The effect of stage of maturity and inclusion rate of processed, whole-crop wheat on the metabolism and performance of dairy cows

Thesis

UNRESTRICTED

THE EFFECT OF STAGE OF MATURITY AND INCLUSION RATE OF PROCESSED, WHOLE-CROP WHEAT ON THE METABOLISM AND PERFORMANCE OF DAIRY COWS

By

Alison Jayne Bond

BSc (Hons) in Agriculture and Food Science, specialising in Animal Science, University of Nottingham, Sutton Bonington
MSc in Animal Nutrition, Aberdeen University

Thesis submitted to the Open University for the award of the Degree of Doctor of Philosophy

July 2006

Harper Adams University College, Edgmond, Newport, Shropshire, TF10 8NB, UK

DATE OF SUBMISSION 14 JULY 2006
DATE OF AWARD 03 JANUARY 2007

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

I declare that this thesis has been composed entirely by myself and it has not been accepted in any previous application for a degree. The work, of which it is a record, has been done by myself. Quotations have been distinguished by quotation marks, and sources of information have been specifically acknowledged.

Alison Jayne Bond

ABSTRACT

A series of experiments were undertaken to determine the effects of stage of maturity and inclusion rate of processed whole-crop wheat (WCW) on crop production, dairy cow performance and metabolism. In experiment one, WCW was harvested at four stages of maturity. Harvesting WCW at approximately 700 g dry matter (DM)/kg significantly increased grain DM yield. Inclusion of processed WCW harvested at 700 g DM/kg and urea-treated resulted in a higher milk yield and lower DM intake compared with animals offered a lower DM, fermented forage or a high DM processed untreated forage. In experiment two, WCW was harvested at 800 g/kg and urea-treated. Processed, urea-treated WCW was included in the ration of dairy cows at differing inclusion rates and the effect on dairy cow performance and apparent digestibility determined. Inclusion of WCW at 0.25 of the forage DM resulted in increased milk yield and protein yield compared with feeding grass silage alone, whilst there was little benefit in feeding WCW at higher inclusion rates. In the third experiment, the effect of rate of inclusion of processed, urea-treated WCW on ruminal fermentation, microbial growth and ruminal digestibility was determined using continuous culture fermentors. The inclusion of processed, urea-treated WCW had no effect on pH, total volatile fatty acid concentration or the proportion of acetate and butyrate. However, the ratio of acetate to propionate increased with rate of inclusion of processed, urea-treated WCW from 2.5 in vessels receiving 0.25 whole-crop wheat to 3.2 in those receiving 0.75 whole-crop wheat. Dry matter, organic matter and neutral detergent fibre digestibilities were not affected by WCW inclusion whilst starch digestibility increased. There was no effect of inclusion on microbial protein synthesis, although numerically diets containing 0.25 WCW had higher values. It is therefore recommended that processed, urea-treated WCW be harvested at a minimum of 700 g DM/kg or as soon thereafter as practically possible and urea-treated and that it should replace 0.25 of the silage in the ration.

ACKNOWLEDGEMENTS

I would like to thank the following people, without whose help this project would not have been possible:

Firstly, Dr's Liam Sinclair, Russell Readman and Jim Huntington for all their help and guidance throughout the experiments and write-up of this thesis.

The animal technicians, Dave Ferguson and Giles Vince, along with all the placement students for their time and patience. The laboratory technicians and Dr John Clement for their time and trouble, along with all the Honours students that have been involved throughout. Kevin Sinclair of Nottingham University for the insulin assay and Debbie Allen of IGER who helped with the ^{15}N analysis.

Finally, thanks to my family and close friends, especially Mum, Dad and Nik for all their encouragement and support throughout everything.

Part of this thesis has appeared previously;

Bond, A.J., Readman, R.J., Huntington, J.A. and Sinclair, L.A. (2004) The effect of stage of maturity and method of preservation of processed, whole-crop wheat on the intake and milk production in dairy cows. *Proceedings of the Winter meeting of the British Society of Animal Science*, p 49.

Bond, A.J., Readman, R.J., Huntington, J.A. and Sinclair, L.A. (2005) The effect of rate of inclusion of processed, urea treated whole-crop wheat on the intake, milk production and apparent digestibility in dairy cows. *Proceedings of the Winter meeting of the British Society of Animal Science,* p 22.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

Feed costs are the largest single variable input on dairy farms, and the cost of concentrates is approximately twice that of conserved forage on a DM basis (Phipps *et al.*, 1992). One way to facilitate a reduction in feed costs is to reduce concentrate input and attempt to place greater reliance on high quality, home grown forages with high intake potentials (Phipps, 1996). In the past, grass has provided the most important single source of grazed and conserved forage for ruminant production in the UK (Phipps *et al.*, 1995). Although its nutritional quality has improved, the higher genetic merit and production potential of the UK dairy herd (Brigstocke, 2001) would indicate that grass silage may not be capable of supporting the high yielding dairy cow (Phipps, 1994). In addition, attention has recently focused on the need to improve nitrogen (N) efficiency in dairy production systems and alternative forages, such as whole-crop cereals can be produced with greater N efficiency compared with grass silage (Hameleers, 1998). Consequently, alternative forages to grass silage have been considered, the two most popular options being maize silage and whole-crop cereals.

Maize silage overcomes many of the problems of grass silage, with its potentially lower forage costs, increased forage intake and improved product quality (Phipps, 1996; Heron, 1996). Phipps *et al.* (1995) demonstrated that when offered to dairy cows along with grass silage, DM intakes were increased, along with higher milk yields (kg/d) and milk protein concentrations (g/kg). Northern and western regions of the UK and Ireland are however, considered as marginal growing areas for maize due to the moist, cool climate. Consequently, it is often harvested at an earlier stage of maturity which results in lower DM intakes and milk production than when maize is fed at higher maturities (O'Mara *et al.*, 1998; Hameleers, 1998).

Whole-crop cereals are a versatile forage, providing several harvesting options depending on farm requirements and winter forage stock (Bastiman and Pullar, 1992). They tend to be of consistent nutritional quality even in a dry season (Heron, 1996) whilst producing higher DM yields from one cut compared to three cuts of grass silage (Bastiman and Pullar, 1992). Almost any species of autumn or spring sown cereal may be used, but due to its greater yield, however, winter wheat has been most popular and the cereal most widely reported (Bax, 1997a).

Work conducted on whole-crop wheat (WCW) has focused on its utilisation by the dairy cow. Results have indicated increased DM intakes but similar milk production and composition to grass silage (Phipps *et al.*, 1995; Sutton *et al.*, 1997; Sutton *et al.*, 2002). The reduced utilisation of WCW has been attributed to a low digestibility, especially of the starch fraction. Abdalla *et al.* (1999) suggested that some form of physical processing of the grain may be beneficial, particularly at higher DM values. Recently, a forage processor has been developed that cracks the grains at harvest and has been shown to increase whole tract digestibility of the starch component and improve efficiency of forage utilisation (Jackson *et al.*, 2004).

Chapter 1

LITERATURE REVIEW

1.1. WHOLE-CROP CEREALS

Whole-crop cereals are produced by conserving the part of the plant above stubble height under alkaline or acidic conditions (Adesogan *et al.*, 1998b). These forages are increasingly being used for winter feeding of ruminant livestock (Adesogan *et al.*, 1998b). They offer the potential of a higher yielding forage than grass, particularly in dry years when grass production is low and unpredictable, or in climates where alternative forages such as maize are difficult to grow (Sinclair *et al.*, 2003). Whole-crop cereal silages also provide flexibility, as the decision of the quantity of cereal crop to ensile can be made after the first and second cut grass silages and therefore according to a farms requirement and winter forage stock, whilst the remainder of the cereal can then be harvested as grain (Garnsworthy and Stokes, 1993). In addition, the high DM yield from one harvest is an efficient use of land and labour (Bastiman and Pullar, 1992), whilst there is little or no effluent production and lower N fertiliser requirements (Castejon and Leaver, 1994).

Almost any species of autumn or spring-sown cereal may be used, and oats (*Avena sativa*), rye (*Secale cereale*), triticale (*Triticale hexaploide*), barley *(Hordeum sativa)* and wheat (*Triticum aestivum*) have all been evaluated as potential forage sources (McDonald *et al.*, 1991). However, winter wheat tends to be the most commonly used and reported as it generally produces a higher yield (Table 1.1). It also is more drought resistant, and has a high DM yield and grain: straw ratio (Raymond and Waltham, 1996).

Table 1.1. Maximum DM yields of winter cereals (Tetlow, 1992)

	DM yield (t/ha)
Barley	11.9
Wheat	15.0
Oats	15.2
Triticale	18.1

1.2. WHOLE-CROP WHEAT PRODUCTION

1.2.1. The crop

In practice, the production of WCW is very similar to that of the same crop grown for high yielding grain production (Raymond and Waltham, 1996). Shorter strawed, higher yielding feed wheat varieties with good standing ability and disease resistance are most suitable for WCW (Heron, 1996). Furthermore, the relationship between the proportion of grain in the total DM and modified acid detergent fibre (MADF) concentration of the whole plant (Figure. 1.1), influences nutritional quality and illustrates the importance of selecting varieties with the highest grain content (Weller *et al.*, 1995).

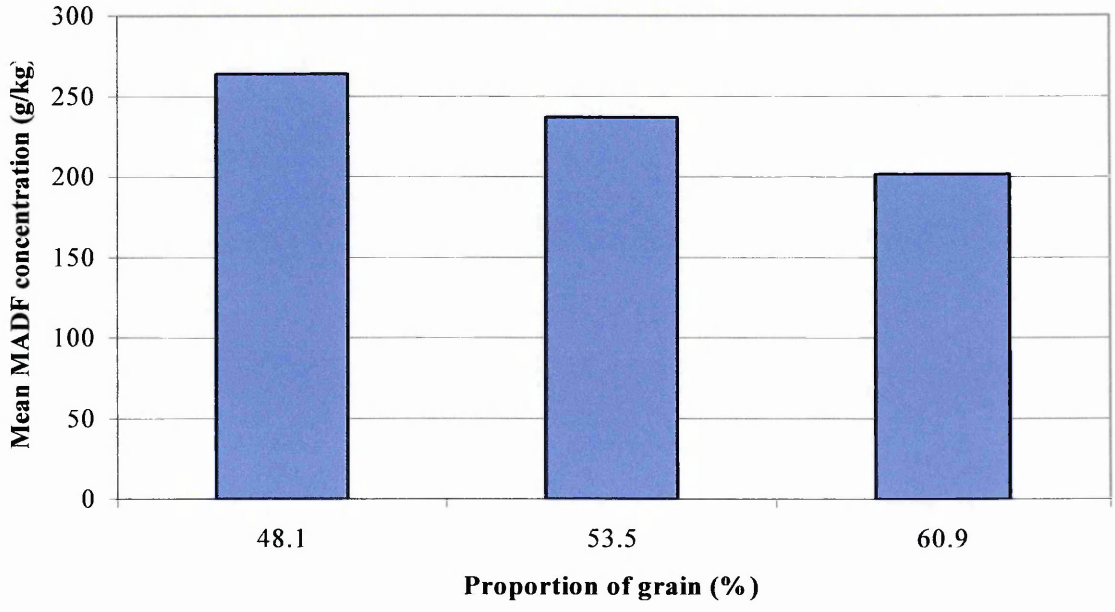

Figure 1.1. The relationship between the proportion of grain in the total DM (%) and modified acid detergent fibre (MADF) concentration of the whole plant (g/kg)

Winter wheat is normally drilled between September and March (Wiseman *et al.*, 2000) at a row width of 10-18 cm with a depth of 2.5 cm and at a rate of 150-250 kg/ha (Wiseman *et al.*, 2000) with the aim to establish 250-350 plants per square metre by the end of the winter (Wiseman *et al.*, 2000). Potassium and phosphorus are commonly applied to the seedbed whilst N is applied in the early spring as a top dressing (Stoskopf, 1981). Wheat is usually sprayed for weed control, with both grass and broad-leaved weeds being common (Boatfield, 1983). Winter wheat for whole-crop can be harvested from 350 g DM/kg onwards depending on whether it is to be fermented or alkaline treated (Bax, 1997a).

1.2.2. Harvesting

To enable effective preservation of a crop for WCW it is important to have some knowledge of the DM of the standing crop prior to harvest (Harvey, 1992). Commonly the Zadoks decimal code (Tottman and Broad, 1987) is used for monitoring the growth stages of cereals. However, Harvey (1992) reported a difficulty in using this key on farm and therefore recommended crop colour and grain texture as an alternative measure of crop DM. Table 1.2 shows the growers guide to the DM content of WCW compiled by Harvey (1992) which includes crop colour and grain texture, in addition Zadoks growth stages have been included (Tottman and Broad, 1987).

Whole-crop wheat has traditionally been direct cut with a self-propelled harvester and modified header (Heron, 1996) which helps to minimise grain loss (Redman and Knight, 1992). Whole-crop wheat to be fermented should be cut at a target length of 25 mm, to aid consolidation, ensure good compaction and reduce aerobic spoilage (Heron, 1996). Alternatively, alkaline-treated WCW need only be moderately consolidated to allow ammonia dispersion but must be sealed completely to ensure retention of the ammonia and prevent early ingress of air (Maize Growers Association, 1992).

Table 1.2. Growers' guide to the DM content and grain growth stages (GS) of whole-crop wheat (Harvey, 1992)

DM (g/kg)	Crop colour	Grain texture
320-350	Green	Soft brie; some grains milky. GS 71-83, late milk to early dough stage
360-380	Green	Soft brie. GS 78-83
390-420	Green, ears turning yellow	Soft cheddar, the grain does not hold fingernail impressions. GS 75-85
430-460	Green going yellow	Soft cheddar. GS 83-87
470-540	Yellow, hint of green	Hard cheddar, with some harder grains. GS 85-92
550-650	Yellow, hint of green on stem	Hard cheddar, with some grains impossible to penetrate with thumbnail. GS 87-92
660-700	Yellow/brown, traces of green at nodes	Very hard, with grains impossible to penetrate with thumbnail. GS 92-93
710-800	Yellow/brown	Too hard to penetrate with thumbnail; loosening in daytime. GS 93.

1.2.3. Factors affecting the yield of whole-crop wheat

i. Effect of wheat variety

A comparison of ten winter wheat varieties by Weller *et al.* (1995) demonstrated large differences between varieties for maximum DM yield and the date on which maximum yield was reached (Fig 1.2). Similarly, the later maturing cultivar Ariel, had a higher DM yield (16.4 t DM/ha) than the earlier maturing variety Bet Hashita (15.2 t DM/ha) (Ashbell *et al.*, 1997).

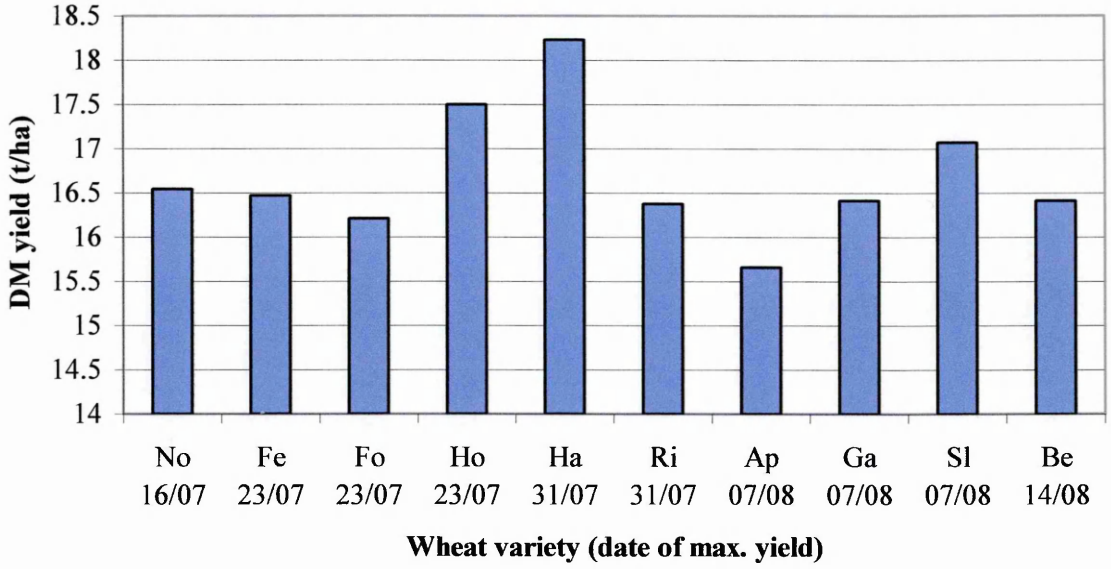

Figure 1.2. The effect of wheat variety on maximum DM yield (t/ha) and date of maximum yield. Wheat varieties: No, Norman; Fe, Fenman; Fo, Fortress; Ho, Hornet; Ha, Haven; Ri, Riband; Ap, Apollo; Ga, Galahad; Sl, Slepjner; Be, Beaver (Weller *et al.*, 1995)

ii. Effect of stage of maturity

Dry matter yields increased from 10.2 t DM/ha to 15.5 t DM/ha when WCW was harvested at growth stages 49 and 87 respectively (Hill and Leaver, 1999a). Similarly, Sinclair *et al.* (2003) reported an increase in DM yields from 12.1 to 15.6 tDM/ha when WCW was harvested at growth stages 71 and 85 (320 and 400 g DM/kg, respectively). Corral *et al.* (1977) concluded that the optimum DM yield was achieved at the medium-dough growth stage, 550-600 g DM/kg.

iii. Effect of cutting height

The effect of varying stubble height on DM yield of WCW has been investigated in a number of studies as summarised in Table 1.3. Increasing the stubble height of WCW at harvest decreases DM yield (Weller *et al.*, 1995; Sinclair *et al.*, 2003). Furthermore, Jackson *et al.* (2004) reported that at increased stages of maturity, the reduction in DM yield was greater.

Table 1.3. Dry matter (DM) yield (t DM/ha) of whole-crop wheat (WCW) cut at different stubble heights (cm)

Reference	DM (g/kg)	Stubble height	DM yield
Weller *et al.* (1995)	580	10.0	15.7
	580	25.0	14.1
	580	40.0	12.4
Sinclair *et al.* (2003)	450	17.9	10.6
	450	38.2	8.90
Jackson *et al.* (2004)	696	17.8	18.2
	696	37.3	14.9

1.2.4. Factors affecting ensiling losses of whole-crop wheat

The extent of DM losses is influenced by the intensity of fermentation (Bolsen *et al.*, 1983), although whole-crop wheat produces very little effluent (Castejon and Leaver, 1994). Crops which contain higher amounts of readily available sugars, higher amounts of less lignified cell-wall and higher moisture content are liable to have a more extensive fermentation, and hence higher ensiling DM losses (Adogla-Bessa and Owen, 1995; Table 1.4). In general, therefore, DM losses decrease with increasing maturity (Tetlow, 1992; Hill and Leaver, 1999a) whilst the addition of urea has been reported to further decrease DM losses of WCW harvested at growth stages 49 and 87 (Hill and Leaver, 1999a).

Table 1.4. Dry matter (DM) loss (g/kg DM) during fermentation for whole-crop wheat harvested at different stages of maturity

Reference	DM (g/kg)	DM loss
Bolsen *et al.* (1983)	270	11.4
	393	8.60
	472	9.70
Adogla-Bessa and Owen (1995)	310	22.0
	405	26.0
	520	14.0
	680	12.0

1.3. WHOLE-CROP WHEAT PRESERVATION

Whole-crop wheat may be preserved by two distinctly different methods; either by fermentation at lower plant maturities, 250 – 500 g DM/kg (Kristensen, 1992) or by alkali

(sodium hydroxide, ammonia or urea) treatment at higher maturities (>500 g DM/kg; Tetlow and Mason, 1987).

1.3.1 Fermented whole-crop wheat

Preservation of forages by fermentation is a naturally occurring process based on acid bacteria converting released plant sugars (water soluble carbohydrates) into organic acids (Filya *et al.*, 2000; Merry *et al.*, 2000). Homo-fermentative lactic acid bacteria (*Lactobacillus plantarum*, *Pediococcus* and *Enterococcus* species) promote a rapid reduction in pH due to the production of lactic acid. Less importantly, hetero-fermentative lactic acid bacteria produce acetic and lactic acid (Merry *et al.*, 2000). The reduction in pH reduces enzyme activity and undesirable micro-organisms, which are not tolerant of low pH (Merry *et al.*, 2000). Whole-crop wheat to be fermented is harvested between 350 and 550 g DM/kg, equivalent to the 'soft Cheddar cheese' grain stage, (growth stages (GS) 71 – 85; Tottman and Broad, 1987). This is approximately three to four weeks before the crop is ready for combine harvesting and the crop colour will be changing from green to yellow (Harvey, 1992). At 350 g DM/kg sugar content is high, and there is little risk of poor fermentation (Heron, 1996), however, yield has not reached its maximum potential (Tetlow and Mason, 1987). Harvested at the higher end of the DM range (approximately 500 g DM/kg), yield is increased and starch levels are higher, but decreased water-soluble carbohydrate levels may result in an inferior fermentation (lowered lactic: butyric acid ratio; Adogla-Bessa and Owens, 1995).

i. The effect of additives on fermented whole-crop wheat

To improve the ensiling process, various additives have been developed (Bolsen and Heidker, 1985). Both chemical and biological additives are available. Chemical additives, such as formic acid, inhibit or stop fermentation and increase aerobic stability (Merry *et*

al., 2000; McDonald *et al.*, 1991), whilst biological additives, such as lactic acid bacteria and enzymes, stimulate lactic acid fermentation, which results in a faster reduction in pH and a more efficient fermentation (higher lactic acid, lower acetic and butyric acid, lower ammonia) with a stable pH and reduced losses (Raymond and Waltham, 1996; Merry *et al.*, 2000).

The majority of biological inoculants contain more than one species of lactic acid bacteria, due to the development of the lactic acid bacterial population being pH dependant and successional (Merry *et al.*, 2000). Weinberg *et al.* (1993) reported that the addition of *Streptococcus faecium* to wheat harvested at flowering, milk and dough stages of maturity resulted in a more rapid decrease in forage pH in the initial stages of ensiling (Table 1.5). However, this organism is active only in the pH range 6.0 to 4.5. The addition of a second inoculum, *Lactobacillus plantarum*, together with *S. faecium* resulted in a faster drop in forage pH and to somewhat lower values (Weinberg *et al.*, 1993). Similarly, Filya *et al.* (2000) reported that the addition of *Lactobacillus plantarum* plus *Enterococcus faecium* and *Lactobacillus pentosus* to fresh and wilted wheat harvested at the milk stage improved fermentation parameters in both silages with reduced pH and increased lactic acid levels (Table 1.5). The pH of all inoculated silages decreased faster and to a greater extent than for the control silages (Filya *et al.*, 2000).

Unfortunately, the addition of lactic acid bacteria to wheat impaired the aerobic stability of forages (Weinberg *et al.*, 1993; Filya *et al.*, 2000). Filya *et al.* (2000) suggested that the decreased aerobic stability was a result of homo-fermentative lactic acid bacteria producing only lactic acid whilst using less water soluble carbohydrates, which are substrates for yeasts and moulds. Furthermore, only small amounts of inhibitory volatile fatty acids (VFA; acetic, butyric and propionic acids) are produced, resulting in silages treated with lactic acid bacteria deteriorating faster when exposed to air (Weinberg *et al.*,

1993). By contrast, Ohyama *et al.* (1975) reported improved aerobic stability of silage inoculated with *Lactobacillus plantarum*, which was probably due to the presence of low concentrations of oxygen at ensilage that resulted in a shift from homolactic fermentation to heterolactic fermentation and production of organic acids, such as acetic and butyric, which inhibit the development of yeasts and moulds.

Table 1.5. The effect of applying lactic acid bacteria at ensiling on pH, water-soluble carbohydrate (WSC) and lactic acid (LA) content of fermented whole-crop wheat

Reference	Treatment		pH	WSC (g/kg)	LA (g/kg)
Weinberg *et al.* (1993)	Flowering	Control	5.0	nd	14
		Inoculum[a]	3.8	60	65
	Milk	Control	3.9	62	31
		Inoculum[a]	3.8	91	33
	Dough	Control	4.0	35	10
		Inoculum[a]	4.0	40	21
Filya *et al.* (2000)	Fresh	Control	4.4	43	8
		Inoculum[b]	3.9	26	35
		Inoculum[c]	3.9	25	28
	Wilted	Control	5.2	46	2
		Inoculum[b]	4.0	27	23
		Inoculum[c]	4.0	44	19

[a] *Streptococcus faecium*
[b] *Lactobacillus plantarum and Enterococcus faecium*
[c] *Lactobacillus pentosus*

The ensiling process may also be improved by the addition of plant cell wall degrading enzymes, such as cellulases, hemi-cellulases, pectinases and amylases (Weinberg *et al.*, 1993). Cellulolytic enzymes have successfully been used to hydrolyse forage polysaccharides in grass silage (Huhtanen *et al.*, 1985). They function by increasing the supply of fermentable carbohydrates that are important for ensiling, through the hydrolysis of cellulose, as well as improving the digestibility of the silage by partial hydrolysis of the cell wall (Weinberg *et al.*, 1993; Adogla-Bessa and Owen, 1995). Combined with *Streptococcus faecium*, cellulase, hemicellulase and pectinase addition to wheat decreased forage pH throughout the ensiling period (Weinberg *et al.*, 1993). Furthermore, the addition of cellulase-hemicellulase based enzymes to WCW at harvest reduced DM losses

on exposure to air and tended to decrease neutral detergent fibre (NDF) and acid detergent fibre (ADF) values when harvested at 310, 405, 520 and 680 g DM/kg. However, increasing enzyme application did not progressively increase fermentation acids or reduce fibre content, the authors concluded that this was probably attributable to all the available substrate being completely hydrolysed at the lowest enzyme application. Enzyme treatment also enhanced fermentation, which was indicated by increased lactic acid and VFA production resulting in a lower pH in forages treated with the enzymes (Adogla-Bessa and Owen, 1995).

1.3.2 Alkaline treated whole-crop wheat

Harvesting at a later stage of maturity helps to maximise DM yield but can result in extensive lignification of the stem internode and leaf sheaf which leads to decreased digestibility (Weinberg *et al.*, 1991). Furthermore, unless the pericarp of the grain is disrupted, starch digestion may also be low (Ørskov, 1979). To counteract this decreased nutritive value various alkali treatments have been investigated. Alkali treatment increases digestibility by dissolving the lignocellulosoic cross-linking of the cell wall of the straw fraction as well as disrupting the testa of the grain (Tetlow and Mason, 1987). Wheat for alkaline treatment is traditionally harvested between 500 and 600 g DM/kg (GS 85 – 92; Tottman and Broad, 1987) when the grains are at the 'hard Cheddar cheese' stage (Bax, 1997b). At this stage the grains will take a thumbnail impression with difficulty, and the crop is almost entirely yellow in colour with some green material visible at the second node (Harvey, 1992). The crop is maturing very rapidly with the DM increasing by about 10 – 20 g/kg per day depending on weather conditions (Heron, 1996) and as a result there is only a small harvest window.

i. Effect of sodium hydroxide treatment of whole-crop wheat

Previous work has demonstrated that sodium hydroxide (NaOH) treatment is an effective method for upgrading low quality straws (Ørskov and Grubb, 1978) and increasing grain digestibility (Ørskov and Greenhalgh, 1977). At DM contents below 400 g/kg, NaOH treatment of WCW increased the production of fermentation acids in the clamp (Tetlow and Mason, 1987) but decreased ammonia-N content and pH (Deschard *et al.*, 1987). Furthermore, NaOH treatment stimulated primary and secondary fermentation in the silo but had no beneficial effect on aerobic stability (Deschard *et al.*, 1987). By contrast, at higher DM values (above 400 g/kg) the addition of NaOH reduced fermentation acid production in the clamp (Tetlow and Mason, 1987). Similarly, addition of NaOH to WCW harvested at a DM value of 690 g/kg increased forage pH and decreased lactic acid content (Tetlow *et al.*, 1987). In addition, the NaOH-treated WCW remained stable on exposure to air, which suggests that treatment may impart a considerable degree of aerobic stability at higher DM values (Tetlow *et al.*, 1987). Tetlow and Mason (1987) concluded that WCW directly harvested at a DM content of approximately 600 g/kg can be effectively preserved and upgraded by ensilage with NaOH. However, a disadvantage is that NaOH is dangerous and impractical to handle on farm (Tetlow, 1992).

ii Effect of ammonia treatment of whole-crop wheat

Ammonia treatment of whole-crop cereals has also been shown to efficiently preserve forages (Ørskov *et al.*, 1983). The advantage of using a nitrogenous alkali in comparison with NaOH is the increased N supply. The microbial requirement for N is higher when the digestibility of the fibre fraction is increased and this can be supplied by the added ammonia (Ørskov *et al.*, 1983). Treatment of WCW harvested at 410 g DM/kg with either anhydrous or aqueous ammonia (35 kg NH_3/t DM) increased forage pH to approximately pH 8.8, whilst lactic acid and ethanol production was greatly reduced and clostridial fermentation curtailed (Deschard *et al.*, 1987; Table 1.6). Similarly, at higher DM levels

(637 g DM/kg) anhydrous ammonia increased pH relative to the control and the production of lactic acid, butyric acid and ethanol were decreased (Deschard *et al.*, 1988). However, if sugar levels are high, as in lower DM WCW, ammonia treatment may cause the formation of toxic imidazoles (Nielsen *et al.*, 1986). In addition, like NaOH, ammonia treatment is difficult and can often be dangerous to handle and apply on farm (Tetlow, 1992).

Table 1.6. Effect of ammonia treatment (NH_3 and NH_4OH) of whole-crop wheat (WCW) on pH, lactic acid (LA), acetic acid (AA), butyric acid (BA) and ethanol production

Reference	Treatment	DM (g/kg)	pH	LA	AA	BA	Ethanol
Deschard *et*	Control	406	4.0	56	13	0	18
al. (1987)	NH_3	466	8.8	23	19	0	1.0
	NH_4OH	384	8.9	17	14	0	1.0
Deschard *et*	Control	593	4.9	32	13	2	4.3
al. (1988)	NH_3	637	8.7	8.2	17	0	0.3

iii. Effect of urea treatment of whole-crop wheat

Compared with ammonia, urea is safer to handle and this makes it an attractive alternative to NaOH and ammonia (Ørskov *et al.*, 1983). Consequently, urea application is currently the principal method of conservation for WCW harvested at approximately 500 g DM/kg (Hill and Leaver, 1999a). In the presence of moisture, urease, a naturally occurring enzyme, hydrolyses urea to release gaseous ammonia (Equation 1.1), which spreads throughout the clamp delignifying the cell walls and raising the pH above 8.0. This increase in pH prevents the development of moulds, yeasts and harmful bacteria (Tetlow, 1992) which inhibits aerobic deterioration (Givens *et al.*, 1993).

$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3 \qquad \text{(Equation 1.1)}$$

The optimum stage of maturity for urea application is between 450 and 550 g DM/kg (Tetlow, 1992) when the crop contains sufficient moisture levels for urease activity and ammonia release (Adesogan *et al.*, 1998a). At a DM content of only 410 g/kg urea

application exerted some control over fermentation and silage stability, but caused pungent silages and was unpalatable to sheep (Deschard *et al.*, 1987). Deschard *et al.* (1987) therefore concluded that it was inappropriate to use urea as an additive when DM content was below 400 g/kg. Hill and Leaver (1999a) indicated that the effect of urea treatment may be reduced in conserved forages with DM values below 600 g/kg due to the rapid initial fermentation of available substrates, such as water-soluble carbohydrates and production of short chain fatty acids, which result in a rapid decline in silo pH. At higher DM values (603 g/kg DM) aerobic stability of urea-treated WCW was similar to that of sodium hydroxide-treated WCW (Deschard *et al.*, 1988). Urea application also provided a valuable source of supplementary N for microbial growth (Deschard *et al.*, 1988) whilst increasing DM digestibility (Ørskov *et al.*, 1983). If DM content is above approximately 650 g/kg insufficient moisture may be present to hydrolyse the urea and ammonia will not be produced rapidly enough to prevent moulding in the stored crop (Hill and Leaver, 1999a).

The conversion of urea to ammonia in the clamp relies on the presence of moisture and bacterial ureases (Hill and Leaver, 1999a). Urease from other sources, such as soya bean, have been used to facilitate urea-treatment of high DM straws (Sahnoune *et al.*, 1991; Williams *et al.*, 1984). The use of a combined urea and urease additive for the treatment of mature WCW has been suggested to allow a rapid and more complete conversion of urea to ammonia, allowing the crop to be harvested at higher DM values. Recent work has harvested wheat for urea-treatment at DM levels as high as 800 g/kg (Sinclair *et al.*, 2005). The resulting forage was stable with a pH of 8.0 and ammonia N level of 165 g/kg total N, indicating an effective release of ammonia (Sinclair *et al.*, 2005).

The optimal level of urea application will vary according to the DM content of the forage (Hill and Leaver, 1999a), and is usually between 20 and 40 kg/t DM. Hill and Leaver

(1999a) reported that the optimal rate of application for aerobic stability was 20 g urea/kg DM. This level of application resulted in a similar level of dairy cow performance to a 40 g urea/kg DM application (Leaver and Hill, 1995). However, at the lower level it was particularly important that the urea was evenly spread at application (Leaver and Hill, 1995). By contrast, Adesogan *et al.* (1998a) reported that the greatest improvement in digestibility of urea-treated WCW was at a higher level of urea application (40 g urea/kg DM). Furthermore, O'Kiely and Moloney (1995) demonstrated that increasing urea addition from 56 to 111 kg/t DM increased forage pH from pH 5.6 to 7.6. In addition, urea application represents a nitrogen input of 250 kg N/ha, which when added to the 120 - 150 kg N/ha that is normally applied to grow the crop (Raymond and Waltham, 1996) represents a considerable N input (Leaver and Hill, 1992), much of which will be excreted, mainly in the urine (Raymond and Waltham, 1996).

1.4. CHEMICAL COMPOSITION OF WHOLE-CROP WHEAT

The varying nutritive value of WCW is well recognised with several factors affecting the composition (Adamson and Reeve, 1992). The major sources of variation are the type of preservation, grain: straw ratio, variety (Weller *et al.*, 1995), crop DM content (Weinberg *et al.*, 1991) and cutting height (Sinclair *et al.*, 2003; Jackson *et al.*, 2004).

1.4.1. Effect of preservation method on the chemical composition of whole-crop wheat

Table 1.7 shows the average chemical composition of fermented and urea-treated WCW. It is apparent that for both fermented and urea-treated WCW there is a wide range in DM. In general, the DM value of a crop at harvest will determine the type of preservation undertaken although there may be a certain amount of overlap.

Table 1.7. Average DM (g/kg), pH, Ammonium-N (g/kg total N), crude protein (CP; g/kg DM) and neutral detergent fibre (NDF; g/kg DM) composition of fermented whole-crop wheat (FWCW) and urea-treated whole-crop wheat (UWCW) treated with 20 g/kg DM urea (20) and 40 g/kg DM urea (40)

Reference	Treatment	DM (g/kg)	pH	Ammonium -N	CP	NDF
Adamson and Reeve (1992)	FWCW	337	4.0	70.0	102	483
	UWCW	549	7.7	250	244	438
Hameleers (1998)	FWCW	557	4.0	33.0	89.0	nd
	UWCW - 40	791	8.4	73.0	183	nd
Adesogan *et al.* (1998a)	FWCW	645	5.7	4.70	122	513*
	UWCW - 20	781	8.5	13.2	183	504*
	UWCW - 40	620	8.5	11.4	200	538*
Sutton *et al.* (2002)	FWCW	511	5.0	74.0	104	522
	UWCW - 40	584	7.3	62.0	199	414

nd not determined
* neutral detergent fibre and amylase

The addition of urea to the higher DM forages and its conversion to ammonia in the clamp is reflected in the higher crude protein and ammonium N levels (Adamson and Reeve, 1992; Hameleers, 1998). Furthermore, crude protein content increases with higher inclusion of urea (Adesogan *et al.*, 1998a). By contrast, Sutton *et al.* (1997 and 1998) reported inconsistent increases in crude protein content; the application of urea at the rate of 20 kg/t DM resulted in a crude protein content of 220 g/kg DM (Sutton *et al.*, 1997), whilst in a further study application of urea at 40 kg/t DM resulted in a crude protein content of only 171 g/kg DM (Sutton *et al.*, 1998). A large proportion of N in urea-treated WCW is in a rapidly soluble form and there is increasing evidence that this may be released too rapidly in the rumen (Abdalla *et al.*, 1999). If the excess N is not captured by the rumen microbes it may be excreted and potentially act as a pollutant (Raymond and Waltham, 1996).

The treatment of wheat straws with NaOH decreased NDF (Table 1.8), mainly because of a reduction in hemicellullose content, which resulted in cellulose and lignin constituting a greater proportion of the remaining cell wall (Moss *et al.*, 1990). Similarly, ammonia

treatment of WCW resulted in a reduction of the NDF fraction, due to the partial solubilisation of hemicellulose (Haddad *et al.*, 1995; Fondevila *et al.*, 1994).

Table 1.8. The chemical composition (g/kg DM, unless otherwise stated) of sodium hydroxide (NaOH) and untreated wheat straw (Moss *et al.*, 1990)

	Untreated	NaOH treated
Dry matter (g/kg)	841	762
Crude protein	44	39
Neutral detergent fibre	799	681
Cellulose	407	398
Hemicellulose	276	171
Lignin	91	95

The pH value of WCW forage gives a good indication of its method of preservation (Adamson and Reeve, 1992). Fermented WCW has a pH value similar to grass silage of an equivalent DM, but the production of fermentation acids and their subsequent burden to the rumen, otherwise known as the buffering capacity, is much lower (Heron, 1996). By contrast, urea-treated WCW has an alkaline pH, between pH 7.0 and 8.0 depending on DM content and urea application rate (Adamson and Reeve, 1992). The high pH values indicate that an alkaline state has been reached thus preventing fermentation from occurring (Adogla-Bessa *et al.*, 1999). The alkalinity of urea-treated WCW will also buffer rumen acidity (Adogla-Bessa *et al.*, 1999) and may provide a more conducive environment for cellulolysis and growth of rumen bacteria and protozoa (Mould and Ørskov, 1983).

1.4.2. Effect of stage of maturity on the chemical composition of whole-crop wheat

The chemical composition of WCW harvested at different DM contents as recorded in various studies is shown in Table 1.9. Whole-crop wheat DM increases with maturity (Tetlow and Mason, 1987; Crovetto *et al.*, 1998; Adesogan *et al.*, 1998a) as does the proportion of grain DM in the whole-crop DM (Hill and Leaver, 1999a).

Table 1.9. The DM (g/kg), crude protein (CP, g/kg DM), neutral detergent fibre (NDF; g/kg DM), water soluble carbohydrate (WSC; g/kg DM), starch (g/kg DM) and pH composition of whole-crop wheat harvested at differing stages of maturity

Reference	DM	CP	NDF	WSC	Starch	pH
Ashbell *et al.* (1997)	319	109	664	nd	nd	4.63
	328	94.0	655	nd	nd	4.32
	343	151	584	nd	nd	4.36
	405	71.0	620	nd	nd	4.69
Hill and Leaver (1999a)	316	118	356	176	3	6.50
	445	112	416	62.0	110	6.50
	589	109	488	38.0	241	6.70
Sutton *et al.* (2002)	301	116	610	31.0	32	4.00
	511	104	522	2.00	170	5.00
Sinclair *et al.* (2003)	296	104	595	54.0	52	3.90
	371	78.0	487	10.0	221	4.00

nd not determined

The crude protein and ammonium N content of untreated WCW decreased with maturity (Crovetto *et al.*, 1998; Ashbell *et al.*, 1997). The maturation of the wheat crop is associated with an increase in the lignification of the stem and leaves (Leaver and Hill, 1995) and generally, NDF concentration increases with maturity (Leaver and Hill, 1992; Heron, 1996). However, in wheat, unlike in grass, NDF does not rise substantially (Weinberg *et al.*, 1991) as the starch content compensates for the increase in lignocellulose formation (Arieli and Adin, 1994).

The water-soluble carbohydrate content of wheat will remain high until 4-5 weeks after ear emergence, at which point the content will drop as they are converted to starch during grain formation (Heron, 1996; Tetlow and Mason, 1987; Tetlow, 1992). Consequently, starch content of WCW increases with DM content (Sinclair *et al.*, 2003; Adesogan *et al.*, 1998a).

1.4.3. Effect of cutting height on the chemical composition of whole-crop wheat

Altering the cutting height at harvest may improve the nutritive value of WCW (Jackson *et al.*, 2004). Weller *et al.* (1995) demonstrated that increasing the cutting height at harvest from 10 cm to 40 cm increased the proportion of grain within the forage from 0.48 to 0.61. Furthermore, there was a corresponding decrease in the MADF content from 250 to 195 g/kg DM. Neutral detergent fibre levels decreased as cutting height increased, whilst starch content increased (Sinclair *et al.*, 2003; Jackson *et al.*, 2004; Table 1.10). However, a comparison of tall and short wheat varieties (Cadenza vs Slepjner) demonstrated no difference between starch content (231 and 237 g/kg DM; Adesogan *et al.*, 1998a).

Table 1.10. The DM (g/kg), crude protein (CP; g/kg DM), neutral detergent fibre (NDF; g/kg DM) and starch (g/kg DM) composition of whole-crop wheat harvested at differing stubble heights (cm)

Reference	Stubble height (cm)	Crop height at harvest (cm)	DM	CP	NDF	Starch
Sinclair *et al.* (2003)	17.9	87.0	296	104	595	52
	38.2	87.0	371	78	487	221
Jackson *et al.* (2004)	17.8	66.6	713	137	425	369
	37.3	66.6	707	140	331	417

1.5. UTILISATION OF WHOLE-CROP WHEAT

In recent years there has been a considerable amount of interest in the utilisation of urea-treated and fermented WCW for ruminants, to act as an alternative or supplementary forage to grass and maize silage (Sutton *et al.* 1997).

1.5.1. Effect of preservation method on intake and animal performance

i. Fermented whole-crop wheat

Previous work has shown that a range of inoculants to fermented whole-crop cereals, including *Lactobacillus buchneri,* may improve animal performance (Kung and Muck, 1997). By contrast, the addition of *Lactobacillus buchneri* to WCW harvested at 494 g

DM/kg and fermented had no effect on food intake, milk production or milk composition of dairy cows (Sutton *et al.*, 2002: Table 1.11).

Table 1.11. Average forage DM intake (DMI; kg/d), milk yield (kg/d) and milk composition (g/kg) of dairy cows offered fermented whole-crop wheat treated with *Lactobacillus buchneri* (Sutton *et al.*, 2002)

Treatment	Forage DMI	Milk yield	Milk fat	Milk protein
Control	11.7	28.5	46.3	32.9
Inoculum	11.7	28.8	46.6	32.2

ii. Sodium hydroxide-treated whole-crop wheat

The treatment of WCW with NaOH may improve starch digestibility by increasing digestibility of the husk of the wheat grain thereby increasing access to the starch (Sutton *et al.*, 2001). Previously, the treatment of WCW with NaOH was shown to increase digestibility (Tetlow, 1992) and resulted in increased live-weight gain by steers (Deschard *et al.*, 1988). In sheep, NaOH treatment led to improved digestibility and voluntary intake (Tetlow *et al.*, 1987). Unfortunately, NaOH also stimulated water consumption and increased urine production in sheep (Tetlow *et al.*, 1987) and steers (Deschard *et al.*, 1988) to unacceptable levels. Furthermore, at DM levels above 450 g/kg, NaOH addition at 50 g/kg DM increased the N requirements of the rumen bacteria for protein synthesis (Tetlow *et al.*, 1987). In dairy cows, NaOH treatment of wheat straw and grain decreased forage DM intake compared with animals offered urea-treated and fermented WCW (Leaver and Hill, 1995). While milk yield, composition and constituent yield were unaffected by treatment (Leaver and Hill, 1995). By contrast, Sutton *et al.* (2001) reported increased DM intakes, which resulted in a small but consistent increase in milk yield as a result of NaOH treatment of WCW. Neutral detergent fibre digestibility was increased, but starch digestibility decreased, meaning NaOH treatment had no effect on OM digestion. Consequently, increased milk yields were due to enhanced food intake and not increased starch digestion or improved energy utilisation (Sutton *et al.*, 2001). Sodium hydroxide

treatment also reduced the concentration of ammonia in the WCW and as a consequence, reduced the rumen ammonia concentrations and losses of ammonia in urine. This did not result in increased milk N or retained N, but did reduce the potential N pollution compared with feeding urea-treated WCW. However, NaOH treatment led to higher water consumption and therefore increased urine production in dairy cattle (Sutton *et al.*, 2001).

iii. Urea-treated whole-crop wheat

Urea treatment is currently the principal method of conservation for WCW harvested at approximately 500 g DM/kg (Hill and Leaver, 1999a). The addition of urea to forages may buffer rumen acidity and thus provide a conducive environment for cellulolysis and the growth of rumen bacteria and protozoa (Mould and Ørskov, 1983). In addition, an improvement in synchronisation of supply of additional volatile N from urea, and the fermentable energy in WCW, may contribute to enhanced microbial growth (Adogla-Bessa *et al.*, 1999) and animal performance (Sinclair *et al.*, 1993). Previous work has reported that the partial replacement of grass silage with urea-treated WCW in dairy cow rations increased DM intake without significantly affecting milk yield or composition (Leaver and Hill, 1995; Phipps *et al.*, 1995; Hameleers, 1998; Sutton *et al.*, 2002; Table 1.12).

Table 1.12. The forage DM intake (DMI; kg/d), milk yield (kg/d) and composition (g/kg) of dairy cows offered grass silage alone (GS) or with either fermented whole-crop wheat (FWCW) or urea-treated whole-crop wheat (UWCW; 40 g urea/kg DM)

Reference	Treatment	Forage DMI	Milk yield	Milk fat	Milk protein
Leaver and Hill (1995)	GS	9.4	28.0	40.4	31.3
	FWCW	11.1	28.7	40.5	31.3
	UWCW	11.6	29.6	39.8	31.2
Phipps *et al.* (1995)	GS	9.3	23.0	41.7	29.9
	FWCW	10.6	24.2	41.7	30.8
	UWCW	10.2	24.0	42.1	30.8
Hameleers (1998)	GS	10.6	27.4	48.9	34.1
	FWCW	12.2	27.1	49.0	34.0
	UWCW	13.1	26.9	48.1	34.3
Sutton *et al.* (2002)	FWCW	11.7	28.5	46.3	32.9
	UWCW	13.7	29.2	47.4	34.2

iv. Comparison of fermented and urea-treated whole-crop wheat

Comparisons of fermented and urea-treated WCW have demonstrated that intakes are generally higher for animals offered urea-treated WCW (Leaver and Hill, 1995; Hameleers, 1998; Sutton *et al.*, 2002; Table 1.12). Live-weight gain was not significantly affected by treatment (Hameleers, 1998; Sutton *et al.*, 2002), although ME intakes were higher for those animals offered fermented or urea-treated WCW as a result of the increased intakes (Hameleers, 1998).

