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Effects of commercial feed enzymes in wheat-based diets on egg and egg shell quality in imported strains of laying hen

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by Juliet R. Roberts

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Researcher Contact Details
Associate Professor Juliet R. Roberts
Animal Physiology, School of Rural Science
and Agriculture, University of New England,
Armidale, NSW 2351
Phone: (02) 6773 2506
Fax: (02) 6773 3234
Email: jrobert2@metz.une.edu.au

In submitting this report, the researcher has agreed to AECL publishing this material in its edited form.

AECL Contact Details
Australian Egg Corporation Limited
A.B.N: 6610 2859 585
Suite 502, Level 5
12-14 Ormonde Parade
HURSTVILLE NSW 2220
PO Box 569
HURSTVILLE NSW 1481

Phone:	02 9570 9222
Fax:	02 9570 9763
Email:	irene@aeia.org
Website:	http://www.aecl.org

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Foreword

This study was conducted to evaluate the benefits of adding commercial feed enzyme preparations to the diets of laying hens. The benefits of enzyme use with broilers are well-established. However, at the time of commencement of this project, very little research had been conducted using layers and results were not consistent.

The addition of enzymes to layer diets involves a significant, if relatively minor, cost to the producer. Therefore, it is important for producers in the Australian Egg Industry to have access to scientific data which evaluate the benefits so that producers can, themselves, carry out a cost-benefit analysis.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Irene Gorman
Research Manager
Australian Egg Corporation Limited

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Mrs. Wendy Ball provided invaluable technical support to the project. Mrs. Ball conducted all the egg and egg shell quality analyses, assisted with the digesta viscosity analyses and was involved with all aspects of the project.

The studies on apparent metabolisable energy (including feed intake and excreta moisture) were conducted by Ms. Elfira Suawa as part of her degree of Master of Science in Agriculture. Ms. Suawa assisted with the digesta viscosity analyses.

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The assistance of Ridley AgriProducts, Tamworth, Australia for formulation and manufacture of the diets is gratefully acknowledged. Particular thanks are due to Mr. Scott O'Brien, Mr. David Curtis and Mr. Ian Fairbairn.

I wish to thank the following personnel from companies that market enzyme preparations who cooperated in the study. Dr. Susan Davidson and Mr. Geoff Ross of Roche Vitamins provided information about BioFeed Wheat and supplied the Roxazyme G2 Granular used in the study because this product was not available in Australia at the time. Mr. Malcolm Mottram of FinnFeeds provided information about Avizyme 1302 and donated extract viscosity analyses of the grains used in the study. Mr. Rick Carter of Kemin Industries provided information about Kemzyme W dry.

The pullets were purchased from commercial pullet growers, Mr. and Mrs. Max and Shirley Whitten, who provided full details of the rearing conditions of the birds.

Associate Professor Mingan Choct conducted the NSP analyses and some of the extract viscosity analyses on a fee-for-service basis.

Mr. Bob Hughes assayed some AME samples during the time that the UNE bomb calorimeters were out of order, on a fee-for-service basis.

I thank colleagues and staff of the University of New England for advice and assistance.

The Animal Ethics Committee of the University of New England approved the studies reported here. The relevant Animal Ethics Committee Approval numbers are: AEC 2000/0075, AEC 01/059.

Abbreviations

AV	Avizyme 1302
BF	Biofeed Wheat
C	control diet
D	diet
D*E	diet-enzyme interaction
E	enzyme
KM	Kemzyme W dry
N	normal wheat
NS	not statistically significant
NSP	non-starch polysaccharide
P	pinched wheat
RX	Roxazyme G2 granular
W	wheat
W*E	wheat-enzyme interaction
W+R	wheat plus rye

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Executive Summary

The aim of this project was to investigate the efficacy of adding commercial feed enzyme preparations to wheat-based diets in laying hens. A previous study found that commercial feed enzymes improved egg shell quality when added to wheat-based diets but that there were some negative effects on shell colour and Haugh Units (Roberts and Choct, 1999; Roberts *et at.,* 1999). Wheat-based diets were chosen because wheat is a common ingredient in layer diets in Australia. In addition, wheat had been shown to create problems at some times of some years because of high levels of non-starch polysaccharides (NSP) (Annison, 1990; Choct *et at.,* 1995, 1999). Non-starch polysaccharides have been shown to increase the digesta viscosity in poultry, leading to wet, sticky droppings and reduced efficiency of utilisation of feed ingredients. However, most of the studies related to this "new season wheat phenomenon" have been conducted in broilers. Relatively little is known about the situation in layers.

In the present study, every attempt was made to locate new season wheat that was high in NSP levels. Advice was sought from researchers at the University of New England who were involved in the Premium Grains for Livestock Project funded by the Grains Research and Development Corporation and various intensive livestock RDCs, including RIRDC. For Trial 1, two wheats were selected: a "normal" wheat which appeared to have received ample water prior to harvest and a "pinched" wheat which had been water deprived and which was predicted to be high in non-starch polysaccharides. Diets were formulated and manufactured using these two wheat types and different commercial enzyme preparations were added to the diets. Disappointingly, upon analysis, the two wheats were found to be very similar in extract viscosity (where the grain or diet is digested in a manner similar to what would happen in the gut of the bird), and the levels of non-starch polysaccharides. The "pinched" wheat was higher in crude protein (per unit of weight) and resulted in a finished feed that was also higher in crude protein.

The enzymes used were: Biofeed Wheat, Avizyme 1302, Roxazyme G2 granular and Kemzyme W dry. These enzymes were selected because they or similar products have been used in Australia in broiler diets. The levels of inclusion of these enzymes were as recommended for "normal" wheat by the relevant representative of the commercial companies which market these products in Australia.

These experimental diets were fed to the birds in Trial 1 from 25 to 50 weeks of age. The Apparent Metabolisable Energy (AME) of the two diets produced from the "normal" and "pinched" wheat were very similar when measured at 35, 40, 45 and 50 weeks of age. The same birds were used for Trials 1 and 2 for measurement of AME and the experimental diets were fed to these birds throughout the trials. There were no mortalities amongst these birds so there was no need for substitution of birds. The AME of the diets in Trial 1 was not significantly affected by either the type of wheat on which the diet was based, nor by the inclusion of enzymes. In addition, feed intake and excreta moisture were not significantly affected by either the type of wheat on which the diets were based nor the use of enzyme preparations. These findings suggest that enzymes do not improve AME and litter quality in layers, in the absence of high levels of NSP.

For Trial 1, production changed with the age of the birds and was slightly better for the diet based on "pinched" wheat but there was no effect of enzyme supplementation. Egg internal quality and egg shell quality were generally better for birds receiving the "normal" wheat. The reason for this is not clear although it is, presumably, due to factors other than the levels of NSP in the diets. Enzyme supplementation of the diets resulted in some effects on egg internal quality and egg shell quality. In general, the effects of diet and enzymes were greatest when the birds were younger. This response may be due to the age of the birds and the maturity of their gastrointestinal systems or it may reflect the amount of time that they have been consuming the diets, or both of these factors. For all the egg collections made during Trial 1, at 27, 30, 35, 40, 45 and 50 weeks of age, the diets based on "normal" wheat resulted in darker shell colour, better egg shell breaking strength, heavier and thicker

egg shells and better albumen quality. Over this same time period, the addition of commercial enzyme preparations was found to affect shell colour, shell breaking strength, percentage shell, shell thickness and yolk colour. Shell colour was slightly lighter for some of the enzymes, particularly Roxazyme and Kemzyme. This is probably not of commercial significance. However, it is an effect which should be monitored when enzymes are used in layers. Shell breaking strength was not consistently improved by the addition of feed enzymes, although there were some beneficial effects with Kemzyme. The percentage shell (ratio of shell weight to egg weight, expressed as a percentage) and shell thickness were best for Kemzyme. Yolk colour varied, being generally lower for the enzyme groups. The reason for this is not clear. The effect is slight and not of commercial significance as all yolk colours were very acceptable. However, it is interesting in terms of the mode of action of the enzymes.

Because a previous study (Roberts and Choct, 1999; Roberts *et at.,* 1999) had found a negative effect of feed enzymes on Haugh Units, it was decided to investigate the effect of adding feed enzymes to the diets on the "keeping power" of the eggs. Keeping power refers to the extent to which albumen height and Haugh Units are maintained at high levels during the storage of the eggs. Albumen height and Haugh Units were measured in fresh eggs at 40 and 45 weeks of age. Eggs collected at the same time were stored at either cool room temperature (10-12°C) or room temperature (23-25°C) for 4 weeks prior to measurement of albumen height and Haugh Units. As would be expected, albumen height and Haugh Units were highest in the fresh eggs, followed by the eggs stored at cool room temperature and, lowest of all, the eggs stored at room temperature. Although there were some effects of diet and enzyme on albumen height and Haugh Units, these "primary" effects were not influenced by the storage treatment itself.

At 45 weeks of age, blood samples were taken from the same birds that were used for the AME measurements. The haematocrit (proportion of red blood cells to the volume of whole blood) and the concentrations of sodium, potassium and ionised calcium (the portion of the calcium in blood that is available for biological activities such as bone and egg shell formation) were measured. There were some interesting effects with haematocrit being higher for Biofeed Wheat than for the other treatment groups and ionised calcium higher for Avizyme. The significance of these findings is not clear but it appears that the feed enzymes have some effects on the physiology of the birds, either directly or indirectly.

