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Effect of rate of substitution of processed, urea-treated whole-crop wheat for grass silage on the intake, milk production and diet digestibility in dairy cows and ruminal metabolism *in vitro*

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The effect of rate of substitution of processed, urea-treated whole-crop wheat (pWCW) for grass silage on intake, performance and whole-tract digestibility was evaluated using 44 dairy cows. Cows received 10.5 kg of concentrates per day and one of the following forage mixtures (dry matter (DM) basis): grass silage alone (W-0); 0.75 grass silage, 0.25 pWCW (W-25); 0.5 grass silage, 0.5 pWCW (W-50) or 0.25 grass silage, 0.75 pWCW (W-75). Forage DM intake increased linearly with inclusion rate of pWCW from 9.7 kg DM per day in cows fed W-0 to 14.6 kg DM per day in W-75. By contrast, milk and protein yield (kg/day) were higher ($P < 0.05$) in cows receiving W-25 compared with W-0, but there was no effect ($P > 0.05$) of treatment on fat yield (kg/day). From week 11 of the experiment onwards, body condition score increased with rate of inclusion of pWCW ($P < 0.05$). Whole-tract apparent digestibility of organic matter (OM) and fibre (kg/kg), decreased linearly with rate of inclusion of pWCW. Assuming a constant digestibility of starch in the other diet components, the apparent digestibility of starch in pWCW was 0.95 kg/kg and was not affected by rate of inclusion ($P > 0.05$). Four continuous culture vessels were used to determine the effect of rate of inclusion of pWCW on ruminal metabolism in four periods, each of 14 d duration with sampling conducted on days 9 to 14. Vessel ammonia concentration increased linearly ($P < 0.05$) with rate of inclusion of pWCW whilst mean pH tended ($P = 0.06$) to decrease. The ratio of acetate to propionate increased from 2.5 in vessels receiving W-0 to 3.2 in those receiving W-75 ($P < 0.001$). There was no effect ($P > 0.05$) of treatment on digestibility (g/g) of OM, fibre or starch or microbial protein flow (g/day). It is concluded that forage DM intake increased linearly with rate of inclusion of pWCW, but there was no further improvement in milk yield from inclusion rates above 0.25 of the forage DM, with body condition score increasing instead. Increasing the inclusion rate of pWCW resulted in a more ketogenic volatile fatty acid profile but did not affect the efficiency of microbial protein synthesis when determined in vitro.

Keywords: dairy cows, digestibility, in vitro *digestion, whole-crop wheat*

Introduction

Whole-crop wheat (WCW) is an alternative forage to grass silage, particularly in temperate grass growing areas where forages such as maize do not grow well. Compared with grass silage, traditional means of preserving whole-crop cereals, either by fermenting at lower dry matter (DM) values (300 to 500 g/kg) or urea-treatment at higher DM values (600 to 700 g/kg), have resulted in a significant increase in DM intake but had little effect on milk yield (Hameleers, 1998; Sutton *et al.*, 2001; Sinclair *et al.*, 2003). Studies that have compared WCW with good-quality maize silage have reported a lower milk yield (Phipps *et al.*, 1995). Abdalla *et al.* (1999) and Sutton *et al.* (1998) demonstrated that ruminal and whole-tract digestibility of fibre and particularly starch was depressed in cows fed diets containing urea-treated WCW, and suggested that improvement of ruminal starch digestibility may enhance performance.

Recently, a processor that is fitted to the forage harvester has been developed that allows the grains in WCW to be cracked prior to ensiling. Jackson *et al.* (2004) reported that processing higher DM WCW at harvest and preserving with urea (pWCW) increased forage starch digestibility from 0.80 to 0.97 kg/kg, and reduced the number of whole grains excreted in the faeces. The cows responded to this greater nutrient availability by decreasing

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forage intake, with little effect on milk yield (Jackson *et al*., 2004). When compared with maize silage, the inclusion of pWCW resulted in a similar milk and milk component yield (kg/day), although forage DM intake was substantially higher (Sinclair *et al*., 2005; Murphy *et al*., 2004).

Inclusion rate of urea-treated WCW has ranged from 0.22 of the forage DM (Hill and Leaver, 1991) to 1.0 (Hill and Leaver, 1999) but there has been no consistent effect on dairy cow performance. Sutton *et al.* (1998) reported that milk yield tended to be highest when cows were fed unprocessed, urea-treated WCW at 0.67 of the forage DM, and this inclusion rate has been used in the majority of recent experiments that have evaluated pWCW (Jackson *et al*., 2004; Murphy *et al*., 2004; Sinclair *et al*., 2005). One objective of the current experiment was therefore to investigate the effects of rate of inclusion of pWCW on the intake, performance and whole-tract digestibility in dairy cows. An additional objective was to examine the effects of inclusion rate of pWCW on ruminal metabolism because despite the large influence of forage digestion in the rumen on animal performance (Abdalla *et al*., 1999), few studies have been conducted in this area. The use of continuous culture fermentors to simulate the ruminal environment enables the study of factors that may affect the microbial ecology and digestion of nutrients (Stern *et al*., 1997) whilst providing a cheaper, more rapid and repeatable alternative to *in vivo* studies (Owens and Goetsch, 1988).

Material and methods

The work described in this paper was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986 (Her Majesty's Stationery Office, 2000).

Forage production

A commercial crop of winter wheat (cv. Equinox) was grown using standard agronomic practice on a sandy clay loam soil following a 3-year grass ley. Crop DM was monitored twice weekly from approximately 4 weeks pre-harvest by cutting two adjacent 0.5 m lengths of row at approximately 15 cm stubble height at six random positions throughout the field and air drying to constant weight at 105 °C. The crop was harvested on the 4 August 2003 (growth stage (GS) 92; Tottman and Broad, 1987) and cut and processed using a forager fitted with a forage mill as described by Jackson *et al.* (2004). The forage was ensiled in a concrete walled roofed silage clamp and a urea and urease additive (Volac Home n'Dry, Volac, Royston, UK) added to provide 20 kg of urea per tonne of forage DM. The clamp was rolled well and sealed using a double layer of plastic sheeting and weighted down with tyres. The grass silage was a first-cut harvested using a self propelled, precision-chop forage harvester (Class Jaguar 800 series, Claas, Bury St Edmonds, UK) on 30 May 2003 from a predominantly perennial ryegrass sward, wilted for 24 h, received no additive and was ensiled in a concrete-walled clamp. The grass silage and pWCW were ensiled for approximately 180 and 110 days respectively prior to feeding.

