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A FEASIBILITY STUDY TO EVALUATE MECHANICAL AND ENZYMATIC METHODS OF IMPROVING CEREAL QUALITY FOR ANIMAL FEEDS

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by

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Final report of a three month project at FMBRA. The work commenced in November 1992 and was funded by a grant of £19,000 from the Home-Grown Cereals Authority (Project No. 0043/1/92).

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A FEASIBILITY STUDY TO EVALUATE MECHANICAL AND ENZYMIC METHODS OF IMPROVING CEREAL QUALITY FOR ANIMAL FEEDS

Project No. 0043/1/92

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Final report on a project of duration 0.25 year commencing 2nd November 1992

ABSTRACT

The project's objective was to investigate the use of some existing technologies to improve the nutritional quality of barley for use in animal-feeds using *in vitro* techniques. The ability to improve starch and protein digestibilities and increase sensitivity to cell-wall degrading enzymes were measured. Two scenarios were investigated: a comparison of barley-meal pellets produced under conditions similar to those presently used in compound-feed plants (conditioning) with pellets produced by an expansion process (expansion). The second scenario evaluated the potential of cell-wall degrading enzymes as a process aid in the in-house production of liquid feed as practised on some farms.

Although both types of pellets had higher starch-digestibilities than the meal, the starch in pellets produced by expansion not only was more digestible but was also digested at a faster rate by pancreatic amylases. Both production methods reduced protein solubility and made the protein more sensitive to attack by intestinal proteases. However, the increase in protein-digestibility was slightly lower in the case of expanded pellets. Processing abolished the increased solubilization of protein seen when meal was treated in dilute suspension with cell-wall degrading enzymes. While addition of some cell-wall degrading enzymes could bring about a similar, but slight increase in protein digestibility in the meal and conditioned pellets, the combination of expansion followed by addition of enzyme led to a synergistic increase in protein-digestibility. With regard to susceptibility towards cell-wall digesting enzymes, processing had varying effects. Where an effect was observed, it was similar for both conditioning and expansion.

In studies investigating the role of cell-wall digesting enzymes as process aids, experiments were performed with the best performing enzyme identified in the pellet studies. The enzyme

was used to treat 40% suspensions of barley meal for 1 or 2 hours at 20° or 40°C. Enzyme treatment had no consistent effect on protein solubility or digestibility. However, it was generally observed that suspending meal at 40% solids frequently increased protein digestibility.

This project demonstrates the possibility of effectively measuring the effects of a number of processing combinations on nutritional parameters in terms of both time and cost. It has also demonstrated that the desirable effects associated with the use of cell-wall digesting enzymes can sometimes be further enhanced by the process used to produce the original feed.

INTRODUCTION

The use of cereals in animal feeds is governed by two factors, market forces and nutritional quality.

Consideration of statistics released by the HGCA in June 1992 revealed that for the first 39 weeks of the year 1991/92, the amounts of wheat and barley used for feed purposes (both by feed-compounders and directly on-farm) were respectively 7 and 5% lower, compared with the previous year. Although this reduction partly reflects contractions in agriculture, it is also a response to the availability of other, more cost-effective ingredients (e.g. sunflower-seed cake).

Many nutrients present in cereals are poorly available to animals. This is, in part, corrected for by processing of the ingredients - typically milling and conditioning. Nevertheless, even with processing, only 70% of the amino acids and 80% of the energy are absorbed. Cereals are also rich in non-starch polysaccharides. Being indigestible in the small intestine, they represent unavailable nutrients. They also have other undesirable properties in particular reducing general nutrient absorbtion and increasing faecal bulk.

Identifying measures which both improve nutritional quality and are cost-effective is therefore desirable in order to reverse declining sales of cereals for feed. This project describes a feasibility study to evaluate the potential of some processes to improve the nutritional quality of barley using *in vitro* predictive assays.

MATERIAL AND METHODS

Barley

The variety of barley used to produce pellets by conditioning was Posaune and purchased from Blackbird Farm, Aldenham. That used for production of expanded pellets was an unknown 6-row variety routinely used by The Brecks Co Ltd, Swillington. Both varieties were converted to meals by hammer milling (3mm sieve). For convenience the barley meals and mashes produced will be referred to by the source of the material *viz* Blackbird or Brecks.

Preparation of Barley Pellets

Conditioned pellets were produced at FMBRA by cooking 20kg barley meal with 4kg water at 80°C in an autoclave for 10 minutes. The conditioned meal was pelleted using an APV Baker MPF 50D twin screw extruder. Pellets were dried overnight at 40°C in a Mitchell drier. Expanded barley-meal pellets were a gift from The Brecks Company and were produced under commercial conditions by extruding under pressure with steam.