Because Trial 1 had been unable to determine the effect of adding commercial feed enzymes to diets which create a high digesta viscosity, it was decided to use a "rye model" for Trial 2 of this study. The "rye model" had been used previously to investigate the "new season wheat" phenomenon where high non-starch polysaccharide levels in wheat result in high viscosity of the intestinal contents. Twenty-five tonnes of the "pinched" wheat had been stored in a silo at the commencement of Trial 1. This wheat was used for all the diets in Trial 2. For the birds which received the diets based on "pinched" wheat in Trial 1, the diets were maintained the same for Trial 2. However, the birds which had received "normal" wheat in Trial 1 were now fed diets based on the "pinched" wheat but with 20% of the wheat substituted with cereal rye. Rye is a grain with a high extract viscosity and was predicted to increase the digesta viscosity, mimicking the effect of a high NSP/low AME "new season" wheat. The same enzymes were used at the same levels of inclusion as in Trial 1. These diets were fed to the birds, including the AME birds, from 50-73 weeks of age. At 65 weeks of age, following the egg collection and the AME measurements, the birds were placed into an induced moult. Full feed was restored at 68 weeks of age and birds were back in full production by 71 weeks of age. Egg collections and AME determinations were made at 55, 60, 65 and 73 weeks of age. Eggs were collected for keeping power studies at 55, 60 and 65 weeks of age and blood samples were taken at 72 weeks of age. At the end of Trial 2, when birds were 72-73 weeks of age, a sample of 50 birds (5 per diet) was used for determination of digesta viscosity in the jejunum and ileum of the gastrointestinal tract.

Although the cereal rye grain had a very high extract viscosity, the diets which were based on wheat plus rye had an extract viscosity only 3 times that of the diets containing wheat only. This finding was surprising and may be due to the presence of endogenous enzymes in other feed ingredients used in the diets. When the levels of non-starch polysaccharides of the wheat and wheat plus rye diets were compared, the wheat plus rye diets were 10-15% higher for soluble, insoluble and total non-starch polysaccharides than were the wheat diets.

As found in Trial 1, feed intake, excreta moisture and the apparent metabolisable energy of the diets were not significantly affected by the type of diet or the inclusion of feed enzymes. Digesta viscosity was higher in both the jejunum and ileum for the wheat plus rye diets as compared with the wheat diets. However, the addition of feed enzymes did not reduce the digesta viscosity in either part of the gut. This finding is surprising, given the higher extract viscosity of the wheat plus rye diets and raises questions about the ability of feed enzymes to reduce digesta viscosity in laying hens.

In Trial 2, production at 55-65 weeks was affected by dietary enzyme supplementation, with production being slightly higher than the control for Biofeed Wheat and Roxazyme and slightly lower for Avizyme and Kemzyme. However, during the period of the induced moult, production was affected by diet but not by the addition of enzymes. For the wheat diets, production dropped more rapidly and to lower levels than for the wheat plus rye diets.

There were some significant effects of the grains on which the diets were based on egg internal quality and egg shell quality. The wheat plus rye diets resulted in higher shell breaking strength and better albumen quality that the wheat diets. There were also significant effects on egg internal quality and egg shell quality of the feed enzymes. Egg weight was higher for the control and lowest for Kemzyme. Shell colour was lighter in the eggs from birds receiving enzymes than it was for the control. Albumen height and Haugh Units were significantly lower for the Kemzyme group. These effects on shell colour and albumen quality are similar to those reported in a previous study (Roberts and Choct, 1999; Roberts *et at.*, 1999). Yolk colour varied among the enzyme treatment groups.

As was found in Trial 2, there were some effects on egg keeping power of the diets and the enzymes. However, again, these were primary effects and the egg storage treatment did not modify them further. The only effect on blood parameters was that haematocrit was lower for the group receiving Roxazyme in the wheat diet.

For the final stage of the study (Trial 3), a new season wheat was sourced for the diets which were fed from 73 to 87 weeks. There were only five diets, containing the same enzyme treatments as for Trials 1 and 2. Birds continued to receive the same enzymes throughout. Despite this wheat being a "new season" wheat, it was not high in soluble, insoluble or total non-starch polysaccharides. Production declined as the birds aged but was not affected by enzymes. The only effect of enzymes on egg internal quality and egg shell quality was that shell colour was lighter than the control for Biofeed Wheat, Avizyme and Kemzyme (but not Roxazyme) and yolk colour was generally lighter for the enzyme groups. The effects of enzymes on the keeping power of eggs were, again, primary effects which were not further modified by the egg storage treatment.

There are several main conclusions that result from this study:

1. New season wheats are not necessarily high in non-starch polysaccharides

2. The "new season wheat" phenomenon appears to occur occasionally, rather than regularly

3. Enzymes do not necessarily reduce litter moisture

4. Enzymes do not necessarily increase apparent metabolisable energy

5. Different wheats produced different levels of egg internal quality and egg shell quality which appeared to be independent of the levels of non-starch polysaccharides and the levels of crude protein

6. Enzymes do not necessarily improve egg shell quality, although they appear to do so under some circumstances

7. The effects of diets and enzymes varied with the age of the bird (and possibly also with the length of time that the birds had been receiving the diets)

8. Enzymes did not alter the keeping power of eggs, beyond any primary effects that they had on albumen quality

9. A moderate elevation in the digesta viscosity resulting from the inclusion of rye in the diets did not have negative effects on bird performance

10. The addition of feed enzymes had some effects on blood parameters, presumably reflecting effects on the birds' physiology

11. Enzymes did not reduce digesta viscosity, at least where digesta viscosity was moderately elevated

12. Enzymes did not affect the performance of hens during an induced moult

Introduction

The use of enzymes in commercial layer diets has become more common in recent years (Acamovic, 2001, Bedford and Morgan, 1996; Leeson and Summers, 1997). Enzymes are employed to increase the digestibility of feed ingredients and reduce the incidence of wet droppings which may result from the presence of non-starch polysaccharides in the diets (Annison, 1990, 1993; Annison and Choct 1991; Choct 2001). Some ingredients present in feed bind other feed components such as phosphorus, calcium and trace minerals. Therefore, use of appropriate enzymes will increase the availability of these feed components, many of which influence egg shell quality (Hurwitz, 1987). Concern has been expressed about reduced egg shell quality resulting from the use of enzymes (Richards, 1998). However, a recent study showed that addition of commercial enzyme preparations improved egg shell quality in wheat- and barley-based layer diets but that there were some negative effects on shell colour and Haugh Units (Roberts and Choct, 1999; Roberts *et at.*, 1999). The types of enzymes, the substrates on which they act, their functions and benefits are summarised in Table 1.

The benefits of adding commercial enzyme preparations to poultry feed have been researched extensively for broilers (Acamovic, 2001). However, there is little information available about the benefits of adding enzymes to layer diets (Berg, 1961; Wyatt and Goodman, 1993; Zhang *et at.,* 2000). Layers and broilers differ in a number of ways including the fact that broilers are immature birds, whereas layers are mature birds. There is evidence that bird age, even within broilers, can influence digestive function (Petersen *et at.,* 1999). Therefore, the present study investigated the benefits of adding commonly used commercial enzyme preparations to layer diets.

The benefits obtained by adding enzymes to feed appear to be related, at least in a large part, to the ability of the enzymes to reduce the viscosity of the intestinal contents (Bedford and Classen, 1992; Smits *et at.*, 1997), although this may not be the only mechanism operating (Choct *et at.*, 1996).

Because the end product of the layer industry is the table egg, the present study focussed on the effect of adding commercial enzymes to layer feed on egg internal quality and egg shell quality. It was important to see if the beneficial effects on egg shell quality reported previously (Roberts and Choct, 1999; Roberts *et at.*, 1999) could be repeated and also to see if the negative effects on shell colour and Haugh Units were a consistent effect.

In Australia, wheat is a common ingredient in layer diets. However, the quality and composition of Australian wheats are variable (Choct and Hughes, 1996). New season wheats have been reported to be high in non-starch polysaccharides which often increase the viscosity of the digesta and reduce the efficiency of utilisation of feed ingredients. Digestibility problems associated with wheat have been researched extensively but mainly in broilers (Annison, 1990; *Beford et at.*, 1998; Choct *et at.*, 1995; Choct *et at.*, 1999; McNab, 1996; Pettersson and Aman, 1989; Ravindran *et at.*, 1999; Rogel *et at.*, 1987; Scott *et at.*, 1998; Wiseman *et at.*, 2000).

General Objectives

This project investigated the effects of adding commercial feed enzyme preparations to two different types of wheat. The initial intention had been to investigate the effect of new season wheat (high in non-starch polysaccharides), with or without enzyme supplementation, on egg and egg shell quality in an imported strain (Isa Brown) of laying hen.

Table 1: Some enzymes that can be used in feed for poultry

Enzymes	Substrates	Function	Benefits or use
β-glucanase	Barley Oats	Viscosity reduction	Enhanced digestion and utilization of nutrients
Xylanase	Wheat, Rye Triticale Rice bran	Viscosity reduction	Enhanced digestion and utilization of nutrients
β-galactosidases	Grain Legumes Lupins	Viscosity reduction	Enhanced digestion and utilization of nutrients
Phytases	Plant Feedstuffs	Release of P from phytate-P	Enhanced phosphate absorption
Proteases	Protein	Hydrolysis of protein	Increase digestion of proteins
Lipases	Lipid	Hydrolysis of fat	Use in young animals
Amylases	Starch	Hydrolysis of starch	Supplemental amylase for young animals

Adapted from Marquardt, 1997.

Table 2: Enzyme preparations used in this study

Product	Inclusion Rate for Layers	Enzyme	*Minimum Activity
BioFeed Wheat CT (Ronozyme WX (CT)	150-200 g/tonne	Fungal xylanase	1000 units/g
Avizyme 1302	375 g/tonne (or as per Avicheck)	Endo-1,4-ß-glucanase Subtilisin (Protease)	5000 units/g 1600 units/g
Roxazyme G2 granular	50-100 g/tonne	Endo-1,4-ß-glucanase Endo-1,3(4)-ß-glucanase Endo-1,4-ß-xylanase	8000 units/g 18,000 units/g 26,000 units/g
Kemzyme W Dry	500-1000g/tonne	Xylanase ß-glucanase Cellulase Protease □-Amylase Lipase	For detail, contact Kemin (Aust.) Pty. Ltd.