Immediately prior to harvesting, the wheat crop DM yield was assessed by cutting an area of 0.72 m^2 to ground level at 10 random positions within the area to be harvested. The material was separated into ensiled material and residual material remaining in the field after harvest by cutting at 19 cm above the stem base. Grain yield was determined by cutting ears from the ensiled material and threshing using a single ear thresher (Wintersteiger-AG, 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 mean crop height prior to harvest was 68.4 (s.d. 3.9) cm, and the mean stubble height post-harvest 19.0 (s.d. 1.9) cm. Total above ground DM yield was 17.2 t DM per ha and mean crop yield 15.2 t DM per ha, with a grain yield (adjusted to 15% moisture) of 10.4 t/ha.

Animals and experimental procedure

Forty-four Holstein-Friesian dairy cows (four prima and 40 multiparous) that were on average 29 (s.d. +/− 5.5) days into lactation were used in a 14-week continuous design. Based on recordings taken when the cows were in week 3 of lactation, animals were blocked according to parity (prima or multi), calving date, milk yield, condition score (Lowman *et al*., 1976) and live weight and randomly allocated to one of four dietary treatments. Cows were housed in cubicles fitted with rubber mattresses, bedded with chopped paper twice weekly with the loafing area cleaned using automatic scrapers four times daily. All animals had continuous access to water.

The four dietary treatments were formulated according to Agricultural and Food Research Council (AFRC, 1995) and varied in their inclusion rate of grass silage and pWCW (DM basis): grass silage alone (W-0); 0.75 grass silage, 0.25 pWCW (W-25); 0.5 grass silage, 0.5 pWCW (W-50) or 0.25 grass silage, 0.75 pWCW (W-75). Two forage samples were taken weekly; one was oven dried at 100 °C to constant weight to maintain the grass silage to pWCW ratio and the remaining sample frozen at −20 °C prior to subsequent analysis. All animals received 7.0 kg/day of a standard dairy concentrate (g/kg: 200 wheat, 150 rapeseed meal, 140 dried sugar-beet feed, 140 palm kernel extract, 120 sunflower meal, 100 soya-bean meal, 60 molasses, 42 Megalac® (Volac UK Ltd, Royston, UK), 20 fatty acid distillate, 13.2 limestone, 10 calcined magnesite, 3.8 salt and 1 mineral and vitamin pre-mix (which provided on a total-concentrate basis (mg/kg): Cu 40; Mn 80; Zn 120; Co 1; I 6; Se 0.5; retinol 2.4; cholecalciferol 0.05; and alpha-tocopherol acetate 30) in two equal meals through out of parlour feeders. Each cow also received 2 kg/day of rapeseed meal, 1.5 kg/day of sugar-beet feed and 0.5 kg/day of Lactofeed (a lactose containing supplement; Volac

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UK Ltd, Royston, UK), which was mixed with the forage mixture and fed through electronic food bins which measured intake by weigh cells located under the bins and individual collar transponders fitted onto each cow (Insentec, Marknesse, Holland). The forage mixture was fed once daily at approximately 0900 h at a rate of 1.05 of the calculated intake, with refusals collected twice weekly.

Cows were milked twice daily at approximately 0630 and 1630 h with yield recorded at each milking and samples taken on a Mon p.m. and Tues a.m. for subsequent analysis. Cows were weighed, condition scored and locomotion scored (Manson and Leaver, 1988) weekly at 1400 h. Blood samples were collected by venipuncture from the coccygeal vein from a subsample of six cows per treatment during weeks 0, 3, 8 and 13 of the experiment. The individual blood samples were taken at 1100 h into two vacutainer tubes containing either potassium oxalate or lithium heparin as anti-coagulants, centrifuged immediately and the plasma stored at −20 °C prior to subsequent analysis. Diet apparent digestibility was measured in five cows per treatment when they were in week 10 of the experiment, using acid insoluble ash as an indigestible marker (Van Keulen and Young, 1977). For 7 days approximately 100 g of faeces were collected at 0900 and 1500 h from each cow by grab sampling and stored at −20 °C prior to subsequent analysis.

In vitro *digestibility and metabolism*

An *in vitro* continuous culture system adapted from Hoover *et al.* (1976) was used. Four double-layered glass *in vitro* fermentor vessels of 1.18 l volume were maintained at 39 °C by continuously pumping water from a bath (Ecoline RE106, Lauda, Germany) around the jacket of each vessel. Each vessel had a 20 mm internal diameter overflow port and artificial saliva (McDougall, 1948), modified to contain 13.8 mg/l ammonium sulphate and diluted 60:40 with deionized water; Rufener *et al.*, 1963) was infused continuously at the rate of 69 ml/h via a port in the head plate which also permitted a continuous flow of CO_2 and monitoring of vessel temperature. Vessel contents were mixed using a motor controlled stirrer (Crouzet Ltd, Hampshire, UK) attached to the head-plate, with the contents mixed 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 absorbed through the raft. Outflow was collected from the overflow port into a container maintained at 3 °C, with contents removed three times daily and stored at −20 °C.

Four wether sheep fitted with permanent rumen cannulae (39 mm) that were group housed and bedded on chopped straw were used. The sheep had continuous access to water and were fed a forage mixture consisting of grass silage and pWCW (50:50 DM basis), twice daily at 0800 h and 1600 h at 1.1 maintenance energy requirements (AFRC, 1995). The animals were fed the basal diet for 2 weeks prior to the first inoculum collection and were not bedded up 2 days prior to rumen fluid collection. Rumen fluid was removed by suction 4 h after the morning feed into pre-heated vacuum flasks under CO_2, pooled between sheep and strained through four layers of muslin cloth at 39 °C. The vessels, which were previously flushed with CO_2 and filled with 510 ml artificial saliva, were then inoculated with 670 ml of rumen fluid. New rumen fluid was used to charge the vessels at the beginning of each experimental period.