Nitrogen intake for animals offered urea-treated WCW was significantly higher than animals offered grass silage or fermented WCW (Hameleers, 1998) as a result of the increased N content of this forage. As no additional milk protein was produced this resulted in a significantly lower N efficiency (Table 1.13). Furthermore, Sutton *et al.* (2002) reported large increases in losses of N in both faeces and urine for those animals offered urea-treated WCW. Plasma urea levels were also increased with urea treatment (O'Kiely and Moloney, 1995). This indicates an inefficient use of dietary N and suggests a need for a rapidly fermentable carbohydrate supplement to facilitate the incorporation of rapidly released ammonia into microbial protein in the rumen (Moloney *et al.*, 1994; Sinclair *et al.*, 1995).

Table 1.13. Nitrogen (N) efficiency of dairy cows offered grass silage alone (GS) or with either fermented whole-crop wheat (FWCW) or urea-treated whole-crop wheat (UWCW)

Reference	Treatment	N intake (g/d)	N milk* (g/d)	N efficiency$^{\Delta}$
Hameleers, 1998	GS	529	145	28.0
	FWCW	528	143	27.2
	UWCW	625	143	22.9

* N milk = milk yield (kg/d) x crude protein (g/kg)/ 6.38

$^{\Delta}$N efficiency = N milk/ N intake

1.5.2. Effect of stage of maturity on intake and animal performance

Increasing crop maturity of fermented WCW produced small but non-significant increases in both forage intake and milk yield but there were no treatment effects on milk fat or protein content (Sutton *et al.*, 2002; Sinclair *et al.*, 2003; Table 1.14). Plasma urea levels decreased for animals offered the higher maturity forage which may reflect the higher starch content and greater supply of fermentable energy to the rumen and subsequent capture of ammonia by rumen microbes (Sinclair *et al.*, 2003). Sutton *et al.* (2002) concluded that there was little evidence that harvesting WCW at such widely different DM contents had any practical implications for feeding dairy cows. However, crop yields per hectare did increase substantially with advancing maturity before stabilising at maturities of about 400g DM/kg (Sinclair *et al.*, 2003)

Table 1.14. Average forage DM intake (DMI; kg/d), milk yield (kg/d) and milk composition (g/kg) of dairy cows offered fermented whole-crop wheat (WCW) harvested at different stages of maturity

Reference	WCW DM	Forage DMI	Milk yield	Milk fat	Milk protein
Sutton *et al.* (2002)	301	10.9	27.9	47.7	33.0
	511	11.7	28.5	46.3	32.9
Sinclair *et al.* (2003)	296	10.7	30.1	40.9	30.7
	371	11.5	30.7	39.7	31.6

1.5.3. Effect of inclusion rate on intake and animal performance

Previously, Leaver and Hill (1992) reported poor milk yield responses when urea-treated WCW constituted 0.56 or 1.0 of the forage DM and concluded that it was due to low utilisation of the metabolizable energy (ME). Further work, in which urea-treated WCW replaced proportionately only 0.33 or 0.40 of the grass silage DM in forage mixtures demonstrated no major imbalance between calculated ME intake and output (Leaver and Hill, 1995). The authors suggested that the previous inefficiency was a reflection of the higher proportion of WCW in the forage (Leaver and Hill, 1995). Increasing the proportion

of urea-treated WCW in the diet from 0.33 to 0.67 significantly increased DM intake whilst only raising milk yield slightly (Sutton *et al.*, 1997, Abdalla *et al.*, 1999, Phipps *et al.*, 1992; Table 1.15).

Table 1.15. Average forage DM intake (DMI; kg/d) and milk yield (kg/d) of cows offered grass silage alone (GS) or urea-treated whole-crop wheat (UWCW; 40 g urea/kg DM) at differing inclusion rates.

Reference	Type	GS: WCW	Forage DMI	Milk yield
Phipps *et al.* (1992)	GS	100	6.30	18.1
	UWCW	75: 25	7.70	18.1
	UWCW	50: 50	7.60	18.2
	UWCW	25: 75	8.00	18.4
	UWCW	100	7.70	18.2
Sutton *et al.* (1997)	GS	100	9.26	20.1
	UWCW	67: 33	10.3	20.5
	UWCW	33: 67	10.7	21.6
Abdalla *et al.* (1999)	GS	100	10.6	21.2
	UWCW	67: 33	11.5	21.2
	UWCW	33: 67	12.4	22.2

*Urea applied at 40 kg/t

Milk fat level was slightly lower for those animals offered the higher level of WCW (Figure 1.3), whilst milk protein was increased for both treatments containing WCW (Sutton *et al.*, 1997, Phipps *et al.*, 1992; Figure 1.4). Furthermore, milk urea concentrations were significantly higher for animals offered the higher levels of urea-treated WCW (Sutton *et al.*, 1997). This would imply that rumen ammonia levels were also increased and, therefore, improvement in dairy cow performance may occur by means of supplementation with a readily fermentable carbohydrate (Sutton *et al.*, 1997).

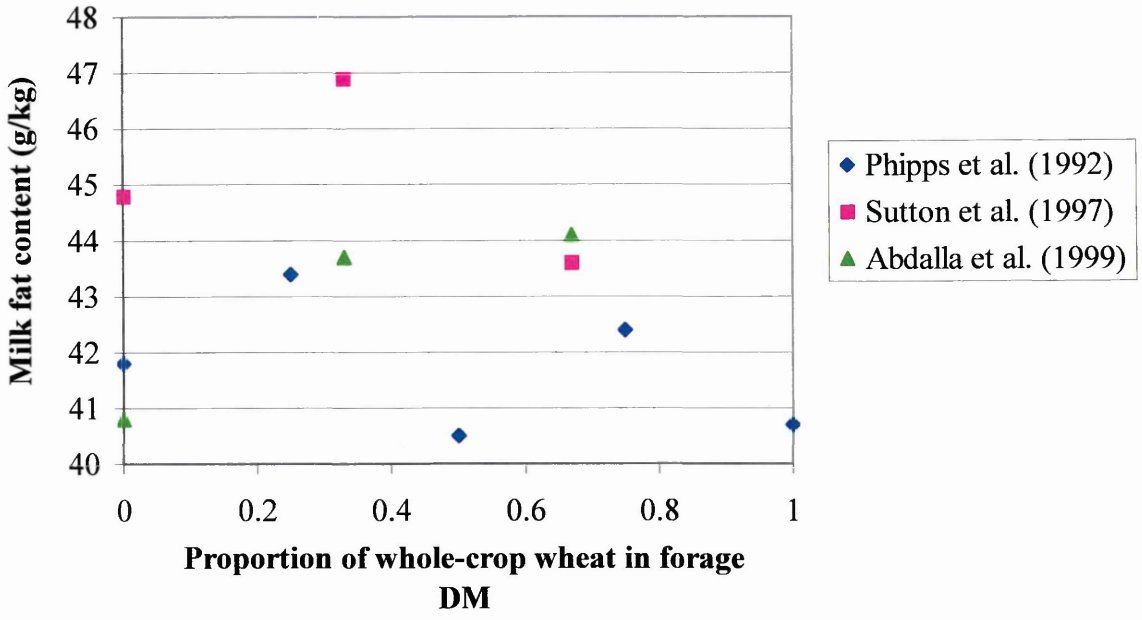

Figure 1.3. Average milk fat level (g/kg) of cows offered grass silage alone or urea-treated whole-crop wheat at differing inclusion rates

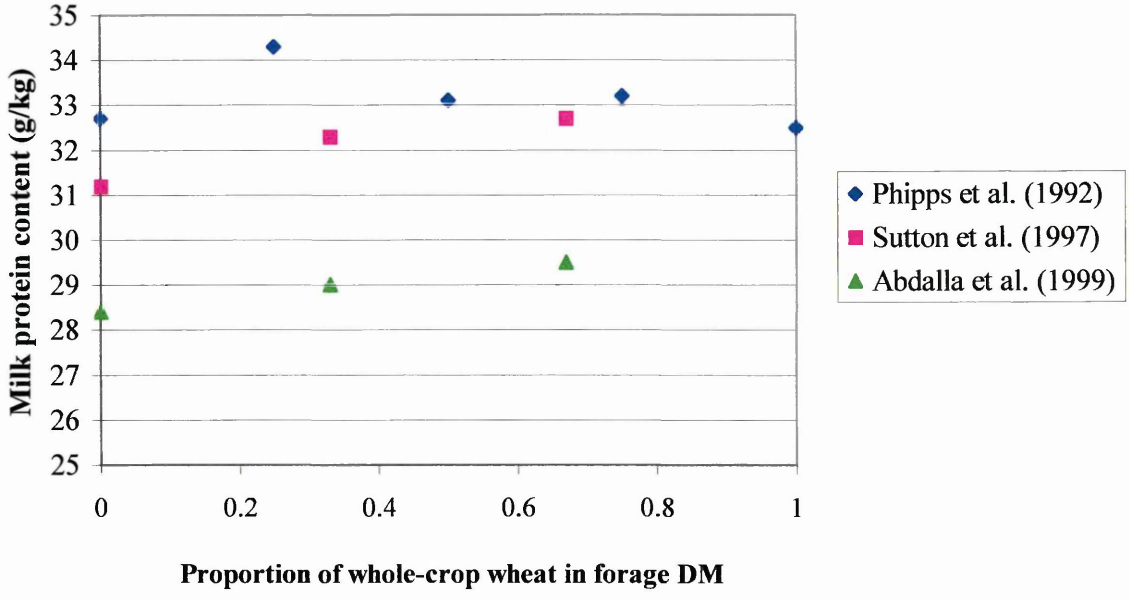

Figure 1.4. Average milk protein level (g/kg) of cows offered grass silage alone or urea-treated whole-crop wheat at differing inclusion rates

Abdalla *et al.* (1999) reported increased proportions of acetate and butyrate in the rumen with increasing inclusion of WCW in the diet of dairy cows, whilst the proportion of rumen propionate was reduced (Table 1.16).

Table 1.16. Average concentrations of total volatile fatty acids (VFA; mmol/l) and molar proportions of acetate, butyrate and propionate (mmol/l) in the rumen of cows offered grass silage alone (GS) or urea-treated whole-crop wheat (UWCW) at differing inclusion rates (Abdalla *et al.* 1999)

Type	GS: WCW	Total VFA	Acetate	Butyrate	Propionate
GS	100	107	638	102	223
UWCW	67: 33	113	647	113	205
UWCW	33: 67	111	659	113	197

This result is unusual as increased starch to fibre ratios are usually associated with a shift in VFA production in the rumen away from acetate towards propionate (Reynolds *et al.*, 1997). By contrast, Overton *et al.* (1995) reported that although the total VFA concentration was unaltered with increasing barley starch in the diet of dairy cows the proportion of acetate decreased whilst propionate increased (Table 1.17). Furthermore, milk fat content decreased with increasing barley starch (Overton *et al.*, 1995).

Table 1.17. Average milk fat content (kg/d), concentrations of total volatile fatty acids (VFA; mM) and molar proportions of acetate, butyrate and propionate (mol/100mol) in the rumen for cows offered differing ratios of corn starch (CS) and barley starch (BS; Overton *et al.*, 1995)

CS: BS	Milk fat	Total VFA	Acetate	Butyrate	Propionate
100: 0	0.97	120	61.1	11.2	24.9
75: 25	0.94	122	58.0	9.80	29.6
50: 50	0.92	121	57.1	9.80	30.5
25: 75	0.85	119	56.1	9.00	32.2
0 : 100	0.86	123	56.1	9.40	31.7

A considerable amount of work has been conducted in the area of milk fat depression as a result of feeding high grain, low roughage diets (Bauman *et al.*, 2001; Bauman and Griinari, 2001). Acetate is the principal carbon source for fat synthesis in the mammary gland and, therefore, a shift in VFA production in the rumen away from acetate towards propionate may cause milk fat depression (Bauman and Griinari, 2000). Furthermore, increased propionate triggers pancreatic release of insulin which depresses lipolysis and enhances lipogenesis, thereby causing a shortage of lipogenic precursors for milk fat synthesis by the mammary gland (Bauman and Griinari, 2001) and increased lipid

deposition in body tissue (Reynolds *et al.*, 1997). However, more recent evidence suggests that milk fat depression as a result of feeding high grain, low roughage diets results from a direct inhibition of milk fat synthesis by trans fatty acids (Bauman and Griinari, 2001). Trans fatty acids are formed as intermediates in the biohydrogenation of unsaturated fatty acids by rumen bacteria (Figure 1.5). Previous work (Griinari *et al.*, 2000) has suggested that the reduction in milk fat yield is correlated with an increase in *trans*-10, $C_{18:1}$, and in particular *trans*-10, *cis* 12 conjugated linoleic acid (CLA; Bauman and Griinari, 2001).

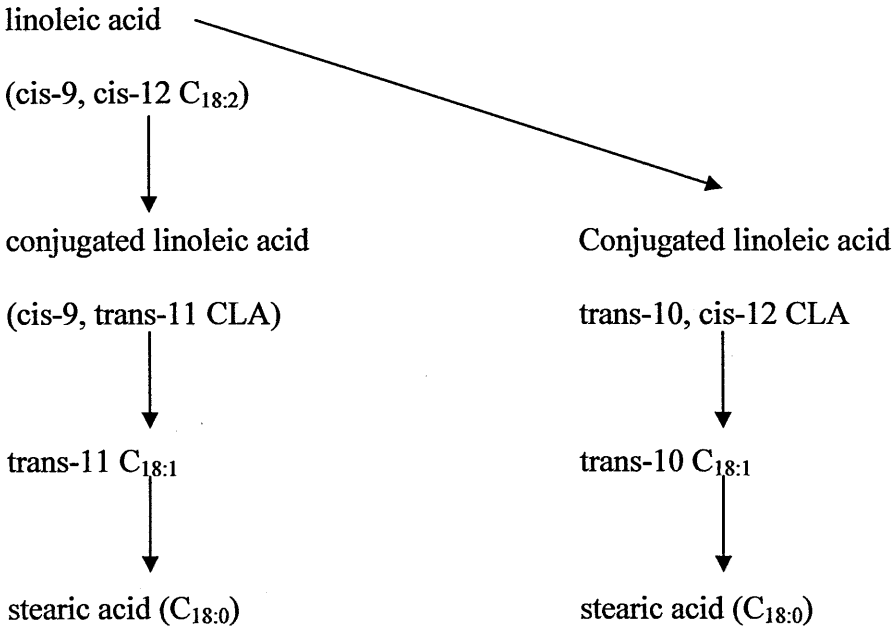

Figure 1.5. Pathways of ruminal biohydrogenation of linoleic acid (Griinari and Bauman, 1999).

In addition, high starch diets may result in rumen acidosis which may cause lameness in dairy cows (Blowey, 1993). Rumen acidosis may cause bacterial endotoxins to enter the circulatory system and produce an allergic reaction in the capillary cells of the corium in the hoof. This can result in an insufficient supply of sulphur amino acids reaching the keratin-producing cells of the epidermis, thus leading to lower levels of disulphide bonding in the keratin tissue and the production of structurally unviable horn (Dougherty *et al.*, 1975). Comparisons of starch (barley) and fibre (sugar-beet pulp) based diets demonstrated

increased lameness in animals offered the starch based diets (Kelly and Leaver, 1990). Furthermore, Bazeley and Pinsent (1984) reported high dietary protein levels or high levels of free ammonia in silage resulted in a higher incidence of laminitis. Similarly, Manson and Leaver (1988) reported a high protein diet significantly increased locomotion scores and the duration and number of observations of clinical lameness. Previously, it has been suggested that thromboses develop in the capillary supply of the hooves as an allergic-histaminotic reaction to high protein levels in the diet (Nilson, 1963). Thromboses may prevent adequate supplies of sulphur amino acids reaching the keratin producing cells, which results in poor horn formation. Alternatively, high protein intakes result in excess rumen ammonia production which may cause liver damage and induce laminitis. In addition, high serum glutamate oxaloacetate transaminase (GOT) levels in animals offered a high protein diet may indicate liver damage (Manson and Leaver, 1988). Although WCW is high in dietary starch, and ammonia if urea-treated, it is also high in fibre, which may decrease the occurrence of lameness. Currently no work has been conducted to determine the effect of feeding WCW on the incidence of lameness in dairy cows.

1.5.4. Effect of supplementation on intake and animal performance

i. Effect of energy supplementation

The addition of readily fermentable carbohydrates, such as molasses, sucrose or starch, to dairy cow diets supplies rapidly available energy to the rumen microbes. This may enable a more efficient utilisation of the rapidly degradable nitrogen in silages and a greater microbial protein synthesis (Sinclair *et al.*, 1993; Sinclair *et al.*, 1995; Richardson *et al.*, 2003), which is a good source of amino acids for milk protein production (Murphy, 1999). Previous work has reported that the addition of sucrose to grass silage based diets for dairy cows increased milk protein concentration (g/kg) but did not alter forage DM intake or

milk yield (Keady and Murphy, 1998; Table 1.18) which would indicate an improved efficiency of microbial protein synthesis in the rumen (Chamberlain *et al.*, 1993).

Table 1.18. Average forage DM intake (DMI: kg/d), milk yield (kg/d) and milk composition (g/kg) for cows offered grass silage alone (SO) or grass silage supplemented with sucrose (SS) (Keady and Murphy, 1998).

Treatment	Forage DMI	Milk yield	Milk fat	Milk protein
SO	14.0	14.4	38.6	30.7
SS	13.7	14.5	38.6	32.2

Sugar cane molasses contains approximately 657 g/kg DM water-soluble carbohydrates (Ministry of Agriculture, Fisheries and Food; MAFF 1992) of which the majority is sucrose (Blake, 1993), and would appear to be a good source of rumen fermentable energy (Murphy, 1999). Increasing the addition of molasses to grass-silage based diets increased forage DM intakes, milk yields and milk protein yields (Murphy, 1999; Yan *et al.*, 1997). Furthermore, reduced blood urea levels for animals offered molasses reflected a more efficient capture of degradable N due to the increased fermentable metabolisable energy intakes (Murphy, 1999). Similarly, supplementation of grass silage with molasses decreased rumen ammonia-N concentration and mean plasma urea-N compared with animals offered a barley-based supplement, whilst also decreasing acetate and increasing butyrate proportions in the rumen fluid (Moloney *et al.*, 1994).

Previously, supplementation of fermented WCW with either wheat or molasses had no consistent effect on forage DM intake, milk production or composition (Sinclair *et al.*, 2003; Table 1.19). Supplementation of WCW with wheat did however tend to decrease plasma ß-hydroxybutyrate (BHB) and non-esterified fatty acid (NEFA) levels which would indicate a more positive energy balance (Sinclair *et al.*, 2003).

Table 1.19. Average forage DM intake (DMI; kg/d) milk yield (kg/d) and milk composition (g/kg) for cows offered soyabean meal (SB) or wheat (W) supplement to fermented whole-crop wheat.

	Treatment	Forage DMI	Milk yield	Milk Fat	Milk protein
Sinclair *et al.* (2003)	SB	9.9	30.3	37.8	30.9
	W	9.7	29.9	36.6	31.4

The feeding of urea-treated WCW as the sole forage in the diet of dairy cows resulted in imbalances in total ME intakes and estimated ME outputs (Hill and Leaver, 1991). It was suggested that the high level of ammonia ingested due to feeding urea-treated WCW may increase the requirement for energy to excrete the ammonia as urea (Hill and Leaver, 1999b). The increased rumen ammonia levels as a consequence of partial inclusion of urea-treated WCW in the diet of dairy cows may offer the possibility for increased microbial protein synthesis and possibly milk production through supplementation with a suitable energy supplement (Sutton *et al.*, 2001; Abdalla *et al.*, 1999). Increasing the supply of ME from the diet, by altering the level of concentrate offered led to reduced forage intakes (Table 1.20) whilst significantly increasing milk yield and milk protein content (Leaver and Hill, 1992; Hill and Leaver, 1999b).

Table 1.20. Average forage DM intake (DMI; kg/d), milk yield (kg/d), milk fat and protein levels (g/kg) of cows offered whole-crop wheat plus differing levels of concentrate (Hill and Leaver, 1999b)

Concentrate inclusion	Forage DMI	Milk yield	Milk fat	Milk protein
6 kg/d	14.9	21.3	39.2	31.8
8 kg/d	13.8	22.4	40.2	32.7
10 kg/d	12.8	23.1	40.0	33.1

Likewise, supplementation of urea-treated WCW with molasses increased forage DM intakes compared with animals offered ground wheat, although there was no benefit in milk production (Table 1.21; Sutton *et al.*, 2001). Furthermore, the daily mean concentration of ammonia in the rumen was unaffected by molasses and there was even some evidence that the peak concentration was higher with molasses than with the control diet (Sutton *et al.*, 2001).

Table 1.21. Average forage DM intake (DMI; kg/d), milk yield (kg/d), milk fat and protein levels (g/kg) of cows offered urea-treated whole-crop wheat (UWCW) supplemented with molasses (M) or wheat (W)

Reference	Treatment	Forage DMI	Milk yield	Milk fat	Milk protein
Sutton *et al.* (2001)	UWCW	13.4	25.7	49.8	36.2
	UWCW M	14.7	25.9	48.3	35.7
	UWCW W	13.9	24.9	49.9	37.3

ii. Effect of protein supplementation

Increasing the supply of crude protein in diets based on urea-treated WCW had little effect on milk yield or composition (Hill and Leaver, 1999b). However, fishmeal supplementation decreased forage DM intakes (Castejon and Leaver, 1994) and significantly increased milk yield but not milk composition (Hill and Leaver, 1999b). It was suggested that this was probably due to the beneficial effects of fishmeal on DM digestibility and improved amino acid supply. High plasma urea levels reflected the high levels of ammonia and degradable nitrogen in the diet, although animals offered WCW supplemented with fishmeal had higher levels compared with those offered the soya-supplemented diet (Hill and Leaver, 1999b). By contrast, Sutton *et al.* (2001) reported that increasing the proportion of digestible undegraded protein in the diet had no effect on milk production (Table 1.22). It was suggested that this may have been due to the unusual pattern of digestion of urea-treated WCW. This was demonstrated by Abdalla *et al.* (1999) who reported a large reduction in the proportion of digestion occurring in the rumen with diets based on urea-treated WCW compared with those based on grass silage which was accompanied by a corresponding increase in dietary protein reaching the duodenum. Therefore, diets based on WCW may contain a higher proportion of digestible undegraded protein than would be expected if rumen fermentation were more normal (Sutton *et al.*, 2001).

Table 1.22. Average forage DM intake (DMI; kg/d), milk yield (kg/d) and milk composition (g/kg) of cows offered urea-treated whole-crop wheat supplemented with soyabean meal (SB) or fishmeal (FM)

Reference	Concentrate protein (g/kg DM)	Protein source	Forage DMI	Milk yield	Milk fat	Milk protein
Hill and Leaver (1999b)	166	SB	18.8	17.3	46.1	36.8
	333	SB	19.0	17.2	42.7	37.3
	329	FM	18.2	19.0	42.8	36.6
Sutton *et al.* (2001)	234	SB	13.4	25.7	49.8	36.2
	233	FM	13.6	26.1	48.8	36.6

iii. Effect of enzyme supplementation

Spraying enzymes directly onto feeds, just prior to feeding may protect them from degradation by ruminal proteases, thus improving nutrient digestion, utilisation and animal productivity (Kung *et al.*, 2000). Fibrolytic enzymes applied to the concentrate portion of dairy cow diets had no effect on DM intakes but significantly increased milk yield, probably as a result of enhanced nutrient digestibility in the total tract (Rode *et al.*, 1999). Similarly, the addition of fibrolytic enzymes to a diet based on corn silage and alfalfa hay prior to feeding improved milk production with no marked effect on milk composition or dry matter intakes (Kung *et al.*, 2000). Enzyme application to the concentrate portion of the diet of dairy cows increased milk production due to enhanced nutrient digestibility, whilst application of enzymes to the total mixed ration prior to feeding improved digestibility, but there was no effect on milk production (Yang *et al.*, 2000). Differences between enzyme combinations would suggest that source and combination of specific enzymes is an important factor in optimizing animal response (Kung *et al.*, 2000). In addition, Yang *et al.* (2000) reported that the method of enzyme application onto diets is crucial in affecting enzyme action in dairy cows.

Sutton *et al.* (2002) applied a cell-wall degrading enzyme to WCW diets immediately prior to feeding and reported that forage DM intake, milk yield, milk composition and live-

weight change were unaffected (Table 1.23). However, NDF digestibility reduced with enzyme application, whilst starch digestibility was unaffected.

Table 1.23. Average forage DM intake (DMI; kg/d), milk yield (kg/d), milk fat and protein content (g/kg) and neutral-detergent fibre (NDF) and starch digestibilities for cows offered WCW alone (WCW) and supplemented with a fibrolytic enzyme prior to feeding (Enzyme; Sutton *et al.*, 2002)

Treatment	Forage DMI	Milk yield	Milk fat	Milk protein	NDF	Starch
WCW	13.1	32.1	41.2	31.6	0.52	0.95
Enzyme	12.4	32.3	41.4	31.3	0.51	0.96

1.5.5. Effect of cutting height on intake and animal performance

It is well established that the grain: straw ratio of wheat will affect the nutritive value of WCW (Weller *et al.*, 1995). This means that an alternative way to increase the energy value of WCW is to increase stubble height at harvest by raising cutting height (Leaver and Hill, 1992). Raising cutting height of low DM (average 462 g DM/kg) fermented WCW from 17.8 cm to 37.3 cm had little effect on forage DM intake (Sinclair *et al.*, 2004; Table 1.24). Furthermore, milk yield and average yield of milk fat and protein were unaffected by cutting height, although there was a trend for animals offered the long straw forages to have the lowest concentration of milk protein (Sinclair *et al.*, 2003).

By contrast, at higher DM values (average 696 g/kg DM) and urea-treated forage DM intake tended to be lower for those animals offered the short straw forage (stubble height 37.3 cm; Jackson *et al.*, 2004). This may be a result of increased dietary starch with the short straw forages which could lead to a greater production of ruminal propionate (Theurer, 1986). Overton *et al.* (1995) reported increased propionate production in dairy cows as a result of increased barley starch in the diet whilst Langhans (1999) and Farningham and Whyte (1993) reported reduced DM intake with increased ruminal propionate. Alternatively, the rapid fermentation of soluble carbohydrates in the rumen may lead to increased production of lactate and a fall in ruminal pH (Bazeley and Pinsent,

1984). This may damage the rumen epithelium and inhibit the activity of celluloytic micro-organisms, which can cause a depression in fibre digestion and may lead to reductions in forage and DM intake (Ørskov, 1976).

In the work of Jackson *et al.* (2004) milk yield was unaffected by cutting height whilst milk fat content (g/kg) and yield (kg/d) were lower for those animals offered the short straw forages (Table 1.24). The reduced milk fat content, along with the increased body condition score in animals fed the short straw WCW is in agreement with the observation that increased dietary starch leads to a depression in milk fat and is generally associated with an increase in body lipid deposition (Reynolds *et al.*, 1997). Furthermore, animals offered short straw forages tended to have lower blood plasma NEFA and BHB concentrations, which may indicate a more positive energy balance (Sinclair *et al.*, 2003; Jackson *et al.*, 2004).

Table 1.24. Forage DM intake (DMI; kg/d), milk yield (kg/d), milk composition (g/kg) and condition score (CS) of animals offered whole-crop wheat cut at different stubble heights (cm)

Reference	Stubble height	Forage DMI	Milk yield	Milk fat	Milk protein	CS
Sinclair *et*	17.9	9.90	30.3	37.8	30.9	2.2
al. (2003)	38.2	9.80	30.4	38.5	31.5	2.3
Jackson *et*	17.8	14.1	30.8	41.8	34.4	2.6
al. (2004)	37.3	13.3	30.0	38.4	33.3	2.8

1.6. DIGESTIBILITY OF WHOLE-CROP WHEAT

1.6.1. Whole tract digestibility of whole-crop wheat

Sutton *et al.* (2002; Table 1.25) reported that the whole tract digestibility of all fractions (DM, organic matter, NDF and starch) of fermented WCW decreased with increasing maturity (301 and 511 g/kg DM) this supports previous findings that dairy cows poorly digest the hard cereal grains of mature WCW. Similarly, the digestible organic matter content in the DM (DOMD) decreased with increasing maturity of fermented WCW

(Sutton *et al.*, 2002; Table 1.26). Sutton *et al.* (2002) suggested that this reflected decreasing fibre digestibility with maturity whilst there was little starch formation. At higher DM values and with urea treatment the digestibility of all fractions, except starch, were similar (Table 1.25). In addition, DOMD values were higher compared with fermented (Adesogan *et al.*, 1998a) or untreated WCW (Sutton *et al.*, 2002; Table 1.26). This increase in digestibility following urea treatment supports the findings of Ørskov *et al.* (1983) who reported an increased feed value of straw and whole-crop barley and oats treated with anhydrous or aqueous ammonia or urea.

Table 1.25. Dry matter (DM), organic matter (OM), starch and neutral-detergent fibre (NDF) digestibility values for fermented whole-crop wheat (F) harvested at different stages of maturity and urea-treated whole-crop wheat (U)

Reference	Crop DM (g/kg)	DM digestibility	OM digestibility	Starch digestibility	NDF digestibility
Sutton *et al.* (2002)	301 – F	0.68	0.70	0.96	0.57
	511 - F	0.66	0.68	0.97	0.52
	584 - U	0.68	0.70	0.93	0.60

Table 1.26 Digestible organic matter content in DM (DOMD) of untreated whole-crop wheat, fermented whole-crop wheat (FWCW) and urea-treated whole-crop wheat treated with 20 g/kg urea (UWCW-20) and 40 g/kg urea (UWCW-40)

Reference	Treatment	DM (g/kg)	DOMD (g/kg)
Adesogan *et al.* (1998a)	Untreated	645	633
	FWCW	593	611
	UWCW	781	622
	UWCW	620	646
Sutton *et al.* (2002)	FWCW	301	658
	F WCW	511	651
	UWCW	584	703

Increasing the proportion of urea-treated WCW in the diet of dairy cows decreased whole tract digestibilities of DM, organic matter and starch (Sutton *et al.*, 1997, Abdalla *et al.*, 1999, Sutton *et al.*, 1998; Table 1.27). Assuming the apparent digestibility of the starch in the concentrate measured on the treatment grass silage remained unchanged in the WCW treatments, the apparent digestibility of WCW starch was calculated by Sutton *et al.* (1997) to be 0.81, 0.79 and 0.63, with an overall average of 0.74. Sutton *et al.* (1997) concluded

that the milk yield response of dairy cows offered urea-treated WCW was restricted by the low digestibility of starch in the grains. This decline in digestibility may also be an indication that whole wheat grains escaped digestion. This was supported by Phipps *et al.* (1992) and Sutton *et al.* (1998) who noted whole grain in the faeces of cows offered diets that contained urea-treated WCW.

Table 1.27. Dry matter (DM), organic matter (OM), starch, and neutral-detergent fibre (NDF) digestibility values for grass silage (GS) alone and combined with urea-treated whole-crop wheat (WCW) treated with 40 g/kg urea.

Reference	GS: WCW	DM digestibility	OM digestibility	Starch digestibility	NDF digestibility
Sutton *et al.* (1997)	100: 0	0.70	0.72	0.97	0.64
	67: 33	0.67	0.70	0.90	0.62
	33: 67	0.64	0.66	0.87	0.59
	0: 100	nd	nd	nd	nd
Sutton *et al.* (1998)	100: 0	0.70	0.72	0.97	0.63
	67: 33	0.67	0.69	0.91	0.61
	33: 67	0.65	0.66	0.90	0.59

nd not determined

1.6.2. Digestibility of whole-crop wheat in sheep and cattle

Sinclair *et al.* (2003) determined the starch apparent digestibility in sheep of fermented WCW cut at two heights to be 0.95 and 0.96 kg/kg for long and short straw respectively. The high digestibility of starch WCW at either of the cutting heights was in agreement with other studies on fermented and urea-treated WCW (Adesogan *et al.*, 1998a; Deschard *et al.*, 1987; Givens *et al.*, 1993) when determined in sheep.

By contrast the digestibility of starch in urea-treated WCW when determined in dairy cows was reported to be between 0.8 and 0.9 kg/kg (Sutton *et al.*, 1997; Sutton *et al.*, 1998). The use of sheep as a model for WCW digestion in dairy cows has been questioned by Adesogan *et al.* (1997) and Sutton *et al.* (2002) who undertook parallel digestibility experiments in sheep and dairy cows (Table 1.28). The higher digestibility obtained using

sheep was suggested to result from greater mastication of cereal grains and greater disruption of the pericarp and subsequently increased availability of starch for ruminal digestion (McDonald *et al.*, 1995). Additionally, the smaller omasal orifice in sheep will reduce the outflow of whole grains from the rumen compared with dairy cows (McDonald *et al.*, 1995), which may contribute to the high digestibility (Sutton *et al.*, 2002). Thus the use of sheep as a model for WCW digestion in cows may be inappropriate (Adesogan *et al.*, 1997).

Table 1.28. Dry matter (DM), neutral detergent fibre (NDF) and starch digestibility of fermented whole-crop wheat and urea-treated whole-crop wheat (UWCW) in sheep and dairy cows

Reference	Treatment	Species	DM digestibility	NDF digestibility	Starch digestibility
Sutton *et al.* (2002)	Low DM	Cow	0.68	0.57	0.96
	High DM	Cow	0.66	0.52	0.97
	UWCW	Cow	0.68	0.60	0.93
	Low DM	Sheep	0.69	0.66	0.98
	High DM	Sheep	0.67	0.66	1.00
	UWCW	Sheep	0.71	0.77	1.00

1.6.3. *In vitro* method for assessing digestibility of whole-crop wheat

In vitro studies provide a less expensive, more rapid and repeatable alternative to *in vivo* studies (Owens and Goetsch, 1988). Commonly used *in vitro* methods include gas production (Menke *et al.*, 1979; Theodorou *et al.*, 1994), rumen fluid-pepsin digestibility (Tilley and Terry, 1963), neutral detergent cellulase digestibility (NCD; Dowman and Collins, 1982, Dowman, 1993) and continuous culture fermentors (Stewart *et al.*, 1961).

i. Gas production technique

The anaerobic digestion of feedstuffs by ruminal microbes produces VFA's, CO_2, CH_4 and traces of H_2 (Adesogan, 2002). Therefore, the *in vitro* gas production technique relies on the inverse relationship between gas accumulation and degradation of the feedstuff

(Theodorou *et al.*, 1994). Feedstuffs are incubated with rumen liquor in glass syringe barrels. As the substrate is fermented, gases are produced and the syringe plunger rises. The rate of fermentation of the feedstuff can then be determined (Menke *et al.*, 1979; Theodorou *et al.*, 1994). Previously Menke *et al.* (1979) have reported high correlations between gas production values *in vitro* and apparent digestibilities *in vivo*. By contrast, Adesogan *et al.* (1998b) reported that the *in vivo* digestible organic matter content (DOMD) of fermented and urea-treated WCW was poorly predicted by *in vitro* gas production. Abdalla *et al.* (1999) reported decreased intra-ruminal digestion of organic matter with increasing inclusion of WCW in grass silage-based diets, therefore, inaccurate gas production/*in vivo* DOMD relationships could be due to *in vitro* differences in the rates of degradation and outflow along with improved post-ruminal utilisation of nutrients escaping fermentation (Menke *et al.*, 1979).

ii. Rumen fluid-pepsin digestibility

The rumen fluid-pepsin method of Tilley and Terry (1963) mimics gastric digestion and can therefore be used to predict the *in vivo* digestibility of many forages. Forages are first incubated anaerobically with rumen liquor followed by a period incubated with pepsin in a weak acid (Tilley and Terry, 1963). Table 1.29 shows the DOMD values for WCW analysed *in vivo* and *in vitro* by the rumen fluid-pepsin technique. Givens *et al.* (1993) and Deschard *et al.* (1988) reported a good agreement between the DOMD values measured using the rumen fluid-pepsin method and those measured in sheep and steers *in vivo*. However, Adesogan *et al.* (1998b) reported *in vivo* DOMD was poorly predicted by rumen fluid DOMD (Table 1.29).

Table 1.29. Digestible organic matter content in DM (DOMD) of fermented whole-crop wheat (FWCW) and urea-treated whole-crop wheat (UWCW) measured *in vivo* (DOMD; g/kg DM) and *in vitro* using the rumen fluid-pepsin method (RFP; g/kg DM)

Reference	Treatment	DM (g/kg)	DOMD	RFP
Deschard *et al.* (1988)	Untreated	593	647	594
	UWCW	603	663	678
Givens *et al.* (1993)	UWCW	520	657	644
	UWCW	589	542	545
Adesogan *et al.* (1998a)	Untreated	645	633	529
	FWCW	593	611	557
	UWCW	781	622	602
	UWCW	620	646	568

iii. Neutral detergent cellulase digestibility (NCD)

Digestibility assays that use enzymes instead of micro-organisms have the advantage of not being dependant on a supply of rumen liquor from fistulated animals (Dowman and Collins, 1982). Neutral detergent cellulase digestibility is defined as the percentage of OM digested by neutral detergent and buffered cellulase solution in the DM of the sample (Dowman, 1993). Previous work by Leaver and Hill (1992), Givens *et al.* (1993) and Castejon and Leaver (1994) have shown a good correlation between DOMD values for urea-treated WCW measured *in vivo* and measured *in vitro* using NCD. In contrast however, Adesogan *et al.* (1998) concluded that *in vivo* DOMD of WCW was poorly predicted by NCD (Table 1.30). The authors suggested that *in vitro*/*in vivo* differences were related to differences in the sites, rates and extents of digestion of the starch and cell wall fractions. Furthermore, the acidic conditions imposed by starch digestion may enhance post-ruminal digestion of the cell walls at the expense of intra-ruminal digestion (Adesogan *et al.*, 1998). This is supported by Abdalla *et al.* (1999) who reported decreased intra-ruminal digestion of OM with increasing inclusion of WCW in the diet of dairy cows.

Table 1.30. Digestible organic matter content in DM (DOMD) of urea-treated whole-crop wheat (UWCW) measured *in vivo* (DOMD; g/kg DM) and *in vitro* using neutral detergent cellulase digestibility (NCD; g/kg DM)

Reference	DM (g/kg)	DOMD	NCD
Givens *et al.* (1993)	520	657	690
	589	542	540
Castejon and Leaver (1994)	654	654	647
	585	635	633
Adesogan *et al.* (1998)	781	622	703
	620	646	569

iv. Continuous culture fermentors

The utilisation of continuous culture fermentors to simulate the ruminal environment enables the study of factors that may affect the microbial ecology and digestion of nutrients in the rumen (Stern *et al.*, 1997). In normal rumen fermentation, end products are removed by flow of ingesta down the digestive tract as well as by absorption through the rumen wall, new substrate is added periodically and a continuous flow of salivary buffer into the rumen helps to maintain a physiologically normal pH range (Stewart *et al.*, 1961). A typical continuous culture replicates most of these functions allowing a continuous buffer and feed input along with one output orifice which outflows a homogenous mixture of solids, liquids, fermentation products and micro-organisms (Stewart *et al.*, 1961). Comparisons of *in vivo* fermentation with continuous culture fermentors reported that the same bacterial groups predominanted in both systems, although bacteria and protozoa numbers were lower in fermentors (Slyter and Putnam, 1967). Comparisons of pH, VFA concentration and rates of production concluded that the continuous culture appeared comparable to rumen cultures (Stewart *et al.*, 1961). However, Barry *et al.* (1977) reported that the major problem of continuous cultures is being able to reduce the build up of VFA's without increasing the flow rate to a level that results in loss of protozoa through washout (Hoover *et al.*, 1976).

The dual-flow continuous culture fermentor allows independent solid and liquid dilution rates to be maintained through partial removal of fermentation media through a filter (primarily liquid) whilst mixed fluid and solid media flows out of an outflow port. This system allows a rapid buffer input to maintain pH and a longer residence time for digestion of solid particles (Hoover *et al.*, 1976a). Furthermore, the maintenance of protozoa numbers in this system was improved (Hoover *et al.*, 1976a). A comparison of ruminal fermentation and dual-flow continuous culture fermentation reported that interpretation of results *in vitro* and *in vivo* was similar for 80 % of individual parameters that were evaluated. It was therefore concluded that the dual flow continuous culture system was an excellent model for studying ruminal microbial fermentation (Mansfield *et al.*, 1995). To date there has been no work undertaken to determine WCW digestibility in a continuous culture system.

1.7. METABOLISABLE ENERGY CONTENT OF WHOLE-CROP WHEAT

Accurate ME measurements are integral for the formulation of diets containing WCW (Adesogan *et al.*, 1999). In addition to digestibility measurements in sheep and cattle, ME may be determined by *in vitro* digestibility studies. Previous studies, where urea-treated WCW partially or totally replaced grass silage in diets for dairy cows reported an imbalance between the estimated ME requirements predicted *in vivo* and recorded animal production (Leaver and Hill, 1992; Phipps *et al.*, 1995; Sutton *et al.*, 1997). A comparison of ME intakes, calculated from DM intake and ME content predicted from *in vivo* digestibility studies, with ME requirements, determined from recorded animal performance, consistently showed intakes to be higher than predicted requirements (Leaver and Hill, 1992). Leaver and Hill (1992) concluded that up to 84 MJ ME/d was unaccounted for when animals were fed 0.56 or 1.00 of the ration as urea-treated WCW when the ME of WCW was assumed as 9.6 MJ/kg DM. The authors suggested that this energy imbalance

was due to the high ammonia intakes resulting from the hydrolysed urea, which required energy to be metabolised and excreted, and this implies that the ME utilisation was lower than the accepted values (Leaver and Hill, 1992). By contrast, Leaver and Hill (1995) demonstrated a reasonable relationship between ME requirements and animal production where only 0.4 and 0.33 of grass silage was replaced with urea-treated WCW (Table 1.31).

Table 1.31. Estimated metabolizable energy (ME) efficiency of dairy cows offered grass silage alone (GS) or with either fermented whole-crop wheat (FWCW) or urea-treated whole-crop wheat (UWCW)

Reference	Treatment	ME intake (MJ/d)	ME requirement (MJ/d)	ME efficiency*
Leaver and Hill (1995)	GS	195	195	1.00
	FWCW	211	206	0.98
	UWCW	219	217	0.99

* ME efficiency = ME requirement/ ME intake

It was concluded that the imbalances previously reported reflected the higher proportion of WCW in the diet. However, Phipps *et al.* (1995) reported poor animal responses when either fermented or urea-treated WCW replaced only one-third of the grass silage DM. Using predictions of ME, based on NCD, energy intake increased by approximately 10 MJ ME/d whilst milk yield increased by only the equivalent of 5 MJ ME/kg milk (Phipps *et al.*, 1995). Sutton *et al.* (1997) measured the effect of widely different ratios of grass silage to urea-treated WCW on diet digestibility. Organic matter digestibility decreased with increasing WCW inclusion and Sutton *et al.* (1997) suggested that the ME value of WCW was overestimated. This overestimation is likely to be a result of many of the *in vitro* techniques used to calculate ME in WCW having been developed for the analysis of grass or grass and maize silages. Adesogan *et al.* (1999) reported these techniques to be unsuitable for predicting the ME content of WCW. Furthermore, Sutton *et al.* (2002) reported ME values derived from *in vitro* measurements to be in contrast with values predicted using the NCD technique. Metabolisable energy values predicted in cows decreased from 10.4 MJ/kg corrected DM (CDM) for low DM WCW (DM 301 g/kg) to

9.3 MJ/kg (CDM) for high DM (DM 511 g/kg) whilst urea-treated WCW had an ME of 9.0 MJ/kg CDM (Sutton *et al.*, 2002). By contrast, values predicted using NCD increased from 9.6 MJ/kg CDM for low DM WCW to 10.4 MJ/kg CDM for high DM and 12.4 MJ/kg CDM for urea-treated WCW. Sutton *et al.* (2002) concluded that the ME values of WCW declined by over 1 MJ/kg CDM from about 10.5 as maturity advances from early soft dough (about 300 g DM/kg) to about 9.0 at hard dough (about 500 g DM/kg) and that lab-based predictions fail to predict this change, therefore overestimating the ME value of urea-treated WCW (Sutton *et al.*, 2002).

Further evidence that lab-based predictions for WCW ME are too high was provided by Sutton *et al.* (1998) who undertook a full energy and N balance with dairy cows. The results showed that the increase in energy intake with WCW inclusion was matched by a similar increase in faecal energy output. As a result there was no difference in digestible energy intake and ME intake (Table 1.32).

Table 1.32. Energy balance (MJ/day) of dairy cows fed grass silage alone (GS) and urea-treated whole-crop wheat (WCW; 40 kg/t DM urea) in different ratios.

		GS: WCW	
	GS	2: 1	1: 2
Intake	300	331	329
Faeces	88	110	120
Methane	21	23	23
Urine	10	10	9
Milk	76	81	82
Heat	111	117	110
Retained	-6	-11	-15
Digestible energy	212	220	209
Metabolisable energy	181	187	177

The efficiency of utilization of ME, calculated from measured energy balances and maintenance values derived according to Agricultural Research Council (1980) are shown in Table 1.33 (Sutton *et al.*, 1998). The efficiency of ME utilization for milk production (Milk energy/ MEp) tended to increase with increasing inclusion of WCW.

Table 1.33. Efficiency of utilization of metabolizable energy (ME; kJ/kg $M^{0.75}$ per day) for dairy cows fed grass silage alone (GS) and urea-treated whole-crop wheat (WCW; 40 kg/t DM urea) in different ratios.

		GS: WCW	
	GS	2: 1	1: 2
ME intake	1537	1553	1490
Tissue mobilization	53	89	128
Total ME	1591	1643	1618
Maintenance	480	488	496
ME_p*	1111	1154	1123
Milk energy	649	674	689
Milk energy/ Me_p	0.58	0.59	0.62

* ME_p ME available for production

By making certain assumptions Sutton *et al.* (1998) were able to calculate ME values for WCW from the measured energy balances. Concentrate ME was calculated according to Agricultural and Food Research Council (AFRC; 1993) and grass silage ME calculated by subtracting the concentrate ME from the diet containing only grass silage. These values were assumed to be constant across treatments, allowing ME values for WCW to be calculated by difference. Sutton *et al.* (1998) concluded that the average ME value for WCW was 8.1 and 7.8 MJ ME/kg CDM at maintenance and production levels respectively. These values reflect the low apparent digestibility of WCW and are much lower than values ranging from 9.6 to 12.4 MJ ME/kg DM reported by Leaver and Hill (1992 and 1995) and Sutton *et al.* (2002) based on *in vitro* digestibility analyses.

1.7.1 Effect of cutting height on metabolisable energy content of whole-crop wheat

Weller *et al.*, (1995) reported an increase in calculated ME from 10.6 to 11.2 MJ/kg DM, by increasing stubble height from 10 cm to 40 cm. Furthermore, Burgess *et al.* (1989) observed increases in the *in vitro* digestibility of wheat when decreasing amounts of straw were added to wheat heads. By contrast, the net effects on ME supply of increasing stubble

height, from 17.8cm to 37.3 cm of fermented WCW at harvest were small and non-significant (Sinclair *et al.*, 2003).