**** Please note that the units of activity are manufacturer's units measured under conditions specific to the enzyme and source organism in question. They are not international units. Therefore, it is not meaningful to compare the units for different enzyme preparations***

1. Different wheats with or without enzymes: Birds 25-50 weeks of age (Trial 1)

1.1 Introduction

The use of enzymes in commercial layer diets has become more common in recent years (Bedford and Morgan, 1996; Leeson and Summers, 1997). Enzymes are employed to increase the digestibility of feed ingredients and reduce the incidence of wet droppings which may result from the presence of non-starch polysaccharides in the diets. Some ingredients present in feed bind other feed components such as phosphorus, calcium and trace minerals. Therefore, use of appropriate enzymes will increase the availability of these feed components, many of which influence egg shell quality (Hurwitz, 1987). Concern has been expressed about reduced egg shell quality resulting from the use of enzymes (Richards, 1998). However, a recent study showed that addition of commercial enzyme preparations improved egg shell quality in wheat- and barley-based layer diets but that there were some negative effects on shell colour and Haugh Units (Roberts and Choct, 1999; Roberts *et at.*, 1999). In Australia, wheat is a common ingredient in layer diets. However, the quality and composition of Australian wheats are variable (Choct and Hughes, 1996). In the present study, four commercial feed enzymes were added to two wheat-based layer diets, formulated to standard commercial guidelines. These diets were fed to Isa Brown laying hens, from 25 weeks of age. Egg and egg shell quality were assessed at 27, 30, 35, 40 and 45 weeks of age.

1.2 Methodology

1.2.1 Bird Rearing

Seven hundred and sixty Isa Brown laying hens were purchased at 16 weeks of age from a commercial pullet grower (Max and Shirley Whitten) in the Tamworth area of New South Wales. Birds received starter crumble to 10 weeks of age, grower crumble from 10 to 15 weeks of age and Superall Layer from 15 to 16 weeks of age. Upon arrival at the University of New England, birds were placed on a Prelayer ration (grower with added calcium and yolk pigment). Birds were vaccinated for Marek's at day-old, IB at day-old and 11 weeks, ILT at 11 weeks, Fowl Pox at day-old and 11 weeks, AE at 11 weeks, MS at 12 weeks, and EDS at 13 weeks. Birds were reared on litter until 8-9 weeks of age when they were placed in cages. They were beak-trimmed at 11 weeks of age.

1.2.2 Bird Maintenance and Diets

Birds were maintained, three to a cage, in a commercial poultry house at the University of New England "Laureldale" Poultry Farm. The different treatment groups were randomised to avoid effects due to position in the poultry house. Birds received a prelayer diet until they reached 5% lay, at which time they were transferred to a commercial layer ration. At 25 weeks of age, birds were placed on to the experimental diets. Two types of wheat were selected: one which had received abundant water prior to harvest ("normal" wheat) and a second wheat that had been water-stressed (described in the Australian industry as "pinched" wheat). Basal diets were formulated to standard commercial specifications, each containing 670 g/kg of either "normal" wheat or "pinched" wheat, as shown in Tables 3 and 4. The other ingredients were identical in the two diets. The basal diets were each used to prepare five experimental diets by adding one of four commercial feed enzyme preparations according to the manufacturers' instructions; a control diet of each wheat type (no enzyme added), Biofeed Wheat (175 g/tonne), Avizyme 1302 (265 g/tonne), Roxazyme G2 granular (100 g/tonne), or Kemzyme W dry (600 g/tonne) (Table 2). All commercial enzyme preparations were purchased through standard commercial channels with the exception of Roxazyme G2 granular. Roxazyme G2 granular was not available commercially in Australia at the time of the study. Roxazyme was supplied by Roche Vitamins. Two tonnes of each of the 10 experimental diets were manufactured.

Table 3: Formulation used for wheat-based diets

Raw Material	Percentage inclusion	kg per 2000 kg of feed
Wheat coarse 12.5%	67.41	1348.2
Meat Meal 55.0%	10.0	200.0
Sunflower Meal 30.0	3.05	61.0
Cottonseed Meal 38.0%	5.0	100.0
Soy Lti Flour	1.3	26.0
Rice Pollard 13.0	5.0	100.0
Limestone B12 + 120 kg	7.5	150.0
Rockphos	0.05	1.0
Salt	0.05	1.0
Sodium Bicarbonate	0.1	2.0
Choline Chloride 75%	0.03	0.6
DL-Methionine	0.08	1.6
L-Lysine Scale 3	0.08	1.6
Rap Std Layer/Pullet PMX (2kg/ton)	0.2	4.0
Rap Std Synth Yolk Colour Premix	0.15	3.0
TOTAL	100.0	2000.0

Table 4: Calculated analysis of wheat-based diets

Ingredient	**Percent**	**Ingredient**	**Percent**
[Volume]	100.0	Lysine	0.77
Protein	18.063	Methionine	0.35
Fat	3.70	Methionine + Cystine	0.67
Fibre	3.63	Threonine	0.57
Metabolisable energy kcal/kg	2702.6	Leucine	1.14
Metabolisable energy MJ/kg	11.31	Isoleucine	0.60
Calcium	3.79	Tryptophan	0.17
Phosphorus	0.84	Arginine	1.17
Available Phosphorus	0.50	Average Lysine for poultry	0.55
Calcium:Phosphorus	4.53	Linoleic Acid	1.10
Calcium:Available Phosphorus	7.57	Choline	1193.25
Sodium	0.16	Legumes	0.0
Chloride	0.18	% Sunflower + Cottonseed Meal	8.05
Sodium + Potassium – Chloride	177.48	% Soy + FF	1.3
Salt	0.30		

All values are percentage, unless otherwise indicated

1.2.3 Egg and Egg Shell Quality Measurements

Egg collections were made at 27, 30, 35, 40, 45 and 50 weeks of age. At each age, 300 eggs were collected, 30 from each of the ten treatment groups. Egg and egg shell quality analyses were completed within 24 hours of the eggs being laid. Measurements taken to assess egg shell quality were egg weight, shell reflectivity (an indication of the colour of the egg shell), egg shell breaking strength (measured by quasi-static compression), deformation (the distance that the egg shell is depressed by the shell breaking strength machine before the shell cracks), shell weight and shell thickness. The percentage shell was calculated as the ratio of shell weight to egg weight, expressed as a percentage. The internal quality of the eggs was assessed as albumen height and Haugh Units as well as yolk colour. Most of the equipment used for these measurements had been purchased from Technical Services and Supplies, U.K., although the shell thickness equipment was manufactured at the University of New England, using a Mitutoyo Dial Comparator gauge.

1.2.4 Feed Intake, Apparent Metabolisable Energy and Excreta Moisture

Apparent metabolisable energy (AME) and excreta moisture were measured every 5 weeks from 30 to 50 weeks of age. AME was determined by the conventional total collection procedure. Birds had received the experimental diets for at least 5 weeks prior to the AME assays which were conducted over 4 days. The same birds were used for Trials 1 and 2 for measurement of AME and the experimental diets were fed to these birds throughout the trials. There were no mortalities amongst these birds so there was no need for substitution of birds.

Feed intake was measured and all excreta collected daily. The amount of feed consumed during the excreta collection period was recorded. Feed consumption per bird was expressed on a daily basis and calculated using the formula:

$$\text{Daily feed intake (g/bird/d)} = \frac{\text{Feed eaten (g)} - \text{Feed residue (g)}}{\text{Length of trial (d)}}$$

Fresh excreta were dried in a forced-drought oven at 80°C for 36 h. Moisture content of excreta was calculated as:

$$\frac{\text{Wet excreta (g)} - \text{Dry excreta (g)}}{\text{Wet excreta (g)}} \times 100$$

The dry-matter (DM) content of the diets was determined gravimetrically following drying at 105°C for 16 hours. Gross energy of excreta and diets were determined using a CP 500 automatic calorimetric processor (Digital Data System Ltd, CP 500, Northcliff, South Africa). Benzoic acid was used to standardise the bomb calorimeter.

Excreta from each replicate were pooled over the collection period for the determination of gross energy (GE). AME of diets was calculated as:

$$\frac{(\text{g of feed eaten x GE of feed}) - (\text{g excreta voided x GE excreta})}{\text{g feed eaten}}$$

1.2.5 Keeping Power of Eggs

A previous study (Roberts and Choct, 1999; Roberts *et at.*, 1999) had found that the addition of commercial enzymes resulted in a reduction in Haugh Units. Therefore, albumen height and Haugh units were determined in fresh eggs and also in eggs which had been stored at either cool room temperature (10-12°C) or room temperature (20-25°C) for a period of 4 weeks. This was to test for any differences in the "keeping power" of the eggs, or the maintenance of Haugh Units, in eggs from birds receiving the different enzyme preparations. Albumen height and Haugh Units were measured as described above. Eggs were collected for determination of keeping power at 40 and 45 weeks.

1.2.6 Plasma Electrolytes

Fifty birds, 5 from each of the 10 diets, were bled at 45 weeks of age for blood and plasma samples. A 2 ml blood sample was collected anaerobically in a 2 ml syringe via venipuncture of the cutaneous ulnar vein. Air was expelled and the syringe sealed immediately with a plastic cap. All samples were put in ice until analysed. Ionised calcium, sodium and potassium were analysed immediately after sample collection using an AVL Electrolyte 984 analyser (AVL Medical Instruments, Switzerland), allowing the analyser to take the centre point of the blood in the syringe. Duplicate haematocrit tubes were filled with blood, sealed and centrifuged in a Hawksley Microhaematocrit Centrifuge at 13,000 RPM for 3 minutes for the measurement of haematocrit.

Data were analysed by ANOVA with wheat type, enzyme treatment and hen age being the main independent variables. Differences between means were assessed by Fisher's (Protected) Least Significance Difference test. Significance was assumed at $P<0.05$.

1.3 Detailed Results

1.3.1 Wheats and Diets

The two wheats selected for this study were expected to differ in the levels of non-starch polysaccharides. However, on analysis, the wheats were found to be similar for extract viscosity (Table 5) and for total, soluble and insoluble non-starch polysaccharides (Table 6). However, the pinched wheat was higher in free sugars. The extract viscosities of the two wheats, and the diets based on the two types of wheat, were relatively low, despite the fact that both wheats were relatively "new season" wheats.

Protein analysis of both wheats and the control diets manufactured from them showed that the protein level was higher for pinched wheat (178 g/kg in the wheat, 228 g/kg in the final diet) than for normal wheat (149 g/kg in the wheat, 185 g/kg in the final diet).