The vessels were fed 30 g DM per day of one of four dietary treatments in a 4×4 Latin-square design with each period lasting 14 days. The diets consisted of (g DM per day) 8.25 dairy concentrate, 2.4 rapeseed meal, 1.8 sugar-beet feed, 0.6 Lactofeed (Volac UK Ltd, Royston, UK) and 16.95 forage mix. The forage mix contained (DM basis) either grass silage alone (W-0); 0.75 grass silage, 0.25 WCW (W-25); 0.50 grass silage, 0.50 WCW (W-50) or 0.25 grass silage, 0.75 WCW (W-75). All feed ingredients were freeze-dried and ground through a 3-mm screen and the feed introduced via a 25-mm aperture in the head plate in three equal meals at 0800, 1600 and 0000 h.

Days 1 to 8 of each period were used as an adaptation period with sampling occurring on days 9 to 14. Protozoa were counted on days 1 and 13 by adding 5 ml of vessel outflow to 4 g/l formaldehyde in saline (0.9 g/l NaCl) and the mixture stored at room temperature prior to manual counting. An outflow sub-sample (10 ml) was collected hourly between 0800 and 1600 h on day 9, the pH measured, and the sample mixed with 1 ml of HCl (2 mol/l) and stored at −20 °C prior to subsequent volatile fatty acid (VFA) and ammonium-nitrogen analysis. At 1200 h on day 9, 5.98 mg of ^{15}N (98% enriched $SO_4(^{15}NH_4)_2$: Sigma Chemical, Poole, UK) was added to each vessel to instantaneously label the ammonia-N pool and a solution of $SO_4(^{15}NH_4)_2$ added to the artificial saliva to replace the ammonium sulphate and result in an infusion rate of 2.93 mg ^{15}N per day per vessel. During days 9 to 14 of each period the total overflow was bulked on a daily basis and stored at −20 °C prior to freeze drying, and on days 12 to 14 a subsample was taken for ^{15}N analysis. On day 14, final vessel contents were homogenised in a blender at a low speed for 1 min prior to isolation of a bacterial pellet as described by Carro and Miller (1999).

Chemical analysis

Feed, faecal and freeze dried vessel effluent outflow samples were analysed as described previously (Jackson *et al.*, 2004) whilst feed and faecal samples were also analysed for acid insoluble ash (Van Keulen and Young, 1977). Particle size distribution of the four total mixed rations were measured using a Penn State Separator fitted with three sieves with screen sizes of 19.0, 8.0 and 1.18 mm (Kononoff *et al.*, 2003) and the content of physically effective fibre (peNDF) calculated as the product of the proportion of DM above 1.18 mm and the neutral-detergent fibre (NDF) content of the diet (Mertens, 1997). Milk samples were analysed for fat, protein and lactose using a Lactoscope FTIR spectrophotometer (Delta Instruments B.V.,

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Drachten, Holland). Blood samples were analysed for urea (Randox Laboratories kit catalogue no. UR 221), albumin (Randox Laboratories kit catalogue no. AB 361), glucose (Randox Laboratories kit catalogue no. GL 1611), β-hydroxybutyrate (BHB; Randox laboratories, kit catalogue no. RB 1008), and non-esterified fatty acids (NEFA; Randox Laboratories kit catalogue no. FA 115) using a Cobas Miras Plus autoanalyser (ABX Diagnostics, Bedfordshire, UK). Plasma insulin concentrations were determined using a ^{125}I-labelled insulin double-antibody radio-immunoassay as described by Richardson *et al.* (2003). The sensitivity of the assay at ED85 was 2.36 μIU per ml and the intra-assay coefficients of variation for low and high-quality controls were 5.9 and 10.4%, respectively.

The continuous culture effluent outflow samples for VFA analysis were centrifuged at 25 000 **g** for 20 min, the supernatant decanted, filtered through cellulose filter paper (Whatman, cellulose nitrate 0.2 μm) and the residue discarded. The filtrate was analysed for VFA by gas-liquid chromatography as described by Witt *et al.* (1999) using a DB-FFAP capillary column of 30 m length and 0.25 mm internal diameter (J & W Scientific, Folsom, California, USA) and phenol as an internal standard. The number of protozoa per ml was determined by mixing 1 ml of preserved fluid with 4 ml of 0.2 mol/l iodine solution and counted as described by Chikunya *et al.* (2004). Freeze-dried effluent overflow samples were treated to remove excess ^{15}N-labelled ammonia ($^{15}NH_3$-N) as described by Firkins *et al.* (1992) and outflow and microbial pellets analysed for ^{15}N 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, UK.

Statistical analysis

Milk yield parameters, intake, live weight, condition score and blood parameters were evaluated by analysis of variance with the treatment degrees of freedom split into main effects of dietary treatment (Treat), linear (Lin) and quadratic responses (Quad) to rate of inclusion of pWCW. Milk and component yield (kg/day) in the week prior to blocking were used, when appropriate, as a co-variate. The *in vitro* measurements were analysed as a Latin-square design with main effects of vessels, periods and treatment, which was further split into main effects of dietary treatment (Treat), linear (Lin) and quadratic responses (Quad) to rate of inclusion of pWCW. All statistical analysis was conducted using Genstat 6 (VSN International Ltd., Oxford, UK).

Results

Forage analysis and animal performance

The pWCW had a higher DM, pH, crude protein and starch content than the grass silage (Table 1). By contrast the grass silage had a higher ash content, whilst fibre levels were similar in both forages. The proportion of feed particles greater than 19.0 mm decreased and that below 8.0 mm increased linearly with pWCW inclusion rate, but the physically effective fibre was not affected by treatment ($P > 0.05$; Table 2). There was no difference ($P > 0.05$) in the DM intake of concentrates between cows fed any of the treatments, which averaged 9.7 kg DM per day. By contrast, there was a linear effect ($P < 0.001$) of treatment on total forage DM intake, which increased from 9.99 kg DM per day in cows fed grass silage alone (W-0) to 14.63 kg DM per day in cows fed 0.75 pWCW (W-75). A similar pattern in total DM intake was observed ($P < 0.001$).