1.8. *IN SITU* DEGRADABILITY OF WHOLE-CROP WHEAT

Rumen degradability of N has been extensively studied for both concentrates and forages using the *in situ* polyester bag technique proposed by Ørskov and McDonald (1979). The *in situ* method can also be applied to study the rumen degradability of DM, OM and fibre fractions. Degradation is determined by placing a small amount of feedstuff in an undegradable porous bag and suspending the bag and contents in the rumen for various time intervals (Huntington and Givens, 1995). Dry matter losses are then plotted against time and a non-linear model fitted (Huntington and Givens, 1995).

1.8.1. Effect of preservation method on *in situ* degradability

The treatment of WCW with 35 g/kg DM of ammonia significantly increased the immediately soluble fraction and potentially degradable fraction of the DM whilst having no effect on the rate of degradation (Everington and Givens, 1988). Similarly, the addition of urea to wheat straw increased the DM immediately soluble fraction without affecting the extent or rate of degradation of the insoluble fraction (Givens *et al.*, 1993). This effect may be due to the alkali treatment breaking the labile arabinose and glucuronic acid side-chain linkages from the lignin (Givens *et al.*, 1993). By contrast, urea treatment (40 g/kg DM) of wheat grains reduced the amount of immediately soluble DM fraction and increased the extent of degradation (Table 1.34). It is possible that the preservative nature of urea has prevented hydrolysis of starch within the wheat grains during storage, thus reducing the concentration of soluble products for hydrolysis in the rumen. This may be beneficial in ruminant diets in preventing the fall in rumen pH often associated with a rapid release of starch and the resulting decreased digestion of cell walls (Givens *et al.*, 1993). The

effective degradability, at an assumed outflow rate of 0.05 /h of straw and grain treated with 40 g/kg urea was 680 and 370 g/kg respectively, giving a calculated average degradability of 480 g/kg for WCW if the grain and straw were mixed in equal portions. This value is similar to that of Moos and Sutton (1998) who reported a lower effective DM degradability (at an assumed outflow rate of 0.042 /h) for urea-treated WCW compared with WCW that was fermented (Table 1.34), and suggested that there may be a greater post-ruminal digestion of urea-treated WCW.

Table 1.34. Dry matter degradability coefficients for wheat straw treated with no urea (S0), 20 g/kg urea (S20), 40 g/kg urea (S40) and wheat grain treated with no urea (G0), 20 g/kg urea (G20), 40 g/kg urea (G40) and fermented (FWCW) and urea-treated (UWCW) whole-crop wheat

Reference	Treatment	Immediately soluble fraction (a)	Potentially degradable fraction (b)	Rate of degradation (c)	Effective degradability*
Givens *et al.* (1993)	S0	167	445	0.031	nd
	S20	182	474	0.027	nd
	S40	202	510	0.025	680
	G0	244	670	0.105	nd
	G20	280	615	0.126	nd
	G40	191	751	0.093	370
Moos and Sutton (1998)	FWCW	376	437	0.033	568
	FWCW	418	398	0.040	607
	UWCW	144	766	0.036	497

* Effective degradability at an assumed outflow of 0.05 /h (Givens *et al.*, 1993) and 0.042/h (Moos and Sutton, 1998).
nd not determined

1.8.2. Effect of stage of maturity on *in situ* degradability

Studies comparing the degradability coefficients for early-maturing and late-maturing wheat cultivars have reported that the DM degradability (immediately soluble and potentially degradable fractions) was higher for the early maturing cultivar as a result of the lower NDF content and higher immediately soluble fraction (Ashbell *et al.*, 1997). Furthermore, comparisons at four stages of maturity (tillering, anthesis, milk and dough) reported the highest DM solubility to be at the dough stage (DM 348 and 405 g/kg; Table

1.35), which can be attributed to the increased grain to biomass ratio as the crop approaches the dough stage (Ashbell *et al.*, 1997). By contrast, Southworth *et al.* (1999) reported reduced effective degradability (assumed outflow rate of 0.05 /h) of fermented WCW with increasing crop maturity (Table 1.35). The effective degradability (assumed outflow of 0.04 /h) of NDF decreased with maturity (Arieli and Adin, 1994) and can be related to cell wall lignification, leading to a reduction in substrate susceptibility to microbial degradation (Tamminga and Van Vuuren, 1988). The NDF effective degradability (assumed outflow rate of 0.04 /h) also tended to be higher in early-harvested wheat compared with later-harvested WCW in the work of Ashbell *et al.* (1997). To date no work has been undertaken to determine the *in situ* degradability of urea-treated WCW at different stages of maturity.

Table 1.35. Immediately soluble (a), potentially degradable (b), rate of degradation (c) and effective degradability of DM and neutral detergent fibre (NDF) effective degradability of whole-crop wheat harvested at different stages of maturity

Reference	Crop DM (g/kg)	a	b	c	Effective degradability*	NDF
Arieli and Adin (1994)	301	282	497	0.03	437	294
	379	316	422	0.02	428	237
Ashbell *et al.* (1997)	302	341	338	0.03	477	320
	319	337	193	0.04	432	320
	328	350	171	0.03	420	254
	343	358	254	0.05	496	374
	347	412	314	0.03	549	398
	348	493	169	0.03	563	297
	357	400	262	0.02	498	312
	405	421	185	0.02	483	293
Southworth *et al.* (1999)	299	275	372	0.04	388	nd
	349	356	325	0.04	340	nd
	420	292	374	0.04	212	nd

* Effective degradability at an assumed outflow rate of 0.04 /h (Arieli and Adin, 1994; Ashbell *et al.*, 1997) and 0.05 /h (Southworth *et al.*, 1999)
nd not determined

1.9. FORAGE PROCESSING

Processing maize silage increased DM intakes, milk production and starch digestion for dairy cows (Bal *et al.*, 2000). By contrast, Eun *et al.* (2004) reported that feeding mechanically processed barley silage had little effect on DM intake, milk yield or milk composition in dairy cows. Eun *et al.* (2004) concluded that the rollers for processing maize silage were unsuitable for processing barley silage. Abdalla *et al.* (1999) suggested that the reduced starch digestion of urea-treated WCW was related to the high content of whole grains in the food and that some form of processing may improve the utilisation of WCW. Recently, a forage processor that is fitted within the harvester and specifically designed for small-grained cereals has been developed (Jackson *et al.*, 2004). As mentioned previously in the introduction, this allows grains to be ground prior to ensiling, breaking the outer tissues of the grain to allow access of rumen micro-organisms and digestive enzymes (Campling, 1991).

1.9.1. Effect of processing whole-crop wheat on apparent digestibility

Processing of urea-treated WCW (pWCW) at harvest had no effect on OM or NDF whole-tract digestibility (Jackson *et al.*, 2004; Table 1.36). However, processing at harvest increased the whole tract digestibility of starch from an average 0.88 kg/kg for the unprocessed dietary treatments to an average 0.97 kg/kg for the processed dietary treatments (Jackson *et al.*, 2004). Assuming that the digestibility of starch for all dietary components other than WCW was constant at 0.97 kg/kg (Sutton *et al.*, 1997), then the apparent digestibility of the starch component in the WCW forages was estimated to be on average 0.84 and 0.97 kg/kg for unprocessed and processed WCW respectively, a value comparable to that of unprocessed WCW when measured in sheep (Sutton *et al.*, 2002). Furthermore, processing decreased the number of whole grains in the forage from an

average 917 grains per 100 g DM unprocessed WCW to 162 grains per 100 g DM processed WCW (Jackson *et al.*, 2004).

Table 1.36. Organic matter (OM), neutral detergent fibre (NDF) and starch apparent digestibility (kg/kg) in dairy cows offered unprocessed, urea-treated whole-crop wheat (UP) or processed, urea-treated whole-crop wheat (pWCW) cut at one of two heights to produce a forage that contained long straw (*) or short straw (+; Jackson *et al.*, 2004).

Treatment	OM digestibility	NDF digestibility	Starch digestibility	Calculated starch digestibility WCW
UP*	0.64	0.59	0.90	0.87
UP^{+}	0.60	0.54	0.85	0.80
pWCW*	0.63	0.56	0.97	0.97
pWCW^{+}	0.64	0.53	0.96	0.96

* long straw (stubble height 17.8 cm); $^{+}$ short straw (stubble height 37.3 cm)

1.9.2. Effect of processing on dry matter intake and animal performance

Mechanical processing of maize silage has been shown to increase DM intake in dairy cows (Johnson *et al.*, 1999). By contrast, processing of urea-treated WCW at harvest significantly reduced forage DM intakes compared with animals offered unprocessed WCW (Jackson *et al.*, 2004; Table 1.37). Similarly, the replacement of whole grains with ground and pelleted grains in dairy diets was associated with a reduced DM intake (Ørskov *et al.*, 1978). This may have been due to increased propionate absorption (Reynolds *et al.*, 1997) or a reduction in ruminal pH and subsequent fibre digestion (Ørskov, 1976). Processing WCW had little effect on milk yield (kg/d) or fat or protein levels (Table 1.37). Processing of WCW at harvest had no effect on live-weight change or body condition score (Jackson *et al.*, 2004; Table 1.38).

Table 1.37. Forage dry matter intake (DMI; kg/d), milk yield (kg/d) and milk composition (g/kg) of animals offered unprocessed, urea-treated whole-crop wheat (UP) or processed, urea-treated whole-crop wheat (pWCW; Jackson *et al.*, 2004).

Treatment	Forage DMI	Milk yield	Milk fat	Milk protein
UP*	14.1	30.8	41.8	34.4
UP^{+}	13.3	30.0	38.4	33.3
pWCW*	13.0	29.9	41.9	34.6
pWCW^{+}	12.0	29.8	35.6	33.0

* long straw (stubble height 17.8 cm); $^{+}$ short straw (stubble height 37.3 cm)

Table 1.38. Average live-weight change (ΔWt; kg/d) and condition score for animals offered grass silage (G), maize silage (M), fermented whole-crop wheat (FWCW) or unprocessed, urea-treated WCW (UP) or processed, urea-treated whole-crop wheat (pWCW)

Reference	Treatment	ΔWt	Condition score
Jackson *et al.* (2004)	UP*	0.33	2.59
	UP^{+}	0.39	2.83
	pWCW*	0.44	2.61
	pWCW^{+}	0.44	2.76

* long straw (stubble height 17.8 cm); $^{+}$ short straw (stubble height 37.3 cm)

i. Comparison of processed whole-crop wheat with other forages

A number of studies have been undertaken to compare pWCW with other forages (Murphy *et al.*, 2004; Patterson and Kilpatrick, 2005; Sinclair *et al.*, 2005; Table 1.39). Inclusion of pWCW in the diet increased forage DM intakes compared with those animals fed grass silage alone, whilst animals fed maize silage had the lowest DM intake.

The partial replacement of grass silage with fermented WCW, pWCW or maize silage increased milk yield (Murphy *et al.* 2004). Furthermore, Sinclair *et al.* (2005) reported milk yields for animals offered maize silage harvested at approximately 300 g/kg DM to be comparable with animals offered pWCW (Table 1.39), whilst Patterson and Kilpatrick (2005) concluded that only the inclusion of maize silage in the diet increased milk yield compared with feeding grass silage alone. Milk fat and protein yields were increased as a result of the inclusion of fermented WCW, processed urea-treated WCW or maize silage (Murphy *et al.*, 2004).

Table 1.39. Forage DM intake (DMI; kg/d), milk yield (kg/d) and milk composition (g/kg) for dairy cows offered grass silage (GS), maize silage (M), fermented whole-crop wheat (FWCW) or processed, urea-treated whole-crop wheat (pWCW)

Reference	Treatment	Forage DMI	Milk yield	Milk fat	Milk protein
Murphy *et al.* (2004)	GS	8.8	27.6	39.6	30.7
	FWCW	12.8	29.7	38.4	31.7
	pWCW	14.8	29.4	37.8	31.7
	Maize	11.2	29.6	40.4	31.2
Patterson and Kilpatrick (2005)	GS	12.5	31.5	38.5	32.8
	FWCW	12.3	29.6	39.0	32.0
	pWCW	12.6	28.7	39.5	32.0
	Maize	13.6	30.9	40.3	32.2
Sinclair *et al.* (2005)	Maize	10.7	34.0	37.7	31.2
	pWCW W*	13.3	34.4	34.3	32.5

* pWCW W processed urea-treated whole-crop wheat supplemented with wheat

Compared with animals offered maize silage, animals offered processed, urea-treated WCW had significantly higher live-weight gains, possibly as a result of the higher DM intakes in some (Murphy *et al.*, 2004) but not all studies (Sinclair *et al.*, 2005; Table 1.40).

Table 1.40. Average live-weight (kg), live-weight change (ΔWt; kg/d) and condition score for animals offered grass silage (GS), maize silage (M), fermented whole-crop wheat (FWCW) or unprocessed, urea-treated WCW (UP) or processed, urea-treated whole-crop wheat (pWCW)

Reference	Treatment	Live-weight	ΔWt	Condition score
Murphy *et al.* (2004)	GS	nd	7.1	nd
	FWCW	nd	16.2	nd
	pWCW	nd	24.1	nd
	M	nd	3.4	nd
Sinclair *et al.* (2005)	M	585	0.30	2.53
	pWCW W	613	0.49	2.71

pWCW W processed urea-treated whole-crop wheat supplemented with wheat
nd not determined

ii. Effect of energy supplementation of processed whole-crop wheat

Processing urea-treated WCW at harvest reduced forage intakes by dairy cows possibly as a result of increased starch availability in the rumen, which may have lowered ruminal pH and reduced the efficiency of microbial protein synthesis (Jackson *et al.*, 2004). Previously

the use of sugar sources has been shown to improve the efficiency of microbial protein synthesis in the rumen and may improve animal performance (Chamberlain *et al.*, 1993).

Dairy cows offered pWCW supplemented with molasses had a higher forage DM intake than those offered rolled wheat (Sinclair *et al.,* 2005; Table 1.41). Furthermore, the lower plasma urea levels for animals supplemented with molasses is in agreement with grass fed cows in the work of Murphy (1999), which implies a greater capture of ammonia in the rumen (Sinclair *et al.*, 2005). The lower plasma NEFA levels and a trend for increased body condition score and live-weight gain for animals supplemented with rolled wheat suggests an improvement in energy balance (Sinclair *et al.*, 2005). Alternative sugar sources, such as lactose, have been shown to result in a more stable rumen pH and greater microbial protein synthesis (Hussain and Miller, 1999). This effect would potentially be most beneficial in diets that contain a large amount of rapidly fermentable starch, such as WCW (Sinclair *et al.*, 2005). Although lactose supplementation of pWCW resulted in a higher milk yield (kg/d) compared with animals supplemented with molasses and rolled wheat, milk protein content was lower (Table 1.41) and there was no effect on fat and protein yield (Sinclair *et al.*, 2005).

Table 1.41. Average forage dry matter intake (DMI kg/d) milk yield (kg/d), milk fat and protein levels (g/kg) of cows offered processed, urea-treated whole-crop wheat (pWCW) supplemented with molasses (M), wheat (W) or lactose (L; Sinclair *et al.*, 2005).

Treatment	Forage DMI	Milk yield	Milk fat	Milk protein
pWCW - W	13.3	34.4	34.3	32.5
pWCW – M	14.6	33.1	38.4	33.3
pWCW - L	12.2	35.6	34.3	31.5

1.9.3. Milk energy outputs and nitrogen efficiency

Milk energy outputs in unprocessed and pWCW have been reported to be similar (96.5 and 93 MJ/d; Jackson *et al.*, 2004). This observation, in combination with the significant decrease in forage DM intake for cows given the processed forages, represented an

improvement in the efficiency of forage use for milk production due to processing (Jackson *et al.*, 2004). Furthermore, work undertaken to compare pWCW with maize silage reported similar milk energy outputs between treatments (Sinclair *et al.*, 2005). Nitrogen efficiency (kg milk N output per kg N intake) was also similar for unprocessed and pWCW treatments (Jackson *et al.*, 2004), although values were higher than those reported by Hameleers (1998) for unprocessed urea-treated WCW.

1.10. CONCLUSIONS

Whole-crop cereals may provide a viable forage alternative to grass silage, as they overcome some of the limitations highlighted for grass silage in dairy cow rations or geographical limitations for maize silage. Almost any species of autumn and spring sown cereal can be used although winter wheat has been the most popular and widely reported, possibly as a result of its higher yield and greater flexibility. Whole-crop wheat can be preserved by one of two methods; fermentation at lower DM contents and alkaline treatment, mainly urea-treatment, at higher maturities.

In recent years, a considerable amount of work has concentrated on wheat for whole-crop and its utilisation by the dairy cow. Results have been varied, but overall they show that a low digestibility of the wheat grain in higher DM, urea-treated WCW resulted in increased DM intake but without any change in milk yield. Work to improve the utilisation of WCW by dairy cows has concentrated on: altering the stage of maturity at which the wheat is harvested; the addition of enzymes at feeding; energy and protein supplementation; altering cutting height; and more recently, processing or grinding the grains at harvest using a forage processor. The recent advent of the forage processor allows wheat to be harvested over a much wider harvest window. Work to determine the utilisation of processed WCW by dairy cows has so far been limited, but has shown increased

digestibility of the starch However, the optimum stage of maturity at which processed WCW should be harvested and inclusion rate in the diet of dairy cows is unclear.

1.11. OBJECTIVES

The objectives of the current work were to determine the effects of harvesting WCW at different stages of maturity and the effects of urea as an additive to mature processed, urea-treated WCW on crop production, dairy cow performance and *in situ* degradability. Secondly, the work aimed to determine the effect of differing rates of inclusion of processed urea-treated WCW on dairy cow performance, apparent digestibility, rumen fermentation, microbial growth and ruminal digestibility.

Chapter 2

GENERAL MATERIALS AND METHODS

2.1. ROUTINE ANALYSIS

Forage and concentrate samples from the dairy cow experiments (1a and 2a) along with the faecal samples from the dairy cow digestibility experiment (2b) were analysed by NRM Laboratories Ltd, Berkshire, UK. The remainder of the analysis (degradability samples; Experiment 1b and effluent outflow samples; Experiment 3) were undertaken at Harper-Adams University College, Newport, Shropshire.

2.1.1. Dry matter

Forage and dairy concentrates from Experiments 1a and 2a were dried in a force-draught oven at 90 °C until a constant weight was obtained. Degradability residues from Experiment 1b and faecal samples from Experiment 2b were dried to a constant weight at 60 °C. Effluent outflow samples from experiment 3 were frozen and subsequently freeze dried (Girovac Ltd, Borehamwood, Hert, UK). Dry matter (DM) content was calculated as;

$$\text{Dry matter (g/kg)} = (\text{dried sample weight (g)}/\text{initial sample weight (g)}) \times 1000 \quad \text{(Equation 2.1)}$$

The grass silage and fermented WCW DM values were corrected for volatile losses according to AFRC (1993);

$$\text{Corrected DM (CDM; g/kg)} = 0.99 \times [\text{Oven DM}] + 18.2 \quad \text{(Equation 2.2)}$$

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

2.1.2. Organic matter

All samples were analysed for organic matter (OM) according to Association of Official Analytical Chemists (AOAC; 2000). Approximately 2 g of dried, ground sample was weighed into a pre-weighed porcelain crucible and heated to 550 °C in a muffle furnace (Gallenkamp muffle furnace, size 3, GAFSE 620, Gallenkamp, Loughborough, UK) for 16h. Samples were cooled in a dessicator and re-weighed. Organic matter was calculated as;

Ash (g/kg DM) = (weight of ash (g)/initial sample weight (gDM)) x 1000 (Equation 2.3)

Organic matter (g/kg DM) = 1000 - ash (g/kg DM) (Equation 2.4)

2.1.3. Nitrogen

Nitrogen content of the forage and dairy concentrates from Experiments 1a and 2a was determined by kjeldahl digestion with an automated kjeldahl procedure using a Tecator 1035 autoanalyser (FOSS UK, Warrington, UK). Approximately 1.0 g of the dried, ground sample was weighed onto a Whatman No. 1 filter paper (Whatman plc, Maidstone, UK) and transferred to a 250 ml digestion tube, to which 2 kjeltab catalyst tablets (C + K Thompson and Capper Limited, Runcorn, Cheshire, UK) were added. Exactly 16 ml of 98% (w/v) low nitrogen, sulphuric acid (Analar, VWR, Lutterworth, UK) was added to each tube. The samples were then digested at 400 °C on a heating block for 45 min and allowed to cool for 10 min before adding 75 ml of distilled water. Nitrogen content was estimated via back titration using 0.2 M hydrochloric acid as the titrant. Crude protein was calculated as;

Crude protein (g/kg DM) = Nitrogen (g/kg DM) x 6.25 (Equation 2.5)

The degradability samples from Experiment 1b and the effluent outflow samples from Experiment 3 were analysed for total N by the Dumas method (Watson and Galliher, 2001)

using an automatic analyser (LECO FP-528, LECO Corp, St Joseph, MI, USA). Approximately 0.2 g of dried, ground sample was weighed in duplicate into metal cups (LECO Corp, USA) prior to analysis. The samples were heated to 1020 °C in a mixture of O_2 and CO_2 and the resulting N oxides reduced to N_2 by warmed copper filings and the N measured with a thermal conductivity detector. Crude protein was calculated according to equation 2.5.

2.1.4. Ammonia Nitrogen

Ammonia N was extracted from forages (Experiments 1a and 2a) according to Ministry of Agriculture, Fisheries and Food (MAFF; 1986). Approximately 20 g of fresh forage along with 100 ml of distilled water were placed in a shaker bottle. The samples were then shaken for 60 min before being filtered through a 150 mm Whatman No. 1 filter paper. The ammonium N content of the sample was then determined using a colorimetric method by reaction with alkaline hypochlorite and phenol to produce indophenol blue (Environment Protection Agency, 1984).

The ammonium N content of the effluent overflow (experiment 3) was determined according to MAFF (1986). Approximately 40 ml of effluent outflow was centrifuged at 26811 g for 22 min at 4 °C (Avanti 30 Centrifuge, Beckman Coulter UK Ltd, Buckinghamshire, UK) to remove particulate material. Exactly 5 ml of the supernatant was transferred to a 250 ml digestion tube and 6 ml of magnesium oxide suspension (17 g of ignited MgO_2 suspended in 100 ml distilled water) added. The resulting solution was steam distilled using a Tecator 1030 (FOSS, UK) and the liberated ammonia bubbled through 25 ml of receiver solution (receiver solution was made by dissolving 50 g of boric acid in 5 l of distilled water, 50 ml of bromocresol green (100 mg in 100 ml methanol), 35 ml of methyl red solution (100 mg in 100 ml methanol), and 1.5 ml of 0.1 M sodium

hydroxide solution was added). The receiver solution was then back titrated with 0.02 M hydrochloric acid (VWR, UK) as the titrant. In addition, an external standard (0.472 g dried ammonium-sulphate dissolved in distilled water and diluted to 1 litre) was analysed. Ammonium N content of forages was calculated as;

$$\text{Ammonium N (g/kg DM)} = (7 \times T \times F \times (120-(0.02 \times DM))/ DM \times 5) \quad \text{(Equation 2.6)}$$

Ammonium N content of rumen effluent was calculated as;

$$\text{Ammonium N (g/kg DM)} = (14.01 \times 0.1 \times T \times 1000)/ 5 \quad \text{(Equation 2.7)}$$

Where, T is titre reading; F is factor, calculated as 0.357/ external standard; DM is dry matter of the sample (g/kg). Ammonium-N (g/kg total N) was calculated as;

$$\text{Ammonium N (g/kg total N)} = (\text{ammonium N (g/kg DM)} \times 1000)/ \text{Total N (g/kg DM)} \quad \text{(Equation 2.8)}$$

2.1.5. Starch

Forage and dairy concentrate samples from Experiments 1a and 2a, along with the apparent digestibility samples (Experiment 2b) were analysed for starch according to the polarimetric method (MAFF, 1982). Samples were first treated with warm diluted hydrochloric acid, clarified, filtered and the optical resolution of the resulting solution determined. The samples were then extracted with 40 % ethanol and filtered. The resulting filtrate was acidified using hydrochloric acid, clarified and re-filtered, again the optical rotation of the resulting solution was determined.

The degradability residues from Experiment 1b and effluent outflow samples from Experiment 3 were analysed for starch according to AOAC (2000) using an assay kit (Megazyme International Ireland Ltd, Co. Wicklow). All samples were ground to pass through a 0.5 mm screen. Exactly 100 mg of milled sample was added to a glass tube (16 x 120 mm), samples wetted with 0.2 ml aqueous ethanol (80 % v/v) to aid dispersion and mixed on a vortex mixer (MT20, Phillip Harris, Shenstone, UK) for approximately 20 secs.

Exactly 3 ml of thermostable α-amylase (3000 units/ ml) suspended in 3-[N-Morpholino] propanesulfonic acid (MOPS) buffer (11.55 g 3-[N-Morpholino] propanesulfonic acid sodium salt was added to 900 ml distilled water and the solution adjusted to pH 7.0 with 1M HCl: 30 ml MOPS buffer added to 1 ml thermostable α-amylase) was immediately added to each tube and vigorously stirred on a vortex mixer for 20 secs. The tubes were then incubated in a boiling water bath for 6 min, and stirred vigorously after 2 and 4 min. The tubes were placed in a water bath at 50 °C and 4 ml sodium acetate buffer (11.8 ml glacial acetic acid added to 900 ml distilled water and adjusted to pH 4.0 by the addition of 1 M sodium hydroxide solution) added, followed by 0.1 ml amyloglucosidase. The tubes were then stirred on a vortex mixer before being incubated at 50 °C for 30 min. The samples were transferred to a 100 ml volumetric flask and adjusted to volume with distilled water. Samples were mixed thoroughly and an aliquot of this solution centrifuged at 3,000 rpm for 10 min. Duplicate aliquots (0.1 ml) of the diluted samples were transferred to glass test tubes and approximately 3.0 ml of GOPOD reagent (50 ml glucose reagent buffer (containing 0.4 % Na Azide) diluted to 1 litre with the addition of the glucose determination reagent (glucose oxide, peroxidase and 4-aminoantipyrine)) was added to each tube and the tubes incubated at 50 °C for 20 min. Glucose controls consisted of 0.1 ml glucose standard solution [100 μg/0.1 ml in 0.2 % benzoic acid] and 3.0 ml of GOPOD reagent. Reagent blanks consisted of 0.1 ml distilled water and 3.0 ml of GOPOD reagent. The absorbance of the samples and glucose controls were measured against the reagent blanks using a spectrophotometer (6305 Jenway Ltd, Essex, UK), at 510 nm. Starch was calculated as;

$$\text{Starch (g/kg)} = (\Delta E \times (F/W) \times 90) \times 1000 \qquad \text{(Equation 2.9)}$$

$$\text{Starch (g/kg; dry weight basis)} = \text{Starch (g/kg)} \times (100/100\text{-moisture content g/kg}) \qquad \text{(Equation 2.10)}$$

Where ΔE = absorbance read against reagent blank; F = 100 (μg glucose)/ absorbance 100μg glucose; W = Weight (mg) of sample; 90 = adjustment from free glucose to anhydroglucose.

2.1.6. Water soluble carbohydrates

Water soluble carbohydrates (WSC) were determined for the forage samples in Experiment 1a and 2a according to MAFF (1986). Exactly 0.2 g of dried ground sample and 200 ml distilled water were added to a 250 ml shaking bottle. Samples were shaken for 1 h, filtered through a Whatman No.1 filter paper, the filtrate retained and 2 ml of the extract pipetted into a test tube kept on ice. Approximately 10 ml of anthrone reagent (760 ml nitrogen-free sulphuric acid (98%w/v H_2SO4), 330 ml of water, 1g of thiourea and 1g anthrone) 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 min. The samples were then removed from the water bath and placed on ice to reduce the sample temperature as quickly as possible. Absorption was read at 620 nm using a Beckman DU640 spectrophotometer within 30 min of being removed from the water bath. Water soluble carbohydrate content (g/kg DM) was calculated from a standard curve produced using 2 ml of 0, 0.04, 0.08, 0.12, 0.16 and 0.20 mg/l of glucose added to 10 ml anthrone reagent following the above procedure.

2.1.7. Neutral detergent fibre

Neutral detergent fibre (NDF) content of the forage and concentrate samples from Experiment 1a and 2a, along with degradability residues and digestibility samples (Experiment 1b and 2b) and outflow samples (Experiment 3) were determined by an adaptation of the method of Van Soest *et al.* (1991). Approximately 0.5 g of dried, 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 crucibles were placed into Fibertec apparatus (Tecator Fibertec 1020 Hot extractor, Foss UK Ltd, Warrington, UK) and 25 ml of cold neutral detergent solution (93 g disodium ethylene tetra-acetate dihydrate (EDTA), 34 g sodium borate, 150 g sodium lauryl sulphate, 50ml 2-ethoxy-ethanol mixed with 22.8 g anhydrous disodium phosphate made up to 5 l and pH adjusted to pH 7) added along with 0.5 ml octanol. Samples were boiled for 30 min, after which the heat was turned off and 2 ml of α-amylase solution (2g α-amylase E.C. 3.2.1.1. from *Bacillus subtilis* in 90 ml water filtered, 10 ml 2-ethoxy ethanol added and stored at 4°C) and 25 ml neutral detergent solution added. Samples were then boiled for a further 30 min before filtering and washing the digest 3 times with 20 ml distilled water at 80 °C. Approximately 25 ml hot water (80 °C) and 2 ml α-amylase solution was then added and samples left to stand for 15 min before repeating the washing procedure. Digested samples were oven dried at 100 °C and cooled in a dessicator before re-weighing and ashing at 500°C for 4 h and the ash weighed. Neutral detergent fibre was calculated as;

$$\text{NDF (g/kg DM)} = ((\text{DM residue (g)} - \text{ash (g)})/ \text{sample weight (g DM)}) \times 1000 \quad \text{(Equation 2.11)}$$

2.1.8. Neutral detergent cellulase gammanase digestibility (NCGD)

Concentrate samples from Experiment 1a and 2a were analysed for NCDG according to the method of MAFF (1993) using Fibertec apparatus (1020, FOSS, Warrington, UK). The method commenced as for NDF determination, and continued until the final wash with distilled water. Crucibles were removed from the Fibertec apparatus and a subaseal placed in the bottom of the crucible. Approximately 25 ml of buffered cellulase and gammanase solution (1.36 g sodium acetate dissolved in 500 ml distilled water to which 0.6 ml of glacial acetic acid was added and the volume made up to 1 l, and the pH adjusted to pH 4.8. Exactly 20 g cellulase (from *Aspergillus niger*, Sigma Aldrich, Gillingham, UK) and 0.1 g chloramephenicol (Sigma, Gilligham, UK) was added to 1 litre of buffer and the solution mixed and incubated at 40 °C for at least 1 h prior to use. Exactly, 900 ml of

buffered cellulase was mixed with 100 ml of Gammanase solution (Gammanase Novozyme, Netherlands) was added. The samples were shaken and incubated at 40 °C for 24 h, shaking twice daily. After 24 h the subaseals were removed and the crucibles placed back in the Fibertec apparatus (1020, FOSS, Warrington, UK). The samples were washed 3 times with hot distilled water (80 °C) and once with 25 ml propanone. The crucibles were dried overnight at 100 °C and cooled in a dessicator before re-weighing and ashing at 550°C for 4 h. Samples were then cooled in a dessicator and re-weighed. The NCGD was calculated as;

NCDG (g/kg DM) = 1000 – (indigestible OM (g/kg)) + total ash (g/kg))
(Equation 2.12)

2.1.9. Acid detergent fibre

Acid detergent fibre (ADF) content of the concentrate and forage samples from Experiments 1a and 2a were determined according to Goering and Van Soest (1970). Approximately 1 g of dried ground sample was transferred into a previously weighed crucible (50 ml borosilicate glass, sinter porosity No.2, Soham Scientific, Cambridge, UK). The crucibles were fitted into the Fibertec apparatus (Tecator Fibertec 1020 Hot extractor, Foss UK Ltd, Warrington, UK). To each sample 100 ml ADF reagent (10 g cetyltrimethylammonium bromide (CTAB) in 1 litre of 0.5 M sulphuric acid) was added. The samples were boiled for 60 min, filtered under gentle vacuum and the residue washed 3 times with 50 ml hot (80 °C) distilled water and once with 20 ml acetone. The crucibles were removed from the Fibertec apparatus and dried in an oven overnight at 100 °C, allowed to cool in a dessicator and re-weighed prior to ashing at 550 °C for 4 h. Samples were then cooled in a dessicator and re-weighed. The ADF content was calculated as;

ADF (g/kg DM) = ((DM residue (g) – ash (g))/ sample weight (g DM)) x 1000
(Equation 2.13)

2.1.10. pH

Forage samples from Experiment 1a and 2a were analysed for pH according to the method of MAFF (1986). Approximately 10 g of fresh forage was placed in a glass shaker bottle and 100 ml distilled water added. Samples were shaken for 60 min (HS501 digital shaker, Kika labourtechnik, Germany). The resultant liquid was filtered through a Whatman No.1 filter paper (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 2 pH solutions; colour key buffer solution yellow pH 7.0 (product number 192403F; VWR Interational Ltd, Poole, UK) and colour key solution red pH 4.0 (product number 192393U; VWR Interational Ltd, Poole, UK).

Vessel fluid pH during Experiment 3 was determined using a pH probe (Russell RL150), which was recalibrated daily as outlined above.

2.1.11. Volatile fatty acids

Grass silage and fermented WCW (Experiment 1a and 2a) VFA contents were determined according to the method of Jones and Kay (1976). After extraction from the forages in water, VFA's were determined using gas chromatography and flame ionisation detection by reference to internal standards (provalic acid, AnalR grade, Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK) and standard acids (acetic, propionic, isobutyric, butyric, AnalR grade, Fisher Scientific UK Ltd, Loughborough, Leicestershire, UK).

Acidified effluent outflow samples from Experiment 3 were defrosted and centrifuged at 26811 g for 22 minutes at 4 °C. Exactly 4.5 ml of the supernatant was pipetted into a 30 ml plastic universal along with 0.5 ml of 25 mmol phenol internal standard (23.5 g phenol 'AnalaR' (Merk Cat. No. 10188 3H) diluted to 1 litre with distilled water). The samples were mixed for 10 sec on a vortex mixer (MT20, Phillip Harris, Shenstone, UK) before

filtering through a nitrocellulose filter (Whatman, Cellulose Nitrate, 0.2 μm). Volatile fatty acid concentrations were estimated using gas liquid chromatography (Perkin-Elmer 8500 with an AS3800 auto-sampler). The column was supplied by J & W Scientific, Fissons (FFAP, 30 m long, internal diameter of 0.25 mm). The initial oven operating temperature was 110 °C rising to 200 °C at a rate of 10 °C per minute after 17.0 min. The total running time was 31 min. Retention times of individual VFAs in effluent were calculated with reference to a calibration curve of an external standard (174.8 mmol acetic acid 'AnalaR' (Merck Cat. No. 10001 4M), 106.9 mmol propionic acid, 10.78 mmol isobutyric acid, 54.4 mmol butyric acid, 9.1 mmol isovaleric acid, 9.2 mmol valeric and 8.0 mmol caproic acid per litre (Sigma Cat. No. P1386, B2503, I1754, V9759, I7128 and C2250, respectively)).

2.1.12 Ether extract

Ether extract of the dairy concentrates from experiments 1a and 2a was determined by the solvent extraction method of MAFF (1986) using the Soxtec apparatus (FOSS, Warrington, UK). Approximately 2 g dried sample was accurately weighed into a cellulose extraction thimble (Whatman, Maidstone, UK) and plugged with defatted cotton wool. Total fat was extracted by boiling the samples in 25 ml 30 – 40 °C petroleum ether (Analar, VWR, Lutterworth, UK) for 30 min. Samples were then rinsed for 30 min, prior to evaporating off the petroleum ether. Ether extract was determined as;

Ether extract (g/kg DM) = (weight of fat (g) / weight of sample (gDM)) x 1000
(Equation 2.14)

2.1.13. Metabolisable energy

The metabolisable energy (ME) content of grass silage and dairy concentrates (experiment 1a and 2a) were estimated using an NIR instrument programmed with industry-approved predictive algorithms.

2.2. ANIMAL MEASUREMENTS

2.2.1. Blood sampling

Blood samples were taken from the coccygeal vein into vacutainer tubes (BD Vacutainer, Becton, Plymouth, UK) containing either potassium oxalate (for the determination of BHB, glucose and NEFA) or lithium heparin (for the determination of urea, albumin and total protein) as an anti-coagulant. Samples were centrifuged immediately at 1118 g for 15 min (Centaur 2, Sanyo, UK) and the plasma removed prior to subsequent analysis. Plasma was pipetted into 1.5 ml micro-centrifuge tubes and analysed on a Bayer Technicon RA 1000 autoanalyser (Bayer plc, Newbury, Berkshire, UK) using a Bayer Diagnostics test kit (kit catalogue number T01-1823-56 and T01-1833-56) for blood urea and glucose, Randox laboratories kit (RB 1008) for BHB analysis and a Wako chemicals test kit (994-75409) for NEFA. Albumin and total protein were determined using Randox laboratories kits (AB 361 and TP 245).

Chapter 3

THE EFFECT OF STAGE OF MATURITY AT HARVEST AND UREA-TREATMENT OF PROCESSED WHOLE-CROP WHEAT ON THE PERFORMANCE OF DAIRY COWS AND *IN SITU* DEGRADABILITY

3.1. INTRODUCTION

Whole-crop wheat may be preserved by two distinctly different methods; when harvested at lower plant maturities (between 350 and 550 g DM/kg; GS 71 – 85; Tottman and Broad, 1987) the crop may be fermented, or at higher maturities, >500 g DM/kg (GS 85 – 92; Tottman and Broad, 1987) the crop may be urea-treated (Heron, 1996). Evaluation of fermented and urea-treated WCW in dairy rations, either included as the sole forage or mixed with grass silage has produced varying results. In general, inclusion of WCW (fermented or urea-treated) has resulted in an increased DM intake with little or no effect on milk yield and varied effects on milk composition (Phipps *et al.*, 1995; Sinclair *et al.*, 2003; Hameleers, 1998). Sutton *et al.* (1997) concluded that the disappointing milk yield response to urea-treated WCW was due to the low digestibility of the grains, particularly the starch fraction. Abdalla *et al.* (1999) therefore suggested that some form of physical processing of the grain may be beneficial, particularly when the crop is harvested at higher DM values. Recently, a forage processor that is fitted within the harvester and specifically designed for small-grained cereals has been developed. Processing reduced forage intake by dairy cows whilst improving the whole tract digestibility of the starch component, thereby improving the efficiency of forage utilisation (Jackson *et al.*, 2004). Furthermore, processed, urea-treated WCW was shown to result in a similar performance as cows fed maize silage although animals ate proportionately 0.20 more forage DM (Sinclair *et al.*, 2005). Improvement of starch digestibility through processing allows wheat to be

harvested over a wider harvest window than was previously possible, although, the optimum stage of maturity is unclear. In addition, at the very high DM values that the forage processor allows WCW to now be harvested (up to 900 g DM/kg), the use of urea as a preservative may not be required.

The objectives of the current experiment were to determine the effects of stage of maturity of WCW and the effects of urea as an additive to mature processed WCW on crop production, dairy cow performance and *in situ* degradability.

3.2. MATERIALS AND METHODS

3.2.1. Forage production

A commercial crop of winter wheat (*c.v.* Consort) was grown on a sandy loam/sandy clay loam soil following potatoes. The crop was sown between late September and early October 2001 at a target seed rate of 185 kg/ha. The seed was treated with a single purpose seed dressing (guazatine + triticonazole) and 74 kg/ha of K_2O was applied as muriate of potash on 1st March 2002. The crop received 200 kg N/ha applied as a split dressing: 48 kg N/ha on 22nd March 2002, 86 kg N/ha on 8th April 2002 and 66 kg N/ha on 1st May 2002. The crop also received routine sprays of manganese tank mixed with crop protection products. Chlormequat growth regulator was applied on 3rd May 2002. Disease control consisted of a two spray programme: kresoxim-methyl + pyraclostrobin + epoxiconazole applied on 3rd May 2002 and kresoxim-methyl + fenpropimorph and epoxiconazole + fenpropimorph applied as a tank mix on 30th May 2002. Weed control consisted of isoproturon and bromoxynil + diflufenican + ioxynil applied as a tank mix on 26th March 2002 to control grass weeds and annual broad leaved weeds, and fluroxypyr applied on 30th May 2002 to control cleavers. The crop was sprayed with deltamethrin against aphids on 26th March 2002.

Harvest date was assessed by monitoring crop DM twice weekly from early July by cutting two adjacent 0.5 m lengths of row at 15 cm stubble height at six random positions throughout the field. The samples were dried at 105 °C overnight and the whole crop DM determined. Prior to harvest the field was sub-divided into 12 plots and forage treatments allocated randomly to each plot to ensure that a representative area was harvested.

Immediately prior to harvesting, crop DM yield at harvest was assessed for each treatment by cutting two adjacent 0.5 m lengths of row to ground level at 20 random positions within

the harvested area. The material was separated into ensiled material and residual material remaining in the field after harvest by cutting at 30 cm above the stem base. Residual material was further separated into potentially harvestable material (straw remaining after forage harvesting that could potentially be mowed and baled) and stubble by cutting at 8cm above the stem base. Grain yield was determined by cutting ears from the ensiled material and threshing using a single ear thresher (Wintersteiger, Austria), modified to allow quantitative recovery of grain and chaff. Grain was then separated by passing the grain and chaff over a grain cleaner fitted with a 3.5 mm top sieve and a 2 mm bottom sieve.

The wheat was cut at three target whole crop DM values: 450 g/kg, 700 g/kg and 850 g/kg (F-45, U-70, U-85 and C-85, respectively). The low DM material was harvested on the 28th July 2002 when the grain was at the early dough stage (GS 83; Tottman and Broad, 1987), treated with an inoculant/enzyme additive (Whole Crop Gold, Biotal Limited, Cardiff, UK) at ensiling at a rate of 4 l/t applied on the forage harvester, and preserved by fermentation. The 700 g DM/kg material was harvested on 12th August 2002 when the grain was at the hard dough stage (GS 87) and preserved using a urea and urease additive (Home n'Dry, Volac Limited, Royston, UK) applied using a fertilizer spreader at ensiling to provide 20 kg of urea per t forage DM. The high DM material was harvested on 17th August 2002 at a target DM of 850 g/kg when the grain was fully mature (GS 92). Half of the material was preserved by urea using Home n'Dry added at ensilage to provide 20 kg urea per t DM, and half was ensiled with no additive. All forages were cut at a target stubble height of 30 cm using a self propelled forager harvester fitted with a combine header and a grain processor (Class Jaguar 800 series, Claas, Bury St Edmonds, 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 min (rpm) and the other at 3100 rpm and with a gap of

approximately 0.5 mm between the two rollers. All forages were ensiled in concrete walled, roofed commercial silage pits. The clamps were rolled well prior to sealing using a double layer of plastic sheeting and weighed down with tyres. To provide a more comprehensive assessment of crop yield, in addition to the three harvest dates outlined above, an additional sample was collected on 8th August at a target DM of 600 g/kg and processed as described above.

At each of the harvest dates a representative sample of approximately 8 kg fresh weight of the appropriate WCW forage was added to four pre-weighed 20 kg drum silos. The silos were lined with a double layer of plastic liner, the forage compacted and the silo double sheeted and weighed down with approximately 6.5 cm depth of sand. The silos were left for 100 days before being re-weighed and DM loss determined.

The grass silage was harvested on 21st May 2001 from a predominantly perennial ryegrass sward. The crop was wilted for 24 h, no additive was applied and the crop was double sheeted and ensiled in a concrete walled clamp.

3.2.2. Cows

Forty-four Holstein-Friesian dairy-cows, (8 primaparous, 36 multiparous) approximately 38 (SD +/- 10 days) days into lactation were used. Animals were blocked and allocated to the four forage treatments according to parity (prima or multi), calving date, milk yield (34.4 kg; SD 0.1 kg), milk composition, condition score (2.67; SD 0.03) and live-weight (621 kg; SD 12.9 kg) recorded during the week prior to allocation. Animals were group housed in an open span building in cantilever and super comfort cubicles fitted with Pasture Mats® (Wilson Agriculture, Bristol, UK). Cubicle passages were scraped out approximately every 3 h using automatic scrapers and cubicles bedded twice weekly with chopped paper and limed weekly. All animals were offered water *ad libitum*. The

experiment commenced on 5th December 2002 and animals remained on treatment for fifteen weeks.

3.2.3. Dietary treatments

There were four dietary treatments in which 0.67 (DM basis) of the grass silage was replaced by either fermented WCW harvested at approximately 450 g DM/kg (F-45), urea-treated WCW harvested at approximately 700 g DM/kg (U-70), urea-treated WCW harvested at approximately 850 g DM/kg (U-85) or WCW harvested at approximately 850 g DM/kg and untreated (C-85). In addition to the forage, cows received (kg/cow/d) 2 rapeseed meal, 1.4 molassed sugarbeet pulp and 0.6 Lactofeed 70 (Volac International Ltd, Royston, Herts, UK), mixed with the forage component. To balance the difference in crude protein content of the four WCW treatments, approximately 150 g/cow/d of feed grade urea was added to the fermented (F-45) and untreated (C-85) treatments. All animals also received 6.5 kg per day of a standard dairy concentrate (Table 3.1), which was fed in two meals at least six hours apart through out-of-parlour-feeders (Insentec, Markenesse, Holland). The out-of-parlour-feeders were calibrated twice weekly on a Tuesday and Friday to +/- 0.1 kg. The forages and straights were mixed and fed daily at approximately 08.00 h using a Keenan Compact Feeder mixer wagon (Richard Keenan Ltd, Warwickshire, UK) calibrated to +/-1 kg. Each forage treatment was fed through six feed bins on electronic weigh cells (Insentec, Markenesse, Holland), resulting in twenty-four feeders in total. The feed bins electronically recorded the weight and cows were allowed access to the appropriate feeder by the use of collar transponders. Feed bins were calibrated weekly to +/- 1kg. Feed was offered at a rate of 1.05 *ad libitum* intake and maintained by weighing and removing refusals twice weekly on a Monday and Thursday.

Table 3.1. Ingredient composition of the dairy concentrate

	kg/t
Wheat	265
Rapeseed meal	100
Sugarbeet pulp	181
Palm kernel extract	75
Maize	31
Soyabean meal	125
Sunflower meal	69
Molasses	77
Oil	23
Megalac	30
Minerals and Vitamins	24

3.2.4. Measurements

Forages were sampled (approximately 500 g) twice weekly; one sample was oven dried overnight at 100 °C and the proportion of grass silage and WCW adjusted to maintain the ratio of 1: 2 respectively and the remaining sample frozen at -20 °C prior to subsequent analysis. Approximately 500 g of the standard dairy concentrate was collected weekly and frozen at -20 °C prior to bulking for subsequent analysis.

Cows were milked twice daily through a 24: 24 direct to line parlour (WestfaliaSurge UK Ltd, Milton Keynes, UK) at approximately 05.00 h and 17.00 h with milk yield recorded electronically at each milking. A representative milk sample was collected on a Monday evening and Tuesday morning milking and two Lactabs (Thompson & Capper Ltd, Cheshire, UK) added per sample prior to subsequent analysis. All cows were weighed using a weigh crate and weigh cell (Tru-Test Ltd, Auckland, New Zealand) and condition scored (Lowman *et al.*, 1976) weekly on a Wednesday after the evening milking by the same operator. Blood samples were taken from the coccygeal vein of a sub-sample of 6 cows per treatment in weeks 0, 3, 8 and 13 of the experiment at 11.00 h and stored as described in Section 2.2.1.

3.2.5. *In situ* degradability

Four wether sheep, aged approximately 7 years and fitted with permanent rumen cannulae (39 mm) were used to determine the degradability of the four whole-crop forages (F-45, U-70, U-85 and C-85) according to Agricultural and Food Research Council (AFRC; 1992). Animals were housed in individual slatted floor pens with free access to water and minerals. The animals received 1.1 times maintenance energy requirements according to AFRC (1993), of a diet consisting of 60: 40 long grass hay: pelleted concentrate (DM basis) (crude protein 160 g/kg DM, oil 36 g/kg DM, fibre 75 g/kg DM and ash 75 g/kg DM: Table 3.2) fed twice daily together at 08.00 h and 16.00 h.

Table 3.2 Ingredient composition of the sheep concentrate

	kg/t
Rolled barley	237.5
Maize gluten	150
Linseed	150
Sugarbeet	100
Molasses	100
Flaked maize	75
Flaked peas	75
Maize distillers	50
Sopralin	25
Vitamins and minerals	25
Limestone	12.5

The equivalent of 4 g DM of fresh forage (chopped to approximately 1 cm length) was placed in a numbered pre-weighed synthetic bag with a pore size of 43 μm and thread diameter of 40 μm. The bags were sealed by threading the neck of the bag through a copper curtain ring, folding the neck against itself and sealing with an elastic band. The bags were connected by the curtain ring to a stainless steel spring clip and incubated within the rumen 30 minutes post feeding. Four bags, one for each feed sample was inserted at one time and samples were retrieved from the rumen after 2, 4, 8, 16, 24, 48 and 72 h. The incubations for each time point were repeated to provide at least two replicates per animal for each time point. On removal from the rumen, bags were immediately placed in a bucket

of cold water and feed particles adhering to the outside of the bag gently rinsed off. The bags were then washed in a domestic washing machine (Electra autowasher AWM 1100B) on a cold water 50-60 min wash cycle with spin before drying at 70 °C for 48 h until a constant weight was reached. The zero-hour time point was detailed by following the above washing procedure using eight bags for each feedstuff. The degradability of each component (DM, NDF, N and starch) at each time point was calculated, and fitted to the first-order model of Ørskov and McDonald (1979):

$$p = a + b\,(1 - e^{-ct}) \qquad \text{(Equation 3.1)}$$

Where *p* is the cumulative amount degraded at time *t*; *a* is the readily soluble fraction; *b* is the potentially degradable fraction in the rumen; *c* is the constant rate of degradation of *b; t* is time (h). In order to determine the presence of a possible lag phase, the data were also fitted to a first-order model containing a lag phase (McDonald, 1981);

$$p = a \qquad \text{up to time } t_0 \qquad \text{(Equation 3.2)}$$

$$p = a + b\,(1\text{-}e^{-ct}) \qquad \text{from time } t_0 \text{ onwards} \qquad \text{(Equation 3.3)}$$

Where *a*, *b* and *c* are described above, t_0 is the lag phase (h). The effective extent of degradation (P) was calculated using the fractional output rate of solids from the rumen (k) as:

$$P = a + ((b \times c)/(c + k)) \qquad \text{(Equation 3.4)}$$

Where *a*, *b* and *c* are described above. For components that contained a lag phase, effective degradability was calculated as;

$$P = a \qquad \text{up to lag} \qquad \text{(Equation 3.5)}$$

$$P = a + ((b \times c)/(c + k)) \times e\,(-(c + k) \times t_0) \qquad \text{beyond lag} \qquad \text{(Equation 3.6)}$$

3.2.6. Chemical analysis

Weekly forage and concentrate samples were bulked every four weeks and a sub-sample analysed for DM, OM, N, ammonia N, starch, water soluble carbohydrates, NDF and ADF by NRM according to the methods outlined in Sections 2.1.1 to Section 2.1.9. Forage

samples were also analysed for pH (Section 2.1.10) and fermented WCW and grass silage analysed for volatile fatty acids (Section 2.1.11). The ME content of the grass silage and dairy concentrate was predicted according to Section 2.1.13.

Milk samples were analysed on a weekly basis for fat, protein and lactose using a Dairylab double beam infrared spectrophotometer (Foss, York, UK). Plasma samples were analysed for total protein, urea, albumin, BHB, glucose and NEFA on a Bayer Technicon RA 1000 auto-analyser (ABX Diagnostics, Bedfordshire, UK). Degradability samples were ground through a Micromark mini grinder (Micromark, London, UK) and bulked for each time point and analysed for total N, starch and NDF (Sections 2.1.3, 2.1.5 and Section 2.1.7 respectively).

3.2.7. Statistical Analysis

Statistical analysis was carried out using Genstat version 5 (VSN Int. Ltd, Oxford, UK). Milk yield parameters, live-weight, condition score and blood parameters were evaluated by analysis of variance as a randomised block design. Milk yield (kg/d) in the week prior to blocking was used as a co-variate where appropriate. *In situ* degradability coefficients were determined and evaluated by analysis of variance.

3.3. RESULTS

3.3.1. Crop production

The ensiled DM, total harvestable DM and total above ground DM all increased as the crop matured from 450 to 700 g DM/kg before decreasing at 850 g DM/kg (Table 3.3), although, none of these differences were significant. Grain DM yield was higher for the crop harvested at 700 g DM/kg in comparison with that harvested at 450 g DM/kg ($P<0.01$), whilst numerically the crop harvested at 700 g DM/kg was higher than material harvested at 850 g DM/kg ($P<0.01$). Harvest index was higher ($P=0.002$) when the crop was harvested at 700 g DM/kg compared with 450 g DM/kg.

Table 3.3. Yield components (t/ha) for whole-crop wheat harvested at target DM values of 450 g/kg, 600 g/kg, 700 g/kg and 850 g/kg

	Forage (g DM/kg)					
	450	600[a]	700	850	s.e.d.	Sign.
Date of harvest	28/07/02	08/08/02	12/08/02	17/08/02		
Ensiled DM	14.6	15.1	15.5	14.2	0.557	0.197
Baleable straw DM	2.29	2.37	2.19	2.05	0.282	0.700
Total harvestable DM	16.9	17.4	17.7	16.2	0.717	0.274
Above ground DM	17.7	18.1	18.8	17.0	0.630	0.101
Grain DM yield	9.52[b]	10.5[bc]	11.5[c]	10.0[bc]	0.364	0.009
Harvest index	0.54[b]	0.58[c]	0.59[c]	0.59[c]	0.010	0.002

[a] additional harvest point but no animal data
[bc] within a row, means without a common superscript differ ($P<0.05$)

Ensiling losses for the fermented treatment (F-45) were higher ($P<0.01$) compared to the other treatments (57.0 g/kg DM), with the urea-treated and untreated forage treatments having relatively low losses (Table 3.4).

Table 3.4. Ensiling losses for whole-crop wheat harvested at target DM values of 450 g /kg and fermented (F-45), 700 g/kg and urea-treated (U-70), 850 g/kg and either urea-treated (U-85) or untreated (C-85)

	F-45	U-70	U-85	C-85	s.e.d.	Sign.
Ensiling loss (g/kg DM)	57.0[b]	1.0[a]	1.0[a]	2.0[a]	13.3	0.01

[ab] within a row, means without a common superscript differ ($P<0.05$)

3.3.2. Forage and concentrate analysis

Chemical composition of the forages is shown in Table 3.5. Within the four forages, DM content was similar to that predicted and increased as the crop matured. Crude protein and ammonia N contents were highest in the urea-treated forages (average of 143 g/kg DM and 147 g/kg total N) and lowest in the C-85 forage (98 g/kg DM and 8.5 g/kg DM respectively). The highest forage pH was obtained in the two urea-treated forages (U-70 and U-85) with the lowest value recorded in the fermented WCW, whilst the untreated forage (C-85) had a neutral pH. Fibre levels were highest in the two mature WCW forages (U-85 and C-85) at approximately 325g/kg DM and lowest in U-70, with the fermented WCW having an intermediate value. The fermented whole-crop had the highest content of water soluble carbohydrates and lowest starch content. The grass silage was well preserved with a low ammonium-N and pH value. The concentrate had an ME value of 13.6 MJ/kg DM and crude protein content of 247 g/kg DM (Table 3.5).

Table 3.5. Chemical composition (g/kg DM, unless otherwise stated) for whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70), 850 g/kg and either urea-treated (U-85) or untreated (C-85), grass silage (GS) and dairy concentrate (Conc)

	F-45	U-70	U-85	C-85	GS	Conc.
DM (g/kg)	472	747	823	850	274	898
Crude Protein	102	140	146	98.0	130	247
Ash	39.0	35.0	40.0	33.1	71.0	99.1
Oil-B	22.4	23.1	25.8	21.6	38.9	96.9
D- Value (g/kg DM)	733	780	732	697	701	nd
ME (MJ/kg DM)	nd	nd	nd	nd	10.8	13.6
ND fibre	308	288	327	320	508	263
Starch	370	434	416	442	28.5	138
Water soluble carbohydrate	28.4	5.7	4.8	4.6	49.4	114
AD fibre	147	149	161	150	313	nd
pH	4.2	8.1	8.3	6.5	3.8	nd
Ammonia-N (g/kg total N)	114	144	149	8.5	81	nd

nd – not determine

3.3.3. Animal performance

Data was excluded from the analysis for three cows, (treatment F–45, U-85 and C-85) which were removed from the experiment for reasons unrelated to the dietary treatments. Total concentrate intake was similar across dietary treatments, averaging 9.4 kg DM per day (Table 3.6). Total forage intake (grass silage and WCW) and total DM intake tended to be highest for animals fed the untreated WCW and the lowest values for animals fed the urea-treated forages, although none of the differences were significant.

Table 3.6. Average intake of concentrates and forage of cows offered whole-crop wheat (WCW) harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85).

	F-45	U-70	U-85	C-85	s.e.d.	Sign.
Dairy concentrates	5.45	5.55	5.46	5.50	0.182	0.945
Total concentrates	9.39	9.32	9.40	9.53	0.306	0.919
Grass silage	4.38	4.20	4.17	4.76	0.267	0.120
WCW	9.00	8.64	8.68	9.86	0.552	0.124
Total forage	13.38	12.83	12.85	14.62	0.818	0.122
Total DMI	22.74	22.18	22.21	24.14	1.078	0.250

Average milk yield (kg/d) was 2.5 kg/d higher ($P<0.05$) in cows offered U-70 compared with animals offered F-45, and 2.7 kg/d higher than those offered C-85 (Table 3.7). This difference was apparent from week 1 of the experiment and continued throughout the experiment (Fig 3.1). There was no difference ($P>0.05$) in milk yield between cows fed U-70 and U-85 or between U-85, F-45 and C-85.

There was no effect of treatment on milk fat, protein and lactose content (g/kg), although animals offered U-70 had the lowest fat and protein content (Table 3.7 and Fig 3.2 and 3.3). Similarly, there was no effect ($P>0.05$) of treatments on milk constituent yield (kg/d), average live-weight, live-weight change or condition score, although cows fed treatment U-85 tended to have a lower live-weight and condition score. Over the experimental period condition score increased for cows on all treatments (Fig 3.4).

Table 3.7. Average milk yield, composition, live weight and condition score of cows offered whole crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85)

	F-45	U-70	U-85	C-85	s.e.d.	Sign.
Milk yield (kg/d)	32.31[a]	34.75[b]	33.53[ab]	32.12[a]	0.992	0.042
Fat (g/kg)	40.55	37.89	39.49	40.04	1.251	0.191
Protein (g/kg)	33.47	32.18	32.90	32.98	0.658	0.290
Lactose (g/kg)	45.57	46.09	45.58	45.88	0.360	0.409
Fat yield (kg/d)	1.32	1.32	1.32	1.28	0.062	0.919
Protein yield (kg/d)	1.08	1.12	1.09	1.07	0.037	0.530
Lactose yield (kg/d)	1.47	1.61	1.53	1.48	0.060	0.125
Live-weight (kg)	628	625	610	635	20.81	0.683
Live-weight change (kg/d)	0.17	0.02	0.22	-0.03	0.165	0.388
Condition Score	2.85	2.75	2.64	2.74	0.150	0.573

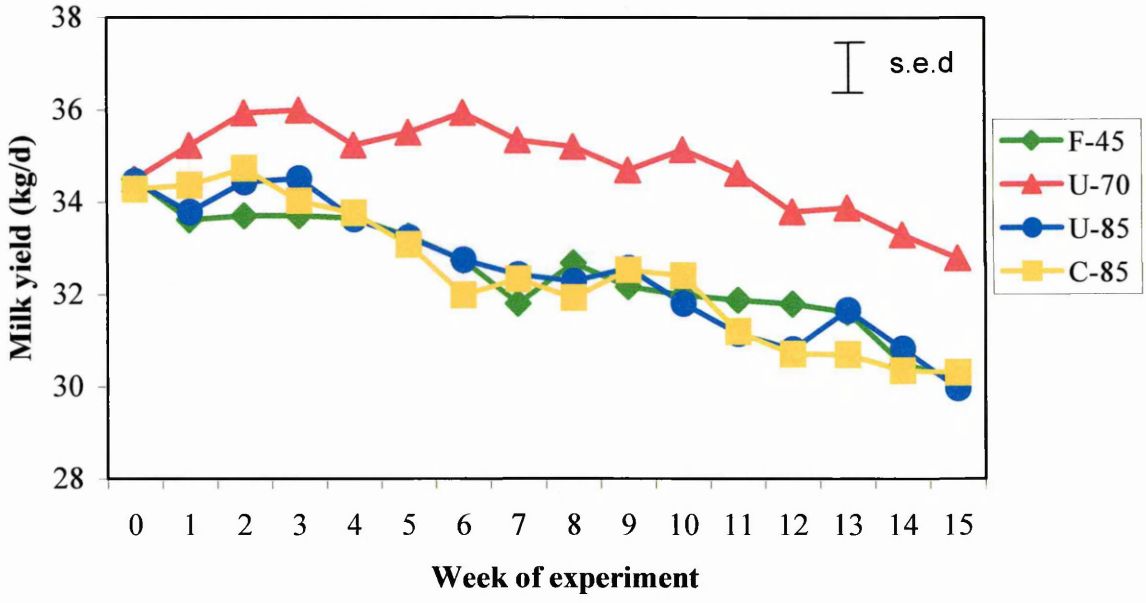

Figure 3.1. Average weekly milk yield of cows offered whole-crop wheat harvested at target dry matter values 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85).

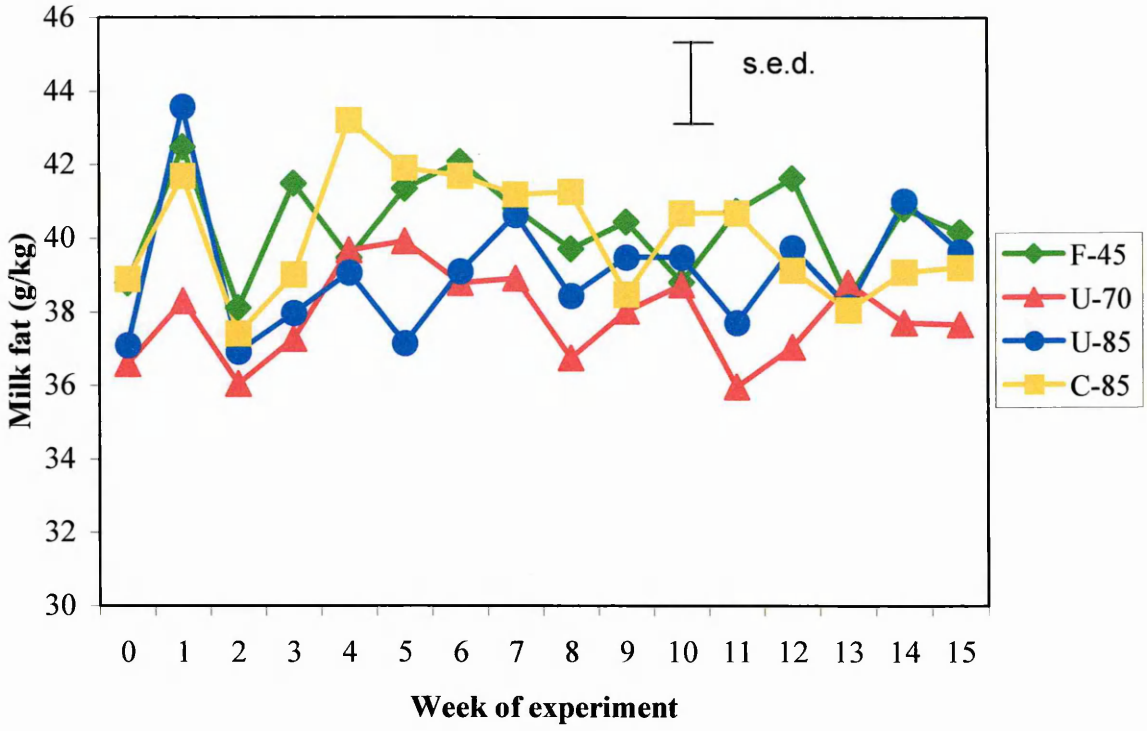

Figure 3.2. Average weekly milk fat content (g/kg) for animals offered whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70), 850 g/kg and urea-treated (U-85) or untreated (C-85).

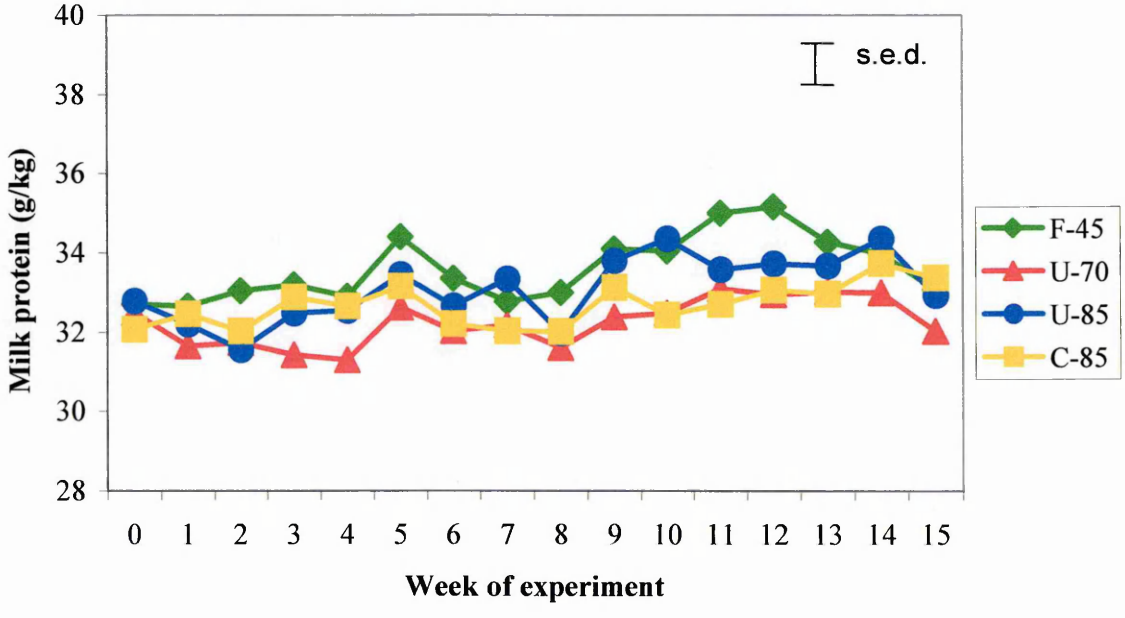

Figure 3.3. Average weekly milk protein content (g/kg) for animals offered whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70), 850 g/kg and urea-treated (U-85) or untreated (C-85).

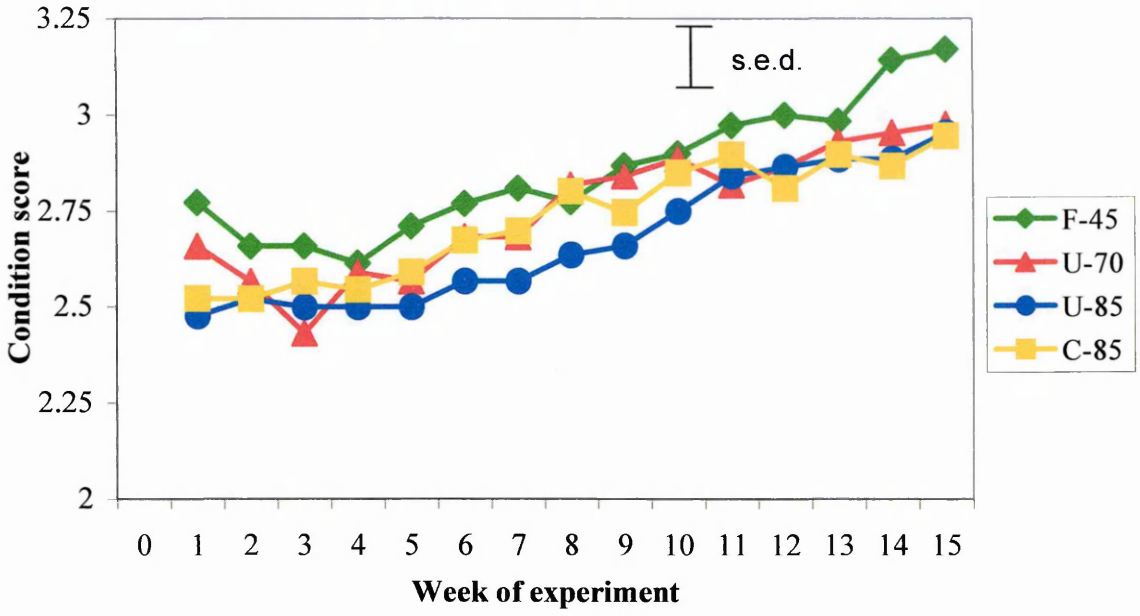

Figure 3.4. Average weekly condition score for animals offered whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70), 850 g/kg and urea-treated (U-85) or untreated (C-85).

Nitrogen intake (Table 3.8) was highest for cows offered the untreated forage (C-85) followed by animals offered the fermented WCW. The lowest N intake was for animals offered U-70. By contrast, milk N output was highest in animals offered the high DM urea-treated forage (U-85) and lowest in animals offered the untreated forage (C-85). Overall N efficiency (defined as daily milk N output (g)/ daily dietary N intake (g)) was similar across all four treatments, averaging 0.32 g/g.

Table 3.8. Estimated nitrogen (N) efficiency of dairy cows offered whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85).

	F-45	U-70	U-85	C-85	s.e.d.	Sign.
N intake (g/d)	660	640	652	674	26.96	0.649
Milk output N (g/d)	206	207	209	202	9.830	0.907
N efficiency (g/g)	0.32	0.33	0.32	0.30	0.018	0.433

3.3.4. Blood metabolite concentrations

Average plasma urea concentrations were higher (P<0.01) in animals fed either of the diets containing WCW harvested at 850 g DM/kg (C-85 and U-85; Table 3.9). The increase in plasma urea levels occurred by week 3 of the experimental period for animals fed the C-85 treatment and remained high thereafter. By contrast, cows fed the U-85 diet had the highest plasma urea level during week 8 of the experiment (Fig 3.5). There were no differences (P>0.05) due to dietary treatment in either average or weekly plasma glucose or BHB levels. Similarly, there were no differences (P>0.05) in plasma NEFA levels between dietary treatments.

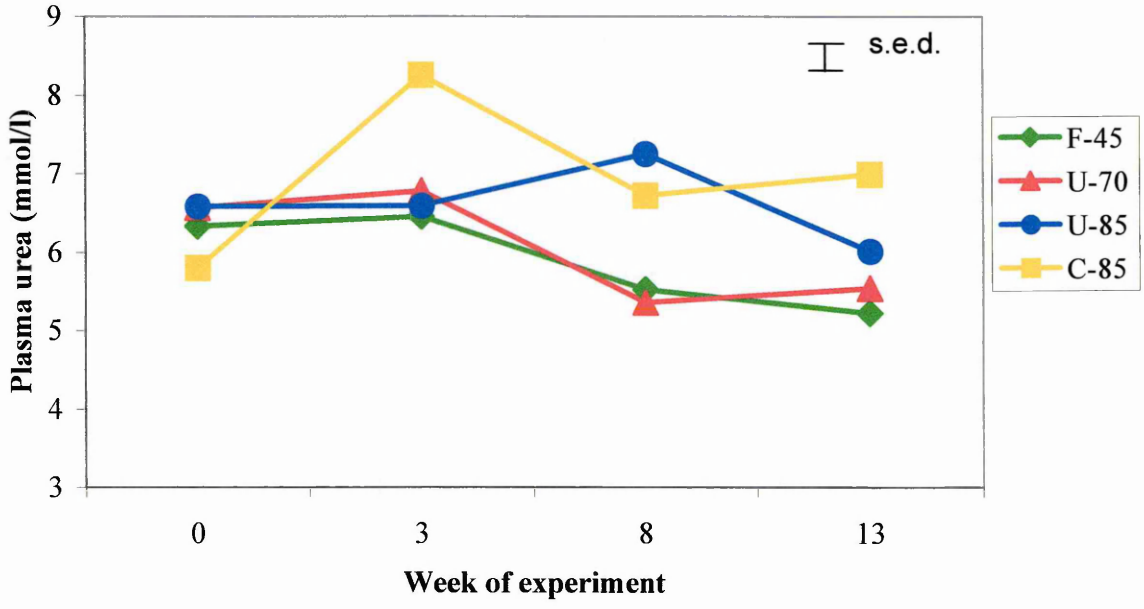

Figure 3.5. Average plasma urea concentrations of cows offered whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70), 850 g/kg and urea-treated (U-85) or untreated (C-85).

Table 3.9. Average plasma concentrations of urea, glucose, β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA; mmol/l), total protein and albumin (g/l) for cows offered whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85).

	F - 45	U - 70	U - 85	C - 85	s.e.d.	Sign.
Urea	5.83^{a}	6.06^{ab}	6.64^{bc}	6.99^{c}	0.294	0.007
Glucose	3.25	3.25	3.31	3.22	0.119	0.890
BHB	0.60	0.66	0.64	0.66	0.069	0.837
NEFA	0.31	0.36	0.29	0.32	0.062	0.786
Tot Protein	83.70	80.21	79.44	81.43	2.011	0.218
Albumin	34.66	33.61	33.94	33.48	1.188	0.755

^{abc} within a row, means without a common superscript differ (P<0.05)

3.3.5. *In situ* degradability

i. Dry matter

The DM degradability coefficients of the four whole-crop forages are presented in Table 3.10. The immediately soluble fraction (intercept, a) of the DM decreased with stage of maturity, with F-45 having the largest immediately soluble fraction whilst there was no difference between the urea-treated forages (U-70 and U-85). The potentially degradable fraction (asymptote, b) of the DM was higher (P<0.001) in U-70, U-85 and C-85 than F-45. The rate of degradation of the potentially degradable fraction (c) was lowest for the fermented forage (F-45) and highest for the high DM urea-treated forage (U-85) whilst the U-70 and C-85 forages had intermediate values (average of 0.08 /h). The calculated effective DM degradability (g/g) at 0.051 outflow rate (Thomas, 2004) decreased with increasing maturity (P<0.001) with both urea-treated forages being similar (average 0.56 g/g).

Table 3.10. Dry matter (DM) degradability coefficients for whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85).

	F-45	U-70	U-85	C-85	s.e.d.	Sign.
Intercept (a) (g/g)	0.53^{c}	0.15^{b}	0.16^{b}	0.09^{a}	0.008	0.001
Asymptote (b) (g/g)	0.33^{a}	0.67^{c}	0.60^{b}	0.64^{c}	0.017	0.001
Fractional rate (c) (/hr)	0.035^{a}	0.082^{b}	0.100^{c}	0.073^{b}	0.051	0.001
a + b (g/g)	0.86^{c}	0.81^{b}	0.76^{a}	0.73^{a}	0.013	0.001
Effective degradability (g/g)*	0.66^{c}	0.56^{b}	0.56^{b}	0.47^{a}	0.007	0.001
R^2	95.9^{a}	97.5^{ab}	95.4^{a}	98.4^{b}	0.911	0.029

^{abc} within a row, means without a common superscript differ (P<0.05)
Where *a* is the readily soluble fraction; *b* is the potentially degradable fraction, *c* is the constant rate of degradation of *b*.
* outflow rate 0.051 (Thomas, 2004)

ii. Neutral detergent fibre

The immediately soluble fraction (a) of the NDF was higher for WCW harvested at 700 g DM/kg and urea-treated than any of the other three treatments (Table 3.11). There was no difference between the fermented and urea-treated forages in the potentially degradable fraction (b), whilst C-85 was lower (P<0.05). Rate of degradation of b (c) was similar for the fermented forage and U-85, averaging 0.027 /h, whilst U-70 and C-85 were higher, averaging 0.049 /h. The high DM untreated forage was the only treatment with a lag phase. Effective degradability was highest for the forage harvested at 700 g/kg DM and urea-treated (P<0.001) and lowest in C-85.

Table 3.11. Neutral detergent fibre (NDF) degradability coefficients for whole-crop wheat harvested at target DM values of 450 g/kg kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85).

	F-45	U-70	U-85	C-85	s.e.d.	Sign.
Intercept (a) (g/g)	0.04^{a}	0.10^{b}	0.03^{a}	0.08^{b}	0.017	0.010
Asymptote (b) (g/g)	0.70^{b}	0.68^{b}	0.67^{b}	0.55^{a}	0.030	0.003
Fractional rate (c) (/hr)	0.028^{a}	0.052^{b}	0.026^{ab}	0.045^{bc}	0.008	0.023
Lag (h)	-	-	-	8.95	-	-
a + b	0.74^{b}	0.77^{b}	0.70^{b}	0.63^{a}	0.027	0.003
Effective degradability (g/g)*	0.28^{a}	0.41^{b}	0.27^{a}	0.25^{a}	0.016	0.001
R^2	95.6	97.1	94.4	94.0	1.667	0.313

^{abc} within a row, means without a common superscript differ (P<0.05)
Where *a* is the readily soluble fraction; *b* is the potentially degradable fraction, *c* is the constant rate of degradation of *b*.
* outflow rate 0.051(Thomas, 2004)

iii. Starch

The immediately soluble starch fraction (a) decreased with increasing stage of maturity and there was no soluble fraction for forage C-85 (Table 3.12). By contrast, the potentially degradable fraction (b) increased from 0.14 for F-45 to 0.93 for C-85 (P<0.001). There was no difference between treatments for the fractional rate of degradation of b (c). Forages U-70 and C-85 contained a lag phase whilst the effective degradability was highest for the fermented forage and lowest for C-85, with the urea-treated forages having intermediate values, averaging 0.81 g/g.

Table 3.12. Starch degradability coefficients for whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85).

	F-45	U-70	U-85	C-85	s.e.d.	Sign.
Intercept (a) (g/g)	0.84^{d}	0.45^{c}	0.32^{b}	0.00^{a}	0.020	<0.001
Asymptote (b) (g/g)	0.14^{a}	0.50^{b}	0.64^{c}	0.93^{d}	0.034	<0.001
Fractional rate (c) (/hr)	0.16	0.23	0.15	0.19	0.049	0.481
a + b	0.99	0.96	0.96	0.93	0.019	0.077
Lag (h)	-	2.10	-	1.11	-	-
Effective degradability (g/g)*	0.95^{c}	0.82^{b}	0.80^{b}	0.69^{a}	0.057	<0.001
R^2	88.8	95.5	91.2	97.7	3.16	0.075

^{abc} within a row, means without a common superscript differ (P<0.05)
Where *a* is the readily soluble fraction; *b* is the potentially degradable fraction, *c* is the constant rate of degradation of *b*.
* outflow rate 0.051(Thomas, 2004)

iv. Nitrogen

The N degradability coefficients are presented in Table 3.13. The immediately soluble (a) fraction decreased with stage of maturity whilst the potentially degradable fraction (b) increased with increasing stage of maturity (P<0.001), although the urea-treated forages were similar at 0.62 and 0.56 g/g for U-70 and U-85 respectively. Similarly, the rate of degradation of b (c) increased with stage of maturity (P<0.001), with the high DM forages (U-85 and C-85) being similar averaging 0.07 g/g. Effective degradability decreased with stage of maturity, although the two urea-treated forages (U-70 and U-85) had similar values.

Table 3.13. Nitrogen degradability coefficients for whole-crop wheat harvested at target dry matter values 450 g/kg kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85).

	F-45	U-70	U-85	C-85	s.e.d.	Sign.
Intercept (a) (g/g)	0.71^{d}	0.24^{b}	0.30^{c}	0.07^{a}	0.008	<0.001
Asymptote (b) (g/g)	0.19^{a}	0.62^{b}	0.56^{c}	0.72^{d}	0.022	<0.001
Fractional rate (c) (/hr)	0.03^{a}	0.07^{b}	0.08^{bc}	0.08^{c}	0.005	<0.001
a + b	0.90^{c}	0.86^{bc}	0.85^{b}	0.79^{a}	0.020	0.002
Effective degradability (g/g)*	0.78^{d}	0.59^{b}	0.63^{c}	0.51^{a}	0.005	<0.001
R^2	84.8^{b}	97.3^{a}	97.9^{a}	98.2^{a}	2.284	<0.001

^{abc} within a row, means without a common superscript differ (P<0.05)

Where a is the readily soluble fraction; b is the potentially degradable fraction, c is the constant rate of degradation of b.

* outflow rate 0.051(Thomas, 2004)

3.4. DISCUSSION

3.4.1. Crop production

Harvesting WCW at approximately 700 g DM/kg compared with 450 g DM/kg increased grain DM yield from 9.5 to 11.5 t DM/ha, whilst leaving the crop to reach 850 g DM/kg resulted in a decrease in grain yield to 10.0 t DM/ha, with a similar trend in harvestable yield. Weller *et al.* (1995), Hill and Leaver (1999) and Sutton *et al.* (2002) reported that crop yield increased substantially with advancing maturity, whilst Corral *et al.* (1977) concluded that the optimum DM yield was attained at 550 - 600 g DM/kg. By contrast, the crop yield for the current experiment continued to increase up to 700 g DM/kg, indicating maximum yield had been reached, with losses occurring at high DM values possibly as a result of carbohydrate respiration (HGCA, 1988). The total harvestable yields for the current experiment were comparable to that of previous work (Kristensen, 1992; Jackson *et al.*, 2004).

Ensiling losses, an aspect most influenced by the DM content of the fresh crop (Bolsen *et al.*, 1983) were significantly higher for WCW ensiled at 450 g DM/kg and fermented (approximately 60 g/kg), although the losses recorded in the current experiment were lower than the 110 g/kg for low DM (300 g/kg) WCW and 95 g/kg for high DM (470 g/kg) WCW reported by Tetlow (1992). Hill and Leaver (1999) suggested that the application of urea significantly reduced storage losses whilst other workers (Bolsen *et al.*, 1983; Tetlow, 1992) have demonstrated decreased losses with advancing maturity of the crop ensiled. The current results indicate that for material ensiled at 850 g DM/kg there was little benefit from the addition of urea, on in silo losses.

3.4.2. Forage and concentrate analysis

The DM contents of the four WCW forages in the current experiment were close to the intended values (472, 747, 823 and 850 g DM/kg) and in agreement with other work (Weller *et al.*, 1995; Hill and Leaver, 1999; Sutton *et al.*, 2002). The DM content of the fermented WCW (F-45) used in the current work was higher than the processed WCW used by Murphy *et al.* (2004) and Patterson and Kilpatrick (2005) but within the range 350 to 550 g DM/kg reported by Tetlow (1992) for fermented WCW and similar to the optimum crop DM levels suggested by Sutton *et al.* (2002) and Sinclair *et al.* (2003). Jackson *et al.* (2004) concluded that the use of a forage processor prior to ensiling enabled WCW to be harvested across a wider harvest window. The high DM processed, urea-treated and untreated WCW forages (U-85 and C-85) in the current work had DM values (823 and 850 g/kg DM) similar to that used by Sinclair *et al.* (2005) whilst the lower DM processed, urea-treated WCW (U-70) was within the range of 653 - 763 g/kg DM reported in other work when processed, urea-treated WCW (Jackson *et al.*, 2004; Murphy *et al.*, 2004; Patterson and Kilpatrick, 2005) or unprocessed WCW (Hameleers, 1998; Abdalla *et al.*, 1999) has been fed to dairy cows. At lower DM values water soluble carbohydrate values are high (Tetlow, 1992) providing a suitable substrate for lactic acid bacteria to convert into organic acids, decreasing pH and preserving the forage against spoilage (Filya *et al.*, 2000). At higher DM values water soluble carbohydrate levels are lower, reducing the fermentation capacity, and starch contents are high (Adogla-Bessa *et al.*, 1999). In the current experiment water soluble carbohydrate values decreased with increasing maturity, whilst starch content increased. The increase in starch compensates to some extent for the increase in NDF and ADF content associated with increased maturity and therefore fibre content of the total crop (g/kg DM) is not substantially changed (Leaver and Hill, 1995). Ammonia N is produced during the breakdown of plant proteins (proteolysis) during fermentation or urea hydrolysis in urea-treated forages (Hill and Leaver, 2002). In the current experiment the elevated ammonia N and crude protein values for the urea-treated

forages reflect the inclusion of urea and its conversion to ammonia in the clamp (Adamson and Reeve, 1992; Hameleers, 1998). The presence of ammonia N in conserved forages inhibits microbial activity during ensiling and subsequent aerobic deterioration (Hill and Leaver, 2002). All the whole-crop forages were well preserved, as reflected by the pH values, with both urea-treated forages having pH values above 8.0 which has been suggested to illustrate successful promotion of an alkaline state, inhibiting the proliferation of fungi and saccharolytic clostridia which often cause aerobic spoilage and DM losses (Adogla-Bessa *et al.*, 1999). Similarly, the low pH value of pH 4.2 in F-45 indicates the successful conversion of water soluble carbohydrates into organic acids, in particular lactic acid, which reduces enzyme activity and undesirable micro-organisms in the forage (Merry *et al.*, 2000). By contrast, C-85 had a neutral pH of pH 6.5. Although aerobic stability was not recorded in the current experiment, Jackson (2005) reported lower yeast counts for untreated WCW harvested at 800 g DM/kg compared with WCW harvested at lower maturities (600 and 700 g DM/kg), a reflection of the high DM inhibiting microbial activity (Adogla-Bessa and Owen, 1995). Furthermore, changes in temperature for WCW harvested at 800 g DM/kg were small (Jackson, 2005). It is therefore likely that in the current experiment the high DM untreated WCW (C-85) was aerobically stable.

The grass silage used in the current experiment appeared to be well preserved, with a low ammonia N level of 81 g/kg total N and low pH of 3.8 although the ME content was moderate at 10.8 MJ/kg DM, suggesting a high intake potential.

3.4.3. Animal performance

The partial replacement of grass silage with either fermented or unprocessed urea-treated WCW in dairy cow rations generally increases DM intake (Phipps *et al.*, 1995; Leaver and Hill, 1995; Hameleers, 1998). By contrast, processing grains of WCW prior to ensiling decreased DM intake but improved the whole tract digestibility of the starch component

compared with unprocessed material (Jackson *et al.*, 2004). The forage DM intakes recorded in the present experiment were within the range reported for fermented and processed, urea-treated WCW (Jackson *et al.,* 2004, Patterson and Kilpatrick, 2005, Sinclair *et al.*, 2005) and were similar across all four treatments. This is in contrast to the findings of Murphy *et al.* (2004) and Patterson and Kilpatrick (2005) who reported increased intakes for animals offered processed, urea-treated WCW compared with fermented WCW. Similarly, a comparison of fermented and unprocessed urea-treated WCW by Hameleers (1998) and Sutton *et al.* (2002) reported animals fed urea-treated WCW had the highest intakes. Forage DM intakes in the current experiment were numerically highest for animals offered the high DM untreated forage. In agreement with these findings, Sutton *et al.* (2002) and Sinclair *et al.* (2003) reported small but non-significant increases in DM intakes with advancing maturity of fermented WCW.

Urea-treatment is the principal method of conservation for WCW harvested at high DM values (Hill and Leaver, 1999). In the presence of moisture the naturally occurring enzyme urease hydrolyses urea to release gaseous ammonia, which spreads throughout the clamp delignifying crop cell walls (Tetlow, 1992) and improving digestibility (Ørskov *et al.*, 1983). Sutton *et al.* (2002) reported an improvement in DM digestibility between high DM WCW (511 g/kg DM) and higher DM urea-treated WCW (584 g/kg DM), possibly as a result of urea-treatment. Although whole-tract digestibility was not measured in the current experiment it is possible that decreased digestibility of the high DM untreated WCW resulted in an increased rate of passage of undigested food from the rumen thus increasing forage DM intake (Abdalla *et al.*, 1999).

Previous work that has examined the utilisation of urea-treated WCW by dairy cows concluded that WCW inclusion had no significant effect on milk yield (Phipps *et al.*, 1995; Leaver and Hill, 1995; Hameleers, 1998). Similarly, in the current experiment, despite the

high forage DM intake by animals offered the untreated high DM forage (C-85), milk yield was not increased. By contrast, Arieli and Adin (1994) reported higher milk yields for cows fed WCW harvested at 315 g DM/kg compared with those fed WCW harvested at 300 g DM/kg. However, animals fed the urea-treated forage harvested at 700 g/kg DM (U-70) had a higher milk yield (kg/d) than those fed F-45, an effect that was consistent throughout the experimental period. The increased milk yield in animals fed U-70 may be due to the addition of urea buffering the rumen acidity thus providing a conducive environment for cellulolysis and the growth of rumen bacteria and protozoa (Mould and Ørskov, 1983), although Abdalla *et al.* (1998) found little effect of urea-treated WCW compared with grass silage on ruminal pH. Alternatively, an improved synchronous supply of additional volatile N from urea and the fermentable energy in WCW may have contributed to an enhanced microbial growth (Adogla-Bessa *et al.*, 1999; Sinclair *et al.*, 1993) and animal performance.

Despite milk yield (kg/d) being greater in cows fed U-70, milk component yield (kg/d) was similar between treatments and animals fed U-70 tended to have the lowest fat content (g/kg). Previously it has been suggested that increased dietary starch concentration can be associated with a shift in VFA production in the rumen from acetate towards propionate resulting in a decrease in milk fat as dietary energy is partitioned towards body lipid (Reynolds *et al.*, 1997). In the current experiment forage starch content and rumen starch degradability were slightly higher in the urea-treated forage harvested at 700 g/kg DM (U-70) compared with U-85 and therefore the lower milk fat content could have been a result of increased dietary starch intake and rumen degradability. In addition, animals offered U-70 tended to also have the lowest milk protein content. This finding is in contrast to work by Sutton *et al.* (2002) who reported that with the appropriate dietary energy source, the higher N intakes associated with urea-treated WCW can increase milk protein levels.

Furthermore, Reynolds *et al.* (1997) also stated that the increased ME intake associated with increased carbohydrate intake often increases milk protein content.

In agreement with previous work (Hameleers, 1998; Hill and Leaver, 1999) there was no difference between the dietary treatments for either live-weight, live-weight change or condition score over the experimental period.

Previous attempts to calculate energy balances for WCW have been varied and it would be misleading to calculate a complete ME balance from the results of the current experiment. However, milk energy outputs, calculated according to Tyrell and Reid (1965) were similar between dietary treatments at 104, 108, 106 and 102 MJ/d (s.e.d. 4.27, P=0.606) for F-45, U-70, U-85 and C-85 respectively. The slightly higher values for the urea-treated forages, along with the decreased DM intakes compared with the high DM untreated forage (C-85) resulted in an improvement in efficiency of forage utilisation for milk production for urea-treated forages, 1.44, 1.63, 1.54 and 1.34 (s.e.d. 0.81, P = 0.008) for F-45, U-70, U-85 and C-85 respectively. Similarly, intake of N was lowest for animals offered the urea-treated forages and highest for those offered C-85. As there was no difference across dietary treatments in milk protein content this resulted in a higher N efficiency for urea-treated forages compared with C-85, although the differences were not significant. By contrast, Hameleers (1998) reported reduced N efficiency for milk production when urea-treated WCW was offered probably as a result of the increased N content of the urea-treated WCW. Overall, the N efficiency values calculated for this experiment (average 0.32 g/g) were higher than those calculated by Hameleers (1998) who reported an N efficiency of 22.9 g/g for unprocessed urea-treated WCW or Jackson *et al.* (2004) who reported an average 0.27 g/g for processed, urea-treated WCW.

3.4.4. Blood analysis

The blood metabolite values measured in the current experiment were within the normal physiological range of the dairy cow during early lactation (Ward *et al.*, 1995). Protein degradation in the rumen along with intake of dietary urea results in an increase in ruminal ammonia concentrations, which if sufficient energy is present is absorbed across the rumen wall and transported to the liver where it is converted to urea (Chesworth *et al.*, 1998). Urea is then recycled back to the rumen, either directly through the blood or via the saliva, where it may be used as a non-protein source of N for microbial amino acid synthesis (Chesworth *et al.*, 1998). Increased plasma urea levels may therefore indicate poor microbial capture of ammonia in the rumen or the inability of the liver to convert ammonia into urea. In the current experiment increased plasma urea levels were associated with increased maturity, irrespective of method of urea inclusion (i.e. at the clamp in U-85 or in the mixer wagon in C-85), suggesting rumen capture of N may have been decreased. Sinclair *et al.* (2003) reported plasma urea levels to be reduced in diets based on fermented WCW when starch was fed as a supplement, an effect attributed to a greater capture of rumen degradable N in the rumen. The current findings therefore suggest that despite a greater content of starch in the 850 g DM/kg forages, rumen capture of N appeared to be decreased, perhaps due to a slower rate of ruminal energy release. Alternatively, although rumen propionate levels were not measured in the current experiment, previous work has suggested that increased dietary starch intake may increase rumen propionate levels (Reynolds *et al.*, 1997) which may reduce hepatic metabolism of ammonia (Choung and Chamberlain, 1995) thus resulting in higher plasma urea levels.

3.4.5. *In situ* degradability

The *in situ* rumen degradability technique measures the rumen degradation of feeds by placing a small amount of feedstuff in an undegradable porous bag and suspending the bag and contents in the rumen (Huntington and Givens, 1995). Previously, Playne *et al.* (1978)

suggested that increased recycling of nutrients in the rumen of cattle may lead to enhanced microbial activity and *in situ* DM disappearance compared with sheep. By contrast, Prigge *et al.* (1984) reported similar *in situ* degradation of forages by wethers and steers suggesting that recycling of nutrients to the rumen was not a major factor between species. Similarly, Huntington and Givens (1997) concluded that mature ruminant species degrade hay, soyabean meal and fishmeal similarly when fed at maintenance and any differences observed between species may relate to differing rumen outflow rates (Huntington and Givens, 1995; Prigge *et al.*, 1984). As a result, the DM, NDF, starch and N degradabilities for the four WCW forages determined using wether sheep in the current study were applicable to dairy cows.

The immediately soluble fraction of the DM decreased with increasing maturity, with the highest value being reported for the fermented forage (F-45), which may reflect the higher content of water soluble carbohydrate in this forage. An effect of increasing the immediately soluble fraction is that this does not occupy rumen space and DM intakes may be increased (Ørskov *et al.*, 1989). This was demonstrated in the current experiment where forage DM intakes were numerically higher for animals offered the fermented WCW (F-45) compared to those offered either of the urea-treated forages (U-70 and U-85). The higher immediately soluble DM fraction observed in the forages treated with urea compared with the untreated forage (C-85) was similar to that reported for urea-treated wheat straw by Givens *et al.* (1993), and for ammonia treatment of mature cereal straw (Everington and Givens, 1988). Givens *et al.* (1993) calculated an effective DM degradability of urea-treated wheat straw plus grain at an assumed outflow rate of 0.05 /h of 0.48 g/g. This value is lower than that reported in the current experiment for the urea-treated forage (average 0.56 g/g) but similar to the effective degradability of the high DM untreated forage (C-85; 0.47 g/g). However, effective degradability decreased with increasing maturity, a finding in agreement with Southworth *et al.* (1999) who reported

that increased maturity of fermented WCW reduced the corrected effective degradability at 0.05 /h assumed outflow rate. Furthermore, Ashbell *et al.* (1997) reported increased DM effective degradabilities were due to a lower NDF content and higher DM solubility. In the current experiment NDF content was lowest for WCW harvested at 700 g/kg DM and urea-treated and as a result effective degradability was higher than for C-85 or F-45.

The immediately soluble fraction for NDF was higher for U-70 compared with the high DM forages and F-45. Furthermore, the effective degradability of U-70 was significantly higher than any of the other forages. Advancing maturity of forages is associated with decreasing NDF degradability in the rumen (Panditharatne *et al.*, 1989; Ashbell *et al.*, 1997) which may be related to cell-wall lignification, which leads to a reduction in substrate susceptibility to microbial degradation (Tamminga and Van Vuuren, 1988). Arieli and Adin (1994) reported a higher NDF effective degradability for forages harvested at an earlier stage of maturity. Furthermore, animals offered the lower maturity forages produced more milk of lower fat concentration, a result attributed to the improved NDF degradability (Arieli and Adin, 1994). By contrast, in the current experiment, the highest NDF degradability (assumed outflow 0.05 /h) was for the WCW harvested at 700 g/kg DM and urea-treated. Furthermore, this forage treatment was associated with an increased milk yield (kg/d) and milk protein yield.

Starch effective degradability was highest for the fermented WCW forage, mainly due to the increase in the readily soluble fraction. Philippeau and Michalet-Doreau (1998) suggested that the increased starch degradability of ensiled corn was due to the solubilization of endosperm proteins during silage fermentation. By contrast, the high DM untreated WCW forage had the lowest starch effective degradability, due to the lack of a readily soluble fraction. In the current experiment, urea-treated forages had higher starch effective degradabilities than the untreated forage, which suggests urea treatment has

increased starch available for degradation. Within the urea-treated forages, WCW harvested at 700 g DM/kg (U-70) had a higher immediately soluble fraction, which may improve microbial protein synthesis and animal performance through improved synchrony with N release (Sinclair *et al.*, 1993). However, starch effective degradabilities were similar for both urea-treated forages (average 0.81 g/g).

Nitrogen degradability coefficients followed a similar pattern to starch degradability coefficients. The fermented WCW harvested at approximately 450 g DM/kg (F-45) had the highest N effective degradability, mainly as a result of the high readily soluble fraction, which decreased with increasing maturity. Similarly, Hoffman *et al.* (1993) reported high crude protein soluble fractions of perennial forages at early maturities, moving towards increased slowly degraded or undegradable fractions at later maturities. The increased readily soluble fraction for F-45 in the current experiment could be due to the solubilization of crude protein during the fermentation stages of ensiling (von Keyerlingk *et al.*, 1996). Surprisingly, the immediately soluble fraction of the urea-treated forages (U-70 and U-85) was low compared with the fermented forage. It would be expected that the urea-treated forages had a higher immediately soluble fraction due to the addition of urea. Furthermore, the WCW harvested at 700 g DM/kg and urea-treated had a lower effective degradability than the higher DM forage (U-85). Previous work has suggested that diets containing less degradable protein would result in higher milk production (Kung and Huber, 1983) and in the current experiment milk yields were highest for animals fed U-70 which had a slightly lower effective degradability compared with U-85.

3.5. CONCLUSIONS

Harvesting WCW at approximately 700 g DM/kg significantly increased grain DM yield with negligible in-silo DM losses. Inclusion of processed, urea-treated WCW harvested at

approximately 700 g DM/kg in dairy cow rations resulted in a significantly higher milk yield and lower DM intake compared to animals offered fermented WCW harvested at approximately 450 g DM/kg or cows fed high DM untreated WCW. Milk fat and protein content and yield were unaffected by stage of maturity or method of preservation.

Chapter 4.

THE EFFECT OF RATE OF INCLUSION OF PROCESSED, UREA-TREATED WHOLE-CROP WHEAT ON THE INTAKE, MILK PRODUCTION AND DIET DIGESTIBILITY IN DAIRY COWS

4.1. INTRODUCTION

The level of inclusion of alternative forages such as WCW into the diets of dairy cows may affect the level of animal performance (Keady, 2005). Previous work by Phipps *et al.* (1992) replaced grass silage with either 0.25, 0.50, 0.75 or 1.0 of urea-treated WCW. Increasing the inclusion of WCW increased DM intakes without any significant change in milk yield. Similarly, Sutton *et al.* (1997) reported that increasing the proportion of urea-treated, unprocessed WCW in the diet significantly increased DM intake but only raised milk yield (kg/d) slightly. This response was attributed to reduced diet digestibility, particularly of the starch fraction indicated by whole wheat grains in the faeces (Sutton *et al.*, 1997). The recent development of a forage processor enables the grains of WCW to be ground prior to ensiling which allows the crop to be harvested over a much wider harvest window than was previously possible. Processing at harvest has been shown to significantly improve whole tract digestibility of the starch component, which in turn results in an improved forage utilisation (Jackson *et al.*, 2004). Previously, processed urea-treated WCW for dairy cows has been included at 0.67 (Jackson *et al.*, 2004, Murphy *et al.*, 2004) or 0.5 of the forage DM intake (Patterson and Kilpatrick, 2005), although the optimal inclusion rate of processed WCW is unclear.

The objectives of the current experiment were to determine the effect of rate of inclusion of processed, urea-treated WCW on intake, milk production and apparent digestibility in dairy cows.

4.2. MATERIALS AND METHODS

4.2.1. Forage production

A commercial crop of winter wheat (*c.v.* Equinox) was grown on a sandy loam soil following a three year grass ley. The field was dressed with farm yard manure at 25 t/ha on 7th November 2002 and the crop was sown on 30th November 2002 using fludioxinil treated seed at a target seed rate of 175 kg/ha. The crop received 130 kg N/ha applied as a split dressing: 44 kg N/ha on 12th February 2003 GS 13; (Tottman and Broad, 1987) and 86 kg N/ha on 17th April 2003 (GS 31). Disease control consisted of a two spray programme: kresoxim-methyl + fenpropimorph and fluquinoconazole + prochloraz applied on 16th April 2003 (GS 31); followed by epoxiconazole + kresoxim methyl + pyraclostrobin and quinoxyfen applied on 20th May 2003 (GS 37). Chlormequat growth regulator was applied on 16th April 2003 (GS 31). Weed control consisted of isoproturon + diflufenican applied 10th February 2003 to control annual meadow grass and annual broadleaved weeds followed by metasulfuron urea applied on 20th May 2003 to control late spring germinating weeds. The crop was sprayed with cypermethrin against aphids on 10th February 2003 and received routine manganese sprays.

Harvest date was determined by monitoring crop DM twice weekly from early July by cutting two adjacent 0.5 m lengths of row at 15 cm stubble height at six random positions throughout the field. Sample fresh weight was recorded and the samples dried at 105°C overnight and the whole crop DM determined. The objective was to cut the wheat at a minimum DM of 700 g/kg or as soon thereafter as practically possible. The crop was harvested on the 4th August 2003 when the grain was at the hard dough stage (DM 830 g/kg; GS 92). Immediately prior to harvest, crop DM yield was assessed by cutting one quadrat (0.7 m^2) to ground level at 10 random positions within the area to be harvested.

Prior to oven drying at 105°C the material was separated into ensiled and residual material by cutting at 30 cm above the stem base. Grain yield was determined by cutting ears from the ensiled material and threshing using a Wintersteiger single ear thresher (F Walter – H Wintersteiger, Austria), modified to allow quantitative recovery of grain and chaff. Grain was then separated by passing the grain and chaff over a grain cleaner fitted with a 3.5 mm top sieve and a 2 mm bottom sieve. Prior to and immediately following harvest average crop and stubble height was determined at 25 random points within each tramline. The crop was preserved using a urea and urease additive (Home n'Dry, Volac Limited, Royston, UK) applied using a fertilizer spreader at ensiling to provide 20 kg of urea per t forage DM. The forage was cut at a stubble height of 19 cm (SD 1.9 cm) using a self propelled forage harvester fitted with a combine header and grain processor (Claas Jaguar 800 series, Claas, Bury St Edmonds, 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 min (rpm) and the other at 3100 rpm and with a gap of approximately 0.5 mm between the two rollers. The forage was ensiled in a concrete walled, roofed commercial silage pit. The clamp was rolled well prior to sealing using a double layer of plastic sheeting and weighed down with tyres. The grass silage was harvested on 30th May 2003 from a predominantly perennial ryegrass sward. The crop was wilted for 24 h, no additive was applied and the crop was ensiled in a concrete-walled clamp covered with a double layer of plastic sheeting.

4.2.2. Cows

Forty-four Holstein-Friesian dairy-cows, (4 primaparous, 40 multiparous) approximately 29 (SD +/- 5.5) days into lactation were used. Animals were blocked according to parity

(prima or multi), calving date, milk yield (mean 36.6 kg; SD 0.19 kg), condition score (mean 2.14; SD 0.05) and live-weight (mean 640 kg; SD 1.62 kg) recorded during week 3 of lactation, and allocated to one of four treatments. Animals were group housed in an area of an open span building in cantilever and super comfort cubicles fitted with Pasture Mats® (Wilson Agriculture, Bristol, UK). Cubicle passages were scraped out using automatic scrapers approximately every 3 h, and cubicles bedded twice weekly with chopped paper and limed weekly. All animals were offered water *ad libitum*. The experiment commenced at the end of November 2003 and animals remained on treatment for fourteen weeks.

4.2.3. Dietary treatments

There were four dietary treatments; U-0: grass silage alone; U-25: 0.25 WCW and 0.75 grass silage; U-50: 0.50 WCW and 0.50 grass silage; U-75: 0.75 WCW and 0.25 grass silage, all on a DM basis. In addition to the forage, cows received (kg/cow/d) 2 rapeseed meal, 1.5 molassed sugarbeet pulp and 0.5 Lactofeed 70 (Volac International Ltd, Royston, Herts, UK) all mixed with the forage component. All animals also received 7 kg/d of a standard dairy concentrate (Table 4.1), which was fed in two equal meals at least six hours apart through out of parlour feeders (Insentec Makenesse, Holland). The out of parlour feeders were calibrated twice weekly on a Tuesday and Friday to +/- 0.1 kg. The forages and straights were mixed and fed daily at approximately 09.00 h using a Keenan Compact Feeder mixer wagon (Richard Keenan Ltd, Warwicksire, UK) calibrated to +/- 1 kg. Each forage treatment was fed through six feed bins on electronic weigh cells (Insentec, Markenesse, Holland), resulting in twenty-four feeders in total. The feed bins electronically recorded the weight and cows were allowed access to the appropriate feeder by the use of collar transponders. Feed bins were calibrated weekly to +/- 1kg. Feed was

offered at a rate of 1.05 *ad libitum* intake which was maintained by weighing and removing feed refusals twice weekly on a Monday and Thursday.

Table 4.1 - Ingredient composition of the dairy concentrate

	Kg/t
Wheat	200
Rapeseed meal	150
Sugarbeet pulp	140
Palm kernel extract	140
Soyabean meal	100
Sunflower meal	120
Molasses	60
Megalac	42
Minerals and Vitamins	48

4.2.4. Measurements

Forages were sampled (approximately 500 g grab sample from the face of the clamp) twice weekly; one sample was oven dried overnight at 100 °C and the proportion of WCW and grass silage adjusted to maintain the appropriate ratio (DM basis) and the remaining sample frozen at -20 °C. Frozen samples were bulked every four weeks prior to subsequent analysis. Approximately 500 g of the standard dairy concentrate was collected weekly and frozen at -20 °C prior to bulking every four weeks for subsequent analysis. Approximately 300 g of each complete diet (forage plus straights) was sampled, along with diet refusals throughout the experiment and frozen at -20 °C prior to analysis for particle size.

Cows were milked twice daily through a 24: 24 direct to line parlour (WestfaliaSurge UK ltd, Milton Keynes, UK) at approximately 05.00 h and 17.00 h with milk yield recorded electronically at each milking. A representative milk sample was collected on a Monday evening and Tuesday morning and two Lactabs (Thompson & Capper, Ltd, Cheshire, UK) added per sample prior to subsequent analysis. All cows were weighed using a weigh crate and weigh cell (Tru-Test, Auckland, New Zealand) and condition scored (Lowman *et al.*,

1976) weekly on a Wednesday at 14.00 h. In addition, cows were locomotion scored at 14.00 h each Wednesday according to the method of Manson and Leaver (1988). Blood samples were taken from the coccygeal vein of a sub-sample of 6 cows per treatment in weeks 0, 3, 8 and 13 of the experiment and stored as described in Section 2.2.1.

Diet apparent digestibility was measured in five cows per treatment during week 10 of the experiment using acid insoluble ash (AIA) as the internal marker according to Van Keulen and Young (1977). For 7 days faecal grab samples were collected from each cow twice daily at 09.00 h and 15.00 h. At each sampling time approximately 100 g of faeces was taken and immediately stored at -20 °C prior to subsequent analysis. Daily feed samples were also taken and stored at -20 °C prior to subsequent analysis.

4.2.5. Chemical analysis

Weekly forage and concentrate samples (approximately 500 g) were bulked every four weeks within each month and a sub-sample analysed for DM, OM, N, ammonia N, starch, water soluble carbohydrates, NDF and ADF by NRM according to the methods outlined in Section 2.1.1. to Section 2.1.9. All of the forage samples were also analysed for pH (Section 2.1.10). Grass silage was analysed for VFA's as outlined in Section 2.1.11 and ME estimated by near infrared reflectance spectroscopy (NIRS). Similarly, the ME of the concentrate was estimated by NIRS (Section 2.1.13).

Particle size and distribution for the total ration (WCW, grass silage, sugarbeet pulp, rapeseed meal and Lactofeed) and refusals were determined using a Penn State Forage Particle Separator (PSPS; Heinrichs and Kononoff, undated). Three sieves were used with mesh sizes of 19 mm, 8 mm and 1.18 mm. Sieves were stacked in decreasing order of mesh size. There was a solid pan on the bottom of the separator. Approximately 300 g of

the ration or refusals was placed on the upper sieve. The separator was shaken on a flat surface in one direction 5 times then rotated one-quarter turn. The separator was shaken at approximately 1.1 shake per sec with a stroke length of 17 cm. This process was repeated 7 times for a total of 8 sets, rotating after each set of 5. After shaking was complete the weight of the material on each sieve and the bottom pan was recorded. The proportion of the diet or refusals retained on each sieve (g/kg DM) was then calculated using the individual DM values for the diet components. Average particle size (mm) was determined using the spreadsheet of Heinrichs and Kononoff (undated). Using the NDF values for the individual diet components the physically effective NDF (peNDF) was calculated by multiplication of ration NDF by the proportion of DM above 1.18 mm (Mertens, 1997).

Milk samples were analysed on a weekly basis for fat, protein and lactose using a Lactoscope FTIR (QuadraChem Laboratories Ltd., Sussez, UK). Plasma samples were analysed for total protein, urea, albumin, BHB, glucose and NEFA using a Cobas Miras Plus autoanalyser (ABX Diagnostics, Bedfordshire, UK). Plasma insulin concentrations were determined using a ^{125}I-labelled insulin double-antibody RIA as described by Richardson *et al.* (2003).

Feed and faecal samples taken during week 10 of the experiment were dried at 80 °C in a forced air oven prior to analysis for AIA according to Van Keulen and Young (1977). Approximately 5 g of dried sample was weighed into a 50 ml crucible (Morgan Crucible Company Plc, UK), dried in a forced air oven at 135 °C for 2 h prior to cooling in a dessicator, re-weighing and ashing overnight at 450 °C in a muffle furnace (Size 3, GAFSE 620, Gallenkamp, Loughborough, UK). The ash was transferred to a 500 ml beaker and 100 ml of 2N HCl added. The mixture was then boiled for 5 min on a hotplate (Cimarec, Barnstead International, Iowa, USA). The hot hydrolysate was filtered through a

Whatman No. 41 filter paper (Whatman plc, Maidstone, UK) and washed free of acid with hot distilled water (85 to 100 °C). The ash and filter paper were then transferred back into the crucible and ashed overnight at 450 °C in a muffle furnace (Size 3, GAFSE 620, Gallenkamp). The crucible and contents were cooled in a dessicator, weighed while containing ash and re-weighed immediately after emptying. Acid insoluble ash (g/kg) content was calculated as:

$$AIA\ (g/kg) = (Wf - We)/Ws \times 1000 \qquad \text{(Equation 4.2)}$$

Where Wf is the weight of the crucible and ash after boiling with acid, filtering and ashing; We is the weight of the crucible after ashing; Ws is the weight of the crucible and ash after initial oven drying. In addition, dried feed and faecal samples were analysed for ash, starch and NDF content outlined in Sections 2.1.2, 2.1.5 and 2.1.7 respectively.

4.2.6. Statistical Analysis

Milk yield parameters, live-weight, condition score, locomotion score, intake, blood parameters and apparent digestibilities were evaluated by analysis of variance as a randomised block design. Milk yield (kg/d) and component yield (kg/d) in the week prior to blocking were used as a co-variate where appropriate. Linear and quadratic effects of the rate of inclusion of WCW were also examined. All statistical analysis was conducted using Genstat version 6 (VSN Int. Ltd., Oxford, UK).

4.3. RESULTS

4.3.1. Crop production results

The average crop height measured on 14th July 2003 was 68.4 cm (SD 3.9 cm), and the mean stubble height post-harvesting was 19.0 cm (SD 1.9 cm). Average ensiled yield was 15.2 t DM/ha with a grain yield (adjusted to 15 % moisture) of 10.4 t/ha.

4.3.2. Forage and concentrate analysis

Chemical composition of the forages and dairy concentrate are presented in Table 4.2. The grass silage had a considerably lower DM than the WCW. Similarly, pH was highest for the WCW, whilst crude protein and NDF contents were similar (average 152 and 510 g/kg DM respectively). The WCW had a starch concentration of 340 g/kg DM whilst starch concentration was not determined for the grass silage. The concentrate had a ME value of 13.7 MJ/kg DM and a crude protein content of 239 g/kg DM whilst the content of sugars and starch were 14.0 g/kg DM and 161 g/kg DM respectively.

Table 4.2. Chemical composition (g/kg DM unless otherwise stated) for the grass silage, processed, urea-treated whole-crop wheat and dairy concentrate.

	Grass silage	Whole-crop wheat	Concentrate
Dry matter (g/kg)	323	836	893
Crude protein	141	163	239
Ash	83	38	95.1
Ammonia-N (g/kg total N)	32	nd	nd
pH	4.0	8.4	nd
D-value (g/kg DM)	688	759	798
ME (MJ/kg DM)	10.9	nd	13.7
Sugars	47	6.2	14.0
ND Fibre	515	505	290
AD Fibre	334	211	163
Oil	32	23	99.4
Starch	nd	340	161
Ethanol	11.5	nd	nd

nd – not determined

4.3.3. Ration and refusals particle size and distribution

The proportion of the diet above 19 mm decreased in a linear fashion with inclusion of WCW (P<.001) with grass silage alone (U-0) having the highest proportion (Table 4.3). There was a similar trend for particles between 19 and 8 mm. The proportion of the diet between 8 and 1.18 mm increased linearly (P<0.001) from 180 g/kg DM for grass silage alone (U-0) to 400 g/kg DM for U-75, whilst the proportion of diet below 1.18 mm was similar for diets U-0 and U-25 (average 30.8 g/kg DM), but increased for diets U-50 and U-75 (average 56.8 g/kg DM). Mean particle size (mm) was similar for U-0 and U-25 at 12.3 mm, whilst particle size for U-50 and U-75 were lower (P<0.001), averaging 8.05mm. Physically effective NDF (peNDF), defined as the proportion of the diet above 1.18mm multiplied by the NDF content of the ration (Mertens, 1997), was lower for grass silage alone (U-0) compared with those diets containing WCW, although the difference was not significant (P>0.05). There were no significant differences between forage treatments for the refusals at any of the size fractions.

Table 4.3. Mean particle size distribution, length of particles (mm) and physically effective neutral detergent fibre (peNDF) of rations and refusals containing grass silage (U-0), or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM along with sugarbeet pulp, rapeseed meal and Lactofeed

	U-0	U-25	U-50	U-75	s.e.d.	Treat.	Lin.	Quad.
Proportion of diet (g/kg DM)								
>19.0mm	362^{b}	332^{b}	213^{a}	161^{a}	39.80	0.001	0.001	0.685
19.0 - 8.0mm	425	430	397	381	25.01	0.219	0.060	0.567
8.0 - 1.18mm	180^{a}	210^{a}	334^{b}	400^{c}	27.31	0.001	0.001	0.368
<1.18mm	34.0^{ab}	27.6^{a}	55.9^{bc}	57.6^{c}	10.50	0.022	0.009	0.594
Particle size (mm)	12.6^{b}	12.1^{b}	8.59^{a}	7.51^{a}	0.975	0.001	0.001	0.648
PeNDF* (g/kg)	391	403	396	397	4.340	0.114	0.497	0.118
Proportion of refusals (g/kg DM)								
>19.0mm	493	500	460	597	134.7	0.766	0.532	0.506
19.0 - 8.0mm	384	349	318	271	83.70	0.594	0.188	0.923
8.0 - 1.18mm	111	139	199	126	45.90	0.292	0.488	0.146
<1.18mm	11.9	12.0	23.3	5.50	9.820	0.375	0.808	0.221
Particle size (mm)	16.6	16.1	14.0	18.5	3.200	0.589	0.727	0.297

^{abc} within a row, means with different superscript letter differ (P<0.05)
*peNDF defined as multiplication of ration NDF by proportion of DM above 1.18mm

4.3.4. Animal performance

Data was excluded from the analysis for four cows (treatments U-0, U-25 and two animals from treatment U-75) due to ill health unrelated to the dietary treatments. There was no significant difference across dietary treatments in the DM intake of straights (sugarbeet pulp, rapeseed meal or lactofeed) or dairy concentrates, with a mean total concentrate intake across all four dietary treatments of 9.73 kg DM per day (Table 4.4). There was however, a linear effect of treatment on total forage DM intake; intake increased from 9.99kg DM/d in cows offered grass silage alone (U-0) to 14.6 kg DM/d in cows offered U-75.

Table 4.4 - Average intake (kg DM per day) of concentrates and forage for cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75	s.e.d	Treat.	Lin.	Quad.
Dairy conc.	6.15	6.14	6.11	6.12	0.063	0.894	0.511	0.772
Straights*	3.50	3.57	3.66	3.68	0.230	0.856	0.402	0.863
Total conc.	9.66	9.71	9.77	9.80	0.253	0.944	0.550	0.932
Grass silage	9.99^{c}	9.12^{c}	6.98^{b}	3.97^{a}	0.600	0.001	0.001	0.008
WCW	0.00^{a}	3.13^{b}	6.66^{c}	10.66^{d}	0.377	0.001	0.001	0.049
Total forage	9.99^{a}	12.25^{b}	13.58^{bc}	14.63^{c}	0.777	0.001	0.001	0.236
Total intake	19.65^{a}	21.97^{b}	23.31^{bc}	24.43^{c}	1.015	0.001	0.001	0.414

^{ab} within a row, means with different superscript letter differ ($P<0.05$)
* Straights; sugarbeet pulp, rapeseed meal and lactofeed

This difference in forage DM intakes was apparent from week one of the experiment and continued throughout (Figure 4.1). A similar pattern was observed in total DM intake, which increased linearly with inclusion rate, being lowest in cows offered U-0 (19.65 kg DM/d) and highest in those offered U-75 (24.43 kg DM/d).

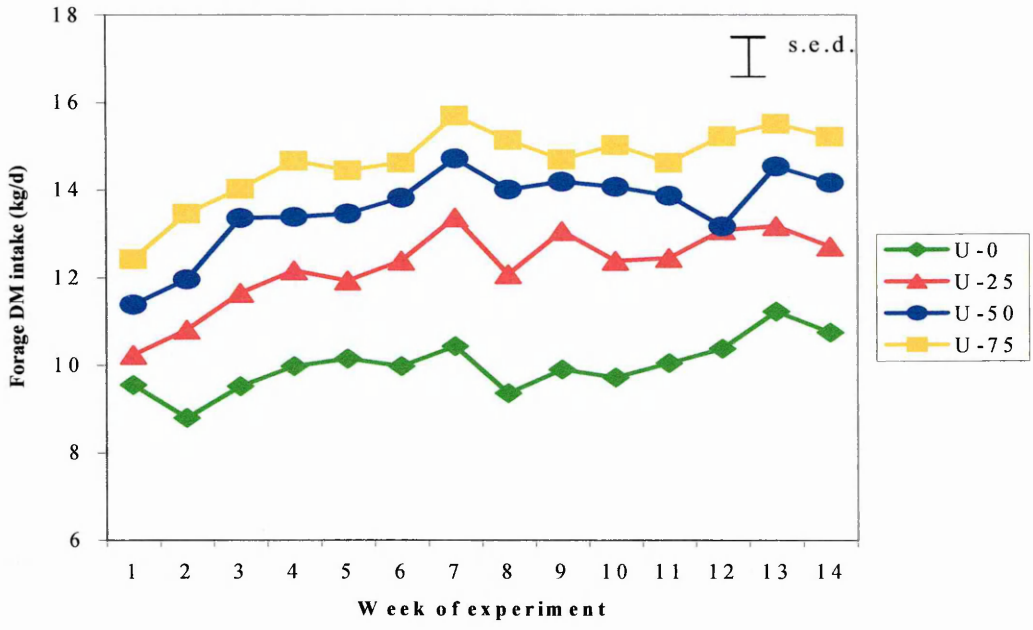

Figure 4.1. Average weekly forage DM intake of cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM.

Milk yield is shown in Table 4.5. Milk yield was 3.6 kg/d higher ($P<0.05$) in cows offered U-25 compared with those offered grass silage alone (U-0). There was no difference ($P>0.05$) in daily milk yield between cows offered U-25, U-50 or U-75, or between U-0, U-50 or U-75, although there was a trend ($P=0.09$) for a quadratic relationship between milk yield and WCW inclusion rate (Figure 4.2). Milk fat and protein content (g/kg) was similar throughout the experimental period (Figure 4.3 and 4.4), although mean fat content (g/kg) was numerically lowest in cows offered U-75 with a significant effect during week 6 ($P<0.05$) of the experiment. Milk protein content (g/kg) tended to increase with the level of inclusion of WCW, with a linear effect during weeks 3 and 5 of the experiment ($P<0.05$). Similarly, there was no effect ($P>0.05$) of treatment on fat or lactose yield (kg/d). Milk protein, however, was 0.11 kg/d higher ($P<0.05$) in cows offered U-25 compared with U-0, although there was no difference ($P>0.05$) between U-0, U-50 and U-75, or between U-25, U-50 or U-75. There was no effect ($P>0.05$) of treatment on live-weight change and

average live-weight was generally constant across the experimental period (Figure 4.5). By contrast body condition score increased over the experimental period (Figure 4.6). Average body condition score was not affected (P>0.05) by treatment; however during weeks 11-14 inclusive there was a linear effect of treatment on body condition score; condition score increased with level of inclusion of WCW. Locomotion score increased with time (Figure 4.7), but differences between treatments were not significant (P>0.05).

Table 4.5 - Average milk yield, milk composition, live-weight, condition score and locomotion score of cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75	s.e.d.	Treat.	Lin.	Quad
Milk yield (kg/d)	34.2^{a}	37.8^{b}	35.2^{ab}	35.8^{ab}	1.216	0.043	0.569	0.092
Fat (g/kg)	39.23	38.00	39.85	35.57	2.480	0.342	0.253	0.392
Protein (g/kg)	30.58	30.88	32.09	31.73	0.883	0.293	0.103	0.598
Lactose (g/kg)	46.46	46.12	46.20	46.59	0.538	0.799	0.779	0.345
Fat yield (kg/d)	1.37	1.41	1.37	1.26	0.090	0.429	0.218	0.270
Protein yield (kg/d)	1.06^{a}	1.17^{b}	1.11^{ab}	1.14^{b}	0.038	0.048	0.135	0.199
Lactose yield (kg/d)	1.59	1.74	1.63	1.67	0.070	0.167	0.559	0.257
LW change (kg/d)	0.08	0.13	0.21	0.36	0.151	0.292	0.067	0.614
Condition Score	2.33	2.31	2.43	2.48	0.116	0.430	0.137	0.696
Locomotion Score	2.36	2.32	2.28	2.42	0.287	0.965	0.894	0.655

^{ab} within a row, means with different superscript letter differ (P<0.05)

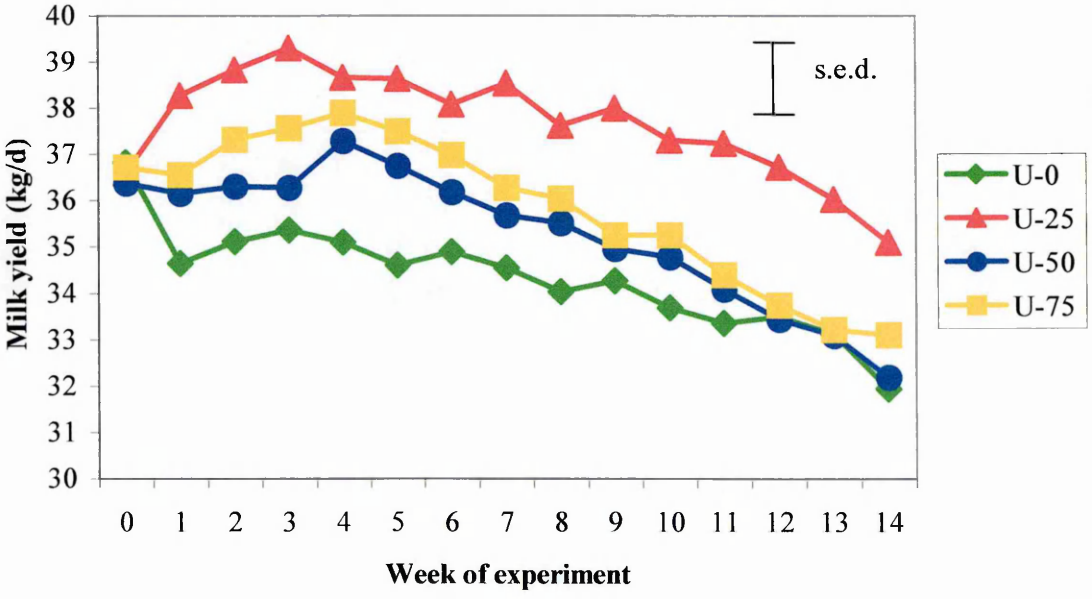

Figure 4.2. Average weekly milk yield of cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM.

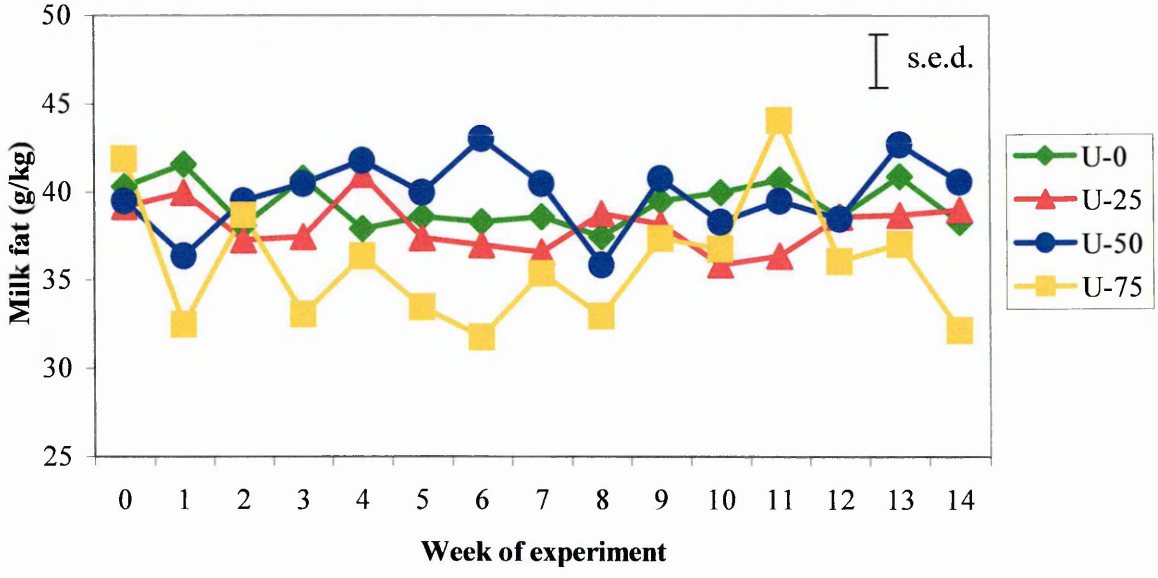

Figure 4.3. Average weekly milk fat concentration of cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM.

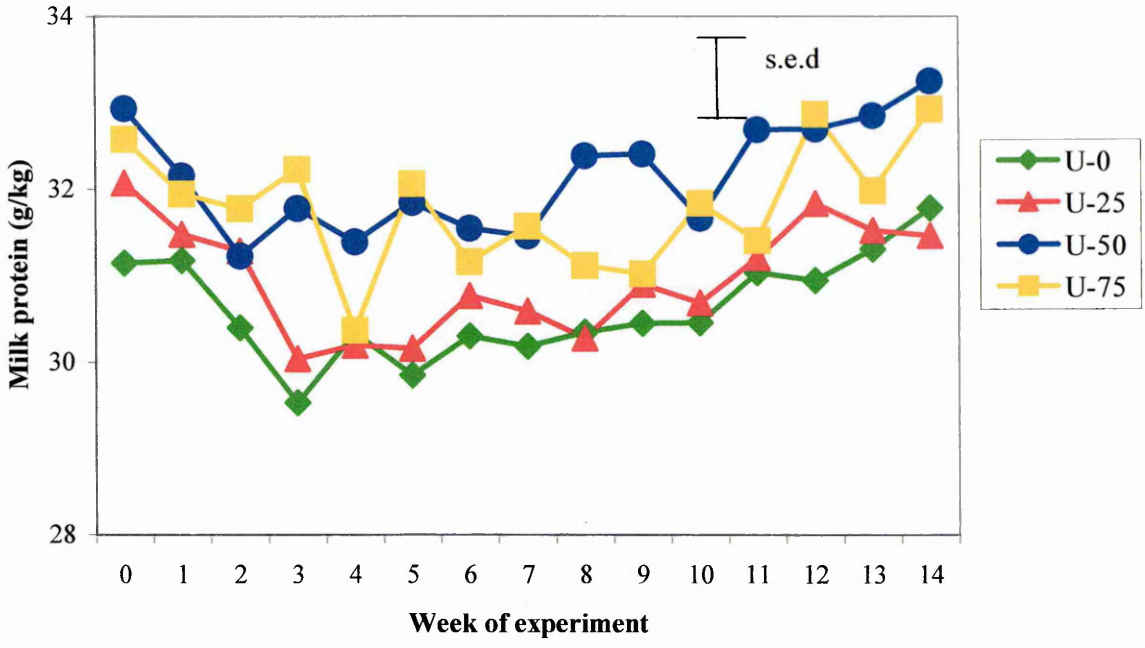

Figure 4.4. Average weekly milk protein concentration of cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM.

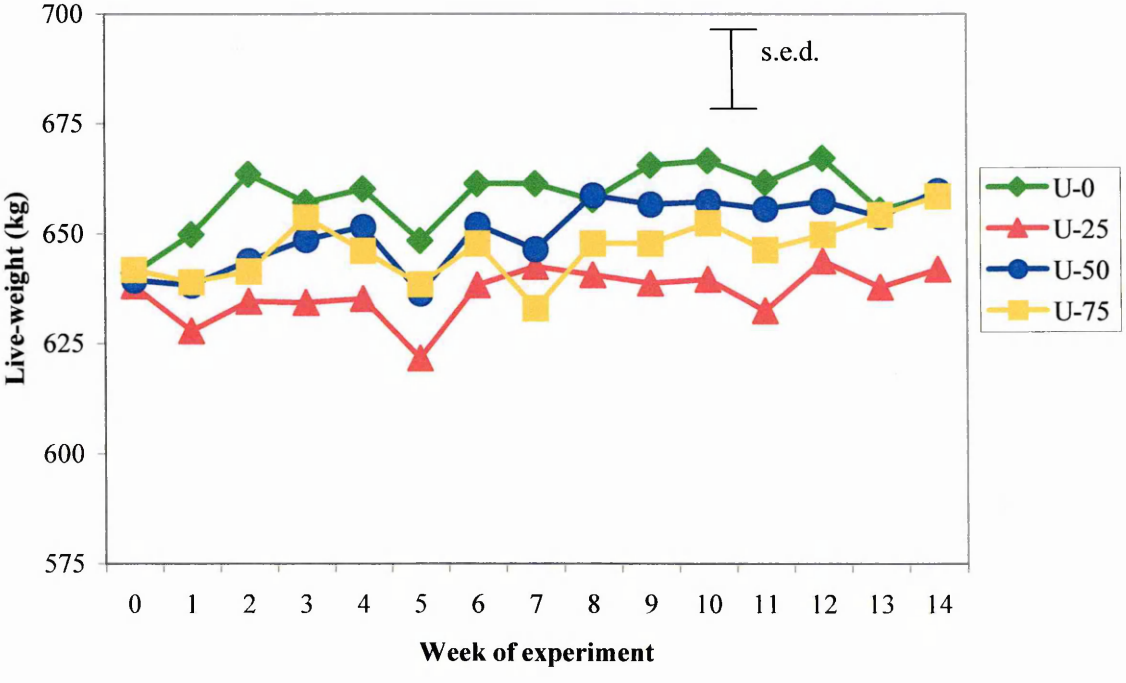

Figure 4.5. Average weekly live-weight of cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM.

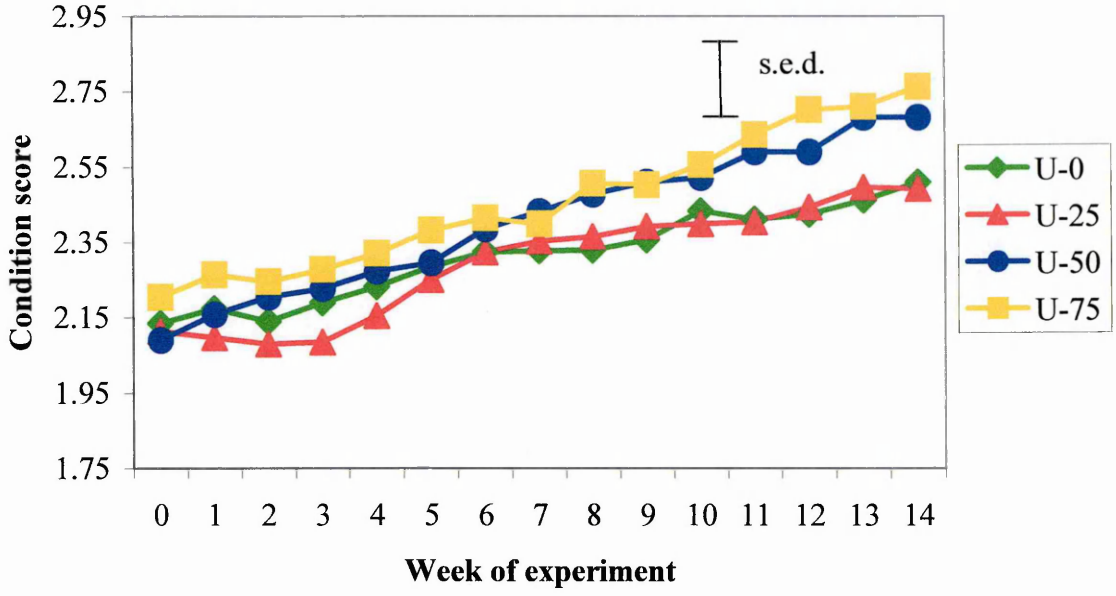

Figure 4.6. Average weekly condition score of cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM.

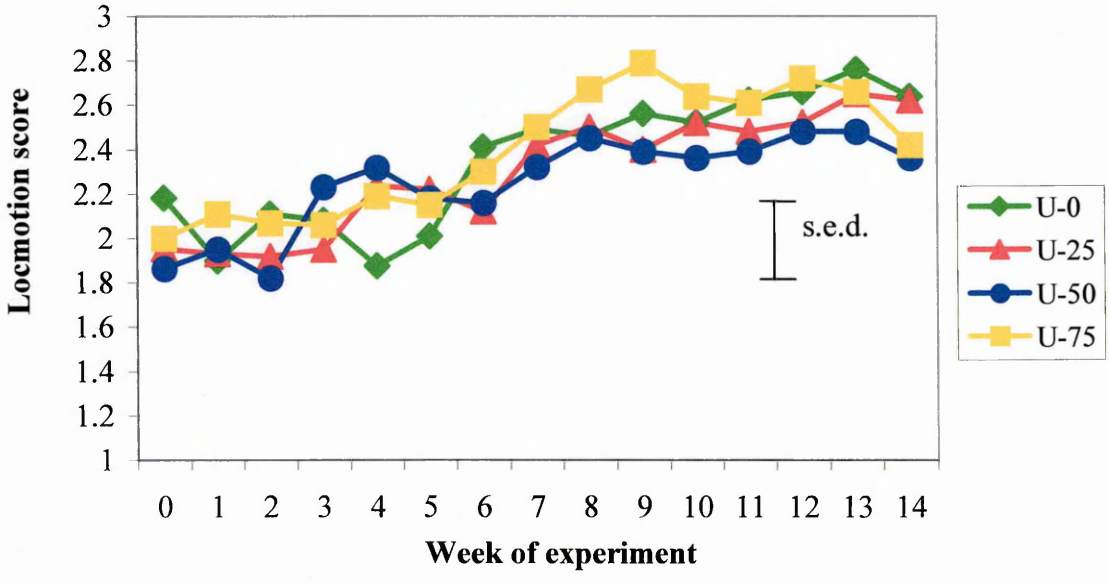

Figure 4.7. Average weekly locomotion score of cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM.

Nitrogen intake (Table 4.6) increased with inclusion of WCW (P<0.001), with cows fed U-75 having the highest N intake (g/d). Milk N output was similar across treatments, averaging 174.8 g/d; this resulted in a lower N efficiency (P<0.05), (defined as daily N output (g)/ daily dietary N intake (g)) for cows offered U-75 compared with U-0.

Table 4.6 - Estimated nitrogen (N) efficiency of dairy cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75	s.e.d.	Treat.	Lin.	Quad.
N intake (g/d)	622[a]	683[b]	720[bc]	745[bc]	29.30	0.002	0.001	0.395
Milk N output (g/d)	169	179	173	178	7.930	0.559	0.451	0.640
N efficiency	0.28[b]	0.26[ab]	0.24[a]	0.24[a]	0.015	0.032	0.004	0.547

[ab] within a row, means with different superscript letter differ (P<0.05)

4.3.5. Blood metabolite concentrations

There was a linear effect of treatment on mean plasma urea levels, which increased with level of inclusion of WCW (Table 4.7). Average plasma albumin levels tended to be highest in cows offered U-50 compared with U-75 (P=0.07), and there was a quadratic effect of level of inclusion of WCW on plasma albumin levels (P<0.05). Similarly, average plasma BHB levels tended to be higher (P=0.06) in animals offered U-50 than those offered U-75, and there was evidence of a quadratic response (P=0.07). Plasma insulin levels were numerically higher for those animals offered U-75 with this being significant (P<0.05) at week 13 of the experiment (Figure 4.8).

Table 4.7 - Average plasma concentrations of urea, glucose, β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA; mmol/l), total protein, albumin (g/l) and insulin (ml/l) for cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U - 25	U - 50	U-75	s.e.d.	Treat.	Lin.	Quad.
Urea	4.46[a]	5.31[b]	5.59[b]	6.04[b]	0.385	0.008	0.001	0.466
Glucose	3.56	3.35	3.51	3.43	0.123	0.404	0.575	0.494
BHB	0.66	0.65	0.81	0.58	0.079	0.062	0.767	0.071
NEFA	0.14	0.15	0.12	0.10	0.030	0.441	0.168	0.622
Tot Protein	81.7	81.4	82.0	83.4	2.446	0.840	0.456	0.629
Albumin	35.5	35.7	36.2	34.1	0.714	0.070	0.130	0.047

Insulin	14.6	15.3	15.9	16.1	3.550	0.974	0.655	0.919

[ab] within a row, means with different superscript letter differ (P<0.05)

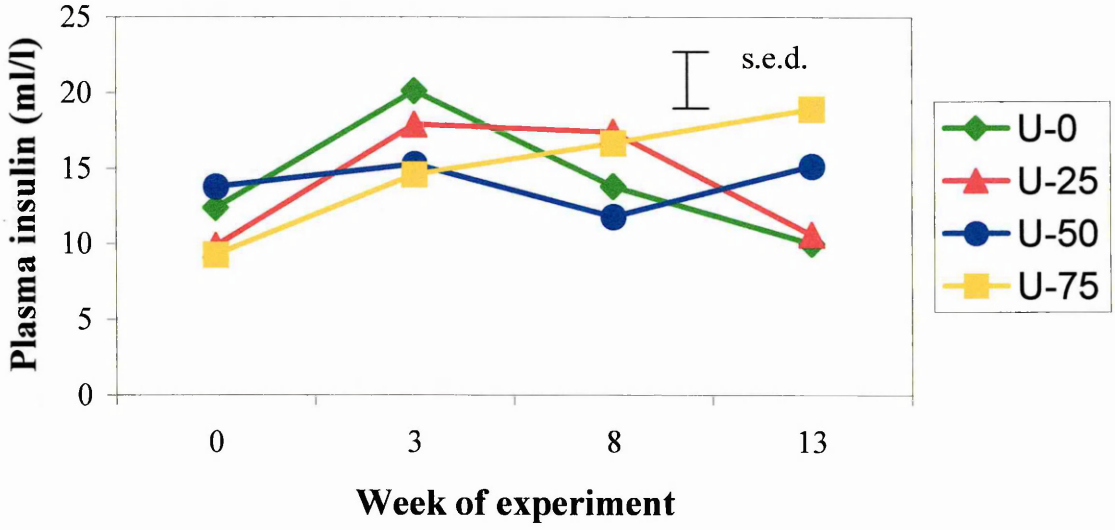

Figure 4.8. Average plasma insulin concentration of cows offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM.

4.3.6. Diet digestibility

Organic matter (OM) intake and faecal output increased with rate of inclusion of WCW, whilst digestibility (kg/kg) decreased (P<0.001) from 0.79 for the grass silage (U-0) to 0.70 for U-75 (Table 4.8). By contrast, intake of digestible OM (kg/d) was not different (P>0.05) between treatments, averaging 15.8 kg/d. Intake and faecal output of NDF also increased with inclusion rate of WCW (P=0.185 and P<0.001 respectively), whilst digestibility decreased (P<0.001), with the intake of digestible fibre being similar across all four treatments at 5.92 kg/d. Similar to OM and NDF, starch intake and faecal output increased with rate of inclusion of WCW, as a consequence the intake of digestible starch (kg/d) increased linearly (P<0.001) with rate of inclusion of WCW whilst starch digestibility decreased linearly (P<0.005). If it is assumed that the digestibility of starch in the concentrates was the same as that measured in U-0 (0.97 kg/kg) and constant across treatments, the digestibility of starch in the WCW can be calculated as 0.97, 0.95, and 0.94

(s.e.d. 0.155) kg/kg for U-25, U-50 and U-75 respectively, and was not different between treatments (P>0.05).

Table 4.8 - Intake, faecal output, digestible (kg/d) and apparent digestibility (kg/kg) of organic matter, fibre and starch in cows offered grass silage (U-0) or processed, urea-treated whole crop wheat at 0.25 (U-25), 0.5 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75	s.e.d.	Treat.	Lin.	Quad.
Organic matter								
Intake	18.5^{a}	21.4^{ab}	22.3^{ab}	23.6^{b}	1.806	0.083	0.016	0.546
Faecal output	4.00^{a}	5.00^{ab}	6.20^{bc}	7.20^{c}	0.521	0.001	0.001	0.993
Digestible	14.5	16.4	16.1	16.4	1.485	0.541	0.266	0.462
Digestibility	0.79^{b}	0.76^{b}	0.72^{a}	0.70^{a}	0.017	0.001	0.001	0.956
NDF								
Intake	7.91	9.27	9.51	9.79	0.850	0.185	0.051	0.394
Faecal output	2.25^{a}	2.80^{ab}	3.56^{bc}	4.16^{c}	0.299	0.001	0.001	0.915
Digestible	5.66	6.46	5.95	5.63	0.712	0.634	0.794	0.289
Digestibility	0.72^{c}	0.69^{c}	0.63^{b}	0.58^{a}	0.028	0.001	0.001	0.512
Starch								
Intake	1.16^{a}	2.30^{b}	3.51^{c}	5.00^{d}	0.206	0.001	0.001	0.245
Faecal output	0.03^{a}	0.07^{ab}	0.14^{b}	0.27^{c}	0.036	0.001	0.001	0.123
Digestible	1.13^{a}	2.22^{b}	3.37^{c}	4.74^{d}	0.198	0.001	0.001	0.362
Digestibility	0.97^{b}	0.97^{b}	0.96^{ab}	0.95^{a}	0.009	0.037	0.005	0.688

^{ab} within a row, means with different superscript letter differ (P<0.05)

4.3.7. Energy balance

The estimated energy balances of the experiment are shown in Table 4.10. The ME of the diet was calculated according to the equation of AFRC (1993);

$$ME\ (MJ/kg\ DM) = 0.0157 \times [DOMD] \qquad \text{(Equation 4.3)}$$

Estimated ME requirements were calculated according to AFRC (1993) and Thomas (2004).

Table 4.9. Estimated intake of metabolisable energy (ME) and ME requirements for recorded production (MJ/d) for animals offered grass silage (U-0) or processed, urea-treated whole crop wheat at 0.25 (U-25), 0.5 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75
ME intake*	235	245	246	247
ME requirement**	249	265	265	273
Difference	14.1	16.9	19.2	23.2
ME requirement***	230	264	258	261
Difference	15.1	15.8	12.1	12.3

* ME intake based on DOMD values (ME = 0.0157[DOMD]

** ME requirements calculated from AFRC (1993)

*** ME requirements calculated from Thomas (2004)

4.3.8. Calculation of ME values for whole-crop wheat

If certain assumptions are made, ME values (MJ/kg DM) for grass silage and WCW can be calculated from the estimated energy balances. The ME value of the dairy concentrate was calculated from AFRC (1993) to be 13.7 MJ/kg DM. The ME values for the straights (rapeseed meal, sugarbeet pulp and lactose) were taken from MAFF (1992). The ME value for grass silage was calculated from treatment U-0 by subtraction of the ME values of the concentrates and straights from the ME intake. The resulting value was 10 MJ/kg DM, a value slightly lower than that recorded by NIRS and stated in Section 4.3.2. If the ME values for the concentrate, straights and grass silage values are assumed to be constant for all treatments, the ME values for U-25, U-50 and U-75 can be calculated as 8.09, 7.14 and 7.32 MJ/kg DM for U-25, U-50 and U-75 respectively, with an overall mean value for WCW of 7.52 MJ/kg DM.

Similarly, it is possible to calculate the ME value of grass silage and WCW by difference from the estimated ME requirements (AFRC, 1993, Thomas, 2004). The ME values for grass silage are 11.3 and 11.5 MJ/kg DM for AFRC (1993) and Thomas (2004) respectively. These values are slightly higher than the previously calculated ME of 10 MJ/kg DM and 10.9 MJ/kg DM stated in the results section. The values for WCW ME can be calculated as; 10.21, 8.79 and 8.85 MJ/kg DM and 9.51, 7.56 and 7.33 MJ/kg DM for U-25, U-50 and U-75 respectively, calculated from AFRC (1993) and Thomas (2004). Overall mean values for WCW are 9.29 and 8.13 MJ/kg DM as calculated from AFRC (1993) and Thomas (2004).

4.4. DISCUSSION

4.4.1. Forage and concentrate analysis

The DM content of the WCW used in the current work was similar to that used by Sinclair *et al.* (2005), although both these DM values were higher than the range of 653 – 763 g DM/kg reported in other work when processed, urea-treated WCW has been fed to dairy cows (Jackson *et al.*, 2004, Murphy *et al.*, 2004, Patterson and Kilpatrick, 2005). This higher maturity was not reflected in the starch content of the WCW, which at 340 g/kg DM was similar to that used by Murphy *et al.* (2004) and Sinclair *et al.* (2005), but lower than the short straw, processed WCW or processed, urea-treated WCW used by Jackson *et al.* (2004) and Patterson and Kilpatrick (2005) respectively. The WCW was well preserved, as reflected by the high pH of 8.4, which has been suggested to illustrate successful promotion of an alkaline state, inhibiting any proliferation of fungi and saccharolytic clostridia which often cause aerobic spoilage and DM losses (Adogla-Bessa *et al.*, 1999). The grass silage used in the experiment had a comparatively high DM at 323 g/kg compared with previous work (Jackson *et al.*, 2004, Murphy *et al.*, 2004), whilst the low pH and ammonia-N values indicate a well-preserved grass silage.

4.4.2. Ration and refusals particle size and distribution

The particle size and distribution of a diet plays an important role in digestion and animal performance (Heinrichs *et al.*, 1999). The PSPS provides a quick and practical method for routine use on farm to evaluate the particle size of forages and total mixed rations (TMR; Lammers *et al.*, 1996). Previously, Lammers *et al.* (1996) recommended that of a TMR, 60 - 100 g/kg DM should be >19 mm, 300 - 500 g/kg DM between 8 - 19 mm, 400 - 600 g/kg DM between 8 - 1.18mm and finally approximately 200 g/kg DM <1.18 mm. In the current experiment DM was not determined for the forage retained on each sieve but calculated

according to the individual DM components. The proportion of diet above 19 mm decreased significantly with increasing inclusion of WCW and was higher than the recommended values (Lammers *et al.*, 1996). As a result, the average particle size (mm) decreased with WCW inclusion. However the proportion of diet between 19 and 8 mm was on average 408 g/kg for all dietary treatments, a value within the range recommended by Lammers *et al.* (1996). By contrast, the proportion of diet between 8 and 1.18 mm and <1.18 mm increased with increasing WCW inclusion. Previous work has suggested that diets containing a higher proportion of smaller particles enter the rumen at a smaller size and therefore leave the rumen at a faster rate. The result is an increase in rumen particulate turnover rate, allowing for increased DM intake (Jaster and Murphy, 1983). It is possible that in the current experiment the increased proportion of smaller particles for the diet containing 0.75 WCW may have increased rumen particulate turnover, thus resulting in an increased DM intake. Furthermore, reduced forage particle size decreases the time spent chewing and causes a trend towards decreased ruminal pH (Woodford and Murphy, 1988) which may affect fibre digestibility (Ørskov, 1976). In the present experiment increasing WCW inclusion resulted in a higher proportion of smaller particles in the diet along with decreased fibre digestibility.

It is well recognised that dairy cows require sufficient fibre, of adequate particle length, to maintain proper rumen function (Yang *et al.*, 2001). The variability of size distribution of fibre particles and thus retention time of fibre in the rumen means the effectiveness of the fibre within a forage is quite variable (Allen, 1997). The PSPS may be used to estimate the physically effective NDF (peNDF) in a ration, otherwise defined by Mertens (1997) as the dietary fibre which effectively stimulates rumination and salivation. Poppi *et al.* (1985) reported that particles retained on a sieve measuring 1.18 mm pass out of the rumen slower than those that are not retained and therefore peNDF can be calculated by multiplication of

ration NDF by the proportion of DM above 1.18 mm. Mertens (1997) suggested that as the particle size of rations increase the peNDF content would also increase, resulting in elevated total chewing activity, salivary buffer secretion and ruminal pH. In the current experiment values were similar for diets containing WCW (U-25, U-50 and U-75), average 397 g/kg DM and higher than diets containing grass silage alone (U-0, 159 g/kg DM). As a result peNDF intakes (kg/d) were 5.38, 6.51, 6.74 and 7.49 for U-0, U-25, U-50 and U-75 respectively. Mertens (2000) recommended 197 g/kg peNDF was needed to maintain milk fat at 34 g/kg and 223 g/kg peNDF was needed to maintain an average ruminal pH of 6.0. Values in the current experiment were higher than those recommended by Mertens (2000), and although milk fat (g/kg) decreased with increasing inclusion of WCW, values were not below 34 g/kg, pH was not measured in the current experiment. Similarly, Yang *et al.* (2001) reported a lack of direct effect of peNDF on pH and therefore suggested that measuring physical characteristics alone cannot be used to predict ruminal acidosis and rumen fermentability of starch may have a larger effect.

The proportion of diet above 19 mm was higher for all dietary treatments than the recommended values of Lammers *et al.* (1996). Particles that are too long may enable animals to sort a ration (Heinrichs and Kononoff, undated), and Beauchemin and Yang (2005) reported that animals offered a ration based on corn silage sorted in favour of long feed particles. The authors therefore concluded that dairy cows may intentionally select long feed particles to meet their need for peNDF, especially when ruminal pH is low (Beauchemin and Yang, 2005). By contrast, Leonardi and Armentano (2003) reported that when offered alfalfa hay dairy cows consistently sorted against longer particles in favour of finer particles. Similarly, in the current experiment analysis of diet refusals demonstrated a higher proportion of the diet above 19 mm and between 8 - 1.18 mm, whilst the proportion of very small particles (<1.18 mm) was lower in the refusals

compared with the TMR initially offered. Furthermore, average particle size (mm) was higher for the refusals, which may suggest a certain amount of diet selection had occurred.

4.4.3. Animal performance

The partial replacement of grass silage with either fermented or unprocessed urea-treated WCW in dairy rations has been generally found to increase DM intakes (Phipps *et al.*, 1995, Leaver and Hill, 1995, Hameleers, 1998), this has been attributed in urea-treated WCW to a lower digestibility, particularly of the starch fraction (Sutton *et al.*, 1998, Abdalla *et al.*, 1999). The use of a forage processor allows the grains of WCW to be ground prior to ensiling, this has been reported to increase digestibility and decrease intakes compared with unprocessed material (Jackson *et al.*, 2004). In the present study, forage DM intakes were within the range reported for processed, urea-treated WCW (Jackson *et al.*, 2004, Murphy *et al.*, 2004, Patterson and Kilpatrick, 2005, Sinclair *et al.*, 2005). Total forage DM intakes increased linearly with inclusion rate. The inclusion of WCW at 0.25 of the forage DM resulted in a higher intake than for those animals offered grass silage alone, although intakes were lower than for those animals offered either U-50 or U-75. Similarly, Sutton *et al.* (1997) and Abdalla *et al.* (1999) reported that increasing the proportion of unprocessed urea-treated WCW in the diet from 0.33 to 0.67 significantly increased DM intakes in dairy cows. In agreement with Heinrichs *et al.* (1999), the decrease in average particle size (mm) and fibre digestibility with inclusion of WCW in the current experiment suggests that there was a higher rumen particulate turnover rate, allowing for increased DM intake but decreased fibre digestibility.

Increasing the proportion of unprocessed urea-treated WCW in grass silage rations significantly increased DM intakes whilst only raising milk yield slightly (Sutton *et al.*, 1997, Abdalla *et al.*, 1999). Furthermore, Sutton *et al.* (1997) reported apparent

digestibility of starch in unprocessed, urea-treated WCW to be only 0.74. The authors therefore concluded that the milk yield response for dairy cows offered unprocessed, urea-treated WCW was restricted by the low digestibility of starch in the grains. Processing of WCW prior to harvest increased apparent digestibility of starch to 0.97 (Jackson *et al.*, 2004). In the current experiment it is likely that processing prior to harvest increased ruminal availability of starch. Increased milk yield for animals offered U-25 compared with those offered grass silage alone may be due to improved synchronisation between energy in the WCW and rapidly degradable N in the grass silage. Previous work has demonstrated animals fed synchronised diets had improved microbial growth and animal performance (Sinclair *et al.*, 1993, Richardson *et al.*, 2003). Milk yields for animals offered U-50 and U-75 were lower than for those offered U-25, this may be due to a rapid release of starch, lowering ruminal pH, resulting in a sub-clinical acidosis reducing microbial growth (Ørskov, 1986) and depressing milk yield.

Phipps *et al.* (1992) and Sutton *et al.* (1997) reported lower milk fat levels for animals offered a higher inclusion of unprocessed urea-treated WCW. Similarly, Jackson *et al.* (2004) reported decreased milk fat (g/kg) and increased condition score for animals offered processed WCW included at 0.7 of the forage DM. Animals fed 0.75 WCW (U-75) in the current study also had the lowest milk fat content (g/kg) and the highest condition score. Increased dietary starch concentration can be associated with milk fat depression due to a shift in VFA production in the rumen from acetate towards propionate and the elevation in peripheral insulin concentration, which depresses lipolysis and promotes lipogenesis (Reynolds *et al.*, 1997). More recently, it has been suggested that milk fat depression as a result of feeding high grain, low roughage diets is associated with changes in rumen biohydrogenation and the formation of inhibitors of milk fat synthesis (Griinari and Bauman, 2003). One such inhibitor is *trans*-10, *cis*-12 conjugated linoleic acid (CLA)

which has been shown to increase with diets associated with milk fat depression (Bauman *et al.*, 2001). Milk fatty acid profiles were not determined in the current study, although it is possible that this may have accounted for the differences in milk fat content reported here.

Milk protein content (g/kg) tended to increase with inclusion of WCW, confirming the work of Sutton *et al.* (2002) who reported that the higher N intakes associated with urea-treated WCW can increase milk protein levels. Furthermore, Reynolds *et al.* (1997) stated that the increased ME intake from increased carbohydrate increases microbial protein synthesis in the rumen and metabolisable protein supply to the small intestine if there are sufficient quantities of rumen degradable protein and non-protein N available. In the experiment reported here, milk protein yield (kg/d) was higher for animals fed diets containing WCW (U-25, U-50 and U-75) compared with those fed grass silage alone.

Intake of N increased linearly with inclusion of WCW, with animals offered U-75 having the highest N intake at 745 g/d. As there were no significant differences between dietary treatments in milk N output (g/d) this resulted in a reduced efficiency of N use (kg milk N output per kg N intake) for milk production with inclusion of WCW. This is in agreement with the work of Hameleers (1998) who suggested that the reduced N efficiency for milk production in dairy cows was a result of the increased N content of the urea-treated WCW. Overall, the N efficiency values calculated for this experiment were similar to those reported by Jackson *et al.* (2004) but lower than those calculated by Hameleers (1998) who fed unprocessed, urea-treated WCW.

Although ruminal pH was not measured in the current study, it has been suggested that a sub-clinical acidosis as a result of a rapid release of starch in the rumen may be a

predisposing cause of lameness in dairy cows (Blowey, 1993). Bacterial endotoxins may enter the circulatory system and produce an allergic reaction in the capillary cells of the hoof corium. This can result in an insufficient supply of sulphur amino acids reaching the keratin-producing cells of the epidermis, thus leading to lower levels of disulphide bonding in the keratin tissue and the production of structurally unstable horn (Dougherty *et al.*, 1975). Kelly and Leaver (1990) reported increased lameness for animals offered a starch supplement in the form of barley compared to those offered fibre as sugar-beet pulp. Furthermore, Bazeley and Pinsent (1984) reported high dietary protein and/or high levels of free ammonia in silage resulted in a higher incidence of laminitis. Similarly, Manson and Leaver (1988) reported a high protein diet significantly increased the number of observations of clinical lameness and their duration. Processed, urea-treated WCW is high in starch and ammonia, therefore locomotion score, as a measure of lameness was undertaken in the current experiment according to the method of Manson and Leaver (1988). There was no significant difference between dietary treatments although over the entire experimental period locomotion score increased from an average 2.0 to 2.5 for all dietary treatments. This demonstrates a moderate to high problem with lameness (Leaver and Webster (1982).

4.4.4. Blood analysis

All values for blood metabolites measured in the current experiment were within the normal physiological range of the dairy cow during early lactation (Ward *et al.*, 1995). Protein degradation in the rumen along with the intake of dietary urea results in increased rumen ammonia which is absorbed across the rumen wall and transported to the liver where it is converted to urea (Chesworth *et al.*, 1998). Urea is then recycled back to the rumen, either directly by the blood or via the saliva (Chesworth *et al.*, 1998). In the current experiment plasma urea levels increased with inclusion of WCW, which may reflect poor

microbial capture of ammonia in the rumen or the inability of the liver to convert ammonia into urea. O'Kiely and Moloney (1995) reported increased plasma urea levels for animals offered urea-treated silages, indicating an inefficient use of dietary N. The authors suggested a need for a rapidly fermentable carbohydrate supplement to facilitate the incorporation of rapidly released ammonia into microbial protein in the rumen (O'Kiely and Moloney, 1995). This was demonstrated by Sinclair *et al.* (2003) who reported reduced plasma urea levels when fermented WCW was supplemented with starch, an effect attributed to a greater capture of rumen degradable protein in the rumen. In the current experiment however, despite increased starch intake for animals offered 0.75 WCW (U-75), plasma urea levels were still high reflecting decreased rumen capture of protein. This may have been as a result of the increased dietary intake of urea or an inability of the liver to convert ammonia. Increased starch to fibre ratios are usually associated with a shift in VFA production in the rumen away from acetate towards propionate (Reynolds *et al.*, 1997) as demonstrated by Overton *et al.* (1995) who reported increased propionate concentrations for those animals offered barley starch. Although rumen propionate levels were not measured in the current study, previous work has suggested that increased rumen propionate may reduce hepatic metabolism of ammonia (Choung and Chamberlain, 1995). Furthermore, Bines and Hart (1984) reported propionate to be a potent stimulator of insulin secretion in the cow. In the current experiment, although not significant, plasma insulin concentrations were numerically higher for those animals fed 0.75 WCW (U-75).

4.4.5. Digestibility

The digestibility of the OM and NDF fractions decreased significantly with increasing inclusion of WCW. This suggests that WCW was less digestible than the grass silage. Alternatively, the rapid release of starch from the WCW may have depressed rumen pH, inhibiting cellulolysis due to a gradual destruction of the microflora associated with

roughage degradation (Mould and Ørskov, 1983). Work by Jackson *et al.* (2004) clearly demonstrated the increased starch digestibility of processed urea-treated WCW compared with unprocessed urea-treated WCW. The starch digestibility values calculated for the current experiment were similar to those reported by Jackson *et al.* (2004) and higher than those given for unprocessed WCW by Sutton *et al.* (1997). Overall, starch digestibility decreased linearly with level of inclusion of WCW, with digestibility being significantly lower for U-75.

4.4.6. Energy balance

Leaver and Hill (1992) calculated energy balances on the basis of measured food intake, milk production and live-weight change of cows and diet ME values derived from digestibility measurements. This approach resulted in up to 84 MJ ME per day unaccounted for in cows consuming diets based on urea-treated WCW at 0.56 or 1.00 of the forage DM. More recent work (Leaver and Hill, 1995) in which urea-treated WCW replaced only 0.33 or 0.40 of grass silage DM resulted in no major imbalance between calculated ME intake and outputs. This was further confirmed by Sutton *et al.* (1998) using energy balances of lactating dairy cows housed in respiration chambers. Metabolisable energy balances indicate that all diets showed a reasonable relationship between estimated ME intake and estimated ME requirements, with an average 18.35 MJ/d being unaccounted for using the AFRC (1993) calculation, whilst 13.83 MJ/d being unaccounted for using Thomas (2004).

4.4.7. Calculation of ME values for whole-crop wheat

Accurate ME measurements are important for the accurate formulation of diets containing WCW (Adesogan *et al.*, 1999). Sutton *et al.* (1998) undertook a full energy and N balance

with dairy cows to determine the ME content of urea-treated WCW. More commonly WCW ME can be calculated from diet digestibility studies or E balance studies.

Overall mean ME values for WCW calculated using the different methods ranged from 7.14 to 10.21 MJ/kg DM. These values are similar to the 8 MJ/kg DM stated by Sutton *et al.* (1998) for unprocessed urea-treated WCW but still lower than those values ranging from 9.6 to 11.6 MJ/kg DM calculated by Leaver and Hill (1992, 1995). This variability in ME values clearly demonstrates the difficulty in calculating a reliable ME value for WCW.

4.5. CONCLUSION

Inclusion of processed, urea-treated WCW at 0.25 of the forage DM results in a higher DM intake along with a higher milk yield and milk protein yield than feeding grass silage alone. Inclusion of WCW at higher levels than 0.25 of the forage DM provided little benefit to milk yield and more energy was partitioned towards body fat compared with grass silage alone.

Chapter 5

THE EFFECT OF INCLUSION RATE OF PROCESSED, UREA-TREATED WHOLE-CROP WHEAT ON *IN VITRO* FERMENTATION, MICROBIAL GROWTH AND RUMINAL DIGESTIBILITY

5.1. INTRODUCTION

The recent development of a forage processor enables the cereal grain in WCW to be ground prior to ensiling which has been shown to significantly improve the whole tract digestibility of the starch component (Jackson *et al.*, 2004). Results from the experiment to determine the effect of rate of inclusion of processed, urea-treated WCW on intake and animal performance in dairy cows (Chapter 4) demonstrated that the inclusion of processed, urea-treated WCW at 0.25 of the forage DM resulted in higher DM intakes along with a higher milk and milk protein yield compared to feeding grass silage alone. Inclusion of WCW at levels above 0.25 of the forage DM provided little benefit in terms of milk yield. Reasons for the highest milk yield occurring at inclusion rate of processed, urea-treated WCW of 0.25 of the forage DM are unclear, but it is possible that processing prior to harvest increased ruminal availability of starch for microbial growth. Increasing the rate of starch release may improve synchronisation between the release of energy and the rapidly degradable nitrogen in grass silage, as improving the degree of synchrony has been shown to improve microbial growth and animal performance in some (Sinclair *et al.*, 1993; Witt *et al.*, 1999) but not all (Witt *et al.*, 2000; Richardson *et al.*, 2003) studies. At higher inclusion rates, processed WCW may result in a very rapid release of starch which may lower ruminal pH, resulting in sub-clinical acidosis and reduced microbial growth efficiency (Ørskov, 1986; Mould and Ørskov, 1983). The utilisation of continuous culture fermentors to simulate the ruminal environment enables factors affecting the microbial

ecology and digestion of nutrients to be studied (Stern *et al.*, 1997). They provide a more rapid, economic and repeatable alternative to *in vivo* studies (Owens and Goetsch, 1988). Continuous culture systems replicate most of the functions within the rumen, allowing a continuous buffer and feed input along with outflow of a homogenous mixture of solids, liquids, fermentation end products and microorganisms (Stewart *et al.*, 1961). Despite the advantages of continuous culture systems as a means to investigate the effect of the inclusion of WCW on rumen metabolism there has been few studies conducted in this area.

The objective of the current experiment was to determine the effect of including processed, urea-treated WCW at four inclusion rates on the *in vitro* ruminal fermentation, microbial growth and ruminal digestibility using a continuous culture system.

5.2. MATERIALS AND METHODS

5.2.1. Forage production and storage

Forages (grass silage and processed, urea-treated WCW) were the same as those described in Chapter 4. Sufficient forage was removed from the grass silage and urea-treated WCW clamps and at the same time sufficient dairy concentrate, rapeseed meal, sugarbeet pulp and Lactofeed 70 (Volac International Ltd, Royston, Herts, UK) were taken and stored at -20 °C prior to freeze drying.

5.2.2. Equipment

The experiment used four glass *in vitro* fermentor vessels (Plate 5.1). The vessels were double layered allowing water from a water bath (Ecoline RE106, Lauda, Germany) to be continuously pumped around each vessel to maintain contents at 39 °C. Each vessel had a 20 mm internal diameter overflow port resulting in a liquid volume of approximately 1.18 l before overflow into a collection container, positioned within ice to maintain a temperature of 3 °C. In addition to the overflow port, the cover of each vessel had one large port (25 mm internal diameter) for feed entry and 9 smaller ports (2 x 12 mm and 7 x 6 mm internal diameter), four of which were used for continuous infusion of artificial saliva by a peristaltic pump (Watson-Marlow Limited, Cornwall, UK), pH monitoring, CO_2 addition, and fermentation media sampling. A motor controlled stirrer (Crouzet Ltd, Hampshire, UK) attached to the cover of each vessel enabled intermittent mixing of the vessel contents and was positioned to act as a foam breaker.

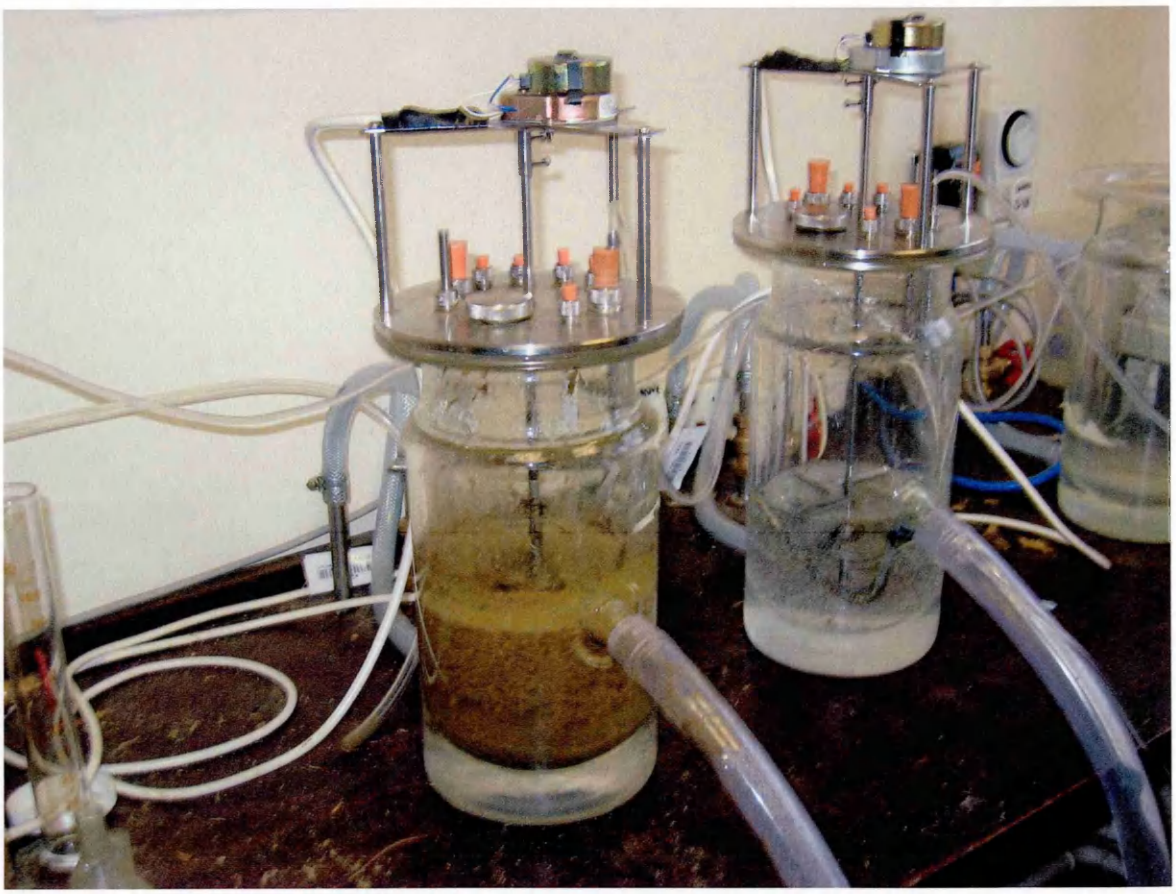

Plate 5.1. Continuous culture *in vitro* fermentor vessel.

5.2.3. Inoculum

Four wether sheep fitted with permanent rumen cannulae (39 mm) were used as donors for the rumen fluid. The sheep were group housed on chopped straw with continuous access to water. They were fed at 1.1 times energy requirements (AFRC, 1993) of a diet consisting of 50: 50, processed, urea-treated WCW: grass silage on a DM basis. The diet was fed twice daily at 08.00 h and 16.00 h. The sheep were fed the experimental diet for two weeks prior to the first inoculum collection and were not bedded up two days prior to collecting rumen fluid. Rumen fluid (approximately 5 l in total) was removed by suction 4 h after the morning feed (at approximately 12.00 h) from at least three of the experimental sheep and carried to the vessels in a pre-heated vacuum flask under CO_2. The rumen fluid was pooled and strained through four layers of muslin cloth at 39 °C under CO_2. Prior to inoculation the vessels were flushed with CO_2 and filled with 510 ml artificial saliva (McDougall, 1948; 9.8 g/l $NaHCO_3$, 9.3 g/l $Na_2HPO_4.12H_2O$, 0.47 g/l NaCl, 0.57 g/l KCl, 0.04 g/l

$CaCl_2$ anhyd., 0.06 g/l $MgCl_2$ anhyd.). Vessels were inoculated with approximately 670 ml rumen fluid within 15 mins of collection. Fresh rumen fluid was used to charge the vessels at the beginning of each experimental period. Immediately after filling the vessels 10 g of the experimental diet was added to each vessel via the vessel cover.

5.2.4. Diet and feeding

Each vessel was fed 30 g DM/d of a total mixed ration consisting of 8.25 g/d dairy concentrate (Table 5.1), 2.4 g/d rapeseed meal 1.8 g/d sugarbeet pulp 0.6 g/d lactose and 16.95 g DM/d forage mix to produce a forage: concentrate ratio of 0.57: 0.43, the average proportion recorded in Chapter 4. The forage mix contained varying proportions, on a DM basis of grass silage and WCW, to provide four dietary treatments; U-0 – grass silage alone, U-25 – 0.75 grass silage: 0.25 WCW, U-50 – 0.50 grass silage: 0.50 WCW, U-75 – 0.25 grass silage: 0.75 WCW. Prior to mixing the diets, all feed was freeze-dried and ground through a 3 mm screen. The diets were mixed on a daily basis and fed in three equal meals at 08.00h, 16.00 h and 00.00 h.

Table 5.1. Ingredient composition of the dairy concentrate

	kg/t
Wheat	200
Rapeseed meal	150
Sugarbeet pulp	140
Palm kernel extract	140
Soyabean meal	100
Sunflower meal	120
Molasses	60
Megalac	42
Minerals and Vitamins	48

5.2.5. Experimental routine

The experiment was a 4 x 4 Latin square design, with each experimental period lasting 14 days. Days 1-8 were used as an adaptation period with sampling occurring on days 9-14. The artificial saliva was that of McDougall (1948), modified to contain 13.82 mg/d

ammonium sulphate per vessel on adaptation days (days 1-8) and 13.09 mg/d $(^{15}NH_4)_2SO_4$ to provide 2.931 mg ^{15}N/d on sampling days (days 9-14). The buffer was diluted 60: 40 with distilled water as used by Rufener *et al.* (1963), and was pumped continuously into the vessels at approximately 1.4 volumes/day (Rufener *et al.*, 1963; Hoover *et al.*, 1976b). The motor controlled stirrer operated intermittently, mixing the contents for 5 min every 0.5 h at approximately 20 turns per min. Additional agitation was achieved by manually stirring the vessel contents after feeding to ensure feed was distributed through the raft which formed above the vessel contents.

5.2.6. Sampling

Throughout the experimental periods, saliva input and overflow effluent were quantified daily. Protozoa counts were undertaken on days 1, 3, 5, 7, 9, 11 and 13. Approximately 5ml of effluent was removed directly from the vessels via a smaller port in the cover using a syringe and preserved by adding 5 ml of 4 g/l formaldehyde in saline (0.9 g/l NaCl) and stored at room temperature prior to counting. At 12.00 h each day the pH of the vessel contents was monitored using a pH probe (Jenway, Model 3510, Essex) and the feed raft depth recorded (cm). During the sampling period (days 9-14) the total overflow volume for each 24 h period was bulked, mixed and 10 ml mixed with 1 ml HCl (2M) and stored at -20 °C prior to analysis for VFA. A further 10 ml sample was frozen at -20 °C prior to ammonium-N determination; the remaining effluent was freeze dried and material collected on days 12, 13 and 14 composited prior to analysis. At the end of each experimental period feed samples were taken and stored prior to analysis. On day 8 of each period pH was measured and samples taken for VFA and ammonium-N analysis hourly between 08.00 h and 16.00 h.

5.2.7. Microbial protein synthesis

The microbial marker ^{15}N was used to determine microbial protein synthesis. On day 9, at 12.00 h 5.98 mg of ^{15}N (98% enriched $(^{15}NH_4)_2 SO_4$: Sigma Chemical, Poole, UK) was added to each vessel to instantaneously label the ammonia-N pool. A solution of $(^{15}NH_4)_2 SO_4$ was then added to the artificial saliva to supply 2.931 mg ^{15}N/d per vessel. During days 12-14 a sub-sample of approximately 350 ml of overflow effluent was frozen, freeze dried and bulked prior to ^{15}N analysis. On day 14 final vessel contents were homogenised in a blender at a low speed for 1 min prior to storage at -20 °C and isolation of the total bacterial pellet for ^{15}N analysis.

5.2.8. Chemical analysis

Fresh samples of effluent were analysed for ammonium-N (Section 2.1.4). Frozen effluent samples treated with HCl were analysed for VFA according to Section 2.1.11. Freeze-dried samples of feed were bulked for the entire experiment and along with effluent samples were analysed for DM (Section 2.1.1), ash (Section 2.1.2), N (Section 2.1.3), starch (Section 2.1.5) and NDF (Section 2.1.7).

Protozoa were diluted and stained by mixing 1 ml of the preserved protozoa with 4 ml of 0.2M iodine solution. Samples were pipetted, drop-wise into a Fuchs Rosenthal counting chamber (Hawksley, Sussex, England) 0.2 mm deep and counted in 80 microscopic fields (5 x 16 squares) under a microscope at 100 x magnification (eyepiece x 10 amplification of lens x 10). The number of protozoa per ml of rumen fluid was calculated;

Protozoa per ml rumen fluid = (Total number/no. squares) x dilution x (5000*) (Equation 5.1)

*5000 is a correction factor, i.e. depth 0.2mm and 1/16sq mm.

Total mixed bacterial pellets were isolated and analysed according to Carro and Miller (1999). Vessel contents were strained through four layers of cheesecloth to remove particulate material. The strained rumen fluid was then centrifuged at 500 g for 10 min at 4°C. The supernatant fraction was then centrifuged at 18,000 g for 25 min at 4 °C to obtain a bacterial pellet. This was washed and re-suspended in saline solution (9 g NaCl/ l) and centrifuged again at 18,000g for 25 min. Finally, the bacterial pellet was washed and re-suspended in distilled water followed by centrifugation at 18,000g for 25 min. Bacterial pellets were freeze-dried and analysed for ash (according to Section 2.1.2) and ^{15}N. Freeze-dried effluent overflow samples were treated to remove excess ^{15}N labelled ammonia ($^{15}NH_3$-N) by wetting 1 g of sample with distilled water, adjusted with 1M NaOH to pH>10 and dried at 90 °C for 16 h (Firkins *et al.*, 1992). Approximately 1.5 mg and 2 mg of bacterial pellet and effluent overflow sample respectively were weighed into aluminium cups and ^{15}N determined on an ANCA/SL 20/20 continuous flow isotope ratio mass spectrometer (IRMS; Europa Scientific Ltd., Crewe, UK) at the Institute of Grassland and Environmental Research, Aberystwyth. The microbial N flow was calculated as;

Microbial N flow (g/d) = (15N: NAN (digesta)/ 15N: N (bacteria)) x NAN flow (g/d) (Equation 5.2)

Control samples allowed correction for background ^{15}N.

5.2.9. Statistical Analysis

Vessel parameters and effluent data were evaluated as a Latin square design with linear and quadratic effects of the ratio of inclusion of WCW. All statistical analysis was conducted using Genstat 6 (VSN Int. Ltd, Oxford, UK).

5.3. RESULTS

5.3.1. Forage and concentrate analysis

Chemical composition of the WCW, grass silage and dairy concentrate are presented in Table 5.2. Crude protein was highest in the dairy concentrate whilst the forages (WCW and grass silage) had similar values (average of 141 g/kg DM). Ash content was considerably lower for the WCW compared with the grass silage or concentrate, whilst NDF values were lowest for the concentrate. Starch content was higher for WCW than the grass silage or concentrate.

Table 5.2. Chemical composition (g/kg DM) for processed, urea-treated whole-crop wheat (WCW), grass silage (GS) and dairy concentrate.

	WCW	GS	Concentrate
Crude protein	148	134	221
Ash	36.8	85.7	96.1
NDF	389	446	254
Starch	342	11.2	72.5

5.3.2. Vessel turnover, temperature, raft depth and pH

There was no difference (P>0.05) between treatments in vessel turnover on days 12, 13 and 14 of each period, with an average turnover of 1.40 volume/d (Table 5.3). Similarly, there was no difference (P>0.05) between treatments in temperature or raft depth. Average pH tended to decrease linearly with inclusion of WCW in the diet (P=0.055).

Table 5.3. Average turnover (volume/d), temperature (°C), raft depth (cm) and pH for *in vitro* vessels given grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75	s.e.d.	Treat.	Lin.	Quad.
Vessel turnover*	1.40	1.39	1.39	1.41	0.029	0.772	0.814	0.339
Temperature	38.3	38.4	38.4	38.3	0.107	0.570	0.896	0.224
Raft	3.73	3.90	3.92	3.95	0.365	0.931	0.613	0.801
pH	6.05	6.03	5.99	5.99	0.030	0.186	0.055	0.485

*Fermentor volume = average 1171 ml

Hourly pH measured on day 8 is presented in Figure 5.1. For all treatments except U-75, pH decreased after 08.00 h, an effect that was significant at 09.00 h. By 15.00 h, pH for vessels fed diets containing WCW had returned to pre-feeding values whilst the pH for vessels fed grass silage alone increased to a level above that at 08.00 h.

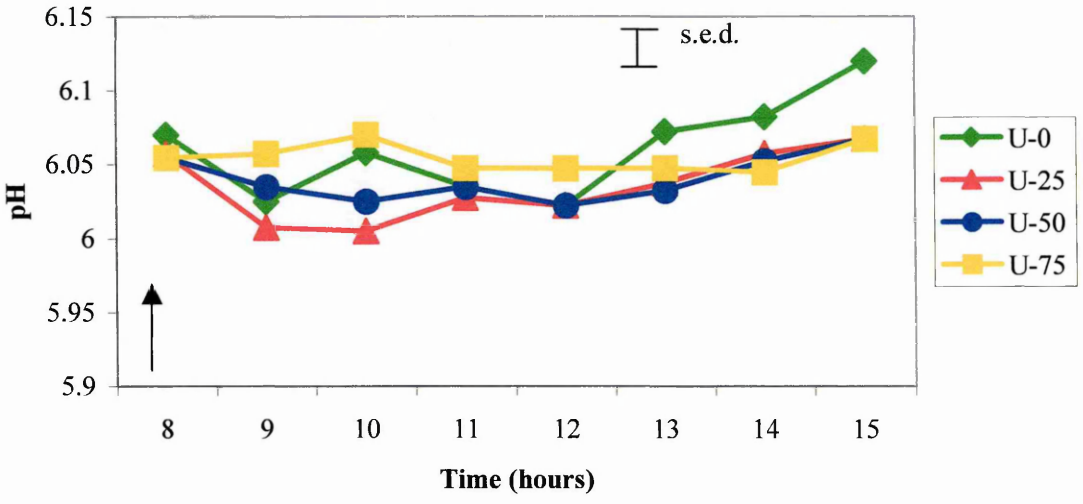

Figure 5.1. Hourly pH for *in vitro* vessels given grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM (↑ indicates feeding time)

5.3.3. Protozoa

Protozoa numbers for all dietary treatments decreased between days 1 and 3, whilst there was a linear effect of inclusion rate of WCW, with vessels receiving U-75 having the highest numbers and those receiving grass silage alone having the lowest (Figure 5.2). This effect was significant on days 5, 7 and 13. Average protozoa counts during the sampling period (day 13) were 4.48, 5.21, 6.25 and 7.34 (x 10^3) protozoa per ml rumen fluid for U-0, U-25, U-50 and U-75 respectively.

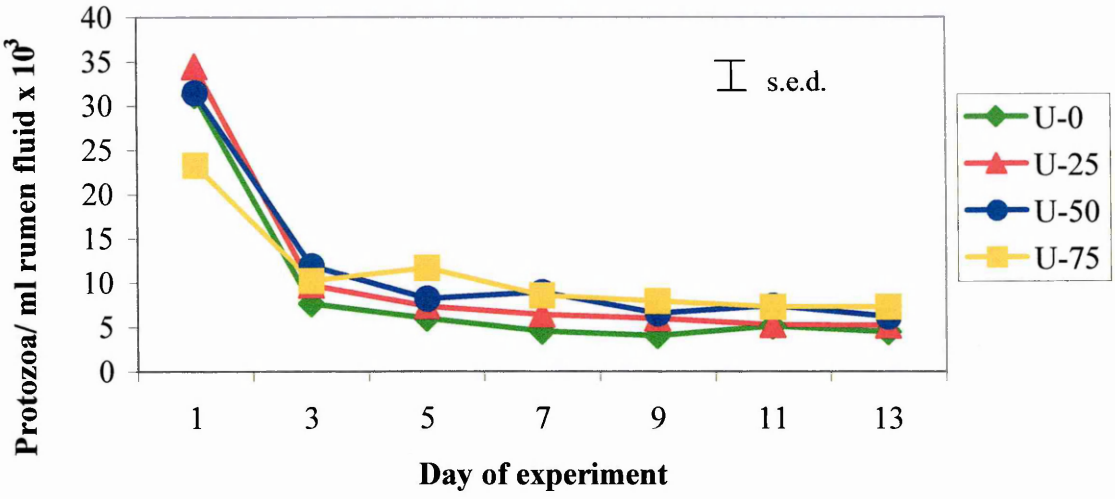

Figure 5.2. Protozoa counts for *in vitro* vessels given grass silage (U-0) processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) and 0.75 (U-75) of the forage dry matter

5.3.4. Ammonia nitrogen and volatile fatty acids

Effluent ammonia-N concentrations increased with inclusion of WCW ($P<0.05$; Table 5.4). By contrast, there was no effect ($P>0.05$) of WCW inclusion on total VFA concentrations (mmol/l) or the proportion of acetic and butyric acids. The proportion of propionic acid decreased linearly with inclusion of WCW ($P<0.001$) whilst the proportion of minor VFA's (isobutyric, isovaleric, valeric and caproic acids) was lower ($P<0.05$) for U-75, compared with vessels fed 0.50 (U-50) or 0.25 (U-25) WCW or grass silage alone (U-0). The ratio of acetic to propionic acid increased ($P<0.01$) with WCW inclusion.

In general, VFA concentration increased post feeding to a maximal value at approximately 3 h post-feeding before decreasing to pre-feeding levels at 5 h post-feeding (Figure 5.3). There was no effect of dietary treatment ($P>0.05$) on VFA concentration at any of the time points measured.

Table 5.4. Ammonia nitrogen (N), total volatile fatty acid concentration (mmol/l) and average molar proportions of acetic, butyric, propionic, and minor acids of total volatile fatty acids of *in vitro* vessels given grass silage (U-0), or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75	s.e.d.	Treat.	Lin.	Quad
Ammonia N (mg/l)	114^{a}	115^{ab}	145^{bc}	153^{c}	12.65	0.045	0.011	0.704
Total VFA (mmol/l)	68.20	67.20	71.30	76.40	7.290	0.617	0.261	0.575
Proportion (mmol/mmol								
Acetic	53.80	53.26	54.10	54.92	0.909	0.398	0.194	0.330
Butyric	16.53	18.18	19.33	20.32	1.124	0.064	0.012	0.690
Propionic	21.37^{c}	20.14^{bc}	18.75^{ab}	17.64^{a}	0.657	0.006	0.001	0.897
Minor VFA*	8.30^{b}	8.42^{b}	7.82^{b}	7.12^{a}	0.280	0.013	0.003	0.083
Acetic: Propionic	2.52^{a}	2.65^{ab}	2.89^{b}	3.16^{c}	0.104	0.004	0.001	0.403

^{abc} within a row, means with different superscript letter differ ($P<0.05$)
*Minor VFA includes isobutyric, isovaleric, valeric and caproic acids

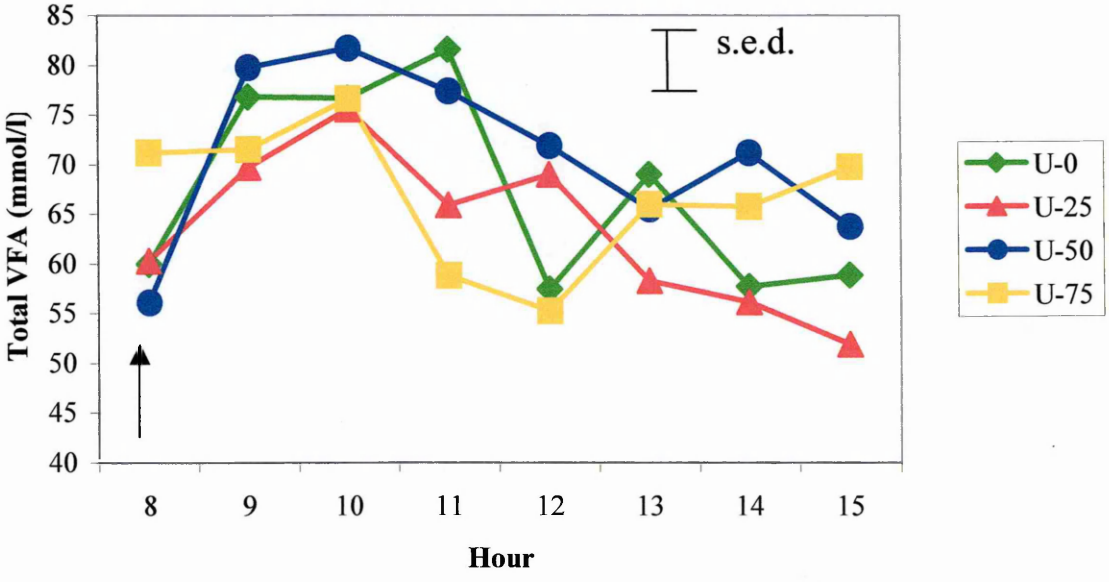

Figure 5.3. Hourly total volatile fatty acid (VFA) concentration (mmol/l) for *in vitro* vessels given grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM measured on day 8 (↑ indicates feeding time)

5.3.5. Digestibility

Dry matter and OM intakes and outputs were similar for all dietary treatments. As a result, DM and OM digestibilities were not affected ($P>0.05$) by inclusion rate of WCW (Table 5.5).

Table 5.5. Average intake, output, digestible (g/d) and apparent digestibility (g/g) of DM and organic matter for *in vitro* vessels given grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75	s.e.d	Treat.	Lin.	Quad
Dry matter								
Intake	50.50	50.10	50.30	50.30	0.347	0.732	0.741	0.451
Output	27.11	26.34	26.88	26.24	1.708	0.946	0.718	0.958
Digestible	23.39	23.76	23.43	24.06	1.790	0.979	0.778	0.921
Digestibility	0.46	0.47	0.47	0.48	0.035	0.973	0.774	0.984
Organic matter								
Intake	28.17	28.37	28.58	28.79				
Output	16.10	15.98	15.36	15.30	0.785	0.664	0.270	0.955
Digestible	12.06	12.39	13.22	13.48	0.785	0.316	0.086	0.955
Digestibility	0.43	0.44	0.46	0.47	0.027	0.452	0.143	0.947

Similarly, NDF digestibility was not affected (P>0.05) by inclusion rate of WCW (Table 5.6). By contrast, starch intake increased linearly from 0.81 g/d for U-0 to 5.03 g/d for U-75. Starch output also increased (P<0.05) with WCW inclusion and starch digestibility was lower (P<0.001) in diets containing grass silage alone (U-0) and increased linearly with WCW inclusion rate (Table 5.6).

Table 5.6. Average intake, output, digestible (g/d) and apparent digestibility (g/g) of neutral detergent fibre and starch for *in vitro* vessels given grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75	s.e.d	Treat.	Lin.	Quad
Neutral detergent fibre								
Intake	10.86	10.62	10.38	10.14				
Output	3.51	3.24	2.58	3.10	0.615	0.532	0.370	0.392
Digestible	7.35	7.38	7.80	7.04	0.615	0.682	0.798	0.392
Digestibility	0.68	0.70	0.75	0.69	0.058	0.615	0.574	0.385
Starch								
Intake	0.81	2.22	3.62	5.03				
Output	0.02^{a}	0.03^{ab}	0.03^{bc}	0.04^{c}	0.005	0.008	0.001	0.939
Digestible	0.80^{a}	2.20^{b}	3.59^{c}	4.99^{d}	0.005	0.001	0.001	0.939
Digestibility	0.98^{a}	0.99^{b}	0.99^{b}	0.99^{b}	0.002	0.006	0.002	0.045

^{abc} within a row, means with different superscript letter differ (P<0.05)

5.3.6. Nitrogen metabolism

Nitrogen intake was similar for all dietary treatments, averaging 0.85 g/d (Table 5.7). Inclusion of WCW did not affect (P>0.05) non-ammonia N (NAN) flow or microbial N

flow (g/d), although for all N fractions values for treatment U-25 were numerically higher. Ammonia-N flow (g/d) increased with inclusion of WCW ($P<0.05$). Similarly, crude protein degradability (CP degrad) increased with inclusion of WCW ($P<0.05$) from 0.82g/kg for grass silage alone to 0.87 g/kg for the treatment containing 0.75 WCW of total forage DM (U-75).

The efficiency of microbial protein synthesis, g N per kg OM apparently and truly digested, was not affected by inclusion of WCW ($P>0.05$), although, values for the treatment containing 0.25 WCW of total forage DM were numerically higher than those containing grass silage alone or 0.50 or 0.75 WCW of total forage DM.

Table 5.7. Nitrogen metabolism for *in vitro* vessels given grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM

	U-0	U-25	U-50	U-75	s.e.d	Treat.	Lin.	Quad
N intake (g/d)	0.84	0.85	0.85	0.86				
N flow (g/d)								
Ammonia	0.19^{a}	0.19^{ab}	0.24^{ab}	0.25^{b}	0.022	0.051	0.012	0.590
NAN	0.51	0.53	0.46	0.46	0.032	0.151	0.067	0.788
Microbial N	0.36	0.39	0.35	0.35	0.025	0.415	0.421	0.491
Dietary UDP	0.18^{b}	0.17^{b}	0.13^{a}	0.13^{a}	0.012	0.008	0.002	0.374
CP Degrad. (g/kg)	0.82^{a}	0.83^{a}	0.87^{b}	0.87^{b}	0.012	0.008	0.002	0.374
Microbial synthesis								
g N/ kg OMAD	28.95	31.33	27.88	27.26	4.970	0.85	0.61	0.68
g N/ kg OMTD	20.87	21.84	20.33	20.00	2.522	0.89	0.62	0.73

^{abc} within a row, means with different superscript letter differ ($P<0.05$)
NAN - non ammonia nitrogen
OMAD - microbial protein synthesis, g N/ kg OM apparently digested
OMTD - microbial protein synthesis, g N/ kg OM truly digested

5.4. DISCUSSION

5.4.1. Forage and Concentrate analysis

The crude protein content of the WCW used in the current work was similar to that used by Jackson *et al.* (2004) and Sinclair *et al.* (2005), although the DM of the crop at harvest (830 g DM/kg) was higher than the range of 653 – 763 g DM/kg reported in other work when processed, urea-treated WCW has been fed to dairy cows (Jackson *et al.*, 2004; Murphy *et al.*, 2004; Patterson and Kilpatrick, 2005). The higher maturity of the WCW used in the current work was not reflected in the starch content which at 342 g/kg DM was similar to that used by Murphy *et al.* (2004) and Sinclair *et al.* (2005) but lower than the short straw, processed WCW or processed urea-treated WCW used by Jackson *et al.* (2004) or the WCW reported by Patterson and Kilpatrick (2005) respectively. The crude protein, ash and starch content of the grass silage used in the current experiment was similar to that used in previous experiments (Jackson *et al.*, 2004; Sinclair *et al.*, 2005). By contrast, the NDF content was lower at 446 g/kg DM than that used in previous experiments evaluating the inclusion of processed WCW (Jackson *et al.*, 2004; Sinclair *et al.*, 2005).

5.4.2. Vessel turnover and feeding

In vivo, liquid turnover rates exceed 1.5 volumes/d (Hungate, 1966). In the current experiment the liquid turnover was the same as the intended rate of 1.4 volumes/d. This rate was lower than that of Stewart *et al.* (1961), and Merry *et al.* (1987) who recorded rates of 1.96 and 1.61 volumes/d respectively but similar to Hoover *et al.* (1976a; 1976b) who reported average liquid turnover rates of 1.41 and 1.45 respectively.

In the current experiment *in vitro* vessels were given 30 g DM/d of a total mixed ration, resulting in a feeding rate (mg per ml total flow) of 18.0, a value similar to that of Hoover *et al.* (1976a; 18.7 mg/ml total flow). The ration was analogous with the diet offered to dairy cows in the feeding experiment outlined in Chapter 4. The proportions of individual dietary components were calculated according to the average intakes recorded for the dairy cows. This provided a constant concentrate: forage ratio of 0.44: 0.56, which was intermediary to the values, recorded in Chapter 4 (0.49: 0.51, 0.44: 0.56, 0.42: 0.58 and 0.40: 0.60 for treatment U-0, U-25, U-50 and U-75 respectively).

5.4.3. Protozoa

Slyter *et al.* (1964) reported that continuous culture systems maintain bacterial numbers similar to those in the rumen. However, protozoa numbers have been reported to decrease markedly compared to ruminal levels (Rufener *et al.*, 1963; Abe and Kumeno, 1973). Slyter *et al.* (1964) reported that in a continuous culture system with a liquid turnover of 1.5 volumes/d, protozoa numbers decreased from 10^5 per ml to 2 x 10^3 per ml in approximately 4 days, after which point numbers remained stable. Similarly, in the current experiment protozoa numbers decreased from approximately 3 x 10^3 to 10^3 per ml rumen fluid in 3 days and remained at a similar level for the remainder of the experiment. The decline in protozoa numbers may be attributed to the dilution rate (Hoover *et al.*, 1976a). The generation time of many protozoa species can be as long as 3 days (Czerkawski and Breckenridge, 1977) and this is greater than the residence time in the fermentor, therefore washout of protozoa may occur (Hoover *et al.*, 1976a). Unfortunately, decreasing the dilution rate to less than 1.0 volume/d has been reported to decrease protozoa numbers due to increased concentrations of fermentation end-products and decreased pH (Abe and Kumeno, 1973). *In vivo,* liquid turnover rates exceed 1.5 volumes/d (Hungate, 1966) and therefore Abe and Kumeno (1973) suggested that protozoa removal from the rumen was probably at a slower rate than fluid flow from the rumen.

Protozoa numbers in the current experiment were higher in vessels fed 0.75 WCW (U-75). Previously, Dennis *et al.* (1983) reported increased ruminal protozoa populations in Holstein heifers when dietary concentrate, (corn starch and dextrose) was increased from 30 to 70 %. Furthermore, Chamberlain *et al.* (1985) reported that supplementation with barley led to an increase in the number of protozoa in the rumen, with the same effect being observed when a supplement of pure starch was fed. It is therefore possible that the higher protozoa numbers in the current experiment for treatment U-75 could have been due to the higher starch intake.

5.4.4. Volatile fatty acids and pH

In general, the total VFA concentration and molar proportions of VFA in the current experiment were similar to those reported previously for *in vitro* continuous culture systems (Hoover *et al.*, 1976b; Slyter *et al.*, 1964). The molar proportions of VFA produced in the current experiment were also close to those which have been observed in ruminal fluid in dairy cows when fed diets containing varying rates of unprocessed urea-treated WCW (Abdalla *et al.*, 1999).

In ruminants, VFA's constitute the major source of energy, providing at least 50 % of the total amount of digested energy (Sutton, 1985). Volatile fatty acids are produced by microbial fermentation of carbohydrates and protein (Dijkstra *et al.*, 1993) with acetic, propionic and butyric acids being the predominant VFA occurring in the rumen fluid. In general, ruminal microbes fermenting starch produce a higher proportion of propionic acid (35 - 45 mol/100mol VFA) than microbes fermenting cellulose or hemicellulose (15 - 20mol/100mol; Ørskov, 1986. As a result, increased dietary starch to fibre ratios are associated with a shift in VFA production in the rumen away from acetate towards propionate (Reynolds *et al.*, 1997). Overton *et al.* (1995) reported a decreased rumen

acetate and elevated propionate proportion with increasing inclusion rate of barley starch in dairy cow diets. By contrast, Abdalla *et al.* (1999) reported an increased ruminal proportion of acetic and butyric acid with increasing inclusion rate of WCW in the diet of dairy cows, whilst the proportion of rumen propionic acid was reduced. The net result of these changes was a substantial increase in the ratio of acetic plus n-butyric acids to propionic acid (Abdalla *et al.*, 1999). In the current experiment, inclusion rate of processed, urea-treated WCW had no significant effect on acetic or butyric acid proportions, although numerically, these were higher for diets with 0.75 WCW. By contrast, the proportion of propionic acid decreased with increasing rate of WCW inclusion. As a result the ratio of acetic to propionic acid was increased. Furthermore, although not significant, the total VFA concentration in the current experiment increased with increasing WCW inclusion which may reflect increased OM digestion at the higher inclusion rates (Abdalla *et al.*, 1999).

Hourly total VFA concentrations (mmol/l) increased immediately after feeding for the diets containing grass silage alone, 0.25 and 0.50 WCW (U-0, U-25 and U-50, respectively) compared with U-75, which had a high and stable VFA concentration. Maximum total VFA concentrations were reached approximately 3 h post feeding, at which point total VFA concentrations decreased to levels similar to 08.00 h. Similarly, Sinclair *et al.* (1993; 1995) reported maximum total VFA concentrations 3 h post feeding for sheep fed diets differing in their relative rate of energy and nitrogen release in the rumen.

Ruminal pH is affected by two principal factors; the amount of saliva entering the rumen and the amount of organic acids, particularly VFA's, that accumulate in the rumen contents (Church, 1976). In the current experiment, increasing inclusion rate of WCW had no significant effect on average vessel pH, although numerically pH decreased with inclusion

of WCW, with diets containing 0.75 and 0.50 WCW having the lowest values at pH 5.99. Sub-acute ruminal acidosis occurs when ruminal pH is less than 5.6 (Owens *et al.*, 1998), and is often the result of excessive consumption of fermentable carbohydrates and the resultant production of large quantities of VFA as well as other acids (such as lactic; Slyter, 1976). Maekawa *et al.* (2002) reported saliva secretion in dairy cows to decrease from 4.43 ml/g feed to 1.19 ml/g for diets based on silage and concentrate respectively. This would suggest concentrates have a weaker buffering power compared with that of forages (Slyter, 1976). Sustained periods of low pH can reduce digestion, ruminal motility, appetite and microbial growth (Allen, 1997). A rumen pH of less than pH 5.8 - 6.0 may be enough to inhibit the growth of cellulolytic bacteria (Hungate, 1966) and microbial growth efficiency (Ørskov, 1986; Mould and Ørskov, 1983). It is therefore possible that the small drop in vessel pH for diets containing 0.50 and 0.75 WCW (U-50 and U-75 respectively) may have affected digestibility. However, the decrease was small and therefore the difference in pH between treatments reported in the current experiment was not significant and may be a result of the infusion rate of artificial saliva buffering the pH effect. Furthermore, Abdalla *et al.* (1999) reported an average minimum ruminal pH value of pH 5.8 for animals offered unprocessed, urea-treated WCW. In addition, pH values for treatments containing increasing amounts of urea-treated WCW were similar and the authors suggested that the alkaline pH of WCW would be expected to raise ruminal pH rather than lower it (Abdalla *et al.*, 1999). It is possible therefore that the lack of pH effect in the current experiment may be due to the alkalinity of the WCW buffering the increased starch.

Hourly vessel content pH decreased after feeding for treatments containing grass silage alone (U-0), 0.25 and 0.50 WCW (U-25 and U-50), whilst there was little change for the treatments containing 0.75 WCW (U-75) which may reflect the higher buffering capacity of urea-treated WCW (Adogla-Bessa *et al.*, 1999). Minimum pH values for U-0, U-25 and

U-50 were reached approximately 2 h post feeding, at which point pH values increased to values similar to 08.00 h. Similarly, Abdalla *et al.* (1999) reported daily minimum ruminal pH at 1.0 to 1.5 h post feeding for animals offered processed, urea-treated WCW.

5.4.5. Digestibility

i. Dry matter and organic matter

The average DM digestibility in the current experiment was similar to that reported previously for *in vitro* continuous culture systems (Vazquez-Anon *et al.*, 2000; Vallimont *et al.*, 2004). Previously, De Veth and Kolver (2001) reported lower DM and OM digestibilities *in vitro* as ruminal pH decreased. However, vessel pH was not significantly different between treatments although OM digestibility tended ($P = 0.014$) to increase with WCW inclusion rate. By contrast, Abdalla *et al.* (1999) reported that OM apparent digestibility decreased in the rumen with a higher inclusion of urea-treated WCW in the diets of dairy cows.

ii. Neutral detergent fibre and starch

The accumulation of acid in the rumen and the resultant drop in pH when cereal starches are digested can damage the rumen epithelium and inhibit the activity of cellulolytic micro-organisms (Ørskov, 1976) which may result in a depression in fibre digestion (Reynolds *et al.*, 1997). Strobel and Russel (1986) reported that *in vitro*, the optimal pH for cellulose digestion by major cellulolytic ruminal bacteria was near pH 6.5. As rumen fluid pH declined, ruminal fibre digestion also decreased, an effect that was more apparent when pH decreased below pH 6.0. In the current experiment, NDF digestibility was not significantly affected by WCW inclusion rate. By contrast, Abdalla *et al.* (1999) reported lower ruminal NDF apparent digestibilities with increasing WCW inclusion in the diets of dairy cows.

Starch intake and digestibility in the present experiment increased with WCW inclusion rate. Similarly, Koenig *et al.* (2003) reported increased starch intake and apparent ruminal digestibility in steers offered increased concentrate levels in the diet. By contrast, Abdalla *et al.* (1999) reported decreased ruminal starch apparent digestibilities for dairy cows offered increased inclusion rates of unprocessed, urea-treated WCW. Furthermore, the reduction in rumen apparent digestibility was accompanied by a large reduction in the contribution of the rumen to total starch apparent digestibility (Abdalla *et al.*, 1999). Starch digestibilities in the current experiment averaged 0.99 g/g, a value higher than that previously reported in dairy cows by Abdalla *et al.* (1999) for unprocessed, urea-treated WCW. The higher starch digestibilities in the current experiment may have been due to the processing of WCW at harvest. It is more likely that the high digestibilities reported in the current experiment were due to the grinding of the diets prior to feeding.

5.5. Ammonia and nitrogen metabolism

Abdalla *et al.* (1999) reported an increased total N and ammonia-N intake with WCW inclusion rate in dairy cows, resulting in an increased outflow of total N and non-ammonia N (NAN) to the duodenum. Similarly, N intake and ammonia-N flow in the present experiment increased with WCW inclusion reflecting the higher ammonia-N content of WCW compared to grass silage. Sutton *et al.* (1997) reported an increased milk urea concentration with increasing levels of urea-treated WCW inclusion, implying that rumen ammonia concentrations were elevated by WCW inclusion. Furthermore, Abdalla *et al.* (1999) suggested that increased ammonia values with WCW diets may be a consequence of the reduced OM digestion which reduced the capacity of the rumen microflora to utilise the increased supply of non-protein N. However, in the current experiment OM digestibilities were not significantly affected by WCW inclusion whilst ammonia-N flow increased. Crude protein degradation in the current experiment also increased with WCW

inclusion, which may have contributed to the increased ammonia-N concentration (Hoover *et al.*, 1982). Koenig *et al.* (2003) suggested that lower ruminal ammonia-N levels could also be related to lower protozoa levels. This was attributed to fewer protozoa resulting in less protozoal predation of bacteria and therefore less recycling of bacterial N through the ammonia N pool, lower protein degradation due to reduced deaminase activity, a lower rate of deamination and less protozoal lysis and degradation of protozoal protein by the bacteria pool (Koenig *et al.*, 2003).

5.6. Microbial protein synthesis

There were no significant differences in microbial protein synthesis (g N/kg OM apparently or truly digested) between dietary treatments. However, microbial protein synthesis (g N/kg OM apparently and truly digested) was higher for treatments containing 0.25 WCW (U-25) though this was not significant. Rumen microbial protein synthesis requires an adequate supply of N to achieve maximum efficiency. If the level of N is not adequate, uncoupled fermentation may occur and this may result in fermentation without useful ATP production (Buttery, 1977; McMeniman *et al.*, 1976). In contrast, if the N level is excessive, energy may be the limiting factor for efficient utilization of N (Stern and Hoover, 1979). Therefore, for maximal efficiency of microbial growth to occur N and energy availability in the rumen must be balanced (Stern and Hoover, 1979). It is possible that in the current experiment, the inclusion of 0.25 WCW (U-25) provided a balance between energy in the WCW and rapidly degradable N in the grass silage. It has been proposed that poor synchronization of N and energy release is one of the reasons for the low efficiency of microbial protein synthesis in animals fed grass silage-based diets (Thomas and Thomas, 1989). Siddons *et al.* (1985) suggested that the provision of a synchronous supply of rapidly fermentable carbohydrate may result in 'trapping' of the ammonia liberated from grass silage and result in more efficient microbial production in the rumen. Furthermore, Sinclair *et al.* (1993; 1995) reported higher microbial protein

efficiency for animals fed a synchronous diet. In the current experiment, although not significant, at the higher WCW inclusion rates (U-50 and U-75) it is possible that a rapid starch release lowered slightly the vessel pH and this may have reduced microbial growth efficiency (Mould and Ørskov, 1983; Ørskov, 1986). This is supported by Strobel and Russel (1986) who studied the effect of pH on bacterial protein synthesis in a continuous culture system and reported that the efficiency of microbial protein synthesis was less at pH 6.0 than at 7.0. This was attributed to an increased energy-dependant expulsion of protons at the lower pH and a diversion of energy from growth to non-growth functions (Strobel and Russel, 1986). Furthermore, De Veth and Kolver (2001) also reported a decline in the efficiency of microbial protein synthesis (g N/kg OM truly digested) with decreasing pH in a continuous culture system.

5.7. CONCLUSION

When fed at a constant rate, the inclusion of processed, urea-treated WCW had no significant effect on pH, total VFA concentration (mmol/l) or the proportion of acetic and butyric acids (mmol/mmol) but propionic acid levels decreased. There was no effect of inclusion on microbial protein synthesis, although numerically diets containing 0.25 WCW had higher values (g N/kg OM apparently digested and truly digested).

Chapter 6

FINAL DISCUSSION

6.1. INTRODUCTION

Whole tract digestibility of WCW, particularly that of the starch fraction, has been shown to be low in dairy cows and has been suggested to be responsible for the lower than predicted performance of dairy cows (Sutton *et al.*, 1997). The recent development of a forage processor enables the grain of WCW to be cracked prior to ensiling. Processing reduced forage intake by dairy cows whilst improving the whole tract digestibility of the starch component (Jackson *et al.*, 2004). Furthermore, animals fed processed, urea-treated WCW (pWCW) were reported to have a similar performance as cows fed maize silage (Sinclair *et al.*, 2005). The objectives of the current series of experiments were to examine the effects of stage of maturity and inclusion rate of processed WCW on crop production, dairy cow performance and metabolism.

In the first experiment WCW was harvested at three stages of maturity; approximately 450 g DM/kg and fermented (F-45), 700 g DM/kg and urea-treated (U-70), 850 g DM/kg and urea-treated (U-85) or untreated (C-85). It was found that while harvesting WCW at approximately 700 g DM/kg and treating with urea significantly increased grain DM yield, inclusion in dairy cow rations at 0.67 of the forage DM resulted in a higher milk yield and lower DM intake compared with animals offered F-45 or C-85. As a result, the second experiment examined the effect of rate of inclusion of pWCW. It was clear that inclusion of WCW at 0.25 of the forage DM resulted in an increased milk and milk protein yield compared with feeding grass silage alone, whilst inclusion at higher levels provided little further benefit to milk yield. Finally, the third experiment aimed to determine the effect of rate of inclusion of pWCW on *in vitro* ruminal fermentation, microbial growth and ruminal

digestibility. When fed at a constant rate, the inclusion of pWCW had no significant effect on pH, total VFA concentration, or the proportion of acetate and butyrate (mmol/mmol). Dry matter, OM and NDF digestibilities were also not affected by WCW inclusion whilst starch digestibility (g/g) increased and there was no effect of inclusion on microbial protein synthesis, although numerically diets containing 0.25 WCW had higher values (g N/kg OM apparently and truly digested).

6.2. ANIMAL PERFORMANCE

Work to determine the utilisation of processed urea-treated WCW by dairy cows has so far been limited, but has shown increased digestibility of the starch component and therefore improved efficiency of forage use for milk production.

6.2.1. Effect of stage of maturity and method of preservation

The advent of a forage processor allows wheat to be harvested over a much wider harvest window. Previously, pWCW has been harvested at DM values as high as 823 g DM/kg (Table 6.1). Increasing the crop maturity of fermented WCW produced small but non-significant increases in both forage intake and milk yield but there were no treatment effects on milk fat or protein content (Sutton *et al.*, 2002; Sinclair *et al.*, 2003). However, in experiment 1 (Chapter 3), milk yield increased whilst forage DM intake was lower for animals fed the lower DM pWCW (U-70) compared with those fed the higher DM forage (U-85; Table 6.1).

Table 6.1. Average forage DM intake (DMI; kg/d), milk yield (kg/d), milk fat and protein content (g/kg) for animals offered fermented whole-crop wheat (FWCW) and processed, urea-treated whole-crop wheat (pWCW) harvested at different stages of maturity

Reference	Treatment	DM	WCW: GS	Forage DMI	Milk yield	Milk fat	Milk protein
Chapter 3	FWCW	472	67: 33	13.4	32.3	40.6	33.5
	pWCW	747	67: 33	12.8	34.8	37.9	32.3
	pWCW	823	67: 33	12.9	33.5	39.5	32.9
Jackson *et al.* (2005)	pWCW	653	67: 33	13.0	29.9	41.9	34.6
	pWCW	709	67: 33	12.0	29.8	35.6	33.0
Murphy *et al.* (2005)	FWCW	370	67: 33	14.3	32.8	36.2	30.8
	FWCW	406	67: 33	12.8	29.7	38.4	31.7
	pWCW	733	67: 33	14.8	29.4	37.8	31.7
	pWCW	763	67: 33	16.4	31.2	40.2	31.9
Patterson *et al.* (2005)	FWCW	459	50: 50	12.3	29.6	39.0	32.0
	pWCW	751	50: 50	12.6	28.7	39.5	32.0
Mean (n = 11)		626		13.4	31.1	38.8	32.4
s.e.d.		165		1.30	1.99	1.87	1.04
Comparison of fermented WCW							
Mean (n = 4)		427		13.2	31.1	38.6	32.0
s.e.d.		47.7		0.88	1.69	1.82	1.12
Comparison of processed urea-treated WCW							
Mean (n = 7)		740		13.5	31.0	39.9	32.6
s.e.d.		51.9		1.54	2.28	2.02	1.00

Comparisons of fermented and unprocessed urea-treated WCW have demonstrated that intakes are generally higher for animals offered unprocessed urea-treated WCW although the differences are often not significant (Leaver and Hill, 1995; Hameleers, 1998; Sutton *et al.*, 2002). Comparison of fermented and pWCW demonstrates a similar forage DM intake, milk yield, milk fat and protein contents (Table 6.1). However, the results from experiment 1 demonstrated a lower fat content in milk produced by animals fed pWCW compared with those fed the fermented WCW, which may reflect the higher starch intake. Previously, it has been suggested that increased dietary starch concentration can be associated with a shift in VFA production in the rumen from acetate towards propionate resulting in a decrease in milk fat as dietary energy is partitioned towards body lipid (Reynolds *et al.*, 1997). However, more recently, it has been suggested that milk fat depression may be associated with changes in rumen biohydrogenation and the formation of inhibitors of milk

fat synthesis (Griinari and Bauman, 2003) with one such inhibitor being *trans*-10, *cis*-12 CLA (Bauman *et al.*, 2001). Although milk fatty acid profiles were not determined in the current series of experiments, it is possible that increased dietary starch resulted in increased production of *trans*-10, *cis*-12 CLA, thus causing a drop in milk fat content.

6.2.2. Effect of rate of inclusion of urea-treated whole-crop wheat

Early work reported major energy imbalances between ME intake and output when unprocessed urea-treated WCW constituted 0.56 or 1.0 of the forage DM (Leaver and Hill, 1992). Furthermore, Phipps *et al.* (1992), Sutton *et al.* (1997) and Abdalla *et al.* (1999) reported increased forage DM intake but only small improvements in milk yield when the proportion of unprocessed urea-treated WCW in the diet was increased from 0.33 to 0.67 (Table 6.2). Similarly in experiment 2 (Chapter 4) forage DM intake increased with increasing inclusion of pWCW, however, there was no benefit to milk yield with inclusion of pWCW above 0.25 of the forage DM.

Table 6.2. Average forage DM intake (DMI; kg/d) and milk yield (kg/d) of cows offered grass silage alone (GS) or unprocessed urea-treated whole-crop wheat (UWCW) and processed, urea-treated whole-crop wheat (pWCW) at differing inclusion rates

Reference	Treatment	WCW: GS	Forage DMI	Milk yield
Chapter 4	GS	100	9.90	34.2
	pWCW	25: 75	12.3	37.8
	pWCW	50: 50	13.6	35.2
	pWCW	75: 25	14.6	35.8
Phipps *et al.* (1992)	GS	100	6.30	18.1
	UWCW	25: 75	7.70	18.1
	UWCW	50: 50	7.60	18.2
	UWCW	75: 25	8.00	18.4
	UWCW	100	7.70	18.2
Sutton *et al.* (1997)	GS	100	9.26	20.1
	UWCW	33: 67	10.3	20.5
	UWCW	67: 33	10.7	21.6
Abdalla *et al.* (1999)	GS	100	10.6	21.2
	UWCW	33: 67	11.5	21.2
	UWCW	67: 33	12.4	22.2

Figures 6.1. and 6.2. show the milk fat and protein content (g/kg) respectively for cows offered increasing amounts of urea-treated WCW. Milk fat content decreases slightly with increasing WCW inclusion although the effect was not significant (P=0.364). This is despite the increased dietary starch concentration, which may cause a shift in VFA production in the rumen from acetate towards propionate, depressing lipolysis and promoting lipogenesis (Reynolds *et al.*, 1997). However, the *in vitro* study (Chapter 5) did not confirm any effect of pWCW inclusion rate on VFA proportion and it is more likely that increasing inclusion of WCW in the ration of dairy cows resulted in a larger amount of rumen undegradable starch flowing to the small intestine which can decrease milk fat as a result of increased glucose absorption (Reynolds *et al.*, 2001).

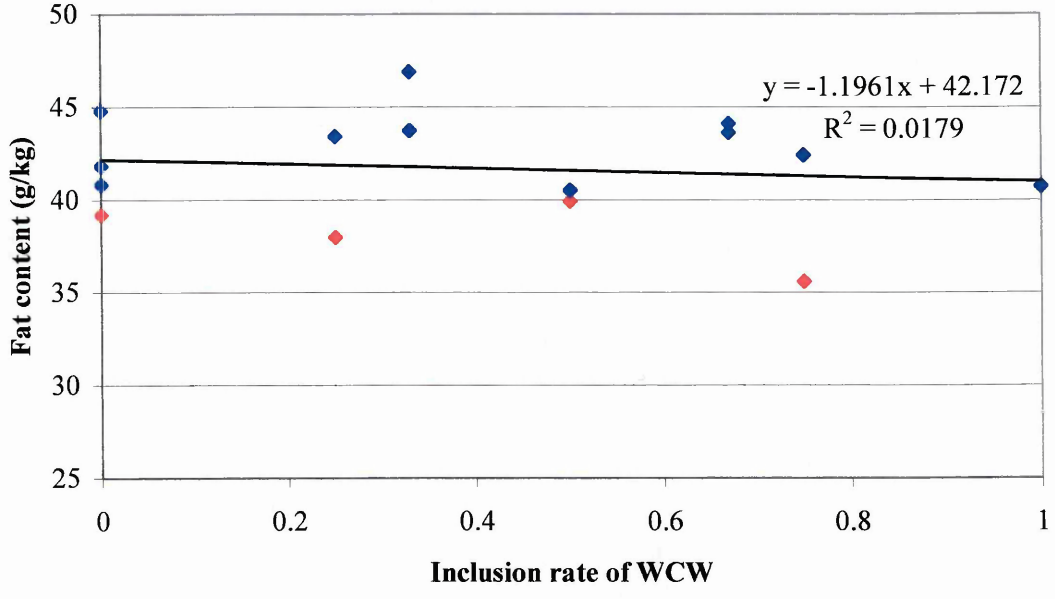

Figure 6.1. Average milk fat content (g/kg) of cows offered grass silage alone or urea-treated whole-crop wheat at differing inclusion rates (Chapter 4 •; Phipps *et al.*, 1992; Sutton *et al.*, 1997; Abdalla *et al.*, 1999 •)

By contrast, milk protein content showed a slight but non significant increase with increasing inclusion of urea-treated WCW (P=0.750; Figure 6.2). Reynolds *et al.* (1997) suggested that if sufficient quantities of rumen degradable protein and non-protein N are available then increased carbohydrate intake in the form of starch may increase microbial protein synthesis in the rumen and thus metabolisable protein supply to the small intestine, resulting in an increased milk protein content.

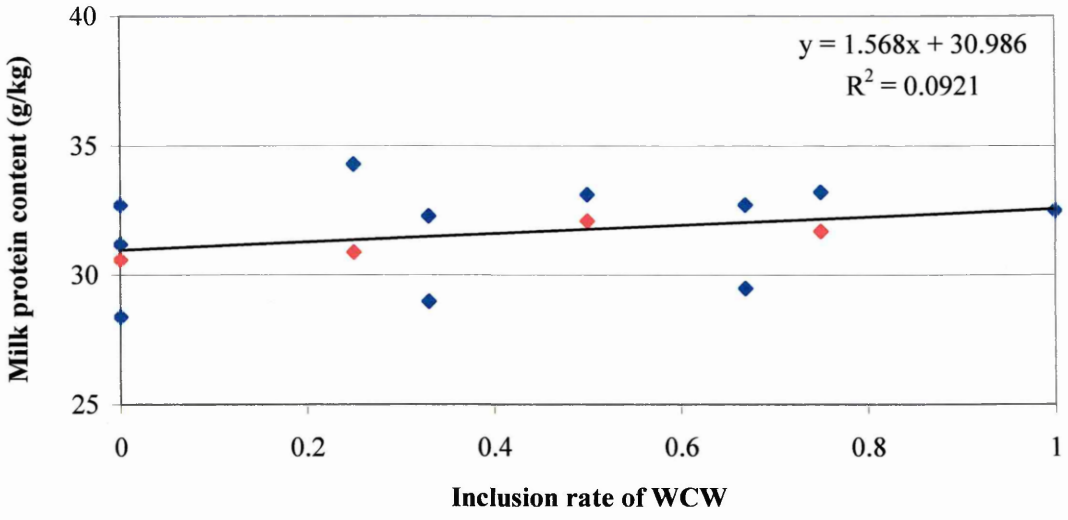

Figure 6.2. Average milk protein content (g/kg) of cows offered grass silage alone or urea-treated whole-crop wheat at differing inclusion rates (Chapter 4 •; Phipps *et al.*, 1992; Sutton *et al.*, 1997; Abdalla *et al.*, 1999 •)

6.3. RUMEN METABOLISM AND STARCH DIGESTIBILITY

By applying the *in situ* starch effective degradability of pWCW harvested at approximately 700 g DM/kg (U-70) calculated for experiment 1 (0.82 kg/kg) along with the whole-tract digestibility of WCW values from experiment 2 of 0.97, 0.95 and 0.94 kg/kg for U-25, U-50 and U-75 respectively, it is possible to calculate the amount of rumen by-pass starch in pWCW and the quantity digested post-ruminally (Table 6.3).

Table 6.3. Rumen by-pass starch and starch digested post-ruminally (kg/d) from processed, urea-treated whole-crop wheat (WCW) for cows offered grass silage alone (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) and 0.75 (U-75) of the forage DM

	U-25	U-50	U-75	s.e.d	Sign	Lin.	Quad.
WCW intake	3.13	6.66	10.7	0.377	0.001	0.011	0.049
Starch intake from WCW	1.07	2.26	3.63	0.147	0.001	0.001	0.502
WCW by-pass starch	0.19	0.41	0.65	0.026	0.001	0.001	0.502
WCW starch digested post-ruminally	0.16	0.29	0.44	0.018	0.001	0.001	0.831

By–pass starch increased from 0.19 kg/d for U-25 to 0.65 kg/d for U-75 ($P<0.001$). Similarly, the amount of starch actually digested post-ruminally increased from 0.16 kg/d for U-25 to 0.44 kg/d for U-75. Nocek and Tamminga (1991) suggested that starch digested post-ruminally may be used more efficiently for milk synthesis than starch digested in the rumen due to the direct production of glucose (Ørskov *et al.*, 1969). Furthermore, Reynolds *et al.* (2001) reported increased milk yield and decreased milk fat with increasing infusion rate of starch into the duodenum of dairy cows. Reynolds *et al.* (2001) suggested that the decreased milk fat may have been as a result of increased glucose absorption and the subsequent peripheral insulin supply, without a concomitant increase in VFA or long-chain fatty acids for fat synthesis. In experiment 2, plasma insulin levels increased with increasing inclusion rate of pWCW, although this effect was not significant. It is therefore possible that in the current series of experiments the increasing amount of starch digested post-ruminally at the high inclusion rates of WCW increased glucose availability, leading to decreased milk fat content and increased live-weight gain and condition score whilst having no effect on rumen VFA proportions. In addition, Knowlton *et al.* (1998) reported increased milk protein in animals infused abomasally with starch, possibly due to the sparing of amino acids through preferential utilisation of glucose by the gut tissue and decrease of hepatic gluconeogenesis. In experiment 2 milk protein content was higher with the rate of inclusion of pWCW, whilst the amount of starch digested post-ruminally also increased ($P<0.001$).

6.4. NITROGEN EFFICIENCY

Recently there has been a need to improve N efficiency within dairy production systems (Hameleers, 1998) and Korevaar (1992) indicated that alternative forages might be an option to maintain profitability and improve N efficiency. In experiments 1 and 2 plasma urea levels were increased with stage of maturity and increasing rate of inclusion of

pWCW. This may suggest an inefficient use of dietary N. Furthermore, there was a reduced efficiency of N use (kg milk N output per kg N intake) for milk production with increasing rate of inclusion of pWCW. Similarly, Hameleers (1998) reported low N efficiency for animals fed unprocessed, urea-treated WCW compared with those fed fermented WCW, probably as a result of the increased N content of the urea-treated WCW. Using the assumptions outlined in Appendix 1, total farm N efficiencies were calculated for the dairy cows in experiments 1 and 2 (Chapters 3 and 4; Table 6.4) as well as for the experiments reported by Jackson *et al.* (2004) and Sinclair *et al.* (2005; Table 6.5).

The increased WCW DM yield and decreased forage DM intake for U-70 in experiment 1 enabled each ha of forage to sustain more animals (livestock units) than the fermented, or high DM forages (F-45, U-85 and C-85). Experiment 2 data resulted in a greater number of animals being sustained per ha when animals were fed grass silage alone, whilst those fed WCW at increasing rates of inclusion were similar (average 9.17 animals/ha). Overall, these values were lower than those reported in the previous experiment due to the decreased DM yield of the wheat crop. Nitrogen efficiencies were marginally higher for the second experiment (average 0.36) compared with the first experiment (average 0.35), due to the lower N fertiliser applied and therefore the lower total N intakes. Nitrogen efficiencies in experiment 1 were highest for those diets containing either fermented or untreated forages (F-45 and C-85), an effect that can be attributed to the lower N input, whilst Chapter 4 data resulted in the highest N efficiencies reported for animals fed only 0.25 pWCW of the forage DM. This result was due mainly to the low N input compared with the fermented forage and higher milk N compared with the high DM forages but may also indicate an improvement in the balance of the ration (Groot *et al.*, 2006).

Table 6.4. Total farm N efficiencies for dairy cattle offered whole-crop wheat harvested at target DM values of 450 g/kg and fermented (F-45), 700 g/kg and urea-treated (U-70) or 850 g/kg and either urea-treated (U-85) or untreated (C-85; Chapter 3) and dairy cattle offered grass silage (U-0) or processed, urea-treated whole-crop wheat at 0.25 (U-25), 0.50 (U-50) or 0.75 (U-75) of the forage DM (Chapter 4; kg N/ha DM, unless otherwise stated)

	Experiment 1				Experiment 2			
	F-45	U-70	U-85	C-85	U-0	U-25	U-50	U-75
Grass area	0.33	0.33	0.33	0.33	1.00	0.75	0.50	0.25
WCW area	0.67	0.67	0.67	0.67	0.00	0.25	0.50	0.75
N fertiliser	90.4	127	137	141	96.9	99.9	103	106
N supplement	1.20	1.69	1.55	1.07	0.00	0.54	1.08	1.62
Livestock units (/ha)	10.6	11.5	10.6	9.41	10.4	9.21	9.10	9.20
Concentrate N	419	451	422	378	413	369	367	372
Total N in	511	580	561	520	510	469	471	479
Milk N	188	212	193	164	179	177	169	172
Farm N surplus	322	368	368	356	331	292	301	307
N efficiency (kg/kg)	0.37	0.36	0.34	0.32	0.35	0.38	0.36	0.36

Calculated according to assumptions outlined in Appendix 1

In the experiment of Jackson *et al.* (2004; Table 6.5) the long straw WCW (LU and LP) sustained more animals per ha compared with the short straw forages (SU and SP; average 11.11 and 10.21 animals/ha) respectively. Furthermore, the decreased forage DM intake for animals fed the processed forages (LP and SP) resulted in more animals per ha (average 11.15 and 10.17 animals/ha) respectively, therefore the long straw processed WCW sustained the greatest number of animals. Overall, livestock units were lower in the study of Sinclair *et al.* (2005), with an average 8.14 animals/ha as a result of the lower forage DM yield (t DM/ha). Total N efficiencies in the experiment of Jackson *et al.* (2004) were highest for the long straw forages (average 0.39 kg N/ha) and processed forages (average 0.38 kg N/ha), whilst in the study by Sinclair *et al.* (2005) efficiencies were highest for the pWCW supplemented with lactose and lowest when supplemented with molasses.

Table 6.5. Total farm N efficiencies for dairy cattle offered 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 (Jackson *et al.*, 2004) and dairy cattle offered processed, urea-treated whole-crop wheat supplemented with wheat (W-WCW), lactose (L-WCW) or molasses (M-WCW; Sinclair *et al.*, 2005; kg N/ha DM, unless otherwise stated)

	Jackson *et al.* (2004)				Sinclair *et al.* (2005)		
	LU	LP	SU	SP	W-WCW	L-WCW	M-WCW
Grass area	0.33	0.33	0.33	0.33	0.33	0.33	0.33
WCW area	0.67	0.67	0.67	0.67	0.67	0.67	0.67
N fertiliser	97.0	90.8	96.4	96.6	110	110	110
N supplement	1.74	1.74	1.42	1.42	1.11	1.11	1.11
Livestock units	10.7	11.6	9.69	10.7	8.13	8.87	7.41
Concentrate N	395	403	347	384	310	332	304
Total N in	494	495	445	482	421	444	415
Milk N	186	197	159	174	150	164	134
Farm N surplus	308	298	286	309	272	280	281
N efficiency	0.38	0.40	0.36	0.36	0.36	0.37	0.32

Calculated according to assumptions outlined in Appendix 1

Overall the total farm N efficiencies reported here are higher than those reported by Hart (2005) for dairy cows offered processed, urea-treated WCW mixed with grass silage in a 50:50 ratio (average 0.36 and 0.26 kg/kg respectively). The reason for this difference is due to the higher grass silage and WCW DM yields (t DM/ha) which resulted in a higher number of animals per ha. Furthermore, milk N values were much higher in the current series of experiments. However, Spears *et al.* (2003) reported an average whole farm N utilisation efficiency of 35.8% for 41 dairy farms in Utah and Idaho, a value similar to the overall average of experiments 3 and 4 of 0.36 kg/kg.

6.5. CONCLUSIONS

A considerable amount of work has previously been conducted to determine the utilisation of unprocessed WCW by dairy cows, the recent development of a forage processor which enables the grains of WCW to be cracked at harvest has been shown to increase the whole tract digestibility of the starch component and therefore improve the efficiency of forage

utilisation. As a result the optimum stage of maturity at which to harvest WCW along with the ideal inclusion rate for dairy cows was unclear.

The main findings from the current study are:

Farmers should aim to harvest the WCW at approximately 700 g DM/kg. At this stage of maturity the total crop and grain yield were maximised. Furthermore, fermentation losses were negligible compared with WCW harvested at 450 g DM/kg and fermented. Inclusion of WCW harvested at 700 g DM/kg and urea-treated in dairy cow rations resulted in a significantly higher milk yield (kg/d) but had little effect on milk fat and protein yield (kg/d). Once the optimum stage of maturity (700 g DM/kg) has been reached it is recommended that the crop be harvested whenever the weather is suitable. The current work demonstrated that although crop and milk yield decreased at 850 g DM/kg the reduction was small. At DM values of 850 g DM/kg a urea-based additive should be applied because although the current work suggests the crop is stable at this stage of maturity, cows ate more but yielded less milk compared to animals offered urea-treated WCW harvested at 850 g DM/kg.

Processed, urea-treated WCW should be included in dairy cow rations at 0.25 of the forage DM. This resulted in a higher milk yield (kg/d) and milk protein yield (kg/d) compared with animals offered grass silage alone. Animals offered WCW at 0.25 of the forage DM ate more than animals offered grass silage alone, however this value was less than animals offered WCW at 0.50 or 0.75 of the forage DM. Processed, urea-treated WCW can be included at higher inclusion rates than 0.25 of the forage DM, but there was little benefit on milk yield compared with grass silage alone, with more energy being partitioned towards body fat. However, there is little evidence of a beneficial or detrimental effect of rate of inclusion of processed, urea-treated WCW on lameness. Furthermore, when fed at a constant amount, the inclusion of processed, urea-treated WCW at 0.25 of the forage DM

had no significant effect on digestibility or microbial growth but resulted in a more ketogenic VFA profile.

REFERENCES

Abdalla, A. L., Sutton, J. D., Phipps, R. H. and Humphries, D. J. (1999) Digestion in the rumen of lactating dairy cows given mixtures of urea-treated whole-crop wheat and grass silage *Animal Science,* **69,** 203 - 212.

Abe, M. and Kumeno, F. (1973) In vitro simulation of rumen fermentation: apparatus and effects of dilution rate and continuous dialysis on fermentation and protozoal population *Journal of Animal Science,* **36,** 941 - 948.

Adamson, A. H. and Reeve, A. (1992) Nutritional evaluation of whole-crop wheat In: *Whole-crop cereals*, (Eds, Stark, B. A. and Wilkinson, J. M.) Chalcombe Publications Caterbury 85 - 95.

Adesogan, A. T. (2002) What are feeds worth?: A critical evealuation of selected nutritive value methods In: *Proceedings 13th Annual Florida Ruminant Nutrition Symposium* 33 - 47

Adesogan, A. T., Owen, E. and Givens, D. I. (1998a) The chemical composition, digestibility and energy value of fermented and urea-treated whole crop wheat harvested at three stages of maturity *Grass and Forage Science,* **53,** 66 - 75.

Adesogan, A. T., Owen, E. and Givens, D. I. (1998b) Prediction of the in vivo digestibility of whole crop wheat from in vitro digestibility, chemical composition, in situ rumen degradability, in vitro gas production and near infrared reflectance spectroscopy *Animal Feed Science and Technology,* **74,** 259 - 272.

Adesogan, A. T., Owen, E. and Givens, D. I. (1999) Prediction of the metabolizable energy value of whole-crop wheat from laboratory-based measurements *Animal Science,* **68,** 427 - 439.

Adogla-Bessa, T. and Owen, E. (1995) Ensiling of whole-crop wheat with cellulase-hemicellulase based enzymes. 1. Effect of crop growth stage and enzyme on silage composition and stability *Animal Feed Science and Technology,* **55,** 335 - 347.

Adogla-Bessa, T., Owen, E. and Adesogan, A. T. (1999) Ensiling of whole crop wheat with cellulase-hemicellulase based enzymes 3. Comparing effects of urea or enzyme treatment on forage composition and stability *Animal Feed Science and Technology,* **82,** 51 - 61.

Agriculture and Food Research Council (1992) Agricultural and Food Research Council: Technical Committee on response to nutrients: Nutritive requirements of ruminant animals: protein *Nutrition Abstracts and Reviews (Series B),* **Report No. 9,** 65 - 71.

Agriculture and Food Research Council (1993) *Energy and protein requirements of ruminants,* CAB International, Wallingford.

Agricultural Research Council (1980) *The nutrient requirements of ruminant livestock,* Commonwealth Agricultural Bureaux, Slough,

Allen, M. S. (1997) Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber *Journal of Dairy Science,* **80,** 1447 - 1462.

Arieli, A. and Adin, G. (1994) Effect of wheat silage maturity on digestion and milk yield in dairy cows *Journal of Dairy Science,* **77,** 237 - 243.

Ashbell, G., Weinberg, Z. G., Bruckental, I., Tabori, K. and Sharet, N. (1997) Wheat silage: effect of cultivar and stage of maturity on yield and degradability in situ *Journal of Agricultural and Food Chemistry,* **45,** 709 - 712.

Association of Official Analytical Chemists (2000) *Official methods of Analysis of AOAC International,* AOAC International, Maryland, USA.

Bal, M. A., Shaver, R. D., Jirovec, A. G., Shinners, K. J. and Coors, J. G. (2000) Crop processing and chop length of corn silage: Effects on intake, digestion, and milk production by dairy cows *Journal of Dairy Science,* **83,** 1264 - 1273.

Barry, T. N., Thompson, A. and Armstrong, D. G. (1977) Rumen fermentation studies on two constrasting diets. 2. Comparison of the performance of an in vitro continuous-culture fermentation with in vivo fermentation *Journal of Agricultural Science,* **89,** 197 - 208.

Bastiman, B. and Pullar, D. (1992) Yields, in-silo losses and feed quality of fermented and urea-treated whole-crop wheat In: *Forward with grass into Europe: Proceedings of the British Grassland Society Winter Meeting* Great Malvern, Wostershire 157 - 159

Bauman, D. E., Corl, B. A., Baumgard, L. H. and Griinari, J. M. (2001) Conjugated linoleic acid (CLA) and the dairy cow In: *Recent advances in animal nutrition,* (Eds, Garnsworthy, P. C. and Wiseman, J.) Nottingham University Press Nottingham 221 - 250.

Bauman, D. E. and Griinari, J. M. (2000) Historical perspective and recent developments in identifying the cause of diet-induced milk fat depression In: *Proceedings Cornell Nutrition Conference for Feed Manufacturers* Cornell Universit, Ithaca, NY, USA 191 - 202

Bauman, D. E. and Griinari, J. M. (2001) Regulation and nutritional manipulation of milk fat: low-fat milk syndrome *Livestock Production Science,* **70,** 15 - 29.

Bax, J. (1997a) *Alternative forages and feeds. 3. Fermented wholcrop cereals for dairy cattle,* SAC,

Bazeley, K. and Pinsent, P. J. N. (1984) Preliminary observations on a series of outbreaks of acute laminitis in dairy cattle *Veterinary Record,* **115,** 619 - 622.

Beauchemin, K. A. and Yang, W. Z. (2005) Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage *Journal of Dairy Science,* **88,** 2117 - 2129.

Bines, J. A. and Hart, I. C. (1984) The response of plasma insulin and other hormones to intraruminal infusion of VFA mixtures in cattle *Canadian Journal of Animal Science,* **64 (Suppl),** 304 - 305.

Blake, J. (1993) Cane molasses - nutritional liquid gold *Feed Compounder,* 30 - 31.

Blowey, R. (1993) The causes and prevention of lameness In: *Cattle lameness and hoof care. An illustrated guide,* (Eds, Farming Press Ipswich, UK 62 -.

Boatfield, G. (1983) *Farm crops,* Farming Press, Ipswich.

Bolsen, K. K. and Heidker, J. (1985) *Silage additives USA,* Chalcombe Publishers, Marlow.

Bolsen, K. K., Tetlow, R. M. and Wilson, R. F. (1983) The effect of calcium and sodium hydroxides and of sodium acrylate on the fermentation and digestibility in vitro of ensiled whole-crop wheat and barley harvested at different stages of maturity *Animal Feed Science and Technology,* **9,** 37 - 47.

Brigstocke, T. (2001) The UK dairy industry. Past, Present and future. *Feed Compounder,* 16-21.

Burgess, P. L., Misener, G. C., McQueen, R. E. and Nicholson, W. G. (1989) Evaluation of barley and wheat head-chop silages for dairy cow *Canadian Journal of Animal Science,* **69,** 947 - 954.

Buttery, P. J. (1977) Aspects of the biochemistry of rumen fermentation and their implication in ruminant productivity In: *Recent Advances in Animal Nutrition,* (Eds, Haresign, W. and Lewis, D.) Butterworth Inc. Boston, M.A.

Campling, R. C. (1991) Processing cereal grains for cattle - a review *Livestock Production Science,* **28,** 223 - 234.

Carro, M. D. and Miller, E. L. (1999) Effect of supplementing a fibre basal diet with different nitrogen forms on ruminal fermentation and microbial growth in an in vitro semi-continuous culture system (RUSITEC) *British Journal of Nutrition,* **82,** 149 - 157.

Castejon, M. and Leaver, J. D. (1994) Intake and digestibility of urea-treated whole crop wheat and liveweight gain by dairy heifers *Animal Feed Science and Technology,* **46,** 119 - 130.

Chadwick, L. (2004) *The Farm Management handbook 2004/05,* SAC, Edinburgh.

Chamberlain, D. G., Robertson, S. and Choung, J. J. (1993) Sugars versus starch as supplements to grass silage: Effects on ruminal fermentation and the supply of microbial protein to the small intestine, estimated from the urinary excretion of purine derivatives, in sheep *Journal of the Science of Food and Agriculture,* **63,** 189 - 194.

Chamberlain, D. G., Thomas, P. C., Wilson, W., Newbold, C. J. and MacDonald, J. C. (1985) The effects of carbohydrate supplements on ruminal concentrations of ammonia in animals given diets of grass silage *Journal of Agricultural Science, Cambridge,* **104,** 331 - 340.

Chesworth, J. M., Stuchbury, T. and Scaife, J. R. (1998) *Agricultural Biochemistry,* Chapman and Hall, Suffolk.

Choung, J. J. and Chamberlain, D. G. (1995) Effects of intraruminal infusion of propionate on the concentrations of ammonia and insulin in peripheral blood of cows receiving an intraruminal infusion of urea *Journal of Dairy Research,* **62,** 549 - 557.

Church, D. C. (1976) *Digestive physiology and nutrition of ruminants. Volume 1: Digestive physiology,*

Corral, A. J., Heard, A. J., Fenlon, J. S., Terry, Cora, P. and Lewis, G. C. (1977) Whole crop silage In: *Grassland Research Institute, Hurley, Technical Report No. 22,* (Eds, 35.

Crovetto, G. M., Galassi, G., Rapetti, L., Sandrucci, A. and Tamburini, A. (1998) Effect of the stage of maturity on the nutritive value of whole crop wheat silage *Livestock Production Science,* **55,** 21 - 32.

Czerkawski, J. W. and Breckenridge, G. (1977) Design and development of a long-term rumen simulation technique (RUSITEC) *British Journal of Nutrition,* **38,** 371 - 384.

De Veth, M. J. and Kolver, E. S. (2001) Digestion of ryegrass pasture in response to change in pH in continuous culture *Journal of Dairy Science,* **84,** 1449 - 1457.

Dennis, S. M., Arambel, M. J., Bartley, E. E. and Dayton, A. D. (1983) Effect of energy concentration and source of nitrogen on numbers and types of rumen protozoa *Journal of Dairy Science,* **66,** 1248 - 1254.

Deschard, G., Mason, V. C. and Tetlow, R. M. (1988) Treatment of whole-crop cereals with alkali. 4. Voluntary intake and growth in steers given wheat ensiled with sodium hydroxide, urea or ammonia *Animal Feed Science and Technology,* **19,** 55 - 66.

Deschard, G., Tetlow, R. M. and Mason, V. C. (1987) Treatment of whole-crop cereals with alkali. 3. Voluntary intake and digestibility studies in sheep given immature wheat ensiled with sodium hydroxide, urea or ammonia. *Animal Feed Science and Technology,* **18,** 283 - 293.

Dijkstra, J., Boer, H., Van Bruchem, J., Bruining, M. and Tamminga, S. (1993) Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume *British Journal of Nutrition,* **69,** 385 - 396.

Dougherty, R. W., Coburn, K. S., Cook, H. M. and Allison, M. J. (1975) Preliminary study of appearance of endotoxins in circulatory system of sheep and cattle after induced grain engorgement *American Journal of Veterinary Research,* **36,** 831 - 832.

Dowman, M. G. (1993) Modifications to the neutral detergent cellulase digestibility method for the prediction of the metabolisable energy of compound feedstuffs containing palm kernel meal *Journal of the Science of Food and Agriculture,* **61,** 327 - 331.

Dowman, M. G. and Collins, F. C. (1982) The use of enzymes to predict the digestibility of animal feeds *Journal of Science and Food Agriculture,* **33,** 689 - 696.

Environment Protection Agency (1984) *Methods for Chemical Analysis of Water and Wastes,* EPA, Cincinnati,.

Eun, J. S., Beauchemin, K. A., Hong, S. H. and Yang, W. Z. (2004) Effects of mechanical processing on the nutritive value of barley silage for lactating dairy cows *Journal of Dairy Science,* **87,** 4170 - 4177.

Everington, J. M. and Givens, D. I. (1988) Degradation characteristics of wheat, barley and oat straws untreated and treated with ammonia in an oven *Animal Production,* **46,** 508 (abstr.).

Farningham, D. A. H. and Whyte, C. C. (1993) The role of propionate and acetate in the control of food intake in sheep *British Journal of Nutrition,* **70,** 37 - 46.

Filya, I., Ashbell, G., Hen, Y. and Weinberg, Z. G. (2000) The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage *Animal Feed Science and Technology,* **88,** 39 - 46.

Firkins, J. L., Weiss, W. P. and Piwonka, E. J. (1992) Quantification of intraruminal recycling of microbial nitrogen using nitrogen-15 *Journal of Animal Science,* **70,** 3223 - 3233.

Fondevila, M., Castrillo, C., Gauda, J. A. and Balcells, J. (1994) Effect of ammonia treatment and carbohydrate supplementation of barley straw on rumen liquid characteristics and substrate degradation by sheep *Animal Feed Science and Technology,* **50,** 137 - 155.

Garnsworthy, P. C. and Stokes, D. T. (1993) The nutritive value of wheat and oat silages ensiled on three cutting dates *Journal of Agricultural Science, Cambridge,* **121,** 233 - 240.

Givens, D. I., Moss, A. R. and Adamson, A. H. (1993) The digestion and energy value of whole crop wheat treated with urea *Animal Feed Science and Technology,* **43,** 51 - 64.

Goering, H.K. and Van Soest, P.J. (1970) *Forage fiber analysis (apparatus, reagents, procedures and some applications).* Agricultural handbook no. 379, US Department of Agriculture, Washington, DC

Groot, J.C.J., Rossing, W.A.H. and Lantinga, E.A. (2006) Evolution of farm management, nitrogen efficiency and economic performance on Dutch dairy farms reducing external inputs *Livestock Science*, **100,** 99 - 110

Griinari, J. M. and Bauman, D. E. (1999) Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants In: *Advances in Conjugated linoleic acid research, Vol. 1*, (Eds, Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W. and Nelson, G. J.) AOCS Press, Champaign, IL 180 - 200.

Griinari, J. M. and Bauman, D. E. (2003) Update on theories of diet-induced milk fat depression and potential applications In: *Recent Advances in Animal Nutrition*, (Eds, Garnsworthy, P. C. and Wiseman, J.) Nottingham University Press Nottingham

Griinari, J. M., Bauman, D. E., Chilliard, Y., Perajoki, P. and Nurmela, K. (2000) Dietary influences on conjugated linoleic acids (CLA) in bovine milk fat In: *3rd meeting of the European Section of AOCS. Oils and Fats, from basic research to Industrial applications* Helsinki, Finland

Haddad, S. G., Grant, R. J. and Klopfenstein, T. J. (1995) Digestibility of alkali-treated wheat straw measured in vitro or in vivo using holstein heifers *Journal of Animal Science,* **73,** 3258 - 3265.

Hameleers, A. (1998) The effects of the inclusion of either maize silage, fermented whole crop wheat or urea-treated whole crop wheat in a diet based on a high-quality grass silage on the performance of dairy cows *Grass and Forage Science,* **53,** 157 - 163.

Hart, K.J. (2005) Evaluation of ensiled whole-crop legumes for ruminants. Harper-Adams University College, Edgmond, Newport, Shropshire

Harvey, J. J. (1992) Assessing whole-crop cereal maturity in the field In: *Whole-crop cereals*, (Eds, Stark, B. A. and Wilkinson, J. M.) Chalcombe Publications Kent 39-57.

Heinrichs, A. J., Buckmaster, D. R. and Lammers, B. P. (1999) Processing, mixing and particle size reduction of forages for dairy cattle *Journal of Animal Science,* **77,** 180 - 186.

Heinrichs, A. J. and Kononoff, P. (undated) Evaluating particle size of forages and TMRs using the New Penn State Forage Particle Separator. http://www.das.psu.edu/dcn/catforg/PARTICLE/pdf/DAS0242.pdf

Heron, S. (1996) *Wholecrop cereals,* Zeneca Bio Products,

HGCA (1988) In: *Assessments of wheat growth to support its production and improvement (volume 1).* (Eds, Sylvester-Bradley, R., Scott, R. K., Clare, R. W. and Duffield, S. J.) University of Nottingham/ADAS Centre of Research in Agronomy, ADAS Boxworth, Cambridge

Hill, J. and Leaver, J. D. (1991) Replacement of whole crop wheat by grass silage in the diet of dairy cows *Animal Production,* **52,** 606.

Hill, J. and Leaver, J. D. (1999a) Effect of stage of growth at harvest and level of urea application on chemical changes during storage of whole-crop wheat *Animal Feed Science and Technology,* **77,** 281 - 301.

Hill, J. and Leaver, J. D. (1999b) Energy and protein supplementation of lactating dairy cows offered urea treated whole-crop wheat as the sole forage *Animal Feed Science and Technology,* **82,** 177 - 193.

Hill, J. and Leaver, J. D. (2002) Changes in chemical composition and nutritive value of urea treated whole crop wheat during exposure to air *Animal Feed Science and Technology,* **102,** 181 - 195.

Hoffman, P. C., Sievert, S. J., Shaver, R. D., Welch, D. A. and Combs, D. K. (1993) In situ dry matter, protein, and fiber degradation of perennial forages *Journal of Dairy Science,* **76,** 2632 - 2643.

Hoover, W. H., Crawford, R. J. and Stern, M. D. (1982) Effects of solids and liquid flows on fermentation in continuous cultures. III. Solids retention time *Journal of Animal Science,* **54,** 849 - 854.

Hoover, W. H., Crooker, B. A. and Sniffen, C. J. (1976a) Effects of differential solid-liquid removal rates of protozoa numbers in continuous cultures of rumen contents *Journal of Animal Science,* **43,** 528 - 534.

Hoover, W. H., Knowlton, P. H., Stern, M. D. and Sniffen, C. J. (1976b) Effects of differential solid-liquid removal rates on fermentation parameters in continuous cultures of rumen contents *Journal of Animal Science,* **43,** 535 - 542.

Huhtanen, P., Hissa, K., Jaakkola, S. and Poutiainen, E. (1985) Enzymes as silage additives. Effect on fermentation quality, digestibility in sacco and performance in growing cattle. *Journal of Agricultural Science, Finland,* **57,** 284-292.

Hungate, R. E. (1966) *The rumen and Its Microbes,* Academic Press, New York.

Huntington, J. A. and Givens, D. I. (1995) The in situ technique for studying the rumen degradation of feeds: A review of the procedure *Nutrition Abstracts and Reviews (Series B),* **65,** 63 - 93.

Huntington, J. A. and Givens, D. I. (1997) Studies on in situ degradation of feeds in the rumen: 1. Effect of species, bag mobility and incubation sequence on dry matter disappearance *Animal Feed Science and Technology,* **64,** 227 - 241.

Hussain, A. and Miller, E. L. (1999) Effect of supplementation of sucrose and lactose with sodium bicarbonate on rumen metabolism and microbial protein synthesis in sheep In: *Proceedings of the Winter Meeting of the British Society of Animal Science* 28

Jackson, M. A. (2005) Improving the nutritive value of whole-crop wheat for dairy cows Harper Adams University College, Newport, Shropshire.

Jackson, M. A., Readman, R., 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,** 487 - 476.

Jaster, E. H. and Murphy, M. R. (1983) Effects of varying particle size of forage on digestion and chewing behaviour of dairy heifers *Journal of Dairy Science,* **66,** 802 - 810.

Johnson, L. M., Harrison, J. H., Hunt, C., Shinners, K., Doggett, C. G. and Sapienzas, D. (1999) Nutritive value of corn silage as affected by maturity and mechanical processing: A contemporary review *Journal of Dairy Science,* **82,** 2813 - 2825.

Jones, D.W. and Kay, J.J. (1976) Determination of volatile fatty acids C_1-C_6, and lactic acid in silage juice. *Journal of the Science of Food and Agriculture* **27:** 1005 - 1014

Keady, T. W. J. (2005) Ensiled maize and whole crop wheat forages for beef and dairy cattle: effects on animal performance In: *Proceedings of the XVth International Silage Conference* Belfast, Northern Ireland 65 - 82

Keady, T. W. J. and Murphy, J. J. (1998) The effects of ensiling and supplementation with sucrose and fish meal on forage intake and milk production of lactating dairy cows *Animal Science,* **66,** 9 - 20.

Kelly, E. F. and Leaver, J. D. (1990) Lameness in dairy cattle and the type of concentrate given *Animal Production,* **51,** 221 - 227.

Knowlton, K. F., Dawson, T. E., Glenn, B. P., Huntington, G. B. and Erdman, R. A. (1998) Glucose metabolism and milk yield of cows infused abomasally or ruminally with starch *Journal of Dairy Science,* **81,** 3248 - 3258.

Koenig, K. M., Beauchemin, K. A. and Rode, L. M. (2003) Effect of grain processing and silage on microbial protein synthesis and nutrient digestibility in beef cattle fed barley-based diets *Journal of Animal Science,* **81,** 1057 - 1067.

Korevaar, H. (1992) The nitrogen balance on intensive Dutch dairy farms: a review *Livestock Production Science,* **31,** 17 - 27.

Kristensen, V. F. (1992) The production and feeding of whole-crop cereals and legumes in Denmark In: *Whole-crop cereals*, (Eds, Stark, B. A. and Wilkinson, J. M.) Chalcombe Publications Canterbury 21 - 37.

Kung, L. and Huber, J. T. (1983) Performance of high producing cows in early lactation fed protein of varying amounts, sources, and degradability *Journal of Dairy Science,* **66,** 227 - 234.

Kung, L. and Muck, R. E. (1997) Animal response to additives In: *Proceedings of the silage: field to feedbunk North American Conference* Hershey, Pennsylvania

Kung, L., Treacher, R. J., Nauman, G. A., Smagala, A. M., Endres, K. M. and Cohen, M. A. (2000) The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows *Journal of Dairy Science,* **83,** 115 - 122.

Lammers, B. P., Buckmaster, D. R. and Heinrichs, A. J. (1996) A simple method for the analysis of particle sizes of forage and total mixed rations *Journal of Dairy Science,* **79,** 922 - 928.

Langhans, W. (1999) Appetite regulation In: *Protein metabolism and nutrition,* (Eds, Lobley, G. E., White, A. and MacRae, J.) Wageningen Press 225 - 251.

Leaver, J. D. and Hill, J. (1992) Feeding cattle on whole-crop cereals In: *Whole-crop cereals,* (Eds, Stark, B. A. and Wilkinson, J. M.) Chalcombe Publications Kingston, Near Canterbury, Kent 59 - 69.

Leaver, J. D. and Hill, J. (1995) The performance of dairy cows offered ensiled whole-crop wheat, urea-treated whole-crop wheat or sodium hydroxide-treated wheat grain and wheat straw in a mixture with grass silage *Animal Science,* **61,** 481 - 489.

Leaver, J. D. and Webster, D. M. (1982) Assessment of lameness in dairy cattle *West of Scotland Agricultural College, Chrichton Royal Farm Report 1982,*, 32 - 34.

Leonardi, C. and Armemtano, L. E. (2003) Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows *Journal of Dairy Science,* **66,** 557 - 564.

Lowman, B. G., Scott, N. A. and Somerville, S. H. (1976) Condition scoring of cattle *East of Scotland College of Agriculture bulletin No. 6.*

Maekawa, M., Beauchemin, K.A. and Christensen, D.A. (2002) Effect of concentrate level and feeding management on chewing activities, saliva production and ruminal pH of lactating dairy cows *Journal of Dairy Science,* **85,** 1165 - 1175

Maize Growers Association (1992) Wholecrop cereals. Technical Bulletin No. 29 Cedar, Reading.

Mansfield, H. R., Endres, M. I. and Stern, M. D. (1995) Comparison of microbial fermentation in the rumen of dairy cows and dual flow continuous culture *Animal Feed Science and Technology,* **55,** 47 - 66.

Manson, F. J. and Leaver, J. D. (1988a) The influence of concentrate amount on locomotion and clinical lameness in dairy cattle *Animal Production,* **47,** 185 - 190.

Manson, F. J. and Leaver, J. D. (1988b) The influence of dietary protein intake and of hoof trimming on lameness in dairy cattle *Animal Production,* **47,** 191 - 199.

McDonald, I. (1981) A revised model for the estimation of protein degradability in the rumen *Journal of Agricultural Science,* **96,** 251 - 252.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D. and Morgan, C. A. (1995) *Animal Nutrition,* Longman Scientific and Technical, Essex.

McDonald, P., Henderson, A. R. and Heron, S. J. E. (1991) *The biochemistry of silage,* Chalcombe Publications, Bucks.

McDougall, E. O. (1948) Studies on ruminany saliva. I. The composition and output of sheep's saliva *Biochemistry Journal,* **43,** 99.

McMeniman, N. P., Ben-Ghedalia, D. and Armstrong, D. G. (1976) Nitrogen-energy interations in rumen fermentation In: *Protein metabolism and nutrition,* (Eds, Cole, D. J. A., Boorman, K. N., Buttery, P. J., Lewis, D., Neale, R. J. and Swan, H.) Butterworth Inc. Boston, M.A.

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Schneider, W. (1979) The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liqour in vitro *Journal of Agricultural Science,* **93,** 217 - 222.

Merry, R. J., Jones, R. and Theodorou, M. K. (2000) The conservation of grass In: *Grass. Its production and utilization,* (Eds, Hopkins, A.) Blackwell Science Ltd Oxford 196 - 224.

Merry, R. J., Smith, R. H. and McAllan, A. B. (1987) Studies of rumen function in an in vitro continuous culture system *Archives of Animal Nutrition,* **37,** 475 - 488.

Mertens, D. R. (1997) Creating a system for meeting the fiber requirements of dairy cows *Journal of Dairy Science,* **80,** 1463 - 1481.

Mertens, D. R. (2000) Physically effective NDFand its use in the dairy rations explored *Feedstuffs,* 11 - 14.

Ministry of Agriculture, Fisheries and Food (1982) *The feedingstuffs (Sampling and Analysis) Regulations Statutory Instrument No. 1144,* HMSO, London.

Ministry of Agriculture, Fisheries and Food (1986) *The analysis of Agricultural materials. MAFF reference booklet 427,* HMSO, London.

Ministry of Agriculture, Fisheries and Food (1992) *Feed composition. UK tables of feed composition and nutritive value for ruminants,* Chalcombe Publications, Kent.

Ministry of Agriculture, Fisheries and Food (1993) *Prediction of the energy values of compound feeding stuffs for farm animals.* Ministry of Agriculture Fisheries and Food, London

Moloney, A. P., Almiladi, A. A., Drennan, M. J. and Caffrey, P. J. (1994) Rumen and blood variables in steers fed grass silage and rolled barley or sugar cane molasses-based supplements *Animal Feed Science and Technology,* **50,** 37 - 54.

Moos, T. C. and Sutton, J. D. (1998) The in situ degradability, neutral detergent fibre and starch in whole crop wheat harvested at three stages of maturity *Proceedings of the British Society of Animal Science,* 147.

Moss, A. R., Givens, D. I. and Everington, J. M. (1990) The effects of sodium hydroxide treatment on the chemical composition, digestibility and digestible energy content of wheat, barley and oat straws *Animal Feed Science and Technology,* **29,** 73 - 87.

Mould, F. L. and Ørskov, E. R. (1983) Manipulation of rumen fluid pH and its influence on cellulolysis in sacco, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate *Animal Feed Science and Technology,* **10,** 1 - 14.

Murphy, J. J. (1999) The effects of increasing the proportion of molasses in the diet of milking dairy cows on milk production and composition *Animal Feed Science and Technology,* **78,** 189 - 198.

Murphy, J. J., Kavanagh, S. and Fitzgerald, J. J. (2004) Comparative evaluation of grass silage, fermented whole crop wheat silage, urea-treated processed whole crop wheat silage and maize silage in the diet of early lactation cows. In: *Proceedings of the 55th Annual Meeting of the EAAP, Paper N 4.4* 95

Nielsen, T. K., Wolstrup, C., Witt, N., Kristensen, V. F. and Kraul, I. (1986) 4-methyl-imidazole in ammoniated roughage. Preliminary investigations In: *International Conference on Production Disease in Farm Animals* Belfast 293 - 296

Nilson, S. A. (1963) Clinical, morphological and experimental studies of laminitis in cattle *Acta Veterinaria Scandinavia,* **4,** 9 - 304.

Nocek, J.E. and Tamminga, S. (1991) Site of digestion of starch in the gastrointestinal-tract of dairy-cows and its effect on milk-yield and composition *Journal of Dairy Science* **74:** 3598 - 3629

Ohyama, Y., Morichi, T. and Masaki, S. (1975) The effect of inoculation with lactobacillus plantarum and addition of glucose at ensiling on the quality of aerated silages *Journal of the Science of Food and Agriculture,* **26,** 1001 - 1008.

O'Kiely, P. and Moloney, A. P. (1995) Performance of cattle offered whole-crop barley or wheat silage *Irish journal of Agricultural and Food Research,* **34,** 13 - 24.

O'Mara, F. P., Fitzgerald, J. J., Murphy, J. J. and Rath, M. (1998) The effect on milk production of replacing grass silage with maize silage in the diet of dairy cows *Livestock Production Science,* **55,** 79 - 87.

Ørskov, E. R. (1976) The effect of processing on digestion and utilization of cereals by ruminants *Proceedings of the Nutrition Society,* **35,** 245 - 252.

Ørskov, E. R. (1979) Recent advances in the understanding of cereal processing for ruminants In: *Recent Advances in Animal Nutrition,* (Eds, Haresign, W. and Lewis, D.) Butterworths London 123 - 132.

Ørskov, E. R. (1986) Starch digestion and utilization in ruminants *Journal of Animal Science,* **63,** 1624 - 1633.

Ørskov, E. R., Fraser, C. and Kay, R. N. B. (1969) Dietary factors influencing the digestion of starch in the rumen and small and large intestine of early weaned lambs *British Journal of Nutrition,* **23,** 217 - 226.

Ørskov, E. R. and Greenhalgh, J. F. D. (1977) Alkali treatment as a method of processing whole grain for cattle *Journal of Agricultural Science, Cambridge,* **89,** 253 - 255.

Ørskov, E. R. and Grubb, D. A. (1978) Validation of new systems for protein evaluation in ruminants by testing the effect of urea supplementation on intake and digestibility of

straw with or without sodium hydroxide treatment *Journal of Agricultural Science, Cambridge,* **91,** 483 - 486.

Ørskov, E. R., Kay, M. and Reid, G. W. (1989) Prediction of intake of straw and performance by cattle from chemical analysis, biological measurements and degradation characteristics In: *Evaluation of straws in ruminant feeding,* (Eds, Chenost, M. and Reiniger, P.) Elsevier Applied Science London 155 - 162.

Ørskov, E. R. and McDonald (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage *Journal of Agricultural Science,* **92,** 499 - 503.

Ørskov, E. R., Reid, G. W., Holland, S. M., Tait, C. A. G. and Lee, N. H. (1983) The feeding value for ruminants of straw and whole-crop barley and oats treated with anhydrous or aqeous ammonia or urea *Animal Feed Science and Technology,* **8,** 247 - 257.

Ørskov, E. R., Soliman, H. S. and MacDearmid, A. (1978) Intake of hay by cattle given supplements of barley subjected to various forms of physical treatment or treatment with alkali *Journal of Agricultural Science, Cambridge,* **90,** 611 - 615.

Overton, T. R., Cameron, M. R., Elliott, J. P. and clark, J. H. (1995) Ruminal fermentation and passage of nutrients to the duodenum of lactating cows fed mixtures of corn and barley *Journal of Dairy Science,* **78,** 1981 - 1998.

Owens, F. N. and Goetsch, A. L. (1988) Ruminal fermentation In: *The ruminant animal. Digestive physiology and nutrition,* (Eds, Church, D. C.) Prentice Hall New Jersey

Owens, F. N., Secrist, D. S., Hill, W. J. and Gill, D. R. (1998) Acidosis in cattle: A review *Journal of Animal Science,* **76,** 275 - 286.

Panditharatne, S., Allen, V. G., Fontenot, J. P. and Jayasuriya, M. C. N. (1989) Effect of stage of growth and chopping lenth on digestibility and palatibility of Guinea - 'A' grass silage *Journal of Animal Science,* **66,** 1005.

Patterson, D. C. and Kilpatrick, D. J. (2005) The effects of maize and whole crop wheat silages and quality of grass silage on the performance of lactating dairy cows In: *Proceedings of the XVth International Silage Conference* Belfast, Northern Ireland 165

Philippeau, C. and Michalet-Doreau, B. (1998) Influence of genotype and ensiling of corn grain on in situ degradation of starch in the rumen *Journal of Dairy Science,* **81,** 2178 - 2184.

Phipps, R. H. (1994) Complementary forages for milk production In: *Recent Advances in Animal Nutrition,* (Eds, Garnsworthy, P. C. and Cole, D. J. A.) Nottingham University Press Nottingham 215 - 230.

Phipps, R. H. (1996) A crop from over there that's doing rather well over here: Forage maize in the diet of the lactating dairy cow *Journal of the Royal Agricultural Society of England,* **157,** 103 - 115.

Phipps, R. H., Sutton, J. D. and Jones, B. A. (1995) Forage mixtures for dairy cows: the effect on dry-matter intake and milk production of incorporating either fermented or urea-treated whole-crop wheat, brewers' grains, fodder beet or maize silage into diets based on grass silage *Animal Science,* **61,** 491 - 496.

Phipps, R. H., Weller, R. F. and Siviter, J. W. (1992) Whole-crop cereals for dairy cows In: *Whole-crop cereals*, (Eds, Stark, B. A. and Wilkinson, J. M.) Chalcombe Publications Canterbury 51 - 57.

Playne, M. J., Khummalthong, W. and Echevarria, M. G. (1978) Factors affecting the digestion of oesophageal fistula samples and hay samples in nylon bags in the rumen of cattle *Journal of Agricultural Science,* **90,** 193 - 204.

Poppi, D. P., Hendricksen, R. E. and Minson, D. J. (1985) The relative resistance to escape of leaf and stem particles from the rumen of cattle and sheep *Journal of Agricultural Science,* **105,** 9 - 14.

Prigge, E. C., Baker, M. J. and Varga, G. A. (1984) Comparative digestion, rumen fermentation and kinetics of forage diets by steers and wethers *Journal of Animal Science,* **59,** 237 - 245.

Raymond, F. and Waltham, R. (1996) *Forage conservation and feeding,* Farming Press, Ipswich.

Redman and Knight (1992) Machinery for whole-crop cereals In: *Whole-crop cereals*, (Eds, Stark, B. A. and Wilkinson, J. M.) Chalcombe Publications Kent

Reynolds, C. K., Cammell, S. B., Humphries, D. J., Beever, D. E., Sutton, J. D. and J.R., N. (2001) Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows *Journal of Dairy Science,* **84,** 2250 - 2259.

Reynolds, C. K., Sutton, J. D. and Beever, D. E. (1997) Effects of feeding starch to dairy cattle on nutrient availability and production In: *Recent advances in Animal Nutrition*, (Eds, Garnsworthy, P. C. and Wiseman, J.) Nottingham University Press Nottingham 105 - 134.

Richardson, J. M., Wilkinson, R. G. and Sinclair, L. A. (2003) Synchrony of nutrient supply to the rumen and dietary energy source and their effects on the growth and metabolism of lambs *Journal of Animal Science,* **81,** 1332 - 1347.

Rode, L. M., Yang, W. Z. and Beauchemin, K. A. (1999) Fibrolytic enzyme supplements for dairy cows in early lactation *Journal of Dairy Science,* **82,** 2121 - 2126.

Rufener, W. H., Jr., Nelson, W. O. and Wolin, M. J. (1963) Maintenance of the rumen microbial population in continuous culture *Applied Microbiology,* **11,** 374.

Sahnoune, S., Besle, J. M., Chenost, M., Jouany, J. P. and Combes, D. (1991) Treatment of straw with urea. 1. Ureolysis in a low water medium *Animal Feed Science and Technology,* **34,** 75 - 93.

Siddons, R. C., Nolan, J. V., Beever, D. E. and MacRae (1985) Nitrogen digestion and metabolism in sheep consuming diets containing contrasting forms and levels of N *British Journal of Nutrition,* **54,** 175 - 187.

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R. and Buttery, P. J. (1993) Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep *Journal of Agricultural Science,* **120,** 251 - 263.

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R. and Buttery, P. J. (1995) Effects of synchronizing the rate of dietary energy and nitrogen release in diets with a similar

carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep *Journal of Agricultural Science,* **124,** 463 - 472.

Sinclair, L. A., Jackson, M. A., Huntington, J. A. and Readman, R. J. (2005) The effects of processed, urea-treated whole-crop wheat or maize silage and supplementation of whole-crop wheat on the performance of dairy cows *Livestock Production Science,* **95,** 1 - 10.

Sinclair, L. A., Wilkinson, R. G. and Ferguson, D. M. R. (2003) Effects of crop maturity and cutting height on the nutritive value of fermented whole crop wheat and milk production in dairy cows *Livestock Production Science,* **81,** 257 - 269.

Slyter, L. L. (1976) Influence of acidosis on rumen metabolism *Journal of Animal Science,* **43,** 910 - 929.

Slyter, L. L., Nelson, W. O. and Wolin, M. J. (1964) Modifications of a device for maintenance of the rumen microbial population in continuous culture *Applied Microbiology,* **12,** 374 - 377.

Slyter, L. L. and Putnam, P. A. (1967) In vivo vs. in vitro continuous culture of ruminal microbial populations *Journal of Animal Science,* **26,** 1421 - 1427.

Southworth, J. E., Gilman, K. M., Raeside, A. J., Wilkinson, R. G., Sinclair, L. A. and Huntington, J. A. (1999) The effect of stage of maturity and endosperm type on the in situ dry matter degradability of fermented whole crop wheat silage *Proceedings of the British Society of Animal Science.*

Spears, R.A., Kohn, R.A. and Young, A.J. (2003) Whole-farm nitrogen balance on western dairy farms *Journal of Dairy Science* **86,** 4178 - 4186

Stern, M. D., Bach, A. and Calsamiglia, S. (1997) Alternative techniques for measuring nutrient digestion in ruminants *Journal of Animal Science,* **75,** 2256 - 2276.

Stern, M. D. and Hoover, W. H. (1979) Methods for determining and factors affecting rumen microbial protein synthesis: A review *Journal of Animal Science,* **49,** 1590 - 1603.

Stewart, D. G., Warner, R. G. and Seeley, H. W. (1961) Continuous culture as a method for studying rumen fermentation *Applied Microbiology,* **9,** 150 - 156.

Stoskopf, N. C. (1981) *Understanding crop production,* Reston Publishing Company, Reston, Va.

Strobel, H. J. and Russel, J. B. (1986) Effect of pH and energy spilling on bacterial protein-synthesis by carbohydrate-limited cultures of mixed rumen bacteria *Journal of Dairy Science,* **69,** 2941 - 2947.

Sutton, J. D. (1985) Symposium - Energy nutrition and metabolism of the lactating cow - digestion and absorption of energy substrates in the lactating cows *Journal of Dairy Science,* **68,** 3376 - 3393.

Sutton, J. D., Abdalla, A. L., Phipps, R. H., Cammell, S. B. and Humphries, D. J. (1997) The effect of the replacement of grass silage by increasing proportions of urea-treated whole-crop wheat on food intake and apparent digestibility and milk production by dairy cows *Animal Science,* **65,** 343 - 351.

Sutton, J. D., Cammell, S. B., Beever, D. E., Humphries, D. J. and Phipps, R. H. (1998) Energy and nitrogen balance of lactating dairy cows give mixtures of urea-treated whole-crop wheat and grass silage *Animal Science,* **67,** 203 - 212.

Sutton, J. D., Phipps, R. H., Cammell, S. B. and Humphries, D. J. (2001) Attempts to improve the utilization of urea-treated whole-crop wheat by lactating dairy cows *Animal Science,* **73,** 137 - 147.

Sutton, J. D., Phipps, R. H., Deaville, E. R., Jones, A. K. and Humphries, D. J. (2002) Whole-crop wheat for dairy cows: effects of crop maturity, a silage inoculant and an enzyme added before feeding on food intake and digestibility and milk production *Animal Science,* **74,** 307 - 318.

Tamminga, S. and Van Vuuren, A. M. (1988) Formation and utilisation of end products of lignocellulose degradation in the rumen *Animal Feed Science and Technology,* **21,** 141.

Tetlow, R. M. (1992) A decade of research into whole-crop cereals at Hurley In: *Whole-crop cereals,* (Eds, Stark, B. A. and Wilkinson, J. M.) Chalcombe Publications Canterbury 1 - 19.

Tetlow, R. M. and Mason, V. C. (1987) Treatment of whole-crop cereals with alkali. 1. The influence of sodium hydroxide and ensiling on the chemical composition and in vitro digestibility of rye, barley and wheat crops harvested at increasing maturity and dry matter content *Animal Feed Science and Technology,* **18,** 257 - 269.

Tetlow, R. M., Mason, V. C. and Deschard, G. (1987) Treatment of whole-crop cereals with alkali. 2. Voluntary intake and digestibility by sheep of rye, barley and wheat crops ensiled with sodium hydroxide *Animal Feed Science and Technology,* **18,** 271 - 281.

Theodorou, M. K., Williams, B. A., Dhanoa, M. S., McAllan, A. B. and France, J. (1994) A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds *Animal Feed Science and Technology,* **48,** 185 - 197.

Theurer, C. B. (1986) Grain processing effects on starch utilization by ruminants *Journal of Animal Science,* **63,** 1649 - 1662.

Thomas, C. (2004) *Feed into Milk,* Nottingham University Press, Nottingham.

Thomas, C. and Thomas, P. C. (1989) Response to supplementary feeding In: *British Grassland Society Occassional Symposium* 49 - 60

Tilley, J. M. A. and Terry, R. A. (1963) A two-stage technique for the in vitro digestion of forage crops *Journal of the British Grassland Society,* **18,** 104 - 111.

Tottman, D. R. and Broad, H. (1987) The decimal code for the growth stages of cereals with illustrations *Annals of Applied Biology,* **110,** 441 - 454.

Tyrell, H. F. and Reid, J. T. (1965) Prediction of the energy value of cow's milk *Journal of Dairy Science,* **48,** 1215 - 1223.

Vallimont, J. E., Bargo, F., Cassidy, T. W., Luchini, N. D., Broderick, G. A. and Varga, G. A. (2004) Effects of replacing dietary starch with sucrose on ruminal fermentation and nitrogen metabolism in continuous culture *Journal of Dairy Science,* **87,** 4221 - 4229.

Van Keulen, J. and Young, B. A. (1977) Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies *Journal of Animal Science,* **44,** 282 - 287.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991) Methods for dietary fiber, neutral detergenr fiber and nonstarch polysaccharides in relation to animal nutrition *Journal of Dairy Science,* **71:** 3583-3597

Vazquez-Anon, M., Cassidy, T. W., McCullough, P. and Varga, G. A. (2000) Effects of alimet on nutrient digestibility, bacterial protein synthesis, and ruminal disappearance during continuous culture *Journal of Dairy Science,* **84,** 159 - 166.

von Keyerlingk, M. A. G., Swift, M. L., Puchala, R. and Shelford, J. A. (1996) Degradability characteristics of dry matter and crude protein of forages in ruminants *Animal Feed Science and Technology,* **57,** 291 - 311.

Ward, W. R., Murray, R. D., White, A. R. and Rees, E. M. (1995) The use of blood biochemistry for determining the nutritional status of dairy cows In: *Recent advances in animal nutrition 1995*, (Eds, Garnsworthy, P. C. and Cole, D. J. A.) Nottingham University Press Nottingham 29 - 51.

Watson, M. E. and Galliher, T. L. (2001) Comparison of dumas and kjeldahl methods with automatic analyzers on agricultural samples under routine rapid analysis conditions *Communications in Soil Science and Plant Analysis,* **32,** 2007 - 2019.

Weinberg, Z. G., Ashbell, G., Azrieli, A. and Bruckental, I. (1993) Ensiling peas, ryegrass and wheat with additives of lactic acid bacteria (LAB) and cell wall degrading enzymes *Grass and Forage Science,* **78,** 70 - 78.

Weinberg, Z. G., Ashbell, G., Hen, Y. and Harduf, Z. (1991) Ensiling whole wheat for ruminant feeding at different stages of maturity *Animal Feed Science and Technology,* **32,** 313 - 320.

Weller, R. F., Cooper, A. and Dhanoa, M. S. (1995) The selection of winter wheat varieties for whole-crop cereal conservation *Grass and Forage Science,* **50,** 172 - 177.

Williams, P. E. V., Innes, G. M. and Brewer, A. (1984) Ammonia treatment of straw via the hydrolysis of urea. II. Additions of soya bean (urease), sodium hydroxide and molasses; effects on the digestibility of urea-treated straw *Animal Feed Science and Technology,* **11,** 115 - 124.

Wiseman, A. J. L., Finch, H. J. S. and Samuel, A. M. (2000) *Lockhart and Wiseman's crop husbandry,* Woodhead Publishing Ltd, Cambridge.

Witt, M. W., Sinclair, L. A., Wilkinson, J. M. and Buttery, P. J. (1999) The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on the metabolism and growth of ram lambs given food at a restricted level *Animal Science,* **69,** 627 - 636.

Witt, M. W., Sinclair, L. A., Wilkinson, J. M. and Buttery, P. J. (2000) The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on milk production and metabolism of ewes offered grass silage based diets *Animal Science,* **71,** 187 - 195.

Woodford, S.T. and Murphy, M.R. (1988) Effect of forage physical form on chewing activity, dry matter intake, and rumen function of dairy-cows in early lactation *Journal of Dairy Science* **71:** 674 - 686

Yan, T., Roberts, D. J. and Higginbotham, J. (1997) The effects of feeding high concentrations of molasses and supplementing with nitrogen and unprotected tallow on intake and performance of dairy cows *Animal Science,* **64,** 17 - 24.

Yang, W. Z., Beauchemin, K. A. and Rode, L. M. (2000) A comparison of methods of adding fibrolytic enzymes to lactating cow diets *Journal of Dairy Science,* **83,** 2512 - 2520.

Yang, W. Z., Beauchemin, K. A. and Rode, L. M. (2001) Effects of grain processing, forage to concentrate ratio and forage particle size on rumen pH and digestion by dairy cows *Journal of Dairy Science,* **84,** 2203 - 2216.

Zadoks, J. C., Chang, T. T. and Konzak, C. F. (1974) A decimal code for the growth stages of cereals *Weed Research,* **14,** 415 - 421.

APPENDIX 1: Total farm Nitrogen efficiencies

Areas of grass silage and WCW were calculated according to the ratio of DM in the diets offered, assuming that grass silage yielded 10.8 t DM/ha (Chadwick, 2004) and the WCW in the Chapter 3 yielded 16.9, 17.7, 16.2 t DM/ha for fermented WCW (F-45), urea-treated WCW harvested at approximately 700 g DM/kg (U-70) and 850 g DM/kg (U-85 and C-85) respectively. In Chapter 4 WCW yielded 15.2 t DM/ha. The long straw WCW (LU and LP) in the study reported by Jackson *et al.* (2004) yielded 18.2 t DM/ha whilst the short straw WCW (SU and SP) yielded 14.9 t DM/ha. The WCW in the study reported by Sinclair *et al.* (2005) yielded 11.6 t DM/ha. Fertiliser N application was calculated assuming grass silage required 300 kg N/ha (Chadwick, 2004). Whole-crop wheat required 200 kg N/ha (Chapter 3), 130 kg N/ha (Chapter 4), 155 kg N/ha (Jackson *et al.,* 2004) and 136 kg N/ha (Sinclair *et al.,* 2005). Livestock units (per ha) were calculated according to the mean daily forage intake of cattle over 105 d (length of the experiment) where one livestock unit is equivalent to one dairy cow (Chadwick, 2004). Urea-treated forages were treated with 20 kg urea/t DM of a urea plus urease additive (Home n'Dry, Volac, Royston, UK) which was assumed to provide 7.12 g N/kg DM (Jackson, 2005). Prilled urea, assumed to provide 7.2 g N/kg DM (Jackson, 2005) was added to the fermented (F-45) and untreated (C-85) WCW forages at a rate of 150 g/cow/d in Chapter 3. Total N provided from the urea was multiplied up to the number of animals per ha. The concentrate and straights N was calculated from the crude protein concentration of the concentrates fed during the experiments and multiplied up to the number of animals per ha. Milk N (kg/d) was calculated by multiplying daily average milk yield (kg/d) by milk protein (g/kg) and divided by the conversion factor 6.38 (ARC, 1980). Milk N was multiplied up to give a milk N output value (kg N/ha). N efficiencies were calculated by dividing the milk N output by total N input.