Table 5: Feed and grain extract viscosities

Sample	Extract Viscosity cPs
Feed based on normal wheat	4.29
Normal wheat	5.71
Feed based on pinched wheat	4.15
Pinched wheat	5.98

Table 6: Non starch polysaccharides (NSP) in wheats and diets (g/kg)

Feed or Grain	Sugar g/kg	Free Sugars g/kg	Insoluble NSP g/kg	Soluble NSP g/kg	Total NSP g/kg
Feed based on "normal" wheat	Rhamnose	0.00	0.27	0.11	0.38
	Fucose	0.00	0.00	0.10	0.10
	Ribose	0.08	0.00	0.27	0.27
	Arabinose	0.59	20.14	3.6	23.73
	Xylose	0.45	30.74	4.12	34.85
	Mannose	2.86	1.35	0.59	1.93
	Galactose	2.78	3.99	2.22	6.22
	Glucose	26.45	33.01	2.40	35.42
	TOTAL	32.87	79.53	11.57	91.09
"Normal" Wheat (whole grain)	Rhamnose	0.00	0.00	0.04	0.04
	Fucose	0.29	0.00	0.05	0.05
	Ribose	0.00	0.000	0.19	0.19
	Arabinose	0.72	24.57	3.58	28.15
	Xylose	0.45	34.4	4.47	38.87
	Mannose	4.36	0.95	0.49	1.44
	Galactose	1.35	3.1	1.74	4.84
	Glucose	45.55	26.98	1.79	28.77
	TOTAL	52.72	79.81	10.60	90.41
Feed based on "pinched" wheat	Rhamnose	0.00	0.25	0.10	0.35
	Fucose	0.26	0.00	0.06	0.06
	Ribose	0.00	0.00	0.29	0.29
	Arabinose	0.37	20.10	2.89	23.00
	Xylose	0.19	26.19	2.87	29.06
	Mannose	2.02	1.45	0.53	1.99
	Galactose	3.13	2.73	1.81	4.55
	Glucose	15.16	30.05	1.46	31.51
	TOTAL	21.00	71.77	8.55	80.32
"Pinched" Wheat (whole grain)	Rhamnose	0.00	0.00	0.05	0.05
	Fucose	0.00	0.00	0.04	0.04
	Ribose	0.00	0.00	0.16	0.16
	Arabinose	0.41	25.31	2.60	27.91
	Xylose	0.33	33.78	2.50	36.28
	Mannose	1.88	1.12	0.49	1.61
	Galactose	2.29	1.53	1.89	3.42
	Glucose	13.24	26.6	1.66	28.26
	TOTAL	18.15	78.31	8.00	86.32

1.3.2 Feed Intake

Feed intake was not significantly affected by wheat type or enzyme inclusion (Table 7). However, there were differences among the ages at which feed intake was measured. Feed intake was influenced by the time of year, owing to the climatic changes in temperature that occurred. The feed intake measured at 30 weeks of age was an overestimate owing to feed "flicking" by the birds. Metal grids were placed on the top of the feed so that feed spillage was minimal for all later measurements.

Table 7: Feed intake of laying hens fed diets containing wheat with or without enzyme supplementation at 30 to 50 weeks of age.

Diets	**Feed intake g/d**				
	30 weeks	35 weeks	40 weeks	45 weeks	50 weeks
Normal Wheat:					
Control	145.3±6.1	112.6±5.2	131.0± 4.9^{a}	120.1±3.3	116.0±8.2
BioFeed Wheat	147.2±5.5	125.8±6.3	118.8±6.5^{ab}	111.2±9.8	105.1±10.9
Avizyme	144.4±5.5	114.2±6.0	122.0±3.8^{ab}	116.7±6.9	116.0±6.1
Roxazyme	149.5±5.2	107.6±5.4	110.6±4.9^{b}	94.2±11.6	98.2±15.5
Kemzyme	141.7±10.0	115.4±6.2	119.8±6.1^{ab}	126.3±7.1	115.6±7.9
Pinched Wheat:					
Control	144.2±12.1	117.9±4.4	116.4±3.9	109.4±7.7	107.5±3.4
BioFeed Wheat	131.4±4.8	103.7±3.8	106.6±5.9	106.3±3.2	106.0±6.2
Avizyme	132.9±10.4	109.3±10.8	102.1±13.0	97.0±19.4	104.5±11.1
Roxazyme	140.3±10.2	113.4±3.2	124.3±5.7	121.6±5.5	128.9±13.2
Kemzyme	138.0±6.4	111.6±3.4	111.2±5.9	116.0±4.9	125.7±8.6
			Statistical Analysis		
Wheat Type			NS		
Enzyme			NS		
Wheat * Enzyme			NS		
Age			P<0.0001		

Values are Mean ± Standard Error of the Mean. NS is not statistically significant.
Within an age and wheat type, values for enzyme treatments with unlike superscripts differ significantly (P<0.05).

1.3.3 Faecal Moisture Levels

Faecal moisture levels were not significantly affected by wheat type or enzyme inclusion (Table 8). However, there were differences among the ages at which faecal moisture levels were measured, most likely resulting from climatic changes in temperature and humidity.

Table 8: Faecal Moisture of laying hens fed diets containing wheat with or without enzyme supplementation at 30 to 50 weeks of age.

Diets	**Faecal moisture g/100 g**				
	30 weeks	35 weeks	40 weeks	45 weeks	50 weeks
Normal Wheat:					
Control	66.40±0.07[b]	72.71±0.18	72.88±0.10[ab]	74.83±0.19	73.63±0.31[ab]
BioFeed	73.26±0.08[a]	71.03±0.12	73.10±0.07[ab]	78.30±0.12	74.16±0.41[ab]
Avizyme	72.20±0.08[a]	74.84±0.08	68.84±0.08[b]	76.98±0.10	71.91±0.26[b]
Roxazyme	70.82±0.26[ab]	73.24±0.13	73.64±0.15[a]	77.11±0.53	80.55±0.64[a]
Kemzyme	72.09±0.16[a]	72.91±0.22	71.75±0.18[ab]	76.70±0.28	74.60±0.14[ab]
Pinched Wheat:					
Control	67.68±0.19	67.37±0.21[b]	69.23±0.27	72.67±0.44	71.58±0.22
BioFeed	71.89±0.07	74.53±0.13[a]	73.37±0.24	77.75±0.24	73.75±0.25
Avizyme	67.75±0.04	69.77±0.24[ab]	71.88±0.79	75.79±1.01	72.46±0.48
Roxazyme	70.18±0.07	70.59±0.20[ab]	72.49±0.16	73.92±0.22	72.57±0.29
Kemzyme	71.28±0.23	69.60±0.15[ab]	69.81±0.16	71.79±0.15	74.93±0.20
			Statistical Analysis		
Wheat Type			NS		
Enzyme			NS		
Wheat*Enzyme			NS		
Age			P<0.0001		

Values are Mean ± Standard Error of the Mean. NS is not statistically significant.
Within an age and wheat type, values for enzyme treatments with unlike superscripts differ significantly (P<0.05).

1.3.4 Apparent Metabolisable Energy of Diets

The apparent metabolisable energies of the ten diets were very similar, irrespective of the type of wheat on which the diet was based and the addition of enzymes. There were, however, significant differences as the result of hen age. The AME values calculated at 30 weeks will be influenced by the feed wastage described above. However, in general, AME values increased to 40 weeks of age and then remained relatively constant.

Table 9: Apparent Metabolisable Energy (AME) for laying hens from 30 to 50 weeks of age of diets containing wheat with or without enzyme supplementation.

Diets	**AME MJ/kg DM**				
	30 weeks	35 weeks	40 weeks	45 weeks	50 weeks
Normal Wheat					
Control	13.07±0.07	13.44±0.18	14.79±0.10[a]	14.41±0.19	13.52±0.31[ab]
BioFeed	13.05±0.08	13.03±0.12	14.22±0.07[b]	13.85±0.12	12.66±0.41[b]
Avizyme	13.03±0.08	13.15±0.08	13.63±0.08[c]	14.2±0.10	13.47±0.26[ab]
Roxazyme	13.22±0.03	12.91±0.13	14.33±0.15[b]	13.49±0.53	13.95±0.64[a]
Kemzyme	12.69±0.16	13.03±0.22	13.48±0.18[c]	13.53±0.28	13.74±0.14[ab]
Pinched Wheat					
Control	12.97±0.19	11.65±0.21[c]	14.78±0.27	14.3±0.44	12.94±0.22[b]
BioFeed	12.96±0.07	13.82±0.13[a]	14.12±0.24	13.84±0.24	13.54±0.25[ab]
Avizyme	13.08±0.04	13.17±0.24[b]	13.97±0.79	13.25±1.01	13.40±0.48[ab]
Roxazyme	12.91±0.07	12.71±0.20[b]	14.65±0.16	14.71±0.22	14.17±0.29[a]
Kemzyme	12.83±0.23	12.65±0.20[b]	14.13±0.45	10.05±3.97	13.80±0.23[ab]
			Statistical Analysis		
Wheat Type			NS		
Enzyme			NS		
Wheat*Enzyme			NS		
Age			P<0.0001		

Values are Mean ± Standard Error of the Mean. NS is not statistically significant.
Within an age and wheat type, values for enzyme treatments with unlike superscripts differ significantly (P<0.05).

1.3.5 Production

Tables 10-12 show the main effects of hen age, wheat type and enzyme addition, respectively, on egg production. Egg production increased to 35 weeks of age after which it decreased (Table 10). Production was slightly but significantly better for the pinched wheat than for the normal wheat (Table 11). However, production was not affected by the addition of commercial feed enzyme preparations (Table 12).

Table 10: Effect of hen age on production at 27-50 weeks of age.