Daily milk yield was 3.6 kg/day higher in cows fed W-25 compared with those fed grass silage alone (W-0; $P < 0.05$), but there was no difference ($P > 0.05$) between cows fed W-25, W-50 or W-75, or between W-0, U-50 or W-75, although there was a trend ($P = 0.09$) for a quadratic relationship between milk yield and pWCW inclusion rate (Table 3). Milk fat, protein and lactose concentrations were similar between treatments although there was a trend ($P = 0.10$) for milk protein content to increase with the level of inclusion of pWCW. Milk protein yield was 0.11 kg/day higher ($P < 0.05$) in cows fed W-25 compared with W-0, although there was no difference ($P > 0.05$) between cows fed W-0, W-50 or W-75, and W-25, W-50 or W-75. When milk yield was corrected to the same energy content there was no difference ($P > 0.05$)

Table 1 *Chemical composition[†] (g/kg dry matter (DM)) of grass silage, processed, urea-treated whole-crop wheat (WCW) and concentrate fed to dairy cows*

	Grass silage	Processed WCW	Concentrate
DM (g/kg)	323	836	893
Crude protein	141	158	239
Ash	83	38	95.1
Ammonia-N (g/kg total N)	32	157	nd
pH	4.0	8.4	nd
DOMD[‡] (g/kg DM)	688	759	798
ME[‡] (MJ/kg DM)	10.9	nd	13.7
Sugars	47	6.2	14.0
NDF[‡]	515	505	290
ADF[‡]	334	211	163
Oil	32	23	99.4
Starch	nd	340	161
Ethanol	11.5	nd	nd
Fermentation acids			
Lactic acid	119	nd	nd
Acetic acid	52	nd	nd
Propionic acid	4.5	nd	nd
Butyric acid	<0.1	nd	nd
Iso-valeric acid	<0.1	nd	nd
n-valeric acid	<0.1	nd	nd

[†] *n* = mean of four replicates per feed.
[‡] Abbreviations are: DOMD = digestible organic matter in the dry matter; ME = metabolisable energy; NDF = neutral-detergent fibre; ADF = acid-detergent fibre.
nd = not determined.

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Table 2 *Mean diet particle size distribution (g/kg dry matter (DM)) and mean intake (kg DM per day) of concentrates and forage by cows fed diets in which grass silage was substituted for 0 (W-0), 0.25 (W-25), 0.5 (W-50) or 0.75 (W-75) of processed, urea-treated whole-crop wheat (pWCW) on a DM basis*

	Diet					Significance[†]		
	W-0	W-25	W-50	W-75	s.e.d.	Treat	Lin	Quad
Proportion of diet[‡]								
> 19.0 mm	362[b]	332[b]	213[a]	161[a]	39.8	< 0.001	< 0.001	0.685
19.0–8.0 mm	424	430	397	381	25.0	0.219	0.060	0.567
8.0–1.18 mm	180[a]	210[a]	334[b]	400[c]	27.3	< 0.001	< 0.001	0.368
< 1.18 mm	34[ab]	28[a]	56[bc]	58[c]	10.5	0.022	0.009	0.594
Mean particle size mm	12.6[b]	12.1[b]	8.6[a]	7.5[a]	0.98	< 0.001	< 0.001	0.648
PeNDF (g/kg DM)[§]	391	403	396	397	4.3	0.114	0.497	0.118
Intake								
Dairy concentrates	6.15	6.14	6.11	6.12	0.063	0.900	0.518	0.795
Straights	3.51	3.57	3.66	3.68	0.229	0.876	0.428	0.894
Total concentrates	9.67	9.71	9.73	9.80	0.247	0.961	0.605	0.952
Grass silage	9.99[c]	9.12[c]	6.98[b]	3.96[a]	0.596	< 0.001	< 0.001	0.007
pWCW	0.00[a]	3.13[b]	6.64[c]	10.66[d]	0.372	< 0.001	< 0.001	0.027
Total forage	9.99[a]	12.25[b]	13.58[bc]	14.63[c]	0.777	< 0.001	< 0.001	0.277
Total DM intake	19.65[a]	21.97[b]	23.31[bc]	24.43[c]	1.015	< 0.001	< 0.001	0.414

[a,b,c,d] Within a row, means without a common superscript letter differ ($P < 0.05$).
[†] Treatment (Treat), linear (Lin) and quadratic responses (Quad) to rate of inclusion of pWCW.
[‡] Total mixed ration only, excludes out of parlour concentrates, $n = 5$.
[§] Calculated as the product of the proportion of DM above 1.18 mm and the neutral-detergent fibre content.

between treatments. There was also no effect ($P > 0.05$) of treatment on mean locomotion score, live-weight change (kg/day) or body condition score, but during weeks 11 to 14 inclusive there was an effect ($P < 0.05$) of pWCW inclusion rate on body condition score (Figure 1).

By week 13 of the experiment there was a linear increase ($P < 0.001$; Figure 2) in plasma insulin concentrations with pWCW inclusion rate, although there was no effect ($P > 0.05$) of treatment on mean levels (Table 4). By contrast, there was a linear effect ($P < 0.001$) of treatment on mean plasma urea levels, which increased with pWCW inclusion rate. There was a quadratic effect ($P = 0.047$) of level of inclusion of pWCW on plasma albumin levels, being highest in cows fed W-50. Similarly, there was a trend towards a quadratic response ($P = 0.07$) in plasma BHB levels, which were highest in cows fed W-50. There was no effect ($P > 0.05$) of treatment on plasma NEFA levels, which averaged 0.126 mmol/l.

Table 3 *Mean milk performance, live weight, condition score and locomotion score of cows fed diets in which grass silage was substituted for 0 (W-0), 0.25 (W-25), 0.5 (W-50) or 0.75 (W-75) of processed, urea-treated whole-crop wheat (pWCW) on a dry-matter basis*

	Diet					Significance[†]		
	W-0	W-25	W-50	W-75	s.e.d.	Treat	Lin	Quad
Milk yield (kg/day)	34.2[a]	37.8[b]	35.2[ab]	35.8[ab]	1.22	0.043	0.569	0.092
Energy-corrected milk yield (kg/day)[‡]	32.8	35.6	34.0	32.9	1.42	0.201	0.703	0.070
Fat (g/kg)	39.2	38.0	39.9	35.6	2.48	0.342	0.253	0.392
Protein (g/kg)	30.6	30.9	32.1	31.7	0.88	0.293	0.103	0.598
Lactose (g/kg)	46.6	46.1	46.2	46.6	0.54	0.799	0.779	0.345
Fat yield (kg/day)	1.37	1.41	1.37	1.26	0.090	0.429	0.218	0.270
Protein yield (kg/day)	1.06[a]	1.17[b]	1.11[ab]	1.12[ab]	0.039	0.048	0.135	0.199
Lactose yield (kg/day)	1.59	1.74	1.63	1.67	0.070	0.167	0.559	0.257
Live-weight change (kg/day)	0.08	0.13	0.21	0.36	0.151	0.292	0.067	0.614
Body condition score	2.33	2.31	2.43	2.48	0.117	0.430	0.137	0.696
Locomotion score	2.36	2.32	2.28	2.42	0.279	0.965	0.894	0.655

[a,b] Within a row, means without a common superscript letter differ ($P < 0.05$).
[†] Treatment (Treat), linear (Lin) and quadratic responses (Quad) to rate of inclusion of pWCW.
[‡] Corrected to 3.1 MJ net energy per kg according to AFRC (1995).

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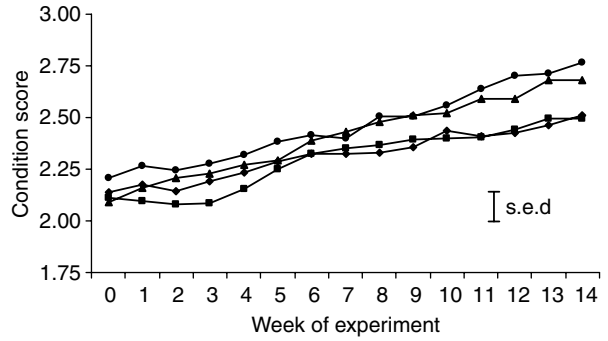

Figure 1 Effect of rate of inclusion of processed, urea-treated whole-crop wheat on the condition score of dairy cows fed diets in which grass silage was substituted for 0 (W-0; ◆) 0.25 (W-25; ■), 0.5 (W-50; ▲) or 0.75 (W-75; ●) of processed, urea-treated whole-crop wheat on a dry-matter basis.

Apparent digestibility

Organic matter (OM) intake and faecal output increased linearly with rate of inclusion of pWCW ($P = 0.08$ and < 0.001 respectively), whilst digestibility (kg/kg) decreased ($P < 0.001$; Table 5). By contrast, intake of digestible OM (kg/day) was similar between treatments, averaging 15.8 kg/day. Similar to OM, faecal output of NDF increased linearly with rate of inclusion of pWCW ($P < 0.001$), whilst digestibility decreased, with the intake of digestible fibre being similar across treatments, averaging 5.92 kg/day. Starch intake increased linearly ($P < 0.001$) with rate of inclusion of pWCW but digestibility was similar among treatments at approximately 0.96 kg/kg resulting in the intake of digestible starch increasing linearly ($P < 0.001$).

In vitro *digestibility and metabolism*

There was no effect of treatment on mean vessel pH, although values tended ($P = 0.06$) to decrease with rate of inclusion of pWCW, whilst vessel ammonia concentration increased linearly ($P < 0.05$; Table 6). There was no effect of treatment on mean total VFA concentration. Similarly, there was no effect of treatment ($P > 0.05$) on the proportion of acetate, but butyrate levels increased ($P < 0.05$) and propionate levels decreased ($P < 0.001$) linearly with rate of inclusion of pWCW; as a consequence, the acetate to propionate ratio increased linearly ($P < 0.001$). The number of protozoa per ml of outflow were similar between treatments on day 1 ($P > 0.05$; mean of 3.0×10^4/ml) but by day 13 numbers had decreased substantially (mean of 5.8×10^3/ml) and there was a linear increase ($P < 0.05$) in numbers with pWCW inclusion rate.

The intake of OM and NDF was similar across treatments and there was no effect of treatment on OM or NDF digestibility (g/g), which averaged 0.45 and 0.70 respectively, whilst the content of digestible starch (g/day) increased with pWCW inclusion rate (Table 7). There was no effect of treatment on microbial N flow (mean value of 0.36 g/day) or microbial efficiency when expressed as either g N per kg organic matter apparently degraded or truly degraded (mean values of 28.8 and 20.8 respectively).

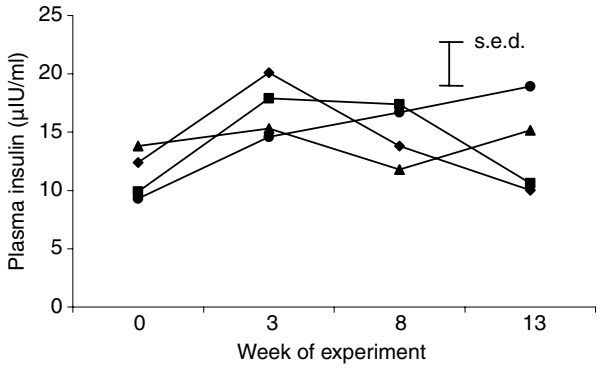

Figure 2 Effect of rate of inclusion of processed, urea-treated whole-crop wheat on plasma insulin concentrations (μIU per ml) in dairy cows fed diets in which grass silage was substituted for 0 (W-0; ◆) 0.25 (W-25; ■), 0.5 (W-50; ▲) or 0.75 (W-75; ●) of processed, urea-treated whole-crop wheat on a dry-matter basis.

Discussion

Intake and performance

The DM content of the WCW used in the current study (836 g/kg) was comparable to the processed and unprocessed WCW used in the studies of Sinclair *et al.* (2005) and Sutton *et al.* (1998) but higher than that used by Jackson *et al.* (2004) or Sutton *et al.* (2001). The optimal DM of pWCW has been shown to be approximately 700 g/kg, although forages with DM contents above this value have only marginally reduced performance (Bond *et al.*, 2004). In the current study there was a large and significant linear increase in forage DM intake with inclusion rate of pWCW. An increase in forage DM intake when grass silage is replaced with WCW in dairy cows is well established. For example, Hameleers (1998) reported a 0.23 increase in forage DM intake when urea-treated WCW replaced 0.4 of the grass silage, whilst Murphy *et al.* (2004) reported a 0.38 increase when processed, urea-treated WCW replaced 0.67 of grass silage. By contrast, the effects of rate of inclusion WCW on intake are less apparent; Phipps *et al.* (1992) and Abdalla *et al.* (1999) reported a modest increase in forage DM intake with inclusion rate of unprocessed WCW, whilst Sutton *et al.* (1997) and Hill and Leaver (1991) reported little effect. In general, feeding more rapidly digested starch sources such as wheat can cause a depression in ruminal pH and may lead to a reduction in DM intake (Grant, 1994). In the current study, despite starch intake increasing from approximately 1 kg/day on W-0 to 4.6 kg/day on W-75, forage DM intake increased, although the highest level of starch intake recorded here was substantially lower than that reported in other studies (Overton *et al.*, 1995; Yang *et al.*, 2001).

Despite the large effect of pWCW inclusion on intake, milk yield was highest when it replaced 0.25 of the grass

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Table 4 *Mean (weeks 3, 8 and 13) plasma concentrations of insulin, urea, albumin, glucose, β-hydroxy-butyrate (BHB, non-esterified fatty acids (NEFA) for cows fed diets in which grass silage was substituted for 0 (W-0), 0.25 (W-25), 0.5 (W-50) or 0.75 (W-75) of processed, urea-treated whole-crop wheat (pWCW) on a dry-matter basis*

	Diet					Significance[†]		
	W-0	W-25	W-50	W-75	s.e.d.	Treat	Lin	Quad
Insulin (μIU per ml)	14.6	15.3	15.9	16.1	3.55	0.974	0.655	0.919
Urea (mmol/l)	4.46[a]	5.31[b]	5.59[b]	6.04[b]	0.385	0.008	<0.001	0.466
Albumin (g/l)	35.5	35.7	36.2	34.1	0.714	0.070	0.13	0.047
Glucose (mmol/l)	3.56	3.35	3.51	3.43	0.123	0.404	0.575	0.494
BHB (mmol/l)	0.66	0.65	0.81	0.58	0.0791	0.062	0.767	0.071
NEFA (mmol/l)	0.138	0.148	0.115	0.104	0.0296	0.441	0.168	0.622

[a,b] Within a row, means without a common superscript letter differ ($P < 0.05$).
[†] Treatment (Treat), linear (Lin) and quadratic responses (Quad) to rate of inclusion of pWCW.

silage, but not at the higher rates that have been used in most other studies (Jackson *et al*., 2004; Murphy *et al*., 2004; Sinclair *et al*., 2005). The effects of processed or unprocessed WCW on dairy cow performance have been inconsistent; in most studies there has either been no significant effect or a trend towards an increase in milk yield (Phipps *et al*., 1995; Sutton *et al*., 1998). By contrast, milk yield was significantly higher when WCW contributed 0.44 of the forage DM in the study of Hill and Leaver (1991) and was increased in some but not all studies that have fed pWCW (Murphy *et al*., 2004). Part of this variation may be explained by the quality of the reference grass silage used, with metabolisable energy (ME) values ranging from 10.0 MJ/kg DM in the study of Hill and Leaver (1991) to 12.0 MJ/kg DM reported by Sutton *et al*. (1998). The lack of a significant increase in milk performance at higher pWCW inclusion rates in the current study may be attributed to a repartioning of dietary energy towards body tissue; body condition score increased with rate of pWCW inclusion, such that by week 11 of treatment the difference was significant. It is well established that increasing dietary starch intake is associated with a partioning of nutrients away from milk fat towards body condition, which may reflect a greater absorption of glucogenic rather than lipogenic precursors (Reynolds *et al*., 1997). This observation is supported by the plasma insulin levels which increased with pWCW inclusion rate during week 13. Increasing the inclusion rate of coarse ground cereal grains has also been shown to increase the flow of undigested starch to the small intestine (Beauchemin *et al*., 2001), and may in theory improve the efficiency of energy utilisation as starch digestion in the small intestine provides more energy than starch digested in the rumen (Reynolds *et al*., 1997). This is unlikely to have had a major impact in the current study as approximately 0.81 kg/kg of the starch in pWCW has been reported to be released within the rumen (Bond,

Table 5 *Intake, faecal output, digestible (kg/day) and apparent digestibility (kg/kg) of organic matter, neutral-detergent fibre and starch in cows fed diets in which grass silage was substituted for 0 (W-0), 0.25 (W-25), 0.5 (W-50) or 0.75 (W-75) of processed, urea-treated whole-crop wheat (pWCW) on a dry-matter basis*

	Diet					Significance[†]		
	W-0	W-25	W-50	W-75	s.e.d.	Treat	Lin	Quad
Organic matter								
Intake	18.5	21.4	22.3	23.6	1.81	0.080	0.016	0.545
Faecal output	4.01[a]	5.00[ab]	6.20[bc]	7.20[c]	0.520	<0.001	<0.001	0.993
Digestible	14.5	16.4	16.1	16.4	1.49	0.541	0.266	0.462
Digestibility	0.786[b]	0.765[b]	0.722[a]	0.695[a]	0.016	<0.001	<0.001	0.802
Fibre								
Intake	7.91	9.27	9.51	9.79	0.851	0.187	0.052	0.394
Faecal output	2.25[a]	2.80[ab]	3.56[bc]	4.16[c]	0.299	<0.001	<0.001	0.903
Digestible	5.66	6.46	5.95	5.63	0.712	0.634	0.794	0.289
Digestibility	0.720[c]	0.695[c]	0.626[b]	0.575[a]	0.027	<0.001	<0.001	0.514
Starch								
Intake	1.16[a]	2.30[b]	3.51[c]	5.00[d]	0.206	<0.001	<0.001	0.248
Faecal output	0.03[a]	0.07[ab]	0.14[b]	0.27[c]	0.0374	<0.001	<0.001	0.129
Digestible	1.13[a]	2.22[b]	3.37[c]	4.74[d]	0.198	<0.001	<0.001	0.362
Digestibility	0.971	0.968	0.959	0.947	0.009	0.101	0.020	0.479

[a,b,c,d] Within a row, means without a common superscript letter differ ($P < 0.05$).
[†] Treatment (Treat), linear (Lin) and quadratic responses (Quad) to rate of inclusion of pWCW.

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Table 6 *Mean pH, ammonia-nitrogen concentration, volatile fatty acid (VFA) proportion and concentration and protozoa numbers for* in vitro *vessels given diets in which grass silage was substituted for 0 (W-0), 0.25 (W-25), 0.5 (W-50) or 0.75 (W-75) of processed, urea-treated whole-crop wheat (pWCW) on a dry-matter basis*

	Diet					Significance[†]		
	W-0	W-25	W-50	W-75	s.e.d.	Treat	Lin	Quad
pH	6.05	6.03	5.99	5.99	0.030	0.186	0.055	0.485
Ammonia-N (mg/l)	114[a]	115[a]	145[ab]	153[b]	12.6	0.045	0.011	0.704
VFA (mmol/mmol)								
Acetic	53.8	53.3	54.1	54.9	0.91	0.398	0.194	0.330
Butyric	16.5	18.2	19.3	20.3	1.12	0.064	0.012	0.690
Propionic	21.4[c]	20.1[bc]	18.8[ab]	17.6[a]	0.66	0.006	<0.001	0.897
Minor VFA[‡]	8.30[b]	8.42[b]	7.82[b]	7.12[a]	0.28	0.013	0.003	0.083
Total VFA (mmol/l)	68.2	67.2	71.3	76.4	7.29	0.617	0.261	0.575
Acetate to propionate ratio	2.52[a]	2.65[ab]	2.89[b]	3.16[c]	0.10	0.004	<0.001	0.403
Protozoa numbers (10^3/ml)								
Day 1	31.2	34.5	31.5	23.3	7.26	0.508	0.287	0.309
Day 13	4.48	5.21	6.25	7.34	1.071	0.137	0.029	0.818

[a,b,c] Within a row, means with different superscript letter differ ($P < 0.05$).
[†] Treatment (Treat), linear (Lin) and quadratic responses (Quad) to rate of inclusion of pWCW.
[‡] Minor VFA includes isobutyric, isovaleric, valeric and caproic acids.

2006) and it is predicted that duodenal starch flow for treatments W-25, W-50 and W-75 compared with W-0 would have been increased by only 0.20, 0.43 and 0.68 kg/day respectively.

There was no effect of treatment on milk fat content or yield, although both were numerically lower in cows fed W-75. This result is somewhat surprising as it is well established that milk fat production decreases as the ratio of starch:fibre in the diet increases (Sutton, 1989), although the physically effective fibre content of the diet, which has been shown to be related to milk fat content (Mertens, 1997), was similar between treatments. The increase in milk protein yield in cows fed W-25 compared with W-0 may be attributed to effects of increasing starch

Table 7 *Mean intake, output, digestible (g/day), apparent digestibility (g/g) of organic matter, neutral-detergent fibre, starch and microbial protein synthesis for* in vitro *vessels given diets in which grass silage was substituted for 0 (W-0), 0.25 (W-25), 0.5 (W-50) or 0.75 (W-75) of processed, urea-treated whole-crop wheat (pWCW) on a dry-matter basis*

	Diet					Significance[†]		
	W-0	W-25	W-50	W-75	s.e.d.	Treat	Lin	Quad
Organic matter								
Intake	28.2	28.4	28.6	28.79				
Output	16.1	16.0	15.4	15.3	0.79	0.664	0.270	0.955
Digestible	12.1	12.4	13.2	13.5	0.79	0.316	0.086	0.955
Digestibility	0.43	0.44	0.46	0.47	0.027	0.452	0.143	0.947
Fibre								
Intake	10.9	10.6	10.4	10.1				
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
Microbial protein synthesis								
Microbial N (g/day)	0.36	0.39	0.35	0.35	0.025	0.415	0.421	0.491
g N per kg OMAD[‡]	28.9	31.3	27.9	27.3	4.97	0.852	0.608	0.684
g N per kg OMTD[‡]	20.9	21.8	20.3	20.0	2.52	0.890	0.623	0.729

[a,b,c] Within a row, means with different superscript letter differ ($P < 0.05$).
[†] Treatment (Treat), linear (Lin) and quadratic responses (Quad) to rate of inclusion of pWCW.
[‡] OMAD = organic matter apparently digested; OMTD = organic matter truly digested.

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intake on microbial protein flow at the duodenum, a greater ruminal propionate absorption rate or a higher ME supply (Reynolds *et al*., 1997). Increased plasma concentrations of insulin have also been shown to be associated with increased milk protein concentrations, providing amino acid supply is adequate (Reynolds *et al*., 1997). Reasons for the lack of a significant response in milk protein output at higher inclusion rates of pWCW are less clear as both starch intake and plasma insulin levels increased with WCW inclusion rate, although Phipps *et al.* (1992) also reported the highest milk protein concentration in cows fed 0.25 of the forage as WCW.

Apparent digestibility

Acid-insoluble ash was used during week 10 of the current experiment as an indirect method of determining whole tract digestibility. By contrast, other studies that have assessed the digestibility of WCW in dairy cows have used a total collection of faeces (Sutton *et al*., 1997, 1998 and 2001) and employed a change-over design, generally with a 3 or 4 week adaptation period prior to faecal collection. Starch digestion has however, been shown to increase substantially in both the rumen and the whole tract when dairy cows received an adaptation period of 42 days (Matthé *et al*., 2003). Additionally, acid-insoluble ash has been shown to have little diurnal variation and result in digestibility coefficients comparable with total collection (Sales and Janssens, 2003).

Sutton *et al.* (1997) reported a linear decrease in the apparent digestibility of DM and OM with inclusion rate of unprocessed, urea-treated WCW, although no consistent effects on fibre digestibility were reported. As a consequence of a greater DM intake with WCW inclusion rate, this resulted in a similar intake of digestible OM and a poor efficiency of utilisation of WCW (Sutton *et al*., 1997). In the current study, digestible OM intake increased by 1.9 kg/day when pWCW replaced 0.25 of the grass silage, although the difference was not significant. Whole-tract apparent digestibility of starch was also similar among treatments. If it is assumed that the digestibility of starch in the concentrates was the same as that measured in W-0 (0.97 kg/kg) and constant across treatments, the digestibility of starch in the pWCW can be calculated as 0.97, 0.95 and 0.94 (s.e.d. 0.155) kg/kg for W-25, W-50 and W-75 respectively, and was not significantly different between treatments. These values are similar to the 0.97 kg/kg reported by Jackson *et al.* (2004) for pWCW but substantially higher than other studies that have fed unprocessed WCW to dairy cows (Sutton *et al*., 1997 and 1998; Jackson *et al*., 2004).

In vitro *digestibility and metabolism*

Sustained periods of ruminal pH below pH 5.8 to 6.0 can reduce ruminal digestion, rumen motility, appetite and cellulolytic bacteria growth and efficiency (Mould and Ørskov, 1983). In the current experiment, there was a trend ($P = 0.06$) for vessel content pH to decrease with pWCW inclusion rate, although the difference between treatments was small. This small difference could be attributed to the constant rate of buffer infusion to all treatments which may have had an ameliorating effect on vessel pH. Ruminal pH *in vivo* is partly mediated by the rate of saliva release which can be affected by factors including particle size, forage to concentrate ratio and moisture content of the feed (Maekawa *et al*., 2002). However, the increase in saliva output due to increased chewing time may not be as great as often assumed, as an increased eating and ruminating time decreases resting time when significant amounts of saliva can be released (Maekawa *et al*., 2002).

Protozoa numbers *in vitro* have been reported to be considerably lower when compared with levels *in vivo* (Rufener *et al*., 1963; Abe and Kumeno, 1973), an effect that may be attributed to a requirement for a large dead space to sequester and find food that is not present in continuous culture systems (Williams and Coleman, 1997). In the current study, numbers of protozoa also decreased substantially after introduction to the vessels, but were higher on day 13 in vessels fed 0.75 WCW, a finding consistent with studies that have fed increasing levels of starch in the diet (Chamberlain *et al*., 1985). There is still no consensus on the value of protozoa to the host animal although their ability to ingest starch and utilize lactic acid may result in a more stable rumen pH, and their presence has been associated with a decrease in the efficiency of bacterial protein synthesis and flow of N to the duodenum (Williams and Coleman, 1997).

The mean VFA concentration in the current experiment of 71 mmol/l was similar to that reported in other studies that have used continuous culture systems (Slyter *et al*., 1964). In general, bacteria that ferment starch produce a higher proportion of propionic acid than those fermenting cellulose or hemicellulose; as a result increased starch intakes are generally associated with a shift in VFA production away from acetate towards propionate (Overton *et al*., 1995). By contrast, in the current study, the proportion of acetate to propionate increased linearly with pWCW inclusion rate. This finding is, however, consistent with that of Abdalla *et al.* (1999) who reported an increased proportion of acetate to propionate when unprocessed WCW replaced grass silage in the diet of dairy cows. Under certain conditions, concentrate diets may encourage the development of a large protozoal population which is accompanied by an increase in butyrate rather than propionate (France and Siddons, 1993). In the current study both butyrate concentrations and protozoal numbers increased linearly with pWCW inclusion rate.

Ammonia-N concentrations were highest in vessels receiving W-75, a finding consistent with the greater content of rumen degradable protein due to the addition of urea as a preservative at ensiling. Abdalla *et al.* (1999) suggested that increased ammonia values in diets containing WCW may also be a consequence of the reduced ruminal digestion of OM with WCW inclusion, although there was no significant effect of treatment on OM digestibility

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in the current study. The lack of an effect on OM digestibility may have been due to a longer retention time *in vitro* than *in vivo* or an effect of the pre-feeding grinding of the diets, both of which may have permitted a greater opportunity for microbial digestion. This is supported by the high digestibility of starch in the current study, which averaged 0.99 kg/kg. There was also no effect of treatment on microbial protein synthesis (g/day) or efficiency. Microbial efficiency has been shown to be negatively correlated with starch digestion in the rumen (Oba and Allen, 2003) whilst McCarthy *et al.* (1989) attributed the greater microbial N passage to the small intestine in dairy cows fed barley compared with maize starch to a greater supply of degradable OM. Despite starch levels increasing with pWCW inclusion rate in the current study, the quantity of OM digested in the rumen was comparable among treatments and would be expected to supply a similar amount of energy for microbial growth.

Conclusions

Forage DM intake increased linearly with rate of inclusion of pWCW, but there was no further improvement in milk yield from inclusion rates above 0.25 of the forage DM, with body condition score increasing instead. Increasing the inclusion rate of pWCW *in vitro* resulted in a more ketogenic absorption profile and there was no effect on the efficiency of microbial protein synthesis. Despite the lack of an increase in milk yield at inclusion rates of pWCW above 0.25 of the forage DM, there may be economic and management justification for higher inclusion rates.

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