Preparation of Barley Mash

Barley mashes were prepared from meals derived from either variety of barley by resuspending in distilled water at 40% solids (weight : volume) with or without a cell-wall degrading enzyme (2mg Biofeed (Novo) per 4g milled barley). The mashes were incubated with constant mixing at either 20 or 40°C for 1 or 2 hours. Following incubation, solids were removed by centrifugation, washed in distilled water, freeze-dried and subsequently evaluated for protein digestibility. The supernatants were analysed for protein and for released carbohydrate.

In Vitro Predictive Assays for Nutritional Quality

Starch content and digestibility

The starch contents (available starch) and digestibilities were determined by the method of Berry (1986). Essentially, available starch was determined by digesting the material with fungal alpha-amylase and measuring the amount of glucose produced. Starch digestibility was estimated by digesting a sample with a cocktail of intestinal amylolytic enzymes and measuring the amount of available starch remaining after known periods. Starch digestibility was expressed in terms of three parameters: rapidly digestible starch (RDS), that digested within 10 minutes; slowly digestible starch (SDS), that digested between 10 and 120 minutes

and indigestible starch (IS), that remaining undigested after 120 minutes.

Protein content and digestibility

Protein concentrations were either derived from Kjeldahl-nitrogen determinations (conversion factor = 5.81) or by the method of Lowry *et al* (1951). Protein digestibility was determined by an adaption of the method of Pedersen and Eggum as described by McDonough *et al* (1990). This assay measures the amount of acid produced when a sample containing 10mg nitrogen is digested with a cocktail of intestinal proteases at 37°C. This value is compared with an internal standard (casein). Digestibility was expressed both in terms of total digestibility (McDonough et al., 1990) by extrapolation of data using an established formula or as a simple ratio with that of the internal standard, casein (relative digestibility).

Soluble carbohydrate

Soluble carbohydrate was measured as free reducing sugars by an adaption of the method of Englyst and Cummings for the colorimetric determination of non-starch polysaccharides (MAFF, 1992). Solutions were heated at 100°C for 10 minutes in the presence of glucose, sodium hydroxide and dinitrosalicylic acid. The intensity of the resultant orange-red colour was measured at 530nm and compared with a standard curve of reducing-sugar solutions of known concentrations.

Screening of cell-wall degrading enzymes (CWDE)

These enzymes were screened specifically for their ability to improve the nutritional quality of non-starch polysaccharides by releasing carbohydrate into solution under conditions *similar to those in the gut*. The substrate material (barley-meal or-pellet) was milled before assay. 50mg of the material was resuspended in 0.1M sodium phosphate buffer pH 7.4 to 2.5% (w/v). It was incubated in the presence or absence of a CWDE for 2 hours at 37°C with continuous mixing. Enzymes were added in the same ratio as recommended by the manufacturer, or if no such information is available at an equivalent level of activity. After incubation, solids were removed by centrifugation and the amount of carbohydrate in solution determined as described in previously.

Effect of CWDE on protein digestibility

The protocol followed was as described for estimating soluble carbohydrate except that the sample size was larger. After enzyme treatment the sample was centrifuged. The supernatant

was analysed for protein content and carbohydrate. The insoluble residue was washed with distilled water and freeze-dried. The protein digestibility of the residue was determined as described previously.

RESULTS

Barley-Meal and Pellets

Analysis

Details of the nitrogen, moisture and available starch contents of the meals and pellets prepared from them are shown in Table 1. Barley from Brecks had a slightly lower nitrogen and higher moisture and starch contents than that from Blackbird Farm.

Starch digestibility

Data for starch digestibility determinations is given in Table 2. Source did not appreciably affect the amounts of the three types of starch present. Conditioning increased the amount of rapidly digestible starch by 3-fold and reduced the amount of indigestible starch by 38%. In contrast expansion led to a 12.2 fold increase in the amount of rapidly digestible starch and reduced the amount of indigestible starch by 74%.

Protein digestibility

Due to concern that ultra-fine milling (as required by the original method) may obscure process-induced changes, only data for rough milled samples are given in Table 3. Although processing led to marginal changes in total digestibility, its effects could be better seen when considering relative digestibility. The largest changes were associated with conditioning although differences due to the two processing methods may, in part, be due to the different digestibilities of the original barley samples.

Susceptibility to CWDE

The CWDE were either samples of commercial preparations supplied by Novo Nordisk Bioindustries UK Ltd., Farnham or semi-purified enzymes (controls) purchased from Sigma Ltd., Poole, Dorset. Details of the enzymes used are given in Appendix 1. The amount of carbohydrate solubilised in the absence of enzymes and the net amounts of carbohydrate released into solution by these enzymes are shown in Table 4.

In the absence of enzyme, conditioning led to a greater increase in the amount of carbohydrate released into solution than did expansion (Table 4a). Of the Novo enzymes studied 'Biofeed' was the most effective and subsequent work concentrated on this enzyme. There appeared to be an interaction between the source of barley and enzyme activity (Table 4b). This was particularly marked in the case of Biofeed and the Sigma hemicellulase, in

both cases the meal and pellets from Brecks were more susceptible. Both conditioning and expansion enhanced the activity of Biofeed, Biofeed Pro, Energex and Sigma hemicellulase, expansion also enhanced the activity of Sigma cellulase.

CWDE and their effects on protein solubility and digestibility

The ability of the Novo enzyme 'Biofeed' to modify protein solubility and digestibility was evaluated as described previously. The increase in soluble carbohydrates released was also measured to ensure that the enzyme functioned under the scaled up condition (2g versus 50mg of substrate). Details of the amounts of carbohydrate released are given in Table 5. As previously seen in the screening trial, processing resulted in increased sensitivity to Biofeed. The effects of processing and/or enzyme on the amount of protein released into solution were determined (Table 6a) as were their effects on protein digestibility (Table 6b).

Treatment with Biofeed appeared to increase protein solubility (Table 6a) in the case of the barley-meal from Blackbird Farm, but reduce it in the case of the Brecks barley meal. While conditioning did not have any effect on solubility, expansion reduced it. With regard to protein digestibility (Table 6b), in the absence of enzyme, conditioning appeared to increase digestibility of insoluble protein. The CWDE Biofeed had little effect on the protein digestibility of either barley-meal, however, in combination with expansion it increased digestibility to the highest values seen in the project.

CWDE As Process Aids in the Manufacture of Barley Mash

Barley mashes (40% w/v) were prepared with or without Biofeed as described in section 2.3 at either 20° or 40°C for 1 or 2 hours. Both the supernatents and insoluble residues were analysed. In the case of barley from Blackbird Farm, no substantial differences due to time or temperature were seen (Table 7). Mash made from Brecks barley was more variable and no consistent trend was seen. This may be a reflection of the higher amounts of carbohydrate released into solution in the absence of enzyme. For both mashes no consistent effect on either protein solubility or digestibility could be attributed to time, temperature or whether or not an enzyme was present (Tables 8 and 9).

DISCUSSION AND CONCLUSIONS

General

This study has demonstrated the feasibility of screening a range of process variables for their effects on predictors of nutritional quality using laboratory assays. Using these assays (or variants) it will be possible to screen large numbers of processes prior to final evaluation in feeding trials with live-stock.

Process-Effects on Nutritional Quality

The most notable difference in nutritional quality was the greater increase in starch-digestibility, seen with the use of expansion. Not only was the amount of indigestible starch (IS) greatly reduced but most of the starch was converted to the rapidly digestible form (RDS). This feature is of particular importance to weanling pigs, who immediately after weaning produce lower amounts of pancreatic α-amylase, required to digest starch in the intestine. Both conditioning and expansion appear to increase the amount of carbohydrate released by cell-wall degrading enzymes (CWDE). The different susceptibilities of the two barley meals (Blackbird and Brecks) to CWDE makes it difficult to determine whether there was a process-dependent effect. A similar point can be made with regard to protein digestibility. In the case of this parameter, what was interesting was the way that the combined effect of expansion and added CWDE increased protein digestibility to the highest value seen in the whole study.

Design of Future Experimental Protocols

Whilst this study has demonstrated that this type of programme is feasible it has also highlighted areas where further controls will have to be applied. These are discussed individually below.

Assay conditions

Preparation of the test material (e.g. additional milling) and the amount of material used in the assay have to be controlled. Process-linked differences in protein digestibility can be obscured if materials are milled too finely. Similarly the magnitude of response and behaviour of the material can change when assay conditions are scaled up (consider the case of the CWDE, Biofeed). These assays are being redesigned to avoid this problem.

Assay design

The assays described here were designed to evaluate purely nutritional parameters (ability to digest a particular nutrient or liberate more carbohydrate). Processing (either mechanically or treating with CWDE) can also have other beneficial effects with regard to physiology. A major case in point is the ability of CWDE to reduce the viscosity of feeds with high soluble non-starch polysaccharide contents. The original reason for the incorporation of CWDE into feeds was to break down the nonstarch polysaccharides naturally present. This has two physiological effects; it reduces the viscosity of gut contents, improving nutrient uptake and it also reduces faecal bulk. These enzymes were not primarily intended to convert nonstarch polysaccharides to low molecular weight sugars of nutritional potential. Thus the low activity of some of the CWDE observed only suggests that they have little ability to release carbohydrate into solution and makes no comment on their ability to reduce viscosity. Taking these points into account, plus those concerning assay conditions, the CWDE assay will be modified and take into account effects on viscosity.

Variation

Notwithstanding the above, analysis of historical control values reveals low variation on a between-assay basis. One major variable appears to be source of raw material. From the point of view of feeding processed product to the animal this may or may not be a problem. Evidence supporting a contributory role for cereal-source in nutritional quality is controversial. Of greater importance in the design of future projects will be variability in manufacture. While evaluating parameters such as particle size and shape should provide little problem, products from processes which might be scaled up to plant-level will have to be evaluated from more than one production run. In terms of cost and effort this is a major deviation from the approach used here, which simply compared one batch of pellets produced by conditioning with another produced by expansion.

ACKNOWLEDGEMENTS

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REFERENCES

Berry, C.S. (1986). Resistant starch : Formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary-fibre, J. Cereal Sci **4**, 301-314.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193,** 265-275

McDonough, F.E., Sarwar, G., Steinke, F.H., Slump, P., Garcia, S., Boisen, S (1990). In vitro assay for protein digestibility : Interlaboratory study. J. Assoc. Off. Anal. Chem, **73**, 622-625

Ministry of Agriculture Fisheries and Food (1992) MAFF Validated Methods for the the Analysis of Foodstuffs, **VI**: Dietary Fibre (colorimetry) HMSO, London.

Table 1 : Nitrogen, moisture and available starch contents of barley-meal and pellets used*

	N_2	Moisture	Available Starch
Barley-meal (Blackbird)	1.88	12.9	40
Conditioned pellets	2.02	10.3	45
Barley-meal (Brecks)	1.75	14.2	49
Expanded pellets	1.81	10.8	52

*Data expressed as g per 100g.

Table 2 : In vitro digestibility of available starch

	% Available Starch as:		
	RDS*	SDS	IS
Barley-meal (Blackbird)	8.0	54.3	37.7
Conditioned pellets	24.2	52.4	23.4
Barley-meal (Brecks)	6.2	63.4	30.4
Expanded pellets	75.7	16.4	7.9

*RDS, starch digested within 10 minutes; SDS, starch digested between 10 and 120 minutes; IS, starch still undigested after 120 minutes exposure to enzymes.

Table 3 : Total and relative protein digestibilities of marley-meals and pellets prepared from them*

	Total Digestibility* (%)	Relative Digestibility*
Barley-meal (Blackbird)	77.4	0.15
Conditioned pellets	83.9	0.41
Barley-meal (Brecks)	79.9	0.24
Expanded pellets	81.6	0.31

*The definitions of the terms total digestibility and relative digestibility are given in the text.

Table 4 : Carbohydrate Solubility and the effect of cell-wall degrading enzymes

4a Carbohydrate solubilized in the absence of CWDE*

Barley-Meal (Blackbird)	Conditioned Pellets	Barley-Meal (Brecks)	Expanded Pellets
15	35	20.7	25.8

*Data expressed as μg reducing-sugar equivalent solubilized per mg dry weight.

4b Additional Carbohydrate solubilized in the presence of CWDE**

CWDE	Barley-Meal (Blackbird)	Conditioned Pellets	Barley-Meal (Brecks)	Expanded Pellets
Novo				
Biofeed (Novo)	13.9	24.6	34.6	40.1
Biofeed Plus	4.7	3.7	4.4	0
Biofeed Pro	3.3	2.0	2.6	6.9
Energex	2.3	4.8	1.9	0
Sigma				
Hemicellulase	2.8	16.5	146	199
Cellulase	71.6	76.5	75.3	100

**Data expressed as μg net reducing-sugar equivalents released per mg dry weight.

Table 5 : Solubilization of carbohydrate in the presence of the CWDE 'Biofeed' (Novo) under scaled-up conditions (2g substrate, 2.5% w/v suspension)

5a Carbohydrate solubilized in the absence of CWDE*

Barley-Meal (Blackbird)	Conditioned Pellets	Barley-Meal (Brecks)	Expanded Pellets
3.7	6.1	5.1	18.4

*Data expressed as μg reducing-sugar equivalents solublized per mg dry weight.

5b Carbohydrate solubilized in the presence of CWDE Biofeed**

Barley-Meal (Blackbird)	Conditioned Pellets	Barley-Meal (Brecks)	Expanded Pellets
2.8	12.6	2.3	103.6

**Data expressed as μg net reducing-sugar equivalents solubilized per mg dry weight.

Table 6 : Effect of the CWDE Biofeed and/or processing on protein solubility and digestibility

6a Solubility (100 x soluble protein/total protein)

	Barley-Meal (Blackbird)	Conditioned Pellets	Barley-Meal (Brecks)	Expanded Pellets
No enzyme	22	22	41	18
Plus Biofeed	32	23	29	19

6b Digestibility

	Total Digestibility (%)*		Relative Digestibility*	
	no enzyme	+Biofeed	no enzyme	+Biofeed
Barley-meal (Blackbird)	84.5	83.5	0.31	0.32
Conditioned pellets	88.5	86.3	0.50	0.44
Barley-Meal (Brecks)	86.5	86.3	0.45	0.44
Expanded pellets	87.7	90.9	0.46	0.64

*The definitions of the terms total digestibility and relative digestibility are given in the text.

Table 7 : Carbohydrate solubilities of barley mashes and the effect of the CWDE Biofeed following incubation at either 20° or 40°C for 1 or 2 hours

7a Carbohydrate solubilized in the absence of CWDE*

	Source	
	Blackbird	**Brecks**
20°C		
1 hour	392	334
2 hour	278	375
40°C		
1 hour	344	471
2 hour	375	415

*Data expressed as μg reducing-sugar equivalent solublized per mg dry weight.

7b Additional carbohydrate solublized in the presence of Biofeed**

	Source	
	Blackbird	**Brecks**
20°C		
1 hour	94.6	151
2 hour	97.8	25.2
40°C		
1 hour	97.5	0
2 hour	76.0	33.6

**Data are expressed as μg net reducing-sugar equivalents released per mg dry weight.

Table 8 : Effects of time, temperature and the CWDE Biofeed on the solubility of protein in barley mashes*

	Source			
	Blackbird		Brecks	
	no enzyme	+ Biofeed	no enzyme	+ Biofeed
20°C				
1 hour	12.2	13.3	12.0	14.9
2 hour	11.7	11.2	18.3	16.6
40°C				
1 hour	14.4	14.4	18.3	20.0
2 hour	19.1	20.2	21.7	18.3

*Solubility expressed as 100 x soluble protein/total protein.

Table 9 : Effects of time, temperature and the CWDE Biofeed on the digestibility of protein in barley mashes

		Total Digestibility (%)*		Relative Digestibility*	
		no enzyme	+ Biofeed	no enzyme	+ Biofeed
Blackbird					
20°C	1 hour	89.4	88.6	0.52	0.49
	2 hour	91.8	93.0	0.64	0.70
40°C	1 hour	85.0	85.5	0.46	0.52
	2 hour	82.7	81.1	0.39	0.33
Brecks					
20°C	1 hour	85.5	85.5	0.41	0.41
	2 hour	81.2	82.7	0.22	0.29
40°C	1 hour	81.9	83.3	0.31	0.26
	2 hour	93.3	92.9	0.72	0.76

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Appendix 1 : Cell wall degrading enzymes

Enzyme and source	Contents (per g)	Recommended level of Incorporation into Feed
Novo		
Biofeed	75 FBU (fungal β glucanase) 35 KU (α amylase) 10^4 VHCU (hemicellulase) 1500 PTU (pentosanase)	0.5kg/tonne
Biofeed plus	800 FXU (fungal xylanase) 2 X 10^4 VHCU (hemicellulase) 800 NCU (cellulase) 2500 PTU (pentosanase)	0.5kg/tonne
Biofeed pro	1.5 Anson units (protease)	0.5kg/tonne
Energex	75 FBU (fungal β glucanase) 1.5 x 10^5 VHCU (hemicellulase) 10^4 PSU (pectinase) 400 EGU (endoglucanase)	0.5kg/tonne
Sigma		
Cellulase	5 100 sigma units	not applicable
Hemicellulase	26 sigma units	not applicable