Age of Hens (weeks)					**P Value**
27-30 wks	30-35 wks	35-40 wks	40-45 wks	45-50 wks	
90.24 ±0.31	96.18 ±0.27	94.99 ±0.35	93.47 ±0.33	90.93 ±0.42	<0.0001

Values are Means ± Standard Errors of the Means

Table 11: Effect of wheat type on production at 27-50 weeks of age

Type of Wheat on which diet based		**P Value**
Normal Wheat	Pinched Wheat	
90.45 ±0.43	91.08 ±0.40	0.0472

Values are Means ± Standard Errors of the Means

Table 12: Effect of enzyme treatment on production at 27-50 weeks of age.

Enzyme Treatment					**Statistical Analysis**
Control	Biofeed Wheat	Avizyme	Roxazyme	Kemzyme	P Value
90.69 ±0.67	91.24 ±0.67	90.41 ±0.71	90.98 ±0.62	90.49 ±0.70	NS

Values are Means ± Standard Errors of the Means. NS is not statistically significant.

Detailed production figures for each age bracket, in relation to wheat type and enzyme treatment, are shown in Tables 13-17. For each individual age bracket, corresponding to the time between egg collections, there were no significant effects on production of wheat type or enzyme addition and no significant interaction between these two main factors.

Table 13: Production from 27-30 weeks of age

Wheat Type	Treatment Group						Statistical Analysis P Values		
	C	BF	AV	RX	KM	**Mean**	W	E	W*E
Normal	89.08 ±1.06	90.30 ±1.05	90.32 ±1.07	89.03 ±1.21	89.53 ±0.71	**89.65 ±0.44**	NS	NS	NS
Pinched	90.75 ±0.72	91.68 ±0.87	90.50 ±1.20	90.59 ±0.70	90.65 ±1.24	**90.83 ±0.41**			
Mean	**89.91 ±0.66**	**90.99 ±0.69**	**90.40 ±0.76**	**89.81 ±0.71**	**90.09 ±0.70**				

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

Table 14: Production from 30-35 weeks of age

Wheat Type	Treatment Group						Statistical Analysis P Values		
	C	BF	AV	RX	KM	**Mean**	W	E	W*E
Normal	95.95 ±0.66	96.14 ±0.44	96.44 ±0.72	94.45 ±1.52	97.19 ±0.44	**96.03 ±0.39**	NS	NS	NS
Pinched	96.75 ±0.32	95.33 ±1.18	96.13 ±0.65	97.26 ±0.75	96.20 ±1.02	**96.33 ±0.37**			
Mean	**96.35 ±0.37**	**95.74 ±0.61**	**96.28 ±0.46**	**95.85 ±0.93**	**96.70 ±0.55**				

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

Table 15: Production from 35-40 weeks of age

Wheat Type	Treatment Group						Statistical Analysis P Values		
	C	BF	AV	RX	KM	**Mean**	W	E	W*E
Normal	94.90 ±1.63	95.70 ±0.80	94.12 ±0.49	94.30 ±0.98	95.29 ±0.75	**94.86 ±0.43**	NS	NS	NS
Pinched	95.42 ±1.44	94.56 ±1.49	94.81 ±1.65	95.95 ±0.31	94.86 ±1.49	**95.12 ±0.57**			
Mean	**95.16 ±1.03**	**95.13 0.82**	**94.47 ±0.82**	**95.13 ±0.56**	**95.07 ±0.79**				

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.

C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

Table 16: Production from 40-45 weeks of age

Wheat Type	Treatment Group						Statistical Analysis P Values		
	C	BF	AV	RX	KM	**Mean**	W	E	W*E
Normal	93.51 ±0.94	92.99 ±0.96	93.43 ±1.63	93.48 ±0.98	92.71 ±0.53	**93.23 ±0.44**	NS	NS	NS
Pinched	94.33 ±1.02	93.61 ±1.17	93.13 ±1.12	94.23 ±0.98	93.29 ±1.62	**93.72 ±0.50**			
Mean	**93.92 ±0.67**	**93.30 ±0.72**	**93.28 ±0.94**	**93.86 ±0.67**	**93.00 ±0.81**				

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

Table 17: Production from 45-50 weeks of age

Wheat Type	Treatment Group						Statistical Analysis P Values		
	C	BF	AV	RX	KM	**Mean**	W	E	W*E
Normal	89.73 ±1.12	92.04 ±0.91	91.28 ±1.94	90.72 ±1.34	89.06 ±1.80	**90.56 ±0.64**	NS	NS	NS
Pinched	91.66 ±1.16	91.96 ±1.57	89.92 ±1.35	91.59 ±1.03	91.33 ±1.39	**91.29 ±0.56**			
Mean	**90.70 ±0.83**	**92.00 ±0.86**	**90.60 ±1.13**	**91.16 ±0.81**	**90.19 ±1.14**				

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

1.3.6 Egg and Egg Shell Quality

Firstly, the main overall findings from 27 to 50 weeks of age will be discussed and then each collection will be considered separately.

As shown in Table 18, there were statistically significant effects of hen age on all measurements except deformation. As hens aged, eggs became larger, shell colour became lighter and shell breaking strength generally decreased. Although shell weight increased and shells generally became thicker, this did not compensate adequately for the increased egg weight so that percentage shell generally decreased. Albumen height and Haugh Units decreased with age of hen and yolk colour fluctuated.

There were significant main effects of wheat type on most measurements of egg and egg shell quality (Table 19). In general, egg internal quality and egg shell quality were better for the normal wheat which resulted in darker shells, higher shell breaking strength, shell weight, percentage shell and shell thickness, as well as higher albumen height and Haugh Units.

Enzyme treatment had significant effects on shell colour, shell breaking strength, shell thickness and yolk colour (Table 20). Shell colour was lighter for most of the enzymes than it was for the control, with Kemzyme producing the lightest coloured shells. Shell breaking strength was not generally improved by enzyme addition although Kemzyme resulted in increased mean breaking strength. Shell thickness was improved by Kemzyme but not by the other enzymes. Yolk colour was lighter for all enzymes than for the control.

At 27 weeks of age, the birds had been receiving the experimental diets for only two weeks (Table 21). Egg weight, deformation, shell weight, percentage shell and shell thickness were not affected by either wheat type or enzymes. However, shell colour was lighter for the birds receiving pinched wheat and also for Roxazyme and Kemzyme. Egg shell breaking strength was significantly lower for pinched wheat but was not significantly affected by addition of feed enzymes. Albumen height and Haugh Units were lower for pinched wheat although the addition of feed enzymes had no significant effect. Yolk colour was higher for birds receiving normal wheat and was significantly lower for both wheats when commercial feed enzymes were added.

At 30 weeks of age, when birds had been receiving the experimental diets for 5 weeks, the only measurement not affected by either wheat type or enzyme was shell colour (Table 22). Birds receiving normal wheat had lower egg weight, higher shell breaking strength, deformation, percentage shell and shell thickness, higher albumen height and Haugh Units. However, the addition of commercial feed enzymes had significant effects only on shell weight and yolk colour. Shell weight was significantly higher for Kemzyme than for the control group and Biofeed Wheat and Avizyme. Yolk colour was significantly lower for Avizyme than for Biofeed Wheat, Roxazyme or Kemzyme.

At 35 weeks of age, there was no significant effect of either wheat type or enzyme on shell colour, deformation, shell weight or shell thickness (Table 23). As was found at 30 weeks of age, shell breaking strength, albumen height and Haugh Units were higher for normal wheat. However, yolk colour was higher for pinched wheat than for normal wheat at 35 weeks. Egg weight was significantly lower, and percentage shell significantly higher, for Kemzyme than for all other treatment groups. In addition, yolk colour was highest for Kemzyme and lowest for Avizyme, with the control, Biofeed Wheat and Roxazyme being intermediate.

When birds were 40 weeks of age and had been receiving the experimental feeds for 15 weeks, there were few effects on egg and egg shell quality (Table 24). The only statistically significant effects of wheat type were for percentage shell and yolk colour. For normal wheat, percentage shell was greater and yolk colour was generally lower. Also, for normal wheat, egg weight tended to be lower ($P=0.08$), shell colour tended to be darker ($P=0.08$) and shell breaking strength tended to be higher ($P=0.07$). There were no statistically significant effects of the enzyme preparations on egg and egg shell quality at 40 weeks. However, there was a tendency for shell colour to be lighter for Kemzyme, especially with normal wheat (P=0.06), breaking strength to be lowest for Biofeed Wheat and Avizyme (P=0.09) and percentage shell to be higher for Roxazyme and Kemzyme (P=0.08).

By the time the birds were 45 weeks of age, they had been receiving the experimental diets for 20 weeks. The only effect of the experimental diets at this stage was an effect of enzyme preparations on Haugh Units (Table 25). When the treatment groups were analysed separately for each wheat type, Haugh Units were statistically higher for all enzyme preparations, than for the control for normal wheat (P=0.02). However, there was no difference between the control and enzyme groups for pinched wheat.

At 50 weeks of age, the only statistically significant main effect of wheat type or enzyme treatment was on yolk colour which was higher for the normal wheat and higher for the control than for the enzyme-supplemented groups (Table 26).

Table 18: Effect of hen age on egg and egg shell quality at 27-50 weeks of age.

Egg Quality Measurement	**Age of Hens (weeks)**						**P Value**
	27	30	35	40	45	50	
Egg Weight (g)	59.25 ±0.23	62.09 ±0.23	65.01 ±0.25	67.12 ±0.25	68.52 ±0.26	68.63 ±0.26	<0.0001
Shell Reflectivity (%)	30.19 ±0.23	31.61 ±0.26	32.00 ±0.23	32.18 ±0.23	32.77 ±0.24	33.28 ±0.27	<0.0001
Breaking Strength N	40.31 ±0.38	38.87 ±0.39	37.50 ±0.36	35.49 ±0.39	36.32 ±0.40	36.10 ±0.37	<0.0001
Deformation (μm)	257.1 ±3.57	252.2 ±3.65	251.5 ±4.45	245.2 ±3.70	249.1 ±4.81	243.5 ±4.56	NS
Shell Weight (g)	5.74 ±0.03	5.89 ±0.03	6.05 ±0.03	6.20 ±0.03	6.43 ±0.04	6.31 ±0.04	<0.0001
Percentage Shell %	9.70 ±0.03	9.51 ±0.04	9.32 ±0.04	9.25 ±0.04	9.38 ±0.04	9.20 ±0.05	<0.0001
Shell Thickness (μm)	404.0 ±1.33	397.5 ±1.45	385.5 ±1.28	406.4 ±1.43	409.5 ±1.77	411.8 ±1.98	<0.0001
Albumen Height (mm)	9.76 ±0.07	9.37 ±0.08	9.28 ±0.08	8.87 ±0.07	8.50 ±0.07	8.11 ±0.07	<0.0001
Haugh Units	98.03 ±0.34	95.31 ±0.47	94.41 ±0.37	91.90 ±0.37	89.43 ±0.41	87.18 ±0.44	<0.0001
Yolk Colour Score	11.04 ±0.05	10.82 ±0.05	11.37 ±0.05	11.38 ±0.05	11.40 ±0.05	11.64 ±0.06	<0.0001

Values are Means ± Standard error of the mean. NS is not statistically significant

Table 19 : Effect of wheat type on egg and egg shell quality at 27-50 weeks of age.

Egg Quality Measurement	**Type of Wheat**		**P Value**
	Normal	Pinched	
Egg Weight (g)	65.07 ±0.19	65.14 ±0.18	NS
Shell Reflectivity (%)	31.78 ±0.15	32.22 ±0.14	.0271
Breaking Strength N	38.29 ±0.22	36.59 ±0.23	<0.0001
Deformation (μm)	252.5 ±2.41	247.1 ±2.38	NS
Shell Weight (g)	6.14 ±0.02	6.07 ±0.02	0.0105
Percentage Shell %	9.45 ±0.03	9.34 ±0.94	0.0011
Shell Thickness (μm)	405.3 ±0.95	399.6 ±0.94	<0.0001
Albumen Height (mm)	9.13 ±0.05	8.83 ±0.04	<0.0001
Haugh Units	93.42 ±0.28	92.00 ±0.25	<0.0001
Yolk Colour Score	11.28 ±0.03	11.27 ±0.03	NS

Values are Means ± Standard error of the mean. NS is not statistically significant.

Table 20: Effect of enzyme treatment on egg and egg shell quality at 27-50 weeks of age.

Egg Quality Measurement	**Enzyme Treatment**					**P Value**
	Control	BF	AV	RX	KM	
Egg Weight (g)	65.12 ±0.29	65.44 ±0.29	65.09 ±0.29	65.17 ±0.28	64.69 ±0.29	NS
Shell Reflectivity (%)	31.61 ±0.22	31.40 ±0.22	31.80 ±0.23	32.02 ±0.21	33.16 ±0.25	<0.0001
Breaking Strength N	37.84 ±0.33	36.56 ±0.34	36.80 ±0.37	37.78 ±0.37	38.19 ±0.36	0.0019
Deformation (µm)	254.0 ±3.76	247.2 ±3.21	245.4 ±3.77	251.9 ±4.16	250.3 ±4.00	NS
Shell Weight (g)	6.08 ±0.03	6.07 ±0.03	6.08 ±0.03	6.12 ±0.03	6.17 ±0.03	NS (0.0602)
Percentage Shell %	9.35 ±0.04	9.30 ±0.03	9.35 ±0.04	9.40 ±0.04	9.57 ±0.04	<0.0001
Shell Thickness (µm)	401.5 ±1.40	400.0 ±1.26	401.3 ±1.52	402.3 ±1.69	407.3 ±1.54	0.0033
Albumen Height (mm)	8.97 ±0.08	9.08 ±0.07	9.04 ±0.07	8.93 ±0.08	8.89 ±0.07	NS
Haugh Units	92.49 ±0.46	93.20 ±0.39	93.12 ±0.38	92.29 ±0.46	92.45 ±0.36	NS
Yolk Colour Score	11.48 ±0.05	11.25 ±0.05	11.03 ±0.05	11.27 ±0.05	11.35 ±0.05	<0.0001

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

Table 21: Egg and egg shell quality for different wheats and enzyme treatments at 27 weeks of age.

Egg Quality Measure	**Wheat Type**	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	Mean	W	E	W*E
Egg Weight (g)	N	59.5	59.9	59.8	59.0	58.4	**59.3**	NS	NS	NS
	P	59.5	59.6	59.5	58.2	59.1	**59.2**			
	Mean	**59.5**	**59.8**	**59.6**	**58.6**	**58.8**				
Shell Reflectivity (%)	N	29.5	28.7	29.3	29.6	31.2	**29.7**	0.017	0.002	NS
	P	29.0	30.0	30.3	32.5	31.9	**30.7**			
	Mean	**29.2**	**29.4**	**29.8**	**31.1**	**31.5**				
Breaking Strength (Newtons)	N	41.4	42.3	39.6	40.7	41.9	**41.2**	0.022	NS	NS
	P	40.8	39.3	38.7	40.4	38.0	**39.4**			
	Mean	**41.1**	**40.8**	**39.1**	**40.5**	**40.0**				
Deformation (μm)	N	250.3	250.0	239.0	273.0	245.0	**251.5**	NS	NS	NS
	P	268.0	259.0	246.3	278.3	262.3	**262.8**			
	Mean	**259.2**	**254.5**	**242.7**	**275.7**	**253.7**				
Shell Weight (g)	N	5.80	5.90	5.81	5.65	5.75	**5.78**	NS	NS	NS
	P	5.76	5.72	5.70	5.67	5.64	**5.70**			
	Mean	**5.78**	**5.81**	**5.75**	**5.66**	**5.70**				
Percentage Shell (%)	N	9.77	9.87	9.70	9.59	9.88	**9.76**	NS	NS	NS
	P	9.69	9.60	9.58	9.77	9.55	**9.64**			
	Mean	**9.73**	**9.73**	**9.64**	**9.68**	**9.72**				
Shell Thickness (μm)	N	409.7	407.6	405.1	400.3	406.9	**405.9**	NS	NS	NS
	P	404.1	398.1	404.8	401.3	401.7	**402.0**			
	Mean	**406.9**	**402.8**	**404.9**	**400.8**	**404.3**				
Albumen Height (mm)	N	10.16	9.89	9.95	10.11	9.92	**10.00**	.0008	NS	NS
	P	9.76	9.49	9.49	9.57	9.25	**9.51**			
	Mean	**9.96**	**9.69**	**9.72**	**9.84**	**9.59**				
Haugh Units	N	99.7	98.4	98.7	99.7	99.1	**99.1**	0.001	NS	NS
	P	98.1	96.7	96.8	97.4	95.6	**96.9**			
	Mean	**98.9**	**97.6**	**97.8**	**98.6**	**97.4**				
Yolk Colour Score	N	11.53	11.00	11.03	11.13	11.13	**11.17**	0.009	.0001	NS
	P	11.47	11.10	10.60	10.50	10.93	**10.92**			
	Mean	**11.50**	**11.05**	**10.82**	**10.82**	**11.03**				

N is "normal" wheat; P is "pinched" wheat
Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

Table 22: Egg and egg shell quality for different wheats and enzyme treatments at 30 weeks of age.

Egg Quality Measure	**Wheat Type**	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	Mean	W	E	W*E
Egg Weight (g)	N	61.7	61.8	60.6	62.1	61.7	**61.6**	0.026	NS	NS
	P	61.2	62.8	62.9	62.9	63.2	**62.6**			
	Mean	**61.4**	**62.3**	**61.8**	**62.5**	**62.5**				
Shell Reflectivity (%)	N	31.5	30.2	31.7	31.7	32.6	**31.6**	NS	NS	NS
	P	31.8	31.7	30.5	32.2	32.1	**31.7**			
	Mean	**31.7**	**31.0**	**31.1**	**32.0**	**32.3**				
Breaking Strength (Newtons)	N	38.7	38.7	40.4	41.0	43.4	**40.4**	<.0001	NS	NS
	P	38.7	36.5	35.7	37.4	38.3	**37.3**		0.06	
	Mean	**38.7**	**37.6**	**38.1**	**39.2**	**40.8**				
Deformation (μm)	N	261.7	253.0	263.0	259.7	282.3	**263.9**	0.001	NS	NS
	P	242.7	232.3	233.3	243.0	251.0	**240.5**			
	Mean	**252.2**	**242.7**	**248.2**	**251.3**	**266.7**				
Shell Weight (g)	N	5.82	5.83	5.85	5.93	6.03	**5.89**	NS	0.0301	NS
	P	5.80	5.82	5.85	5.93	6.04	**5.89**			
	Mean	**5.81**	**5.83**	**5.85**	**5.93**	**6.03**				
Percentage Shell (%)	N	9.45	9.47	9.71	9.56	9.80	**9.60**	0.042	NS	NS
	P	9.48	9.28	9.33	9.44	9.58	**9.42**			
	Mean	**9.47**	**9.37**	**9.52**	**9.50**	**9.69**				
Shell Thickness (μm)	N	400.8	404.2	403.7	405.3	409.9	**404.8**	<.0001	NS	NS
	P	390.3	385.7	384.1	394.5	396.6	**390.2**			
	Mean	**395.6**	**394.9**	**393.9**	**399.9**	**403.3**				
Albumen Height (mm)	N	9.22	9.89	9.60	9.74	9.74	**9.64**	0.001	NS	NS
	P	9.22	9.44	9.27	8.55	9.03	**9.10**			
	Mean	**9.22**	**9.67**	**9.43**	**9.14**	**9.38**				
Haugh Units	N	93.4	97.7	97.0	97.4	95.3	**96.6**	0.006	NS	NS
	P	95.3	95.8	94.6	90.7	93.7	**94.0**			0.055
	Mean	**94.3**	**96.8**	**95.8**	**94.1**	**95.6**				
Yolk Colour Score	N	10.90	11.27	10.37	10.97	11.03	**10.91**	NS	0.0097	NS
	P	10.63	10.73	10.67	10.70	10.97	**10.74**			
	Mean	**10.77**	**11.00**	**10.52**	**10.83**	**11.00**				

N is "normal" wheat; P is "pinched" wheat

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.

C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

Table 23:Egg and egg shell quality for different wheats and enzyme treatments at 35 weeks of age.

Egg Quality Measure	**Wheat Type**	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	Mean	W	E	W*E
Egg Weight (g)	N	65.2	64.6	65.1	66.8	64.3	**65.2**	NS	0.019	NS
	P	65.1	66.3	65.5	64.8	62.5	**64.8**			
	Mean	**65.1**	**65.4**	**65.3**	**65.8**	**63.4**				
Shell Reflectivity (%)	N	31.5	30.3	32.8	32.0	33.7	**32.1**	NS	NS	0.030
	P	31.9	32.7	32.3	30.8	31.7	**31.9**			
	Mean	**31.7**	**31.5**	**32.6**	**31.4**	**32.7**				
Breaking Strength (Newtons)	N	39.2	37.4	37.4	37.9	39.3	**38.2**	0.041	NS	NS
	P	35.9	36.1	37.7	37.7	36.5	**36.8**			
	Mean	**37.5**	**36.7**	**37.5**	**37.8**	**37.9**				
Deformation (µm)	N	258.0	233.0	249.7	256.3	269.3	**253.3**	NS	NS	NS
	P	225.7	242.3	250.3	270.7	259.3	**249.7**			
	Mean	**241.8**	**237.7**	**250.0**	**263.5**	**264.3**				
Shell Weight (g)	N	6.04	6.02	6.11	6.15	6.09	**6.08**	NS	NS	NS
	P	5.93	6.06	6.04	6.00	6.12	**6.03**			
	Mean	**5.98**	**6.04**	**6.08**	**6.07**	**6.10**				
Percentage Shell (%)	N	9.27	9.32	9.38	9.22	9.46	**9.33**	NS	0.0004	NS
	P	9.10	9.13	9.20	9.26	9.84	**9.31**			0.06
	Mean	**9.18**	**9.23**	**9.29**	**9.24**	**9.65**				
Shell Thickness (µm)	N	386.9	386.5	387.4	383.8	391.8	**387.3**	NS	NS	NS
	P	378.7	382.5	386.8	383.7	387.4	**383.8**			
	Mean	**382.8**	**384.5**	**387.1**	**383.8**	**389.6**				
Albumen Height (mm)	N	9.31	9.65	9.61	9.81	9.36	**9.55**	0.0002	NS	NS
	P	9.24	9.35	9.17	8.59	8.68	**9.01**			
	Mean	**9.27**	**9.50**	**9.39**	**9.20**	**9.02**				
Haugh Units	N	94.4	96.4	96.2	96.7	95.1	**95.8**	0.0002	NS	NS
	P	94.3	94.6	93.8	90.2	92.4	**93.1**			
	Mean	**94.4**	**95.5**	**95.0**	**93.5**	**93.8**				
Yolk Colour Score	N	11.37	11.13	11.03	11.40	11.30	**11.25**	0.007	0.003	NS
	P	11.23	11.47	11.17	11.67	11.97	**11.50**			
	Mean	**11.30**	**11.30**	**11.10**	**11.53**	**11.63**				

N is "normal" wheat; P is "pinched" wheat
Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

Table 24: Egg and egg shell quality for different wheats and enzyme treatments at 40 weeks of age.

Egg Quality Measure	**Wheat Type**	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	Mean	W	E	W*E
Egg Weight	N	66.6	67.7	67.3	66.2	65.5	**66.7**	NS	NS	NS
(g)	P	67.6	67.7	68.2	67.0	67.3	**67.6**	0.08		
	Mean	**67.1**	**67.7**	**67.7**	**66.6**	**66.4**				
Shell	N	31.3	31.2	31.4	31.3	33.7	**31.8**	NS	NS	NS
Reflectivity	P	32.4	32.2	32.2	32.8	33.4	**32.6**	0.08	0.06	
(%)	**Mean**	**31.9**	**31.7**	**31.8**	**32.0**	**33.6**				
Breaking	N	38.3	34.7	34.3	37.6	36.0	**36.2**	NS	NS	NS
Strength	P	34.5	33.1	34.6	35.6	36.1	**34.8**	0.07	0.09	
(Newtons)	**Mean**	**36.4**	**33.9**	**34.5**	**36.6**	**36.1**				
Deformation	N	270.3	250.3	231.7	241.3	248.3	**248.4**	NS	NS	NS
(μm)	P	245.7	242.3	242.3	235.7	244.0	**242.0**			
	Mean	**258.0**	**246.3**	**237.0**	**238.5**	**246.2**				
Shell	N	6.19	6.23	6.26	6.29	6.18	**6.23**	NS	NS	NS
Weight	P	6.13	6.14	6.15	6.18	6.26	**6.17**			
(g)	**Mean**	**6.16**	**6.19**	**6.20**	**6.23**	**6.22**				
Percentage	N	9.30	9.20	9.29	9.50	9.46	**9.35**	0.0043	NS	NS
Shell	P	9.07	9.09	9.04	9.23	9.30	**9.15**		0.08	0.06
(%)	**Mean**	**9.19**	**9.15**	**9.16**	**9.37**	**9.38**				
Shell	N	408.3	406.3	405.1	413.5	410.5	**408.8**	NS	NS	NS
Thickness	P	401.9	400.4	401.3	408.5	408.4	**404.1**			
(μm)	**Mean**	**405.1**	**403.3**	**403.2**	**411.0**	**409.5**				
Albumen	N	9.06	8.82	8.82	8.90	8.63	**8.49**	NS	NS	NS
Height	P	8.95	9.06	9.26	8.47	8.71	**8.89**			
(mm)	**Mean**	**9.01**	**8.94**	**9.04**	**8.69**	**8.67**				
Haugh	N	93.0	91.7	91.6	92.3	91.0	**91.9**	NS	NS	NS
Units	P	92.3	92.8	93.9	89.5	91.0	**91.9**			
	Mean	**92.7**	**92.3**	**92.8**	**90.9**	**91.0**				
Yolk	N	11.43	11.40	11.10	11.07	11.13	**11.23**	0.002	NS	NS
Colour	P	11.67	11.33	11.53	11.40	11.73	**11.53**			
Score	**Mean**	**11.55**	**11.37**	**11.32**	**11.23**	**11.43**				

N is "normal" wheat; P is "pinched" wheat
Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

Table 25: Egg and egg shell quality for different wheats and enzyme treatments at 45 weeks of age.

Egg Quality Measure	**Wheat Type**	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	Mean	W	E	W*E
Egg Weight (g)	N	68.7	68.2	67.5	69.6	69.9	**68.8**	NS	NS	NS
	P	69.5	68.4	67.5	67.9	68.0	**68.3**			
	Mean	**69.1**	**68.3**	**67.5**	**68.7**	**69.0**				
Shell Reflectivity (%)	N	31.8	31.4	32.6	32.6	34.1	**32.5**	NS	NS	NS
	P	32.4	33.3	32.3	33.4	33.9	**33.0**			
	Mean	**32.1**	**32.4**	**32.5**	**33.0**	**34.0**				
Breaking Strength (Newtons)	N	38.8	35.1	36.8	36.2	37.5	**36.9**	NS	NS	NS
	P	36.2	35.2	35.5	36.2	35.7	**35.8**			
	Mean	**37.5**	**35.1**	**36.1**	**36.2**	**36.6**				
Deformation (μm)	N	246.3	281.3	256.2	237.3	226.0	**249.4**	NS	NS	NS
	P	252.3	239.0	250.0	255.7	247.3	**248.9**			
	Mean	**249.3**	**260.2**	**253.1**	**246.5**	**236.7**				
Shell Weight (g)	N	6.45	6.32	6.30	6.62	6.58	**6.45**	NS	NS	NS
	P	6.46	6.38	6.29	6.41	6.46	**6.40**			
	Mean	**6.45**	**6.35**	**6.29**	**6.52**	**6.52**				
Percentage Shell (%)	N	9.39	9.28	9.29	9.52	9.46	**9.39**	NS	NS	NS
	P	9.29	9.33	9.33	9.45	9.51	**9.38**			
	Mean	**9.34**	**9.31**	**9.31**	**9.49**	**9.49**				
Shell Thickness (μm)	N	409.5	406.3	406.4	414.3	419.4	**411.2**	NS	NS	NS
	P	406.6	408.4	408.3	402.8	413.3	**407.9**			
	Mean	**408.1**	**407.4**	**407.4**	**408.5**	**416.4**				
Albumen Height (mm)	N	7.97	8.63	8.65	8.89	8.81	**8.59**	NS	NS	NS
	P	8.28	8.30	8.43	8.57	8.43	**8.40**			
	Mean	**8.12**	**8.47**	**8.54**	**8.73**	**8.62**				
Haugh Units	N	86.1	90.1	90.6	91.3	91.2	**89.9**	NS	0.043	NS
	P	87.9	88.6	89.4	89.9	89.1	**89.0**			
	Mean	**87.0**	**89.3**	**90.0**	**90.6**	**90.2**				
Yolk Colour Score	N	11.67	11.37	11.20	11.50	11.27	**11.40**	NS	NS	NS
	P	11.50	11.37	11.37	11.43	11.33	**11.40**			
	Mean	**11.58**	**11.37**	**11.28**	**11.47**	**11.30**				

N is "normal" wheat; P is "pinched" wheat
Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

Table 26: Egg and egg shell quality for different wheats and enzyme treatments at 50 weeks of age.

Egg Quality Measure	**Wheat Type**	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	Mean	W	E	W*E
Egg Weight (g)	N	68.6	68.6	69.0	69.8	68.2	**68.9**	NS	NS	NS
	P	68.2	69.7	68.2	67.8	68.1	**68.4**			
	Mean	**68.4**	**69.1**	**68.6**	**68.8**	**68.2**				
Shell Reflectivity (%)	N	32.9	31.9	33.8	32.3	34.7	**33.1**	NS	NS	NS
	P	33.3	33.2	32.4	33.2	35.0	**33.4**		0.051	
	Mean	**33.1**	**32.6**	**33.1**	**32.8**	**34.9**				
Breaking Strength (Newtons)	N	36.6	36.0	36.8	36.7	37.6	**36.8**	NS	NS	NS
	P	35.1	34.2	34.1	35.9	37.9	**35.5**		0.081	
	Mean	**35.9**	**35.1**	**35.5**	**36.3**	**37.8**				
Deformation (μm)	N	265.0	251.0	249.3	243.4	232.0	**248.2**	NS	NS	NS
	P	262.3	233.3	234.3	227.6	236.3	**238.9**			
	Mean	**263.7**	**242.0**	**241.8**	**235.5**	**234.2**				
Shell Weight (g)	N	6.39	6.21	6.35	6.50	6.40	**6.37**	NS	NS	NS
	P	6.17	6.24	6.24	6.08	6.49	**6.24**			
	Mean	**6.28**	**6.28**	**6.30**	**6.29**	**6.45**				
Percentage Shell (%)	N	9.34	9.07	9.20	9.29	9.42	**9.27**	NS	NS	NS
	P	9.04	8.96	9.17	8.97	9.54	**9.13**		0.064	
	Mean	**9.19**	**9.01**	**9.19**	**9.13**	**9.48**				
Shell Thickness (μm)	N	416.6	405.0	411.7	420.2	417.3	**414.1**	NS	NS	NS
	P	404.2	408.5	411.6	399.3	424.1	**409.5**			
	Mean	**410.4**	**406.8**	**411.6**	**409.7**	**420.7**				
Albumen Height (mm)	N	7.81	8.57	8.07	8.18	8.03	**8.13**	NS	NS	0.015
	P	8.61	7.88	8.20	7.75	8.01	**8.09**			
	Mean	**8.21**	**8.23**	**8.13**	**7.97**	**8.02**				
Haugh Units	N	85.0	90.0	87.0	87.4	86.8	**87.2**	NS	NS	0.007
	P	90.3	85.5	87.8	84.9	87.0	**87.1**			
	Mean	**87.7**	**87.8**	**87.4**	**86.1**	**86.9**				
Yolk Colour Score	N	12.57	11.03	11.24	12.40	11.53	**11.76**	0.022	<.0001	<.0001
	P	11.77	11.80	11.00	11.10	11.90	**11.51**			
	Mean	**12.17**	**11.42**	**11.12**	**11.75**	**11.72**				

N is "normal" wheat; P is "pinched" wheat
Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
W is wheat type, E is enzyme, W*E is wheat-enzyme interaction

1.3.7 Keeping Power of Eggs

The "keeping power" of eggs is the extent to which Haugh Units are maintained during storage of eggs, prior to sale. Tables 27-29 show the main effects on egg quality of eggs from hens at 40 weeks of age. Storage conditions had a highly significant effect on egg weight, albumen height and Haugh units with the values being highest in the fresh eggs, lowest in the eggs stored at room temperature with eggs stored in the cool room being intermediate. The normal wheat resulted in slightly higher albumen height and Haugh units (Table 28). However, there was no significant effect of enzyme addition on albumen height, Haugh units or egg weight (Table 29).

Table 27: Effect of egg storage treatment on egg internal quality at 40 weeks of age.

Egg Quality Measurement	**Treatment Temperature**			**P Value**
	Fresh	4 wks cold	4 wks room	
Egg Weight (g)	67.1 ±0.3	65.1 ±0.3	65.02 ±0.4	<.0001
Albumen Height (mm)	8.9 ±0.7	6.1 ±0.8	4.9 ±0.09	<.0001
Haugh Units	91.9 ±0.4	74.9 ±0.7	64.2 ±0.8	<.0001

Values are Means ± Standard Error of the Mean.

Table 28: Effect of diet on egg internal quality at 40 weeks of age.

Egg Quality Measurement	**Wheat Type**		**P Value**
	Normal	Pinched	
Egg Weight (g)	65.8 ±0.3	66.3 ±0.3	NS
Albumen Height (mm)	7.3 ±0.1	7.1 ±0.1	0.0457
Haugh Units	81.4 ±0.8	80.0 ±0.9	0.0078

Values are Means ± Standard Error of the Mean. NS is not statistically significant

Table 29: Effect of enzyme treatment on egg and egg shell quality at 40 weeks of age.

Egg Quality Measurement	**Enzyme Treatment**					**P Value**
	Control	BF	AV	RX	KM	
Egg Weight (g)	66.0 ±0.4	65.7 ±0.4	66.5 ±0.4	66.0 ±0.4	66.6 ±0.4	NS
Albumen Height (mm)	7.2 ±0.2	7.2 ±0.2	7.2 ±0.2	7.2 ±0.2	7.1 ±0.2	NS
Haugh Units	80.6 ±1.4	81.0 ±1.3	80.8 ±1.3	80.9 ±1.2	80.2 ±1.3	NS

Values are Means ± Standard Error of the Mean. NS is not statistically significant
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

When hens were 45 weeks of age, the effects of storage treatment were very similar to those occurring at 40 weeks (Table 30). However, the effects of wheat type were different with egg weight being higher for normal wheat and albumen height and Haugh Units unaffected (Table 31). At 45 weeks, Haugh units were significantly improved in eggs from birds receiving enzyme supplementation in the feed and the effects were greatest for Avizyme (Table 32).

Table 30: Effect of egg storage treatment on egg internal quality at 45 weeks of age.

Egg Quality Measurement	**Treatment Temperature**			**P Value**
	Fresh	4 wks cold	4 wks room	
Egg Weight (g)	68.5 ±0.3	66.1 ±0.3	60.6 ±0.4	<.0001
Albumen Height (mm)	8.5 ±0.7	6.3 ±0.6	2.7 ±0.6	<.0001
Haugh Units	89.4 ±0.4	76.2 ±0.6	38.3 ±0.6	<.0001

Values are Means ± Standard Error of the Mean.

Table 31: Effect of diet on egg internal quality at 45 weeks of age.

Egg Quality Measurement	**Diet**		**P Value**
	Normal	Pinched	
Egg Weight (g)	66.3 ±0.3	65.6 ±0.3	0.0360
Albumen Height (mm)	6.6 ±0.2	6.4 ±0.2	NS
Haugh Units	73.6 ±1.3	73.1 ±1.3	NS

Values are Means ± Standard Error of the Mean.

Table 32: Effect of enzyme treatment on egg and egg shell quality at 45 weeks of age.

Egg Quality Measurement	**Enzyme Treatment**					**P Value**
	Control	BF	AV	RX	KM	
Egg Weight (g)	66.1 ±0.5	65.6 ±0.5	65.5 ±0.5	66.4 ±0.5	66.1 ±0.5	NS
Albumen Height (mm)	6.2 ±0.2	6.5 ±0.2	6.6 ±0.2	6.6 ±0.3	6.6 ±0.2	NS (0.0723)
Haugh Units	^{B}71.4 ±2.0	^{A}73.5 ±2.0	^{A}74.5 ±2.0	^{A}73.8 ±2.1	^{A}73.4 ±2.1	0.0423

Values are Means ± Standard Error of the Mean. NS is not statistically significant
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

1.3.8 Blood Electrolytes

Haematocrit and the concentrations of sodium, potassium and ionised calcium at 45 weeks of age are shown in Table 33. There were no significant effects of wheat type on haematocrit or plasma electrolytes. However, there were significant effects of enzyme treatment on haematocrit and ionised calcium. For both wheats combined, haematocrit was higher for Biofeed Wheat than for all other treatment groups. Ionised calcium levels were highest for Avizyme and lowest for the control and Kemzyme, with Biofeed Wheat and Roxazyme being intermediate in value. There was a significant wheat-enzyme interaction. When each wheat was considered separately, there were significant enzyme effects for normal wheat but not pinched wheat. For normal wheat, Biofeed Wheat resulted in higher plasma ionised calcium levels than all other treatment groups.

Table 33: Effect of enzyme supplementation on haematocrit and plasma electrolyte concentrations in laying hens at 45 weeks of age

	Haematocrit (%)	**Na (mmol/L)**	**K (mmol/L)**	**Ionised Ca (mmol/L)**
Normal Wheat:				
Control	28.2±1.0	153.4±1.1	5.3±0.2	1.61±0.05[b]
BioFeed Wheat	29.7±0.7	152.6±0.7	5.3±0.2	1.69±0.05[b]
Avizyme	28.4±0.7	153.6±0.7	5.3±0.1	1.84±0.03[a]
Roxazyme	27.1±0.7	150.4±1.4	5.0±0.2	1.64±0.07[b]
Kemzyme	28.1±0.8	150.0±1.4	5.1±0.1	1.57±0.04[b]
Pinched Wheat:				
Control	29.9±1.3	149.32±2.5	5.24±0.2	1.56±0.07
BioFeed Wheat	33.2±2.2	151.32±1.4	5.30±0.2	1.59±0.05
Avizyme	28.2±0.4	150.72±1.0	5.44±0.1	1.62±0.04
Roxazyme	27.4±0.9	152.34±1.4	5.52±0.1	1.69±0.04
Kemzyme	29.3±1.3	152.26±0.8	5.39±0.1	1.69±0.02
Statistical Analysis				
Wheat Type	NS	NS	NS (0.07)	NS
Enzyme	0.0094	NS	NS	0.0380
Wheat*Enzyme	NS	NS (0.07)	NS	0.0059

Means ± SE. Means within columns, for a particular wheat type, with no common superscript differ significantly (P<0.05)

1.4 Discussion of Results

Although the two wheats were grown under different conditions and were different in appearance, they were very similar in total, soluble and insoluble NSP levels and had almost identical AME values. However the crude protein level of the “pinched” wheat was higher, resulting in a higher crude protein content of the finished feed. It appears that the “new season wheat” phenomenon occurs occasionally, but not regularly. During the particular season in which the wheats for this study were sourced, it proved impossible to purchase the requisite quantities of high NSP/low AME wheat. This is an important observation which will influence recommendations concerning enzyme addition to layer feeds.

The addition of commercial enzyme preparations had no effect on the AME value of either of the wheat-based diets. This suggests that enzyme addition has limited potential to improve the utilisation of feed ingredients, in the absence of elevated levels of non-starch polysaccharides. In addition, there was no significant effect on excreta moisture levels or feed intake. Addition of feed enzymes to layer diets has been recommended for the benefit of improving litter quality. