | 1.57 | 1.65 | 1.53 | 0.057 | NS |
| Liveweight change kg/day | 0.30 | 0.49 | 0.30 | 0.47 | 0.133 | NS |
| Condition score | 2.53 | 2.71 | 2.60 | 2.50 | 0.161 | NS |

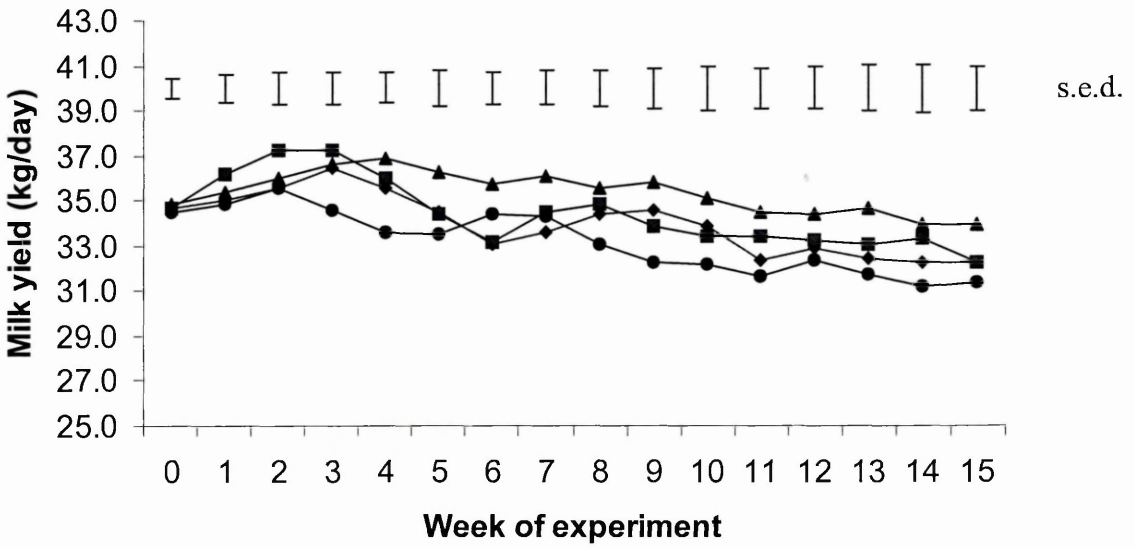


Figure 4.1. Weekly milk yield (kg/day) of cows fed maize silage supplemented with wheat (♦) or whole-crop wheat (WCW) supplemented with wheat (■), lactose (▲) or molasses (●).

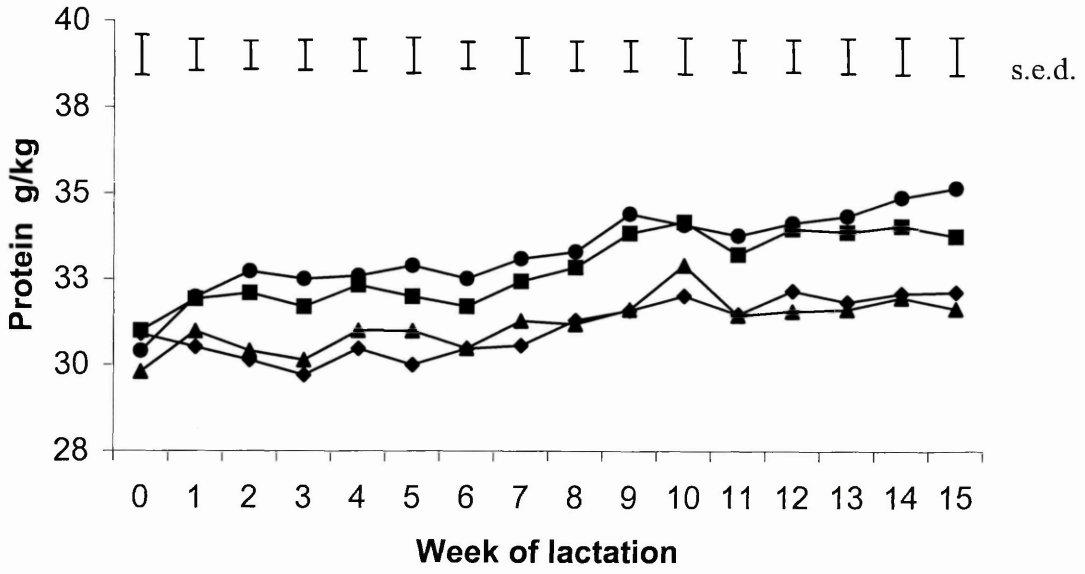


Figure 4.2. Weekly milk protein content (g/kg) of cows fed maize silage supplemented with wheat (♦) or whole-crop wheat (WCW) supplemented with wheat (■), lactose (▲) or molasses (●).

### 4.3.3. Dry matter intake

The intake of concentrates, forage and total DM are presented in Table 4.6. Intake of dairy concentrates was similar between treatments with an average value of 5.5 kg DM/day. Total concentrate intake was also not significantly affected by dietary treatment, averaging 8.9 kg DM/day.

Table 4.6. Average intakes (kg DM/day) of concentrates and forage of cows fed maize silage supplemented with wheat or whole-crop wheat (WCW) supplemented with wheat (W), lactose (L) or molasses (M).

| | Diets | | | | | Significance |
|---|---|---|---|---|---|---|
| | Maize | W-WCW | L-WCW | M-WCW | s.e.d. | |
| †OPF concentrates | 5.5 | 5.4 | 5.5 | 5.4 | 0.097 | NS |
| Total concentrates | 9.2 | 8.9 | 8.8 | 8.6 | 0.24 | NS |
| Grass silage | 3.6 | 4.4 | 4.0 | 4.8 | 0.22 | *** |
| Test forage | 7.1 | 8.9 | 8.2 | 9.8 | 0.46 | *** |
| Total Forage | 10.7 | 13.3 | 12.2 | 14.6 | 0.68 | *** |
| Total DMI | 19.9 | 22.2 | 21.0 | 23.2 | 0.88 | ** |

†OPF = out of parlour

Within supplement type to WCW, forage intake was highest for animals receiving M-WCW and lowest for animals receiving L-WCW (14.6 kg DM/day and 12.2 kg DM/day respectively, $P$ <0.001). This difference was reflected in total DM intake with animals receiving M-WCW having the highest recorded total DM intake of 23.2 kg DM/day and those receiving L-WCW the lowest at 21.0 kg DM/day ($P$ <0.001). Forage intake was higher for animals receiving W-WCW compared to the Maize treatment. This pattern in forage DM intake was reflected in total DM intake with animals receiving W-WCW having a higher total DM intake than those receiving Maize (mean of 19.9 *vs.* 22.2 kg DM/day respectively $P$ <0.01).

### 4.3.4. Blood metabolites

Average plasma glucose content was not significantly different in animals fed any of the treatments, with an average value of 3.57 mmol/l (Table 4.7). There was also no effect of treatment on plasma BHB, with an average value of 0.58 mmol/l. By contrast, animals receiving M-WCW had higher ($P$ <0.05) plasma NEFA concentration (0.42 mmol/l), compared to cows receiving W-WCW (0.32 mmol/l). This difference became apparent after week 3 of the experiment and remained evident for the remainder of the experimental period (Figure 4.3). There was no significant difference between animals fed Maize or W-WCW in plasma NEFA concentration.

Table 4.7. Average plasma concentrations of glucose, ß-hydroxybutyrate (BHB), non esterified fatty acids (NEFA), urea total protein and albumin in cows fed maize silage supplemented with wheat or whole-crop wheat (WCW) supplemented with wheat (W), lactose (L) or molasses (M)

| | Diets | | | | | Significance |
|---|---|---|---|---|---|---|
| | Maize | W-WCW | L-WCW | M-WCW | s.e.d. | |
| Glucose (mmol/l) | 3.47 | 3.54 | 3.68 | 3.59 | 0.117 | NS |
| BHB (mmol/l) | 0.59 | 0.55 | 0.58 | 0.60 | 0.034 | NS |
| NEFA (mmol/l) | 0.33 | 0.32 | 0.38 | 0.42 | 0.029 | * |
| Urea (mmol/l) | 6.11 | 5.94 | 5.68 | 4.97 | 0.314 | * |
| Albumin (g/l) | 33.8 | 33.3 | 32.4 | 33.5 | 1.48 | NS |
| Total protein (g/l) | 80.3 | 81.6 | 83.6 | 81.2 | 3.60 | NS |

Within WCW treatments, supplementation with molasses resulted in a lower plasma urea level of 4.97 mmol/l compared to supplementation with wheat or lactose (means 5.94 and 5.68 mol/l respectively, $P$ <0.05). This difference was already evident at week 3 of the experiment (Figure 4.4) and molasses supplemented cows consistently had a lower plasma urea level for the remainder of the experimental period. No effect of treatment was found on plasma albumin or total protein concentrations, with mean values across dietary treatments of 33.2 and 81.7 mmol/l respectively.

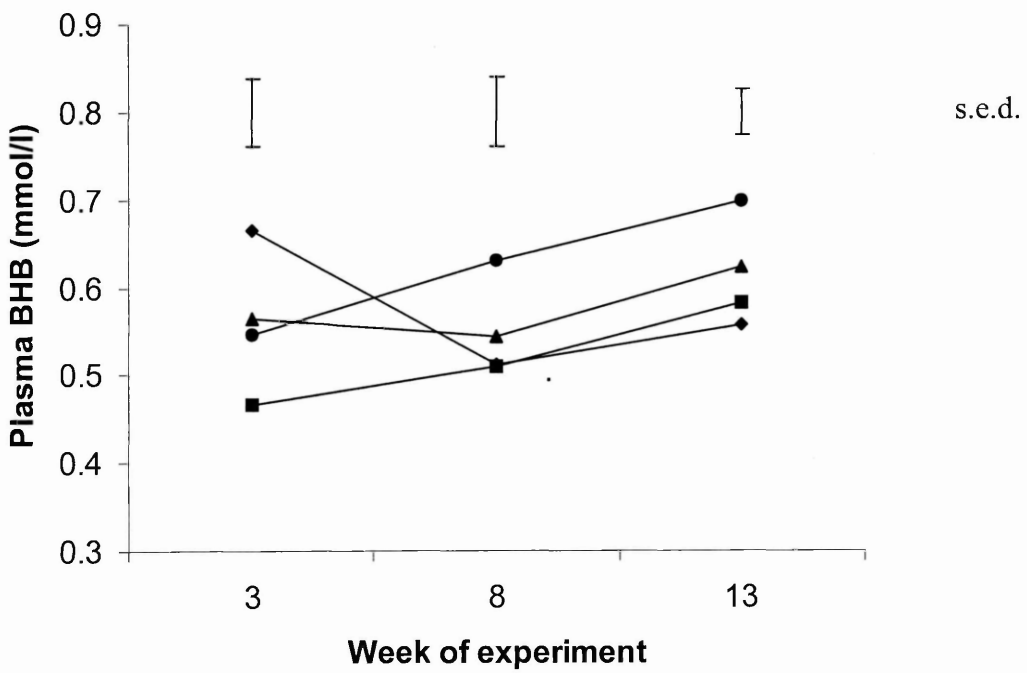


Figure 4.3. Weekly plasma BHB content of cows fed maize silage supplemented with wheat (♦) or whole-crop wheat (WCW) supplemented with wheat (■), lactose (▲) or molasses (●).

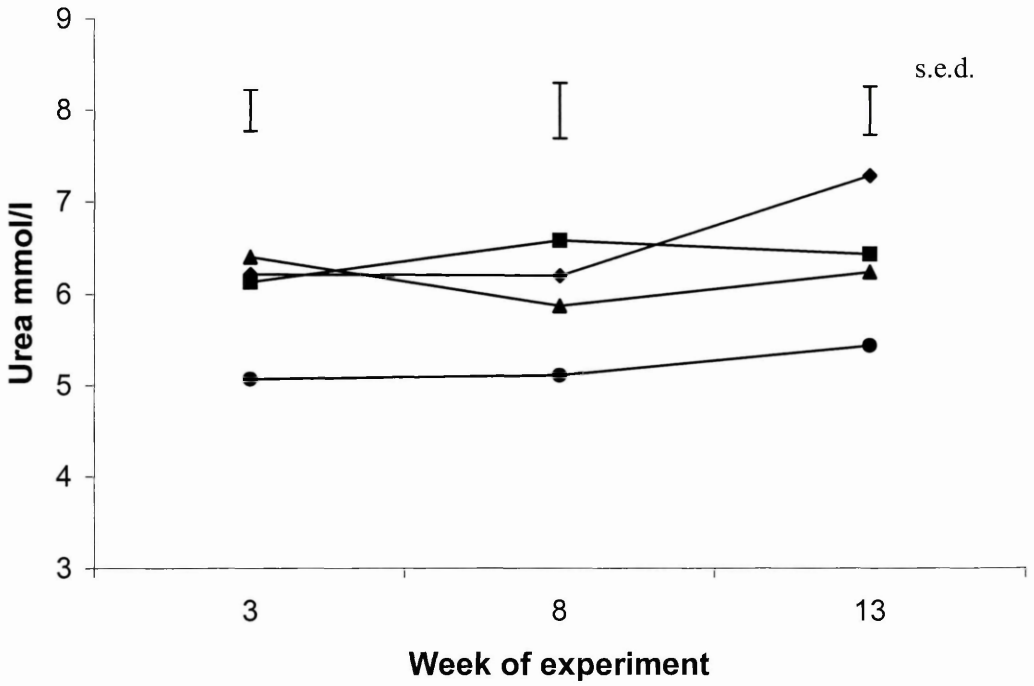


Figure 4.4. Weekly plasma urea content of cows fed maize silage supplemented with wheat (♦) or whole-crop wheat (WCW) supplemented with wheat (■), lactose (▲) or molasses (●).

## 4.4. Discussion

### 4.4.1. Forage analysis

The DM content of the whole-crop wheat, at 823 g DM/kg was comparable to that used by Abdalla *et al.* (1999) of 761 g DM/kg and the 813 g DM/kg reported by Sutton *et al.* (1998), but higher than the 550-700 g DM/kg reported in the majority of other experiments (Pullar, 1992; Sutton *et al.*, 1997; Hill and Leaver, 1999b). The discrepancy in DM contents between the current experiment, and the majority of previously published work, may be attributable to the higher DM's at which it is now possible to harvest urea-treated WCW without the penalty of reduced starch digestibility, as was demonstrated in Experiment 1.

The WCW forage had a starch content of 350 g/kg DM which is lower than the mean value of 387 g/kg DM achieved in Experiment 1, but higher than that reported by Sutton *et al.* (2002) of 306 g/kg DM or the 328 g/kg DM used by Sutton *et al.* (2001). The forage used by Sutton *et al.* (2001;2002) was harvested at 584 g DM/kg and 600 g DM/kg respectively and as has been discussed earlier (Chapter 1), forage starch levels increase with crop growth stage and the discrepancy between the results of the current work and those of Sutton *et al.* (2001:2002) is likely a reflection of this. The WCW NDF content was intermediate between the short straw and long straw of the first experiment (410 g/kg DM), and was similar to that used by Hill and Leaver (1999b), (403 g/kg DM) for material harvested at a cutting height of 7 cm. This cutting height is, however, substantially lower than that of the current experiment (32 cm) and one may have expected that forage NDF levels would be higher for material harvested at a lower stubble height. The similarities in forage NDF content, despite the discrepancy in forage cutting height, may potentially be attributable to the differing starch contents of the forage impacting on forage NDF content. The starch content reported by Hill and Leaver (1999b) was 262 g/kg DM whereas that

recorded for the current experiment was 350 g/kg DM. Decreases in forage NDF levels observed when stage of maturity at harvest increased have previously been ascribed to this effect, namely when starch levels in the forage increase, there is a corresponding decrease in fibre content (Arieli and Adin, 1994).

Forage ADF content, at 237 g/kg DM, was lower than the 334 g/kg DM reported by Sutton *et al.* (1998), but comparable to the 219 g/kg DM used by Hill and Leaver (1999b) despite the previously discussed differences in cutting height. It is possible that the differences in starch content between those reported by Hill and Leaver (1999b) and those of the current experiment may have affected forage ADF as well as NDF content. Ammonia nitrogen levels were lower than those recorded in the Experiment 1 at 165 g/kg N and lower than the 183 g/kg N reported by Sutton *et al.* (1998) for material receiving 20 kg/t DM of urea they were, however, intermediary between the 134 and 206 g/kg N reported by Abdalla *et al.* (1999) for WCW treated with 20 and 40 kg urea/t DM respectively. Crude protein content was similar to that observed in Experiment 1 but lower than the 158 g/kg DM reported by Sutton *et al.* (1998) for material preserved with 20 kg urea/t DM. As has been mentioned earlier (Chapter 3), the application of urea to WCW can result in inconsistent results and in addition, the crude protein content of the initial forage (i.e. pre urea application) will impact on the crude protein content of the urea-treated forage. The factors affecting the crude protein level of wheat as a growing crop include nitrogen application as well as variety and seasonal variation (see Chapter 1). It is possible that one or more of these factors may have resulted in the lower crude protein content observed in this experiment. The WCW forage pH, at pH 8.0 was higher than that achieved in Experiment 1, but within the range reported by Abdalla *et al.* (1996) for forages treated with 20 and 40 kg/t DM urea.

The maize silage DM (310 g DM/kg) was higher than that used in the work of Hameleers (1998) (275 g DM/kg) for maize grown in south west Scotland but lower than that reported by Phipps *et al.* (1995) (354 g DM/kg) grown in southern England. The starch content of the maize silage followed similar tendencies, being within the range reported by Phipps *et al.* (2001) for maize grown in the south of England, but higher than 256 g/kg DM reported by Hameleers (1998) for maize grown in south west Scotland where maize growth is marginal. The NDF content of the maize silage used here was higher than that reported by Mulligan *et al.* (2002) for material harvested at 339 g DM/kg and grown in southern Ireland but lower than that of Phipps *et al.* (1995) for maize harvested at 354 g DM/kg and grown in southern England. These results support the view of Phipps (1994) who stated that growing conditions can have major effects on the quality of the resultant silage, and that producers contemplating growing maize in areas where its growth is marginal need to carefully consider the feasibility of producing good quality maize silage. Ammonia-N content of the maize silage (22 g/kg N) although similar to the 37 g/kg N of Hameleers (1998) was considerably lower than that reported by Phipps *et al.* (2001), or Phipps *et al.* (1995), of 85 g/kg N, indicating that the forage was well preserved (Phipps *et al.,* 1995). Forage crude protein content was lower than the 81 g/kg DM reported by Phipps *et al.* (1995) but similar to the immature forage (crude protein content of 77 g/kg DM) used by Phipps *et al.* (2001). Maize silage pH was, however, higher at pH 4.2 than the 3.8 reported by Phipps *et al.* (1995) or the pH 4.0 of Mulligan *et al.* (2002).

### 4.4.2. Effect of supplement type

Supplement type to whole-crop wheat had no significant effect on milk yield (kg/day). The tendency for lactose to promote a higher milk yield is, however, in contrast to the findings of Allison and Garnsworthy (1997) who reported that the addition of lactose to maize silage based diets had no significant effect on milk yield. It may be that lactose, as

suggested by Abdalla *et al.* (1999), resulted in a more efficient microbial protein production as, due to its rapid rate of degradation in the rumen (Chamberlain and Choung, 1995) it may provide the ruminal microorganisms with a more synchronous rate of release of energy and protein supply. A more synchronous release of N and energy in the rumen has been reported to improve microbial efficiency in some (Sinclair *et al.*, 1993; 1995) but not all studies (Richardson *et al.*, 2003). The higher N efficiency (calculated mean values of 0.235, 0.224, 0.236 and 0.215 milk N output/kg N intake for treatments Maize, W-WCW, L-WCW and M-WCW respectively, s.e.d. 0.0158) for animals receiving lactose supplemented WCW compared to those receiving molasses or wheat, tend to support this conclusion. It is interesting to note that supplementation with molasses resulted in a decrease in milk yield compared to either wheat or molasses, but increased milk protein content. Sutton *et al.* (2001) found no effect on milk yield but a decrease in milk protein content when unprocessed WCW diets were supplemented with molasses. It was suggested by Sutton *et al.* (2001) that the decrease in milk protein content associated with molasses supplementation may be a result of the decreased starch intakes in these diets. The molasses supplemented treatment also resulted in the highest milk fat content. This is in contrast with the findings of Sutton *et al.* (2001) who found a slight, but non-significant decrease in milk fat content when unprocessed WCW was supplemented with molasses.

The increase in forage and total DM intake observed when WCW was supplemented with molasses is in agreement with that of Sutton *et al.* (2001) and Murphy (1999). The reduction in DM intake when WCW based diets were supplemented with lactose is, however, in contrast to Allison and Garnsworthy (1997), who reported an increase in DM intake when lactose was fed. An increase in DMI, as a result of feeding lactose has also been reported by DeFrain *et al.* (2004). However, other authors have reported no effect on DM intake when cows were fed diets containing lactose (Doreau *et al.*, 1987; Maiga *et al.*, 1995). Aside from the current work, there is no published work on the effects of

supplementation of processed WCW with lactose, and it may be possible that the variation attributable to lactose supplementation may be a result of differences in the base forage diet, rather than a variation in the response of cows to lactose. Indeed work by Doreau *et al.* (1987) utilised diets based predominantly on grass hay, whereas that by DeFrain *et al.* (2004) used a mixture of corn silage, alfalfa hay and grass hay and work by Allison and Garnsworthy (1997) incorporated a mixture of grass and maize silage. Supplement type had no significant effect in the current work on either live weight change or body condition score, a finding in accordance with other experiments examining supplementation strategies for WCW diets (Hill and Leaver, 1999b; Sinclair *et al.*, 2003).

Animals receiving molasses had higher plasma NEFA levels than those receiving either wheat or lactose, indicating an increased level of body tissue mobilisation (Nachtomi *et al.*, 1991). The discrepancy between plasma NEFA and BHB levels, which were not significantly different between treatments when diets were supplemented with molasses, indicates that the increase in NEFA may not be wholly dietary related. It has been shown that plasma NEFA levels do not always increase when energy intakes are reduced, particularly when the diet contains a high proportion of cereals (Topps and Thompson, 1984). Additional non-nutritional factors have been observed to have effects on NEFA including excitement at handling (Holmes and Lambourne, 1970). This, when examined in conjunction with the plasma glucose, liveweight and body condition results, indicates that cows were at a similar level of energy status.

It has been suggested that the supplementation of WCW diets with a readily available sugar source may increase animal performance through improved capture of rumen ammonia, the levels of which are increased when WCW diets are fed (Abdalla *et al.*, 1999). Both lactose and molasses are rapidly fermented in the rumen (Czerkawski and Breckenridge, 1969). Plasma urea is mainly produced from protein digestion within the

rumen, at a level at which dietary energy supplies are insufficient to allow the rumen micro organisms to capture all of the ammonia produced (Nocek and Russell, 1988). Blood urea is therefore used as an indicator of an animal's rumen digestible crude protein intake (Manston, 1975). Plasma urea levels were lowest for animals receiving M-WCW, indicating that this supplement was most effective in rumen ammonia capture (Nocek and Russell, 1988). However, caution should be exercised in interpreting these results, as more recent results indicate plasma urea levels have been shown to be a poor indicator of ruminal ammonia levels (Richardson *et al.,* 2003).

### 4.4.3. Maize silage versus urea-treated whole-crop wheat

Cows fed processed urea-treated whole-crop wheat had a similar milk yield to those fed diets containing maize silage. This is in accordance with the findings of Hameleers (1998) who found no difference in milk yield between animals fed maize silage or unprocessed urea-treated whole-crop wheat. However the maize used in the study of Hameleers (1998) was less mature than that in the current study. It has been shown that feeding more mature maize silages (optimum DM 300 g DM/kg) promotes a higher milk yield than lower maturity maize silages (Phipps *et al.*, 2001). Phipps *et al.* (2001) concluded that the optimal DM at harvest for maize silage was approximately 300 g DM/kg, a value comparable to the 310 g DM/kg reported here.

Animals fed maize silage tended to have a higher milk fat content than those receiving processed urea-treated WCW. The maize silage used in the current experiment had a lower starch content (308 g/kg DM) compared to the whole-crop wheat silage (350 g/kg DM) and this coupled with the decreased intakes observed in cows fed maize silage forage, would have resulted in a lower starch supply to the rumen compared to those receiving WCW. In addition the degradability of maize starch is somewhat lower than that reported for wheat

starch and would have further reduced ruminal starch supply (Cone *et al.,* 1989). The changes in starch supply may, therefore, explain the differences in milk fat between the two treatments as milk fat has been shown to be negatively related to starch levels in the diet (Reynolds *et al.*, 1997). This effect was clearly demonstrated by Sutton *et al.* (1977) who reported a clear decrease in milk fat when the level of starchy concentrates in the diet of lactating dairy cows was increased. The reason behind the lower intakes observed in cows fed maize silage compared to that of WCW may not be a case of the maize silage promoting a lower DMI but rather that, in common with a number of experiments with unprocessed WCW (Leaver and Hill, 1995; Sutton *et al.*, 1997) that the processed WCW promoted a higher DMI.

No difference in live weight change in cows fed either maize silage and WCW was observed. This finding is in agreement with Hameleers (1998), but in contrast to that of Phipps *et al.* (1995), who observed a negative effect on live weight change as a result of the inclusion of unprocessed urea-treated WCW compared with maize silage. As the material used by Phipps *et al.* (1995) was more mature, it would be expected that the effect on live weight change may be more pronounced than those obtained using the relatively immature maize of Hameleers (1998). The negative effects observed by Phipps *et al.* (1995) as a result of feeding unprocessed urea-treated whole-crop wheat compared to maize silage were attributed to the low digestibility of the WCW forage. The lack of any difference between the whole-crop wheat and maize silage in the current study is likely to be a reflection of the processing the whole-crop wheat received at harvest which was shown in Experiment 1 to improve forage digestibility.

Cows fed maize silage had marginally lower plasma glucose levels, possibly indicating a deficit in energy supply compared to those receiving W-WCW. It has been reported that plasma glucose is negatively correlated to dry matter intake (Topps and Thompson, 1984)

and would support the low DM intakes observed. Plasma BHB levels were also numerically elevated for cows fed maize, supporting the suggestion of a lower level of energy supply for animals fed maize silage.

## 4.5. Conclusions

Supplementing processed urea-treated WCW with lactose tended to increase milk yield compared with supplementation with wheat. Supplementation with molasses increased milk protein level but no significant difference was observed between supplement type to WCW on milk component yield. Feeding processed urea-treated WCW resulted in a similar level of performance to that achieved from good quality maize silage.

# 5.0. EXPERIMENT 3 EFFECT OF ADDITIVE AND CROP MATURITY ON THE AEROBIC STABILITY, CHEMICAL COMPOSITION AND DIGESTIBILITY OF PROCESSED WHOLE-CROP WHEAT.

## 5.1. Introduction

Earlier work has determined that, through the use of a forage mill at harvest in higher DM, urea-treated WCW, starch digestibility was increased (Experiment 1). This now enables a wider window for harvest of between 500-800 g DM/kg. It has been suggested that the enzyme urease is required to hydrolyse the added urea, as the level and efficiency of plant urease at high dry matters has been questioned (Tetlow, 1992). There is however, little evidence relating to the efficacy of naturally occurring urease at differing forage DM contents and to whether other forms of urea, such as liquid urea or urea prills would be as effective in the preservation of the crop. Indeed there may be benefits in adding liquid urea to the forage in potentially increasing the action of the endogenous urease, as this enzyme requires a small amount of water to be present in order to function (Sahnoune *et al.*, 1991). Bacterial inoculants and enzyme additives have also been used to preserve whole-crop wheat. Work has been carried out by a number of authors utilising *Lactobacillus bunchneri* to improve the aerobic stability of the crop (Weinberg *et al.*, 1999; Driehuis *et al.*, 1999; Adesogan and Selawu, 2004). However this work has generally been carried out on low dry matter material (280-430 g DM/kg), and little work has been conducted at the higher DM contents now possible.

The objectives of Experiment 3a were to determine the effects of three different forms of urea treatment, and one inoculant + enzyme treatment compared with a negative control (i.e. no additive) at two stages of forage maturity on the chemical composition and aerobic stability of processed WCW.

The objectives of Experiment 3b were to determine the effects of two forms of urea treatment and a negative control (no additive) at three stages of forage maturity on the chemical composition, aerobic stability, microbiological composition, intake characteristics and apparent digestibility determined in sheep.

## 5.2. Experiment 3a Materials and Method

### 5.2.1. Crop production

A commercially managed crop of winter wheat (*cv* Equinox) was used. The crop was the same as that used in Experiment 2.

### 5.2.1. Experimental routine

*5.2.1.1. Harvesting procedure*

The crop was harvested at two stages of maturity at target dry matters of 600 g DM/kg and 800 g DM/kg on 31 July 2001 and 17 August 2001 respectively. The mean stubble heights were 35.6 cm (3.97) and 32.0 cm (1.12) on the 31 July and 17 August and the pre harvest crop height was 70.3 cm (2.47). The crop was processed at harvest using a self propelled forage harvester (Claas Jaguar 800 series, Claas, Bury St. Edmunds, UK). For each stage of maturity the treatments consisted of no additive (C), three forms of urea; feed grade urea prills (PU, Barker Hickman, Shifnal, UK), urea + urease enzyme (U+ 'Home 'n' Dry', Volac, Royston, UK) or liquid urea (LU 68.5 l/t DM, 0.438 kg urea/l), or an enzyme + inoculant (Inoc) product (*Lactobacillus bunchneri* and β-gluctanase, xylanase and glucformelanase, Whole Crop Gold Mill, Biotal, Cardiff) applied at 3 litres/t fresh weight. All sources of urea were applied at a rate to provide 30 kg urea/t DM WCW.

Each of the forage treatments was ensiled in four large plastic lined drum silos (80 litres capacity, 57 cm high, 45 cm diameter). The additives were applied by hand (using a knapsack sprayer to apply the liquid urea and the enzyme + inoculant) and the crop mixed thoroughly with a shovel to ensure accurate mixing. Silos were filled to within 10 cm of the top of the silo and compacted manually. The filled silos then were sealed, weighed

down with sand (to a mean depth of 8.5 cm), and remained sealed for a period of 100 days. Upon opening, two samples were taken. One sample was frozen at –20°C for subsequent analysis and the second sample used immediately for aerobic stability determination (as described in section 5.2.1.2). In addition small scale silos were made (28 litres capacity, 50 cm high x 30 cm diameter) for each of the urea treatments for the determination of the hydrolysis of urea. Silos were filled to within 10 cm of the top of the silo and manually compacted. All silos were weighed down with sand (mean depth of 6.5 cm). Duplicate silos were made at each time point and were opened at 4, 8, 12, 45 days after sealing. An additional sample was taken upon ensiling and used as the 0 time point.

*5.2.1.2 Aerobic stability.*

Approximately 40g fresh weight of forage was placed in a polystyrene box (3800cm$^2$ , internal diameter 190 x 125 x 160 mm) containing holes (2 each on the top, bottom and ends of the boxes, 5 on each side, diameter 1.5 cm) to allow passage of air into the silage. The samples (1 box per silo) were kept in a temperature controlled room at 15°C and the temperature of the forage taken daily at 17.00h using a digital thermometer (220 temperature meter, Jenway, Dunmow, Essex) until no further temperature increase was observed. Overall temperature change was calculated over a 21 day period by the addition of the temperature changes after room temperature was subtracted.

*5.2.1.3 Hydrolysis of urea*

Upon opening, sub samples were taken and stored at–20°C prior to analysis as described in section 5.4.1.2.

## 5.3. Experiment 3b Materials and Method

### 5.3.1. Crop production

A commercial crop of winter wheat (*cv* Consort) was grown on a sandy loam/sandy clay loam soil following potatoes. The crop was sown over a four week period from 25 September to 25 October 2001 at a target seed rate of 185 kg/ha. Seed was treated with a single purpose seed dressing (guazatine+triconazole). On 1 March 2002 the crop received 74 kg/ha of $K_2O$ applied as muriate of potash and received 200 kg N/ha applied as a split dressing: 48 kg N/ha on 22 March 2002, 86 kg N/ha on 8 April 2002 and 66 kg/ha on 1 May 2002. The crop also received routine manganese sprays of manganese tank mixed with crop protection products. A growth regulator (chlormequat) was applied on 3 April 2002.

Disease control consisted of a two spray programme: kresoxim-methyl+pyraclostrobin+epoxiconazole applied on 3 March 2002 and kresoxim-methyl+fenpropimorph and epoxicanazole+fenpropimorph applied as a tank mix on 20 April 2002. Weed control consisted of: isoproturon and bromoxynil+diflufenican+ioxynil applied as a tank mix on 26 March 2002 to control grass weeds and annual broad leaved weeds, and fluroxypur applied on 30 April 2002 to control cleavers. The crop was sprayed with deltamethrin against aphids on 26 March 2002. The DM content of the crop was monitored prior to harvest by taking samples twice weekly on a Monday and Thursday.

### 5.3.2. Harvesting procedure

Forage was harvested on 8 August 2002, 12 August 2002 and 17 August 2002 at three stages of maturity and target DM's of 600, 700 and 800 g DM/kg and at growth stages of

87, 91 and 92 respectively (Zadoks, *et al.,* 1974). The forages were processed at harvest using a forage harvester (Class Jaguar 800 series, Class, Bury St. Edmonds, UK). Mean pre-harvest crop height was 75.1 cm (2.39) and mean stubble heights for the forages were 29.44 cm (4.20), 30.64 cm (4.31) and 28.5 cm (3.34) respectively. On each cutting date three additive treatments were applied; a control (C; no additive) or one of two urea treatments which consisted of feed grade urea prills (PU; Barker Hickman, Shifnal, UK), or a urea + urease enzyme product (U+ 'Home 'n' Dry', Volac, Royston, UK). All urea based additives were applied to provide an equivalent of 30 kg urea/t DM of forage. This resulted in 9 dietary treatments (Table 5.1).

Table 5.1. Forage Treatments

| Treatment | Stage of maturity | Additive |
|---|---|---|
| 60C | 600 g DM/kg | none |
| 60U+ | 600 g DM/kg | feed grade urea |
| 60PU | 600 g DM/kg | urea+ urease |
| 70C | 700 g DM/kg | none |
| 70U+ | 700 g DM/kg | feed grade urea |
| 70PU | 700 g DM/kg | urea+ urease |
| 80C | 800 g DM/kg | none |
| 80U+ | 800 g DM/kg | feed grade urea |
| 80PU | 800 g DM/kg | urea+ urease |

The additives were mixed with the forage in a Keenan forage wagon (Compact 70) before being ensiled in triplicate in 1150 litre capacity wooden walled, plastic lined silos (1.2 x 0.7 x 0.8 metres). The silos were then sealed and weighed down with 75 kg sand, and remained sealed for a period of 100 days. Upon opening sub samples were taken for the determination of aerobic stability (as described in section 5.2.1.2) and chemical composition as detailed for Experiment 3a, and for the determination of microbiological composition as described in section 5.4.2.2.

### 5.3.3 Digestibility determination

*5.3.3.1. Animals*

Eighteen Suffolk cross wether lambs (35 kg (5.13)) were used. The animals were housed in individual slatted floor pens ($3m^2$) with continual access to water. The animals were randomly allocated, using an incomplete row and column design, to one of the nine dietary treatments resulting in 6 replicates per treatment. Treatment allocation is presented in Table 5.2. The experiment has three periods each having an adaptation period of 10 days (with the exception of period one which had an adaptation period of 14 days) and a sampling period of seven days.

Animals were offered fresh forage daily at 08.00h at a rate of 105 % of the previous periods calculated intake. During sampling periods refusals were recorded daily. Forages that were not urea-treated were mixed (by hand) with 30 g/kg DM of feed grade urea (dampened with 20 mls water to aid mixing) to balance the diets for crude protein content. During adaptation to the diets, feed refusals were recorded twice weekly on Tuesdays and Fridays. Faecal output was recorded by fitting the animals with collection harnesses that were emptied once daily at 09.30h. Faecal output was weighed and a subsample (10 %) frozen at $-20^0$C for subsequent analysis.

Table 5.2 Allocation of the nine dietary treatments

| | Sheep | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | 60C | 70U+ | 80PU | 80PU | 70U+ | 60C | 70C | 60PU | 80U+ | 80U+ | 70C | 60PU | 70PU | 80C | 60U+ | 70PU | 80C | 60U+ |
| 2 | 80U+ | 60PU | 70C | 60U+ | 80C | 70PU | 60U+ | 80C | 70PU | 60C | 80PU | 70U+ | 60C | 70U+ | 80PU | 80U+ | 60PU | 70C |
| 3 | 70PU | 80C | 60U+ | 70C | 60PU | 80U+ | 80PU | 70U+ | 60C | 70PU | 60U+ | 80C | 80U+ | 60PU | 70C | 60C | 70U+ | 80PU |

## 5.4. Chemical analysis

### 5.4.1. Experiment 3a

*5.4.1.1. Forage analysis*

Forages from the drum silos were analysed for nitrogen, ammonium nitrogen, pH, total ash, starch, water soluble carbohydrate, NDF and ADF by NRM laboratories, (Berkshire UK) using the methods described in Chapter 2. Time point silos were analysed for nitrogen, ammonia nitrogen and pH using the methods described in Chapter 2.

*5.4.1.2. Hydrolysis of urea*

Hydrolysis of urea was determined using the diacetyl method using thiosemicarbazide (Wootton, 1974). Urea was extracted from the forage according to the method of Makkar and Singh (1987). Exactly 0.1 ml of silage effluent (extracted from 1 g of silage with 100 mls of water and shaken for one hour using a laboratory shaker (KIKA Labourtechnik HS501 digital, Germany)), was mixed with 10 ml of distilled water in a 10 ml test tube and vortex mixed using a Fisher brand whirlmixer (Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK). From this, 1.0 ml of the solution was pipetted with 1.0 ml of distilled water into a 10 ml test tube. To the tube was added 2.0 ml of mixed colour reagent (made from mixing 67 ml of colour reagent stock A and 67 ml of colour reagent stock B and making the solution up to 1 litre with distilled water. Colour reagent stock A comprised 20 g of diacetly monoxime (Sigma Aldrich Company Ltd, Poole, Dorset) added to 1 litre of distilled water and the resultant solution filtered through a Whatman No. 1 (Whatman PLC, Maidstone, UK) filter paper. Colour reagent B comprised 5 g thiosemicarbazide (Sigma Aldrich Company Ltd, Poole, Dorset) in 1 litre of water). Exactly 2.0 ml of mixed acid reagent was then added to each tube (0.5 ml of solution C added to 1 litre of solution D, solution D comprises 5 g ferric chloride ($6H_20$) in approximately 20 ml of distilled

water. This was then transferred to a measuring cylinder and 100 ml of phosphoric acid (85 %) slowly added with swirling and the solution made up to 250 ml with distilled water. Solution D comprised 200 ml of concentrated sulphuric acid which was slowly and with cooling, added to 800 ml of water (contained in a 2 litre conical flask)). The test tubes were then thoroughly mixed on a vortex mixer (Fisherbrand Whirlmixer, Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK) before being placed in a boiling water bath for 20 minutes. Tubes were then allowed to cool in the dark before being read using a Beckman DU640 Spectrophotometer (Beckman Coulter (UK) Ltd, High Wycombe, Buckinghamshire, UK) at 520 nm. The testing procedure incorporated a blank test consisting of 2.0 ml of distilled water in place of the sample/water mix and a standard consisting of 200 mg/100 ml aqueous urea solution (2 g GPR grade urea/litre, VWR International Ltd, Poole, UK), and treated as the samples. Urea contained within the effluent was calculated according to the following equation;

$$\text{Effluent urea (mg/100ml)} = \frac{T-B}{S-B} \times 200$$

Where T represents the sample absorbance, B the blank absorbance and S the standard absorbance.

The amount of urea remaining in the silage was calculated according to the following equation;

$$\text{Urea (mg/g forage DM)} = \frac{\text{effluent urea}}{\text{weight of forage used}} \times \frac{(\text{forage DM})}{1000}$$

### 5.4.2. Experiment 3b

#### *5.4.2.1. Forage and faecal sample analysis*

Forages were analysed for nitrogen, ammonium nitrogen, pH, organic matter, starch, oil, water soluble carbohydrate, NDF and ADF using the methods described in Chapter 2. Faecal samples were dried at $60^0$C for 24 hrs before being ground using a Cyclotec 1024 mill (Foss UK Ltd, Warrington, UK) and ground to pass through a 1mm sieve. Samples were then bulked over the sampling period to provide one sample per animal per sampling period and analysed for ash, starch, and NDF as described in Chapter 2.

Metabolisable energy was estimated for the WCW forages according to the following equation;

ME (MJ/kg DM) = 0.01547 [DOMD]

Where DOMD is as g/kg DM (AFRC, 1995)

#### *5.4.2.2. Yeast and mould levels*

The microbiological composition of the forages was determined according to an adapted method of Woolford *et al.* (1982). For each silo, 10 g fresh weight of forage was shaken with 100 mls of distilled water for 10 minutes on a laboratory shaker (KIKA Labourtechnik HS501 digital, Germany). From the resultant effluent, ten-fold solutions were made (as described by Nickin *et al.,* 1998) using quarter strength Ringers solution (Oxoid Ringers solution; Oxoid Ltd, Basingstoke, Hants, UK product Number BR0052G, 1 tablet in 500 ml distilled water and sterilized by autoclaving at 121°C for 15 minutes). Selected dilutions (0.1 ml) were plated in duplicate onto pour plates of oxytetracycline agar (LABM oxytetracyline glucose yeast extract, LABM Ltd, Bury, Lancashire, UK product number LAB 89). These were made by adding 37 g of agar to 1L deionised $H_2O$. The

mixture was allowed to soak for 10 minutes and then swirled to mix. The agar was sterilized by autoclaving at 115°C for 10 minutes. The agar was then cooled aseptically to 47°C and 2 vials (previously reconstituted in 5 ml sterile deionised water) of supplement (product number X089, containing 50 mg oxytetracycline/vial, LABM Ltd, Bury, Lancashire, UK) added. Plates were poured aseptically and allowed to set before use. The plates were incubated at 30 °C for five days and those containing not less than 50 but no greater than 100 colonies were then counted manually.

## 5.5. Statistical analysis

### 5.5.1. Experiment 3a

Chemical composition results were analysed as a 5 (preservative) x 2 (cutting date) factorial design, with means and standard error of the difference (s.e.d) presented. Hydrolysis of urea results were analysed as a 3 (preservative) x 2 (cutting date) factorial design and aerobic stability results were analysed using a generalised linear model with a Poisson distribution with a log link. All analysis was conducted by using Genstat 5 (VSN Int. Ltd, Oxford, UK). Significance is denoted in tables as NS for $P > 0.05$, * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$.

### 5.5.2. Experiment 3b

Chemical composition results were analysed using a 3 (preservative) x 3 (cutting date) factorial design using Genstat 5 (VSN Int. Ltd, Oxford, UK). Aerobic stability and yeast count data was analysed using a generalised linear model with a Poisson distribution with a log link. Lamb intakes and digestibility results were analysed using an incomplete row and

column design using Genstat 5 (VSN Int. Ltd, Oxford, UK). Significance is denoted in tables as NS for $P > 0.05$, * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$.

## 5.6. Results

### 5.6.1. Experiment 3a

#### *5.6.1.1. Chemical composition*

The chemical composition of the forages is provided in Table 5.3. Within the two stages of maturity DM content was not significantly different between treatments. However, there was an interaction ($P$ <0.01) between treatment and ensiling on DM content; when harvested at the earlier maturity, C and PU had the lowest DM content (558 and 547 g DM/kg respectively) but the highest values at the later maturity (833 and 821 g DM/kg respectively).

Crop maturity had an effect on forage crude protein content with the more mature, higher dry matter material having a lower crude protein content than the lower DM material ($P$ <0.01, mean values of 155 *vs.* 167 g/kg DM respectively). Urea treatment (U+, LU and PU) increased ($P$ <0.001) the crude protein content of the resultant forage, (average of 206 g/kg DM for material harvested at 600 g DM/kg and an average of 179 g/kg DM for material harvested at 800 g DM/kg) compared to forage receiving the inoculant or the control (mean values across harvest dates of 109 and 107 g/kg DM respectively).

Forage organic matter content was not ($P$ >0.05) affected by forage treatment but was affected by crop maturity ($P$ <0.001), with material harvested at 600 g DM/kg having a lower organic matter content than material harvested at 800 g DM/kg. Crop maturity also had an effect ($P$ <0.001) on ammonia nitrogen (% total nitrogen), being higher for material harvested at 800 g DM/kg (mean of 13.9 % total N) than for material harvested at 600 g DM/kg (mean of 7.9 % total N, $P$ <0.001).

Table 5.3 Experiment 3a. Effects of additive application on the nutritive value of whole-crop wheat harvested at two stages of maturity (g/kg DM unless otherwise stated)

| | 600 g DM/kg | | | | | 800 g DM/kg | | | | | | Sign. of main effects† | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C‡ | Inoc | U+ | LU | PU | C | Inoc | U+ | LU | PU | s.e.d. | M | A | M x A |
| Dry matter (g/kg) | 558 | 562 | 574 | 567 | 547 | 833 | 812 | 816 | 796 | 821 | 8.3 | *** | NS | ** |
| CP | 109 | 106 | 204 | 200 | 215 | 105 | 112 | 183 | 163 | 210 | 12.2 | * | *** | NS |
| $NH_3N$ (% total N) | 2.0 | 1.5 | 25.0 | 5.3 | 5.3 | 1.3 | 1.0 | 31.5 | 12.7 | 22.8 | 13.91 | *** | *** | *** |
| OM | 962 | 963 | 961 | 964 | 961 | 970 | 969 | 972 | 972 | 974 | 3.9 | *** | NS | NS |
| Sugars | 18.0 | 18.3 | 11.0 | 17.1 | 10.8 | 12.0 | 9.8 | 9.5 | 9.8 | 11.5 | 3.92 | ** | NS | NS |
| NDF | 386 | 413 | 377 | 423 | 426 | 407 | 389 | 352 | 436 | 452 | 18.7 | NS | *** | NS |
| ADF | 182 | 184 | 191 | 190 | 187 | 182 | 153 | 167 | 167 | 166 | 12.5 | ** | NS | NS |
| Starch | 318 | 293 | 263 | 265 | 274 | 383 | 403 | 401 | 363 | 342 | 19.7 | *** | * | NS |
| pH | 3.9 | 3.9 | 8.5 | 4.2 | 4.1 | 6.7 | 6.6 | 9.1 | 8.8 | 8.7 | 0.96 | *** | *** | *** |
| Neutral cellulase digestibility (g/kg) | 732 | 703 | 718 | 758 | 763 | 675 | 719 | 761 | 698 | 738 | 18.7 | NS | ** | ** |

†M = effects of stage of maturity, A = effects of additive, and MxA = interaction between stage of maturity and additive
‡C = Negative control, Inoc = Enzyme + inoculant treatment, U+ = urea + urease additive, PU = prilled urea, LU = liquid urea

Additive application was also observed to affect forage ammonia N with the application of a urea based additive promoting a higher ammonia-N level (mean 17.1 % total N) compared to the negative control or inoculated forages (1.45 % total N, $P$ <0.001). A significant interaction was observed between additive and stage of maturity on ammonia nitrogen content. Ammonia-N levels were low in treatments LU and PU when ensiled at 600 g DM/kg but high when ensiled at 800 g DM/kg.

There was an effect ($P$ <0.001) of both additive and stage of maturity on forage pH. Forages harvested at 600 g DM/kg had a lower pH (mean 4.92) compared to forages harvested at 800 g DM/kg (mean pH value 7.98). The application of urea based additives promoted a higher pH (mean across both harvest dates of pH 7.2) compared to forages receiving no additive or treated with the inoculant additive (mean value of pH 5.3). There was an interaction between additive and stage of maturity with forages harvested at 600 g DM/kg and treated with the LU and PU having a low pH but when harvested at 800 g DM/kg these forages had a higher value.

The lowest NDF content for material harvested at either stage of maturity was for treatment U+. Forage NDF content was significantly affected by additive, with PU having the highest NDF content (average 439 g/kg DM) at both harvest dates. Forage ADF content was affected by stage of maturity with material harvested at 600 g DM/kg having a higher ADF content (average 187 g/kg DM) than material harvested at 800 g DM/kg (167 g/kg DM) with no effect observed as a result of additive application.

A significant effect was observed due to forage maturity on starch content with material harvested at 600 g DM/kg having a lower starch content (average 286 g/kg DM) than material harvested at 800 g DM/kg (average 381 g/kg DM, $P$ < 0.001). There was an effect ($P$ <0.01) of additive on starch content with forages receiving a urea-based additive having

a lower starch content (mean value 321 g/kg DM across both harvest dates) compared to those receiving either no additive or an inoculant (mean value 349 g/kg DM across both harvest dates).

Increasing crop maturity resulted in a significant decrease in sugar content with material harvested at 800 g DM/kg having a lower sugar content (average 10.5 g/kg DM) than forages harvested at 600 g DM/kg (15.1 g/kg DM). There was an interaction between additive and crop maturity on NCD content, with forages receiving PU having the highest value of 763 g/kg DM when harvested at 600 g DM/kg, with the highest NCD for forage harvested at 800 g DM/kg being for forage receiving U+ (761 g/kg DM) ($P$ <0.05).

*5.6.1.2. Hydrolysis of urea*

The effects of form of urea additive on the amounts of urea remaining unhydrolysed within the forage are presented in Figure 5.1 and 5.2. A greater initial level of hydrolysis was observed in forages harvested at approximately 600 g DM/kg ($P$ <0.01). Similar differences as a result of maturity were also observed at time points 4 and 12 days ($P$ <0.001 and $P$ <0.05 respectively). However, no difference in urea hydrolysis was observed at 45 days post ensiling. Source of urea had an effect on urea hydrolysis with prilled urea having the lowest initial level at day 0 ($P$ <0.05, s.e.d. 45.1) for both stages of maturity with the highest levels being for the LU additive. At day 4 and day 12 time points the lowest level of unhydrolysed urea was observed for the U+ additive at both stages of maturity ($P$ <0.01 and $P$<0.05 respectively, s.e.d.'s 6.71 and 18.04 respectively). No significant effect of stage of maturity or form of urea was observed at the 45 day time point.

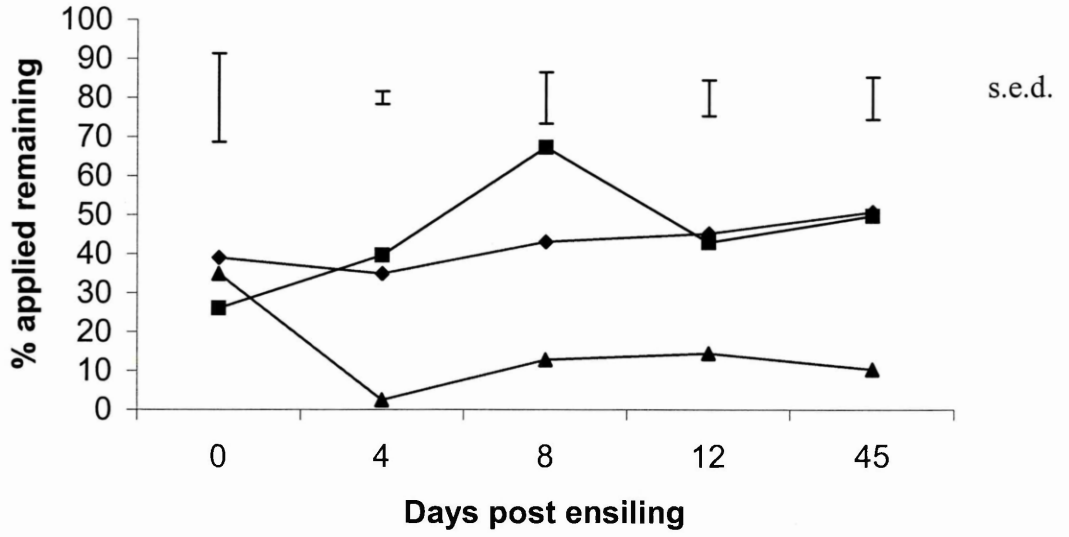


Figure 5.1 Effects of form of urea additive on the levels of unhydrolysed urea for material harvested at 600 g DM/kg and treated with liquid urea (LU, ♦), prilled urea (PU, ■) or urea + urease (U+, ▲).

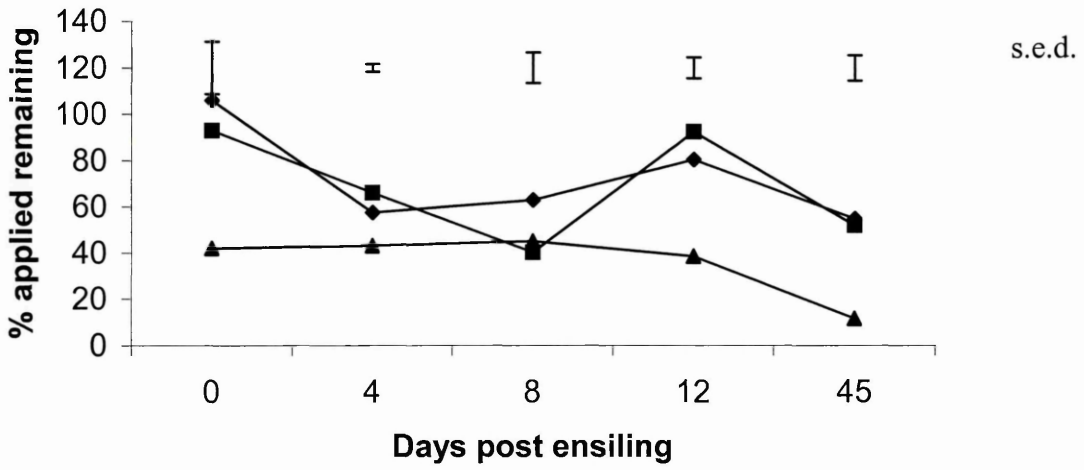


Figure 5.2. Effects of form of urea additive on the levels of unhydrolysed urea for material harvested at 800 g DM/kg and treated with liquid urea (LU, ♦), prilled urea (PU, ■) or urea + urease (U+, ▲).

*5.6.1.3. Aerobic stability*

The effect of stage of maturity and additive on the overall temperature change (calculated by the addition of the temperatures above room temperature over the 21 day exposure period) when forages were exposed to air is presented in Table 5.4. There was no effect of stage of maturity on overall temperature change but there was an effect of additive (*P*

<0.05) with forages receiving any of the four additive treatments having a lower overall temperature change than the control.

Table 5.4 Experiment 3a Effect of additive on overall temperature change (OTC) of whole-crop wheat harvested at two stages of maturity

| | 600 g DM/kg | | | | | 800 g DM/kg | | | | | | Significance† | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C‡ | Inoc | U+ | LU | PU | C | Inoc | U+ | LU | PU | s.e.d. | M | A | MxA |
| OTC§°C | 4.0 | 1.7 | 1.5 | 2.8 | 1.9 | 4.0 | 1.6 | 1.6 | 2.8 | 1.8 | 1.19 | NS | * | NS |

† M = effects of stage of maturity, A = effects of additive and MxA = interaction between stage of maturity and additive
‡C = control, Inoc = Enzyme + inoculant treatment, U+ = urea + urease additive, PU = prilled urea, LU = liquid urea
§ calculated over a 21 day period by the addition of the temperature changes after room temperature was subtracted

### 5.6.2. Experiment 3b

#### *5.6.2.1. Crop yield*

Forage yields were 14.9 (0.82), 15.5 (0.68) and 14.2 (0.60) t DM/ha for material harvested at 600, 700 and 800 g DM/kg respectively, with mean stubble heights of 29.0 cm (4.20), 30.5 cm (4.31) and 28.5 cm (4.57).

#### *5.6.2.2. Chemical composition*

The chemical composition of the forages is presented in Table 5.5. Forage DM increased with harvest date with mean values of 605, 709 and 813 g DM/kg ($P$ <0.001). Crude protein content was affected by stage of maturity being higher for the material harvested at 600 g DM/kg than that harvested at 800 g DM/kg (means of 207 and 145 g/kg DM respectively, $P$ <0.001). Forages receiving a urea based additive had a higher ($P$ <0.001) crude protein content (mean 191 g/kg DM) compared to the control (mean 131 g/kg DM). An interaction was observed between stage of maturity and additive with crude protein being highest for forage receiving U+ at 700 and 800 g DM/kg but, at 600 g DM/kg, the highest value was recorded for forage receiving PU. Ammonia-N content was unaffected by additive application or stage of maturity with a mean value across treatments of 8.8 % total N.

Stage of maturity had an effect on forage pH, being lower ($P$ <0.001) for material harvested at a target DM of 600 g DM/kg (mean pH 5.7) compared to that harvested at 700 g DM/kg or 800 g DM/kg (mean pH's of 7.8 and 8.1 respectively). The application of an additive had an effect on forage pH, being higher ($P$ <0.001) for material treated with a urea based additive (mean pH 7.8) compared to the control (mean pH 6.0). Organic matter content was not affected ($P$ >0.05) by either harvest date or additive application. Similarly forage starch content was not significantly different between additive treatments or stage of maturity. Forage sugar content was, however, higher for treatment PU than for forages receiving the U+ or no additive (C). The lowest sugar content for material harvested at 600 g DM/kg was observed for treatment U+, whereas for material harvested at 700 and 800 g DM/kg, the lowest sugar content was observed in the control. Forage NDF was not affected by either stage of maturity or additive. Similarly no effect of treatment on forage ADF or NCDG was observed.

Table 5.5 Experiment 3b. Effects of additive and stage of maturity on chemical composition of whole-crop wheat (g/kg DM unless otherwise stated)

| | 600 g DM/kg | | | 700 g DM/kg | | | 800 g DM/kg | | | | Significance† | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C‡ | U+ | PU | C | U+ | PU | C | U+ | PU | s.e.d. | M | A | MxA |
| DM (g/kg) | 617 | 595 | 602 | 705 | 732 | 691 | 823 | 807 | 809 | 26.2 | *** | NS | NS |
| CP | 146 | 238 | 237 | 138 | 168 | 173 | 107 | 166 | 162 | 10.5 | *** | *** | ** |
| $NH_3N$ (% total N) | 14.6 | 8.5 | 11.8 | 7.4 | 8.2 | 8.3 | 6.4 | 7.7 | 6.5 | 4.98 | NS | NS | NS |
| OM | 964 | 966 | 957 | 969 | 967 | 971 | 966 | 965 | 971 | 3.57 | * | NS | NS |
| Sugars | 8.9 | 4.4 | 14.7 | 9.4 | 9.7 | 12.1 | 10.9 | 11.5 | 13.2 | 1.50 | * | *** | * |
| NDF | 253 | 282 | 291 | 313 | 332 | 285 | 366 | 344 | 344 | 51.2 | NS | NS | NS |
| ADF | 148 | 150 | 177 | 189 | 180 | 154 | 211 | 185 | 191 | 37.6 | NS | NS | NS |
| Starch | 262 | 252 | 251 | 267 | 249 | 261 | 264 | 282 | 301 | 75.2 | NS | NS | NS |
| pH | 4.75 | 5.33 | 7.16 | 6.31 | 8.63 | 8.36 | 6.82 | 8.80 | 8.62 | 0.562 | *** | *** | NS |
| NCDG | 834 | 814 | 796 | 791 | 789 | 818 | 735 | 786 | 765 | 49.4 | NS | NS | NS |

†M = effects of stage of maturity, A = effects of additive, and MxA = interaction between stage of maturity and additive

‡ N = negative control, U+ = urea + urease additive, PU = prilled urea.

*5.6.2.3. Intake and digestibility*

Intake and digestibility of the whole-crop forages are presented in Table 5.6. Fresh weight intake was affected ($P < 0.05$) by forage maturity, with intakes decreasing with increasing maturity, but was not significantly affected by additive application. No effect was observed of either stage of maturity or additive on daily DM intake when expressed as kg/kg metabolic live weight, with a mean value across treatments of 0.038 kg. No effect was observed in faecal DM output, with a mean value of 0.126 kg/day. There was an effect of stage of maturity on DM digestibility with material harvested at 600 g DM/kg having the highest digestibility (mean across treatments 0.792 kg/kg) and that harvested at 700 g DM/kg having the lowest digestibility (mean across all treatments 0.749 kg/kg). No effect of additive was observed on apparent DM digestibility. Intake of organic matter was not affected by stage of maturity at harvest or by additive, with a mean value across all treatments of 0.549 kg/day. Faecal organic matter output was similarly unaffected by either maturity or additive application. However, there was an effect of stage of maturity on organic matter digestibility, with forage harvested at 600 g DM/kg having a higher digestibility (mean 0.806 kg/kg) than material harvested at 700 or 800 g DM/kg (mean values 0.765 and 0.795 kg/kg respectively, $P < 0.05$). An interaction between maturity and additive was observed with the highest digestibility for material harvested at 600 g DM/kg recorded for forage receiving no additive and the lowest for material receiving PU. However, at 700 and 800 g DM/kg, the highest digestibility was recorded for the U+ forage and the lowest for the control forage. Intake of NDF was not affected by stage of maturity or additive application and no effect was observed on faecal NDF output. Stage of maturity has an effect on NDF digestibility with digestibility being higher ($P < 0.01$) for material harvested at 800 g DM/kg (mean 0.613) compared to that harvested at 600 or 700 g DM/kg (mean values of 0.496 and 0.485 respectively). There was also an effect of additive with forage receiving the U+ additive having a higher ($P < 0.05$) NDF digestibility (mean across all maturities 0.589 kg/kg) than forage receiving either no additive or PU

(mean values 0.530 and 0.474 kg/kg respectively). Stage of maturity or additive application had no effect on starch intake, output or digestibility. Estimated ME was unaffected by stage of maturity or additive application. However an interaction ($P$ <0.05) between maturity and additive was observed with the highest ME for material harvested at 600 g DM/kg being recorded for material receiving no additive (ME content 12.7 MJ/kg DM) whereas the highest ME for material harvested at 700 and 800 g DM/kg was for material receiving the U+ additive (ME contents of 12.0 and 12.5 MJ/kg DM respectively).

Table 5.6 Effects of additive and stage of maturity on intake and digestibility of whole-crop wheat measured in sheep

| | 600 g DM/kg | | | 700 g DM/kg | | | 800 g DM/kg | | | | Significance† | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C‡ | U+ | PU | C | U+ | PU | C | U+ | PU | s.e.d. | M | A | MxA |
| Fwt Intake (kg/day) | 1.000§ | 1.164 | 1.025 | 0.661 | 0.733 | 0.884 | 0.559 | 0.812 | 0.537 | 0.2168 | * | NS | NS |
| DMI/kg Metabolic LWT | 0.034 | 0.046 | 0.040 | 0.031 | 0.037 | 0.039 | 0.032 | 0.045 | 0.029 | 0.0083 | NS | NS | NS |
| Dry matter | | | | | | | | | | | | | |
| Intake (kg/day) | 0.614 | 0.692 | 0.618 | 0.478 | 0.532 | 0.605 | 0.477 | 0.664 | 0.435 | 0.1480 | NS | NS | NS |
| Output (kg/day) | 0.105 | 0.151 | 0.145 | 0.126 | 0.118 | 0.152 | 0.113 | 0.128 | 0.092 | 0.0340 | NS | NS | NS |
| Digestibility (kg/kg) | 0.827 | 0.781 | 0.768 | 0.724 | 0.772 | 0.751 | 0.752 | 0.816 | 0.782 | 0.0281 | * | NS | NS |
| Organic matter | | | | | | | | | | | | | |
| Intake (kg/day) | 0.592 | 0.668 | 0.593 | 0.464 | 0.515 | 0.588 | 0.461 | 0.641 | 0.422 | 0.1431 | NS | NS | NS |
| Output (kg/day) | 0.095 | 0.136 | 0.132 | 0.116 | 0.105 | 0.140 | 0.104 | 0.116 | 0.084 | 0.0312 | NS | NS | NS |
| Digestibility (kg/kg) | 0.839 | 0.796 | 0.782 | 0.740 | 0.791 | 0.763 | 0.766 | 0.826 | 0.795 | 0.0261 | * | NS | * |
| NDF | | | | | | | | | | | | | |
| Intake (kg/day) | 0.148 | 0.196 | 0.176 | 0.175 | 0.173 | 0.165 | 0.196 | 0.250 | 0.148 | 0.0497 | NS | NS | NS |
| Output (kg/day) | 0.068 | 0.099 | 0.099 | 0.087 | 0.067 | 0.099 | 0.078 | 0.082 | 0.061 | 0.0231 | NS | NS | NS |
| Digestibility (kg/kg) | 0.534 | 0.493 | 0.459 | 0.479 | 0.596 | 0.380 | 0.579 | 0.678 | 0.583 | 0.0635 | ** | * | NS |
| Starch | | | | | | | | | | | | | |
| Intake (kg/day) | 0.151 | 0.167 | 0.167 | 0.124 | 0.136 | 0.177 | 0.127 | 0.187 | 0.135 | 0.0507 | NS | NS | NS |
| Output (kg/day) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NS | NS | NS |
| Digestibility (kg/kg) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0 | NS | NS | NS |
| Estimated ME (MJ/kg DM) | 12.7 | 12.1 | 11.8 | 11.3 | 12.0 | 11.6 | 11.6 | 12.5 | 12.1 | 0.40 | NS | NS | * |

† M = effects of stage of maturity, A = effects of additive and MxA = interaction between stage of maturity and additive
‡N = Negative control, U+ = urea + urease additive, P = prilled urea
§ = Intake data calculated from individual silo data and not average data as presented in Table 5.5

*5.6.2.4. Aerobic stability and microbiological levels*

The effects of additive application and stage of maturity on overall temperature change is shown in Table 5.7. Increasing DM at harvest decreased aerobic stability as indicated by an increased temperature change (mean increase of 2.54, 2.85 and 6.97 °C above ambient for material harvested at 600 g DM/kg, 700 g DM/kg and 800 g DM/kg respectively, $P < 0.05$, s.e.d. 0.4202).

The effects of stage of maturity and additive on yeast numbers are presented in Table 5.7. Additive had a significant effect on the number of yeasts with the lowest counts being observed for treatment PU, and the highest for the control ($P < 0.001$). Stage of maturity also had an effect on yeast numbers with the highest counts being obtained for material harvested at 700 g DM/kg and the lowest for material harvested at 800 g DM/kg. An interaction was observed between additive and stage of maturity with the lowest yeast counts being observed for treatment PU for material harvested at 600 and 700 g DM/kg but for material harvested at 800 g/kg the lowest count was observed for treatment U+.

Table 5.7 Experiment 3b Effect of additive application and stage of maturity at harvest on the overall temperature change (OTC) and yeast number of whole-crop wheat

| | 600 g DM/kg | | | 700 g DM/kg | | | 800 g DM/kg | | | | Significance† | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C‡ | U+ | PU | C | U+ | PU | C | U+ | PU | s.e.d. | M | A | MxA |
| OTC§ °C | 1.60 | 1.93 | 4.10 | 4.37 | 2.03 | 2.17 | 6.60 | 6.60 | 7.67 | 0.420 | * | NS | NS |
| Yeasts (per g forage DM) | 68,536 | 4,736 | 2 | 1,278,785 | 525 | 354 | 1,312 | 6 | 11 | 230,968.5 | *** | *** | *** |

†A = effects of additive, M = effects of stage of maturity and AxM = interaction between additive and stage of maturity
‡C = control, U = urea + urease additive, P = prilled urea
§ calculated over a 21 day period by the addition of the temperature changes after room temperature was subtracted

## 5.7. Discussion

### 5.7.1. Chemical composition

The DM's of the two experiments reported here are higher than other authors who have investigated the effects of stage of maturity on chemical composition (Ashbell *et al.,* 1985; Adesogan *et al.,* 1998). Ashbell *et al.* (1985) examined the effects of maturity on whole-crop wheat from 201 to 404 g DM/kg, whilst Adesogan *et al.* (1998) investigated the changes in chemical composition of whole-crop wheat harvested at three stages of growth between 376 g DM/kg and 632 g DM/kg. The current range does not however, differ greatly from the DM's reported for urea-treated WCW by Sutton *et al.* (1998) of 813 g DM/kg, or the 609 g DM/kg of Hill and Leaver, (1999b). In both experiments reported here, forage DM increased with stage of maturity. This is not surprising as crop DM has been shown to increase with crop maturity (Hill and Leaver, 1999a). The presence of an interaction between additive and stage of maturity on forage DM in Experiment 3a is, however, in contrast to the findings of Experiment 3b, where no interaction was observed. In Experiment 3a, the lowest DM at the 600 g DM/kg stage of maturity was recorded for forage receiving the PU additive treatment, and the highest for the forage receiving the U+ additive. Contrastingly when harvested at 800 g DM/kg stage of maturity, the lowest DM was recorded for the forage receiving the LU additive and the highest for the control forage. However, the differences between treatments in terms of DM were small and could be attributed to variations in crop DM at harvest resulting in small changes in ensiled forage DM. The lack of an interaction in Experiment 3b supports this, as the silo volumes were much larger (e.g. 1150 litres in Experiment 3b *vs.* 28 litres in Experiment 3a) and any variation would be more evenly distributed.

Forage crude protein content was significantly affected by additive application in both experiments. The application of a urea based additive resulted in higher CP levels than that recorded for forages receiving either no additive or the inoculant/enzyme treatment, a result consistent with that reported elsewhere (Adesogan *et al.*, 1998; Hill and Leaver, 2002). By contrast increasing stage of maturity resulted in a decrease in forage crude protein level in the first and second experiment. This decrease is in agreement with the findings of Crovetto *et al.* (1998) who, when investigating the effects of stage of maturity of unprocessed, fermented WCW found a decrease in crude protein from 127 g/kg for material harvested at 197 g DM/kg to 79 g/kg for material harvested at 360 g DM/kg. Similar results were observed by Weinberg *et al.* (1991) and Filya, (2003), whilst Adogla-Bessa and Owen (1995a) observed a decrease in forage CP content from 101 g/kg DM for forage harvested at 310 g DM/kg to 70 g/kg DM for forage harvested at 680 g DM/kg.

Both additive and stage of maturity had a significant effect on forage ammonia-N content in Experiment 3a, but not Experiment 3b, where no effect of additive or stage of maturity was seen. Ammonia-N is produced during the breakdown of plant proteins (proteolysis) during fermentation or urea hydrolysis in urea-treated forages (Hill and Leaver, 2002). It would, therefore, be expected that the application of urea would result in higher ammonia N levels than those observed in forages receiving no urea additive, especially at the higher dry matters studies when fermentation is restricted (Adogla-Bessa *et al.*, 1999). Similar results were recorded by Hill and Leaver (2002) who reported an increase in forage ammonia-N levels when unprocessed whole-crop wheat was treated with urea. Levels were also found to increase when the level of urea treatment was increased from 20 to 40 g/kg (Hill and Leaver, 2002) In Experiment 3a, U+ had the highest ammonia-N levels at both stages of maturity, with the other urea treatments (LU and PU) resulting in ammonia-N levels that were higher than the control forage or that of the forage receiving a bacterial inoculant, but lower than the U+ additive treatment. This pattern was also found in

Experiment 3b with the exception of material harvested at 700 g DM/kg where the highest ammonia-N content was observed for material receiving the prilled urea, and at 600 g DM/kg where the highest value was recorded for the control forage. It has been observed that urease is inactivated under acid conditions (Sumner, 1951), and this may explain why ammonia-N levels were higher for the PU additive treated forage in Experiment 3b where a higher pH was recorded than for the U+ additive treated forage.

Forage pH was significantly affected by additive type in both experiments, with the application of a urea based additive increasing pH values. The increase in pH associated with the application of urea has been reported by a number of authors (Adesogan *et al.*, 1998; Hill and Leaver 2002). Increasing stage of maturity at harvest also resulted in an increased forage pH. Similar changes were observed by Adesogan *et al.* (1998) and Hill and Leaver (2002) who reported an increase in pH from 3.87 for forage harvested at GS 49 (and receiving no additive) to pH 6.16 for forage harvested at GS 87. An interaction was observed in Experiment 3a between additive type and stage of maturity, with the pH of forages receiving the LU and PU additives at 600 g DM/kg having a pH similar to C or Inoc forages, whereas forages harvested at 800 g DM/kg and treated with the same additives had higher pH's, similar to those recorded for the U+ forage. Hill and Leaver (2002) observed pH values similar to those of untreated forages for material receiving 20 and 40 g/kg DM urea harvested at 316 g DM/kg and 445 g DM/kg (mean pH 4.6) but higher pH values (mean 8.79) for material harvested at 689 g DM/kg. It would appear that even up to 600 g DM/kg of the crop that the application of urea is not sufficient to prevent fermentation taking place. Indeed it has been stated that in high moisture content forages, ammonia produced during urea hydrolysis is dissolved, forming the ammonium ion and, as a result of this that there would be a reduction rather than a cessation of fermentation (Hill and Leaver, 1999a).

Stage of maturity had an effect on forage organic matter levels in both Experiment 3a and 3b. Organic matter content was observed to increase with increasing crop maturity, but to reduce at 800 g DM/kg compared to values obtained at 700 g DM/kg in Experiment 3b. Contrastingly, a decrease in organic matter content was observed by Hill and Leaver (2002) for material harvested at 318 g DM/kg compared to 669 g DM/kg. The observed changes in the current work were numerically small and unlikely to be of practical significance.

In Experiment 3a forage starch content was observed to increase with increasing crop maturity. This is in agreement with the study of Hill and Leaver (1999a) who reported significant increases in forage starch content when wheat was harvested at a range of growth stages between GS 53 and GS 87 and at DM's of 240 to 672 g DM/kg. Although the DM's used in Experiment 3a fall outside the maximum DM used by Hill and Leaver (1999a), it is conceivable that further increases in crop starch content can occur after this point and indeed the results reported in the current work appear to verify this hypothesis. Although no difference in starch concentration was observed as a result of stage of maturity in Experiment 3b, the variation between samples in starch content was high, which may have masked any physiological changes.

There was a decrease in forage WSC contents with increasing crop maturity in Experiment 3a, a finding in agreement with that of Hill and Leaver (2002). However sugar contents were observed to increase in Experiment 3b with increasing stage of maturity. The increases observed were, however, small (with a maximum increase of 6.8 g/kg DM) and are likely to be of little biological significance.

In Experiment 3a, forage NDF was affected by additive type but not stage of maturity. The forages receiving the U+ additive had consistently lower NDF contents than forages

receiving any of the other additives. This is likely a reflection of the effects of urea treatment. A decrease in forage NDF when urea is applied has been attributed by Van Soest *et al.* (1984) to be a result of the urea partial solublisation of the hemicelluloses contained within the plant. However, in Experiment 3b, no effect of additive was observed at any of the three stages of maturity examined, with forage NDF increasing with maturity. This lack of an effect of stage of maturity on forage NDF is in agreement with the findings of Hill and Leaver (1999a;2002) for unprocessed whole-crop wheat.

Additive type had no effect on forage ADF contents in either Experiment 3a or 3b. However, in Experiment 3a there was an effect of stage of maturity with material harvested at 600 g DM/kg having a higher forage ADF content (mean 187 g/kg DM) than material harvested at 800 g DM/kg (mean 167 g/kg DM, $P < 0.01$). This is in agreement with the findings of Hill and Leaver (1999a) who observed a decrease in forage ADF after growth stage 70-71 (approximately 330 g DM/kg) attributed to increasing crop starch levels. Similar patterns were also observed by Hill and Leaver (2002). In Experiment 3a forage starch content was observed to increase with increasing crop maturity and is the most likely explanation of the changes in forage ADF content.

Additive application had a significant effect on NCDG in Experiment 3a, but had no significant effect in Experiment 3b. However, it has been stated that owing to the changes in crop physiology with maturity (i.e. increasing grain content and increasing lignification of fibrous material) changes in NCDG are minimised (Hill and Leaver, 1999a). As such the lack of a consistent effect in the current experiments is not entirely unexpected.

### 5.7.2. Hydrolysis of urea

In Experiment 3a, U+ resulted in the greatest level of hydrolysis of urea at both stages of maturity, with levels comparable to those obtained by Hill and Leaver (1999a) when using prilled urea on forages harvested at 332 g DM/kg and 531 g DM/kg. Hill and Leaver (1999a) reported almost 100 % hydrolysis after 90 days ensiling for these forages, but such high levels were not observed at either of the two stages of maturity investigated in the current study with either prilled or liquid urea over the 45 day experimental period. The period of 45 days was chosen as this represented the point at which maximal hydrolysis of urea was achieved in the study of Hill and Leaver (1999a). In addition the DM's used in the current experiment were higher than those used by Hill and Leaver (1999a) which may explain the lower levels observed as it has been established that the chemical reaction that turns urea into ammonia is catalysed by water (Sahnoune *et al.*, 1991). It was hypothesised that the provision of additional water through the application of liquid urea may have resulted in a greater level of urea hydrolysis, as it was observed by Williams *et al.* (1984) that the concentration of ammonia released from urea was related to straw moisture content. Excess moisture has been shown to increase the effectiveness of straw treated with anhydrous urea (Sundstol *et al.*, 1978). Indeed a decrease in residual urea was reported by Caneque *et al.* (1998) when straw moisture levels increased. In the current experiment there was no effect of DM on the level of urea hydrolysis after 45 day or to the application of additional water (LU) at ensiling. However, the amount of water added/kg crop DM in treatment LU was low (approximately 68 ml/kg DM). This coupled with the lack of additional urease may explain the results obtained here. It was concluded by Coxworth (1978) that wheat straw does not contain sufficient urease enzyme to affect urea hydrolysis. Indeed, a response to additional urease was reported by Besle *et al.* (1990) who found a higher level of hydrolysis of urea when water and urease was added to wheat straw compared to water alone.

### 5.7.3. Aerobic stability and yeast and mould levels

The application of any of the four additives in Experiment 3a resulted in a decrease in overall temperature change over the exposure period compared to the forage that received no additive. However, changes in temperature were small and likely to be of little practical significance. In Experiment 3b, only stage of maturity had a significant effect on overall temperature change, with temperatures increasing with crop maturity, although changes in temperature were also small. This small increase in temperature across all the treatments is supported by the work by Woolford *et al.* (1982), who found that wheat silage (harvested at 365 g DM/kg) was stable in air with little recorded temperature change. By contrast, Sinclair *et al.* (2003) reported an increase in forage temperature above ambient for fermented WCW (harvested at 296 and 371 g DM/kg) with a peak of 22 °C occurring seven days after the initial exposure of the forage to air.

Microbiological composition of the forages was affected by additive type in Experiment 3b, with the application of urea-based enzymes resulting in lower yeast levels compared to material receiving no additive. This is in agreement with work by Yu *et al.* (1975) who found that ammonia inhibits yeast and fungi growth in silage. A reduction in yeast numbers has been shown to result in a decreased risk of aerobic instability (Woolford, 1984). Stage of maturity also had an effect on microbiological composition of the forages with the lowest yeast counts being recorded for forages harvested at 800 g DM/kg and the highest for forages harvested at 700 g DM/kg. The higher microbial number at 700 g DM/kg could be a reflection of the slightly acidic pH of the untreated forage, being higher than that of the 600 g DM/kg owing to a less extensive fermentation providing an increased availability of nutrients (WSC) for spoilage microrganisms to utilise (Filya, 2000). The lower counts observed at 800 g DM/kg are a likely reflection of the high DM of the forage inhibiting microbial activity (Adogla-Bessa and Owen, 1995a). However,

temperature change values were highest for this stage of maturity. This is unexpected, as temperature increases would be expected to be lower if microbial activity was decreased, not higher as has been reported here.

### 5.7.4. Intake and digestibility

Feed intakes were generally small although comparable to amounts fed by Sutton *et al.* (2002) when expressed on a metabolic live weight basis. The mean intake across all treatments for the current study was 0.038 kg DM intake/kg metabolic live weight compared to 0.038 kg DM intake/kg metabolic live weight in the study of Sutton *et al.* (2002). The lack of a response in apparent DM digestibility in Experiment 3b to urea treatment is surprising as it has been shown that the application of urea to forages and straw results in an increase in DMI and digestibility (Deschard *et al.*, 1988; Fondevila *et al.*, 1994; Al-Masri and Guenther, 1999). A response was seen in OM digestibility attributable to stage of maturity with digestibility being higher at 600 g DM/kg than at 700 or 800 g DM/kg. Most of this difference appears to be attributable to the higher digestibility of the C forage, as the digestibility of the U+ and PU forage were similar to those for the same treatments at 700 and 800 g DM/kg. Changes in OM digestibility linked with stage of maturity have been reported by Adesogan *et al.* (1998). However, Adesogan *et al.* (1998) did not investigate the high DM's at which it is now possible to harvest WCW. The OM digestibilities reported in the current experiment (mean value of 0.789 kg/kg) are higher than those reported by Sutton *et al.* (2002) who observed an OM digestibility of 0.737 kg/kg for urea-treated, unprocessed WCW (with a DM content of 584 g DM/kg) when fed to sheep. However, NDF digestibility was lower (mean value of 0.531 kg/kg) than the 0.7680 kg/kg reported by Sutton *et al.* (2002), although the forage produced by Sutton *et al.* (2002) received a greater level of urea (40 g/kg DM) compared to the 30 g/kg DM in the experiment reported here. The higher level of urea may have

resulted in the higher digestibilities reported by Sutton *et al.* (2002), as it has been observed that when forage is treated with a higher level of urea, NDF digestibility increases (Leaver and Hill, 1995). However, the application of a source of urease alongside urea at ensiling promoted a higher NDF digestibility ($P$ <0.05) than that recorded for forages receiving urea without additional urease. As it has been shown that fibre digestibility decreases with increasing crop maturity (Cherney and Marten, 1982), it seems valid to recommend the application of urease alongside urea at ensiling to enhance NDF digestibility.

The increases in NDF digestibility with increasing crop maturity are in contrast to the reported changes in OM digestibility, namely a decrease with increasing maturity. This difference is a likely reflection of the higher NDF digestibility of the U+ treated forages increasing overall NDF digestibility. At 700 g DM/kg forage receiving the U+ additive had an NDF digestibility of 0.596 kg/kg compared to the control forage of 0.479 and that at forage treated with PU of 0.380. At 800 g DM/kg a similar pattern was observed with the highest digestibility observed for the U+ treated forage (0.678 kg/kg) and the lowest for the control forage (0.579 kg/kg).

When using calorimeters, it had been established that there is the potential for erroneous results when attempting to estimate an ME for urea-treated WCW (as discussed in Chapter 3). The prediction equation used in the current experiment bases its estimate on OM digestibility. This has been shown to be a reliable estimate for most forages, however, WCW presents a number of problems when attempting to estimate ME. Firstly it is not what is traditionally thought of as a forage, as it contains whole, (and in the case of high DM urea-treated material) physiologically mature grains. It is also not preserved via fermentation but through treatment with urea to produce ammonia with resultant changes in the forages chemical composition. In addition, it cannot be entirely thought of as a

concentrate as it contains straw. Equations used for the determination of ME in maize silage have been used but not surprisingly, due to the differences between the two forages, have proved to be inaccurate (Sutton *et al.*, 2002).

Although ME values have been calculated for the current experiment, owing to the potential unreliability of the equations used to estimate ME's for urea-treated WCW, the accuracy of these results is open to question. In addition, the use of sheep to determine ME values of unprocessed WCW for dairy cows has been shown to result in considerable errors (Sutton *et al.*, 2002). However, a direct comparison between sheep and dairy cattle fed processed WCW has not been made.

## 5.8. Conclusions

In Experiment 3a, forages harvested at 600 g DM/kg and treated with either liquid or prilled urea fermented, as indicated by their low pH and ammonia-N levels. Ammonia was released from the U+ additive when applied to forage harvested at 600 g DM/kg and pH elevated. In Experiment 3b, fermentation occurred in the forage treated with urea + urease at 600 g DM/kg, as indicted by the low pH. In Experiments 3a and 3b, prilled urea, liquid urea and urea + urease all had high ammonia levels when applied to forages harvested at 800 g DM/kg. Ammonia was also released from prilled urea and urea + urease at 700 g DM/kg whereas the control forage had a neutral pH and a high microbial load. It would appear, therefore, that the application of an additive to WCW is necessary to limit the growth of spoilage microorganisms. At DM contents of approximately 600 g DM/kg, WCW will ferment, even in certain circumstances when a urease enzyme is added. Dry matter intakes in sheep were not affected by additive or stage of maturity in Experiment 3b, with OM digestibilities being affected by stage of maturity alone. However, as there was a significant increase in NDF digestibility for forage receiving the U+ additive, it would appear that it is beneficial to apply such additives to mature WCW.

# 6.0. DISCUSSION

## 6.1. Introduction

The current series of experiments was designed to determine whether it was possible to improve the utilisation of WCW by dairy cows. The first experiment investigated the effects of processing at harvest and alteration of forage cutting height and showed a clear improvement in digestibility of starch when whole-crop wheat was processed at harvest but there was no effect on milk yield or composition, although forage DMI (kg/day) was decreased. Increasing cutting height at harvest also reduced forage DMI, increased condition score and reduced milk fat content.

The second performance experiment utilised an intermediate cutting height between those used in Experiment one, and investigated the use of carbohydrate supplements as a means of improving performance to processed, urea-treated WCW. It also compared processed urea-treated WCW with maize silage. The main findings were that supplementation of processed urea-treated WCW with lactose resulted in a numerically higher milk yield than supplementation with wheat or molasses but that milk component yield (kg/day) was similar among treatments. It was also observed that the performance of cows fed processed urea-treated WCW was similar to that recorded for cows fed maize silage.

The third experiment comprised two parts: the first investigated the effects of applying three forms of urea on the chemical composition and aerobic stability of processed WCW. All forages fermented at 550 g DM/kg, as evidenced by pH values of less than pH 4.5, except for the urea+urease treatment, which had an alkaline pH of 8.8. When harvested at 800 g DM/kg all forms of urea resulted in an alkaline pH. The second experiment investigated the effect of form of urea within three stages of maturity of WCW on the

chemical composition, microbial composition, aerobic stability and intake and digestibility determined in sheep. All forages were aerobically stable, but the application of an additive reduced microbial numbers. No effect of additive application or stage of maturity was observed on intake. Organic matter digestibility was observed to decrease with increasing crop maturity with additive having no effect. Additive did, however have an effect on NDF digestibility, being highest for forages receiving the U+ additive.

## 6.2. Crop yield

In Experiment 1 forage DM yields were 14.9 t DM/ha and 18.2 t DM/ha for material harvested at a stubble height of 37.3 cm and 17.8 cm respectively. In Experiment 2 the WCW yield was 8.36 t DM/ha with a mean stubble height of 32.0 cm, whereas in Experiment 3b forage yields were 14.9, 15.5 and 14.2 t DM/ha for material harvested at 600, 700 and 800 g DM/kg respectively, with mean stubble heights of 29.0 cm, 30.5 cm and 28.5 cm.

The variation reported in yield can be attributed to crop management and environmental effects. Crop management factors that may have affected the yields recorded include fertiliser application rates. The fertiliser levels applied were 155 kg N/ha, 136 kg N/ha and 200 kg N/ha for Experiments one, two and three b respectively and, although different, were calculated according to the requirements of the individual crop being grown. It has been reported that the level of fertiliser applied to the crop has effects on grain yield (Stone and Savin, 1999) and additional work has shown that there are increases in DM yield attributable to N application rate (Selman, 1975). Although it is feasible that some of the variation reported may have been attributable to the level of N fertiliser application, there are additional factors which were likely to impact on crop yield. Of these factors it is unlikely that soil type had an effect, as the crops were all grown on similar sandy loam

soils. It is, however, possible that seasonal variation may have affected crop yield. Seasonal variation with respect to crop yield have been reported elsewhere, with Selman (1975), reporting yields for the same variety being 9.48 t DM/ha, 11.60 t DM/ha, and 8.03 t DM/ha across three years. Kristensen (1992) has also reported DM yield variation between seasons. It is logical, therefore that the variation reported may well reflect seasonal growing conditions rather than be a direct reflection of crop management strategies. However, the exact source for the variation is unclear and it has been stated by Tennant *et al.* (1991) that the final yield of wheat is an outcome of the interaction between genetic, environmental and crop management factors.

## 6.3. Effects of additive on the chemical composition and aerobic stability of WCW

The application of an additive (urea based or enzyme/inoculant based) was shown in the current work to improve the aerobic stability of processed whole-crop wheat forages. It has been stated that forages with a high DM have a greater potential for aerobic deterioration (Woolford *et al.*, 1982) and increases the importance of applying a suitable additive to WCW that is harvested at the higher DM values now possible. Since aerobic deterioration (and hence aerobic stability) has been attributed to originate from the activities of aerobic microorganisms (Ashbell *et al.*, 2002), the inhibition of these should result in an increased stability. This was clearly shown in Experiment 3b, where the application of either of the two urea-based additives resulted in a lower microbial load and hence a greater aerobic stability. Improved aerobic stability for urea-treated WCW has also been reported by Hill and Leaver (2002), who reported urea to have a beneficial effect on aerobic stability of WCW. It has been shown, therefore, that even at high DM's (700 g DM/kg), WCW still has the capacity to have poor aerobic stability (as characterised by high microbial loads) and the application of an additive to WCW is necessary to prevent nutrient losses as a result of microbial action.

Urea-based additives consistently increased forage CP levels, a finding in agreement with a number of authors (Adesogan *et al.*, 1998; Sutton *et al.*, 2002; Hill and Leaver, 2002). It may be expected that urea would act upon the fibre component of whole-crop wheat and result in an increase in fibre digestibility. The application of alkali to forages such as WCW has long been established to increase their digestibility (Tetlow and Mason, 1987). However, in the current study the level of urea addition of approximately 30 kg/t DM was considerably less than that used in studies that have supplied either ammonia or feed grade urea to enhance the digestibility of straw (Williams *et al.,* 1984: Munoz *et al.,* 1991). The application of urea to straw (at a level of 67 kg urea/tonne DM) was observed by Deschard *et al.* (1987) to increase the digestibility of the fibre (NDF) component from 0.537 kg/kg for untreated material to 0.648 kg/kg for material treated with urea. The digestibilities observed in Experiment 3b (mean across all stages of maturity 0.531 kg/kg) were lower than those of Deschard *et al.* (1987) and were also lower that the 0.768 kg/kg reported for sheep by Sutton *et al.* (2002) who utilised urea at the application rate of 40 kg urea/t DM. It may be that the higher urea addition may have solubilised more of the hemicelulose contained within the forage, hence further increasing fibre digestibility (Van Soest *et al.,* 1984).

As has been stated earlier (Chapter 1), when a crop matures the fibre component increasingly becomes lignified and less digestible (Cherney and Marten, 1982). Without the addition of urea at ensiling there would be a decrease in NDF digestibility with increasing crop maturity (as observed in Experiment 3b). There is, therefore a clear requirement for urea additives when ensiling even high DM material to enhance NDF digestibility. The results from Experiment 3b indicate that, of the forms of urea utilised, the more effective with respect to NDF digestibility was the urea + urease additive. It is, therefore, fair to make the recommendation that this additive be used in preference to feed grade urea to improve NDF digestibility.

Despite the fact that urea hydrolysis did occur in forages treated with all of the urea sources studied, hydrolysis was more rapid and consistently higher in forages that received the U+ additive (urea + urease). However, the level of hydrolysis obtained in Experiment 3a was lower than the 95 % reported by Hill and Leaver (1999a). This could be a result of the high DM's used in the current experiment (600 to 800 g DM/kg) which are considerably higher than those used by Hill and Leaver (1999a) of 240 to 672 g DM/kg. This may have resulted in a lack of available water for the conversion of urea into ammonia (Sahnoune *et al.*, 1991). However, the exact method by which the level of hydrolysis was determined was not stated by Hill and Leaver, (1999a). Assuming that hydrolysis in the work of Hill and Leaver (1999a) was calculated as the difference between crude protein and ammonia N, this may have potentially lead to erroneous results. The chemical analysis used to determine urea in the current study detected urea in its original form and not its presence when transformed into ammonia-N. Hence it could be argued that the variation in levels of hydrolysis between the current experiment and those reported by Hill and Leaver (1999a) may be attributable to differences in chemical analysis rather than the actual levels of hydrolysis.

## 6.4. Intake

In Experiment 1, the highest DM intake was recorded for cows receiving the long straw unprocessed forage, with the lowest observed for those fed the short straw processed forage. The mean average for the processed forages was 21.3 kg DM/day. This compares to treatment averages in Experiment 2 of 22.2, 21.0 and 23.2 kg DM/day for processed WCW supplemented with wheat, lactose and molasses respectively. In Experiment 3b sheep fed WCW had a mean DM intake of 0.568 kg DM/day. If the DM intakes recorded for the sheep are converted to a metabolic live weight they had a mean DM intake of 0.038 kg DMI/kg metabolic live weight. This is in contrast to a mean intake of 0.172 kg DMI/kg

metabolic liveweight and 0.182 kg DMI/kg metabolic liveweight for the cows in the first and second experiments respectively. However, the experiments are not directly comparable as the sheep were fed WCW alone and the dairy cows WCW mixed with grass silage and concentrates. The difference in the intakes between the sheep and cows may, therefore, be a reflection of the mixing of forage promoting a greater intake as has been previously established (Phipps *et al.*, 1995) rather than a direct effect of species. In addition, the sheep in the digestibility experiment, although fed ad-lib, would have had lower requirements for energy and protein than the dairy cows in Experiment 1. It has been established that one of the contributory factors affecting intake is the metabolic demands of the body (Forbes, 1995) and animal requirements would have impacted on DM intake.

Rumen outflow rates would also have been different between the species with values quoted for dairy cows being 0.08/hour compared to 0.02/hour for sheep fed at maintenance (AFRC, 1995). Dry matter intake has been demonstrated to affect rumen outflow rate (Ørskov *et al.*, 1988), with intakes being higher for animals consuming more digestible diets (which have a faster rumen outflow rate (Grovum, 1987)), and may partially explain the results observed here.

The observation that intakes were different between cattle and sheep supports the argument against using sheep as a predictor for the value of feeds for dairy cattle. Previous work by Südekum *et al.* (1995) utilising fermented WCW, observed higher OM intakes in sheep when compared to intakes obtained from steers. Higher digestibilities for urea-treated WCW when fed to sheep compared to digestibilities recorded in dairy cows have also been reported by Sutton *et al.* (2002) with OM digestibilities in sheep reported as 0.737 kg/kg *vs.* 0.695 kg/kg when the same forage was fed to lactating cows. Forage NDF digestibilities were also observed to be lower in cows (0.597 kg/kg *vs.* 0.768 kg/kg) than

that reported for sheep. It would, however, be of interest to determine the intakes of the forages used in Experiment 3b when fed to dairy cattle.

## 6.5. Milk yield/composition

No significant response to processing, forage cutting height or supplementation was observed in milk yield in either of the two dairy cow experiments. Previous experiments utilising unprocessed urea-treated WCW reported no effect of the inclusion of WCW on milk yield (Leaver and Hill, 1995; Hameleers, 1998). The reason behind the lack of a response in milk yield was attributed by Sutton *et al.* (1998) to be a result of the low digestibility of the forage. However, when the digestibility was improved (in Chapter 3) the dairy cows responded to this by decreasing intakes and increasing body fat deposition, rather than milk yield. This has been attributed to the increased digestibility of the starch fraction of WCW that may have promoted the production of glucose via an increase in its precursor, propionate (Bines and Hart, 1986). It has been determined that propionate in the cow regulates plasma insulin levels and if plasma propionate levels increase, insulin secretion is stimulated (Bines and Hart, 1986). The elevated concentration of insulin enhances the uptake of lipogenic precursors and a decrease in fatty acid release by adipose tissue (Bauman and Griinari, 2001). The net effect of these reactions is the stimulation of the partitioning of nutrients towards body tissue gain as opposed to milk production (Beever and Oldham, 1986).

The lack of a significant response in milk yield to forage source (WCW or maize silage) in the second experiment is in contrast to work by Phipps *et al.* (1995) who found that, when compared to maize silage, the feeding of urea-treated WCW resulted in reduced animal performance. However, work to date has focused on unprocessed WCW and not the processed form as in the current series of experiments. Therefore the current work

demonstrates that animals fed processed urea-treated WCW can perform as well as animals fed a good quality maize silage. The comparison between supplementation types was designed to determine the effects of the provision of a readily available carbohydrate source as a supplement to processed urea-treated WCW. Although no significant response was observed, there was a tendency for lactose supplementation to promote higher milk yields than wheat or molasses. It can be concluded from the experiment that the supplementation of processed urea-treated WCW with lactose may have beneficial effects used as a supplement, although further work is required to support this.

The reduction in milk fat concentration and yield in the first experiment as a result of an increase in forage cutting height at harvest has been attributable to an increased availability of starch resulting in changes in nutrient partitioning within the animal, ultimately leading to body lipid deposition and a decrease in milk fat levels (Bines and Hart, 1986). This explanation would also support the lack of a response in the second experiment between WCW treatments in milk fat content as the cutting height of the forage was altered to be intermediate between the long and the short strawed forages (cutting height 32 cm in the second experiment *vs.* 17.8 cm and 37.3 cm in the first experiment for the long and short strawed forages respectively) to avoid the potential acidosis that may be associated with the short strawed, processed forages.

Milk protein content was not significantly different between treatments in Experiment 1, with the effects observed in Experiment 2 attributable to the lower milk yields in the cows offered the molasses supplemented diets, and a greater concentration of milk protein. It has been stated that milk protein increases with increases in energy intake, as long as the energy is in the form of carbohydrate not fat (Reynolds *et al.*, 1997). When ME supply is increased, OM digestion in the rumen would also be expected to increase, and if sufficient

quantities of rumen degradable protein and non protein N are available, microbial protein synthesis will increase, and so will protein supply to the animal (Dewhurst *et al.*, 2000).

## 6.6. Metabolism

It was stated by Phipps (1994) that to justify the use of urea to conserve forage it must be established that the urea was efficiently utilised by the ruminant and not excreted as a pollutant. Hill and Leaver (1993) reported an increase in blood urea as a result of increasing dietary inclusion of unprocessed urea-treated WCW and reported plasma urea levels of 7.15, 6.13 and 9.20 mmol/l for sole grass silage diets, a 50:50 mix of grass silage and urea-treated WCW, and urea-treated WCW as the sole forage respectively. Urea levels recorded in the first experiment (mean value 5.89 mmol/l) were lower than those reported by Hill and Leaver (1993) for forage mixed 50:50 with grass silage (WCW was mixed 2:1 with GS in Experiments 1 and 2). However, in Experiment 2 plasma urea levels, although lower than Experiment 1, were marginally higher for animals receiving processed urea-treated WCW supplemented with wheat and lactose, but were significantly lower for those receiving WCW supplemented with molasses (mean values 5.81, 5.87 and 5.06 mmol/l respectively).

Supplementation of WCW hence offers the potential to reduce negative effects of urea-treated WCW indicated by Phipps (1994). Urinary losses were not measured in Experiment 2 but other authors (Givens *et al.*, 1992) have observed a decrease in urinary losses of N when molasses was included into the diet (which consisted of a mixture of grass silage, barley and soya). This decrease in N loss associated with the inclusion of molasses is supported by Sutton *et al.* (2001) reported a N loss of 204 g/day when diets based on urea-treated WCW were supplemented with molasses compared to 211 g/day for animals fed urea-treated WCW diets which did not include molasses. An improvement in N retention

was also reported by Sutton *et al.* (2001) from –6 g/day in cows fed the control diet *vs.* 3 g/day in cows fed WCW based diets supplemented with molasses. If N efficiencies are considered (Table 6.1) then the efficiency recorded for cows fed urea-treated WCW supplemented with molasses in Experiment 2 (0.215 kg milk N output/kg N intake) is lower than the 0.240 kg milk N output/kg N intake reported by Sutton *et al.* (2001). However, N intake for these animals was substantially higher than those used in the study of Sutton *et al.* (2001) and is likely to be the reason behind these observed differences. Indeed overall intakes of cows supplemented with molasses in Experiment 2 (mean 23.2 kg/day) were higher than the 20.0 kg/day reported by Sutton *et al.* (2001) and further support this theory. The efficiency reported in Experiment 2 for lactose supplemented WCW (0.236 kg milk N output/kg N intake) is higher than the value reported by Sutton *et al.* (2001) and Hameleers (1998) for urea-treated WCW (Table 6.1), indicating that there is potential to improve the N efficiencies of animals fed urea-treated WCW through appropriate supplementation regimes. The effects of supplementation of WCW with a readily available carbohydrate source can at this stage, only be concluded to have a beneficial effect on plasma urea levels, indicative of increased microbial capture of ammonia, rather than necessarily having beneficial effects on whole body nitrogen efficiency. Although it is appreciated that efficiencies have been improved in the current work, which goes some way to address the issue raised by Phipps (1994), further research into appropriate supplementation strategies for processed urea-treated WCW may more completely address this issue.

Table 6.1 Effect of urea-treated WCW on N efficiency

| | Experiment 1 | | | | Experiment 2 | | | Sutton *et al.* (2002) | Sutton *et al.* (2001) | Hameleers (1998) |
|---|---|---|---|---|---|---|---|---|---|---|
| | LU | LP | SU | SP | W-WCW | L-WCW | M-WCW | UT WCW† | WCW+M‡ | UT WCW§ |
| Dietary N intake (g/day) | 593 | 606 | 596 | 559 | 781 | 741 | 805 | 715 | 542 | 625 |
| Milk N output (g/day) | 165 | 159 | 158 | 154 | 176 | 174 | 173 | 161 | 130 | 143 |
| Efficiency of N use (kg milk output/ kg N intake) | 0.269 | 0.262 | 0.268 | 0.276 | 0.224 | 0.236 | 0.215 | 0.225 | 0.240 | 0.229 |

† = Urea-treated WCW treated with 40 kg urea/t DM and mixed 2:1 with grass silage
‡ = Urea-treated WCW treated with 40 kg urea/t DM, mixed 2:1 with grass silage supplemented with 2 kg FWT of molasses
§ = Urea-treated WCW treated with 40 g urea/kg DM and mixed 40:60 with grass silage

Increasing the cutting height of WCW in Experiment 1 resulted in an improved energy balance indicated by higher plasma glucose and lower plasma BHB levels. In Experiment 2, plasma glucose levels were similar to those recorded for animals receiving the short strawed forages in Experiment 1. However mean BHB levels were lower in Experiment 2 than those recorded in Experiment 1, indicating an improved energy balance. This, coupled with the lower plasma urea levels recorded in Experiment 2 (mean across WCW treatments 5.58 mmol/l) indicates an improved efficiency of energy and protein metabolism when compared to the results reported in Experiment 1.

Body condition score was higher in animals fed the short compared with the long strawed forages in Experiment 1. This may be explained by an alteration in nutrient partitioning as a result of increased starch availability (Bines and Hart, 1986). However, no effect of either supplement type or forage source was seen in Experiment 2. It can, therefore, be concluded that supplement type to processed urea-treated WCW has no significant effect on body condition score, despite there being a numerically higher level of body condition when WCW was supplemented with wheat compared to supplementation with lactose. This is likely to be a reflection of the increased starch intakes in animals fed WCW supplemented with wheat, compared to WCW supplemented with lactose, as feeding a higher amount of starch has been suggested to result in a higher level of body condition gain (Bines and Hart, 1986).

No difference was reported between the use of either processed urea-treated WCW or maize silage as a forage source in condition score. This is in contrast to the findings of Phipps *et al.* (1995) who fed unprocessed urea-treated WCW and maize silage. Phipps *et al.* (1995) reported a live weight change of –3 kg across the experimental period whilst animals fed maize silage has a gain of 20 kg across the experimental period. In Experiment 2, however, no difference in live weight change was observed among cows fed any of the

treatments. The contrast between the results of Experiment 2 and the findings of Phipps *et al.* (1995) may well reflect the processed nature of the forage used, and indicates that both processed urea-treated WCW and maize silage are comparable with respect to their effects on animal live weight/condition score change.

## 6.7. Digestibility of WCW in sheep and cattle

Differences were observed between species in digestibility. Mean OM digestibility was 0.635 kg/kg for the processed forages in Experiment 1 in dairy cows compared to 0.789 kg/kg across all stages of maturity and additive treatments in Experiment 3b when WCW was fed to sheep. Notwithstanding between experiment variation, the higher digestibility observed in sheep compared to cows may partly be attributable to differences in rumen outflow rates. It was stated by AFRC (1995) that outflow rates are 0.02/h for animals fed at maintenance and 0.08/h for cows yielding more than 15 kg/day. Differences may also attributable to physiological differences between the two species. It has been well documented that the reticulo-omasal orifice is smaller in sheep compared to that observed in cattle and was attributed by Ørskov (1985) as a reason behind the lower starch digestibility observed in cows when the same feeds are fed to sheep and cattle. Indeed starch digestibility in the third experiment was observed to be 1.00, a value in agreement with the findings of Sutton *et al.* (2002), who fed unprocessed WCW to sheep. When the same forage was fed to lactating dairy cows starch digestibility was measured as 0.966 kg/kg Sutton *et al.* (2002). It can be concluded that, although sheep are a useful indicator of digestibility in larger ruminants, certain aspects of their physiology result in key differences that can have major effects on the subsequent results.

## 6.8. Implications

- If WCW is to be harvested at a mature stage of growth (around 700 g DM/kg) it should be processed at harvest to improve starch digestion.
- Cutting height at harvest will depend on a number of factors but it is advisable not too cut too high as it is likely to result in a reduced crop yield (proportionally 0.19) and lower milk fat levels, (proportionally 0.12) although milk yield will be unaffected and forage DMI reduced, resulting in a more efficient use of forage.
- Supplementation of WCW with lactose appeared to offer the greatest potential to improve performance in the form of milk yield, although further work is required to confirm this.
- The milk yield and composition of animals fed processed urea-treated WCW was similar to those fed maize silage, although forage DMI was proportionally 0.24 higher for animals fed processed WCW.
- The addition of urease at ensiling promotes a rapid level of urea hydrolysis.
- The application of urease alongside urea at ensiling promotes a higher NDF digestibility and hence it would appear to be advantageous to use an additive that contains both urea and urease to maximise the digestibility of the forage.
- There are advantages to leaving the crop to a more mature stage of growth (over 700 g DM/kg) as an alkaline preservation is likely to occur and there are also crop yield benefits.

## 6.9. Conclusions

Processing at harvest resulted in a significant increase in starch digestibility. This enables WCW to be harvested at higher DM's (650-800 g DM/kg) without the problem of reduced starch digestibility. Alteration of cutting height offers the potential to alter the nutritive value of WCW and allows producers to tailor the forage to their individual requirements. If a nutrient dense forage (i.e. a higher starch and a lower fibre content) is required then the cutting height can be raised whereas if a greater volume of forage is required (i.e. a higher fibre and lower starch content forage) then it can be lowered. However increasing cutting height (i.e. harvesting to leave a higher stubble) resulted in a reduced forage intake and lower milk fat concentration as well as a reduced crop yield. Processed, urea-treated WCW has been shown to be comparable to a good quality maize silage and improvements in performance may be achieved through the development of greater precision of supplementation regimes to urea-treated WCW as it has been shown that the inclusion of 0.7 kg/day lactose into dairy cow diets tended to increase milk yield. There is still a necessity to apply additives at high DM's (600-700 g DM/kg) to prevent the growth of spoilage microorganisms, high levels of which were observed for untreated forages. There is a requirement for additional urease at ensiling as a more rapid and extensive hydrolysis of urea was observed than when no urease was added. There are also additional benefits in the form of an enhanced NDF digestibility for forages treated with urea and urease at ensiling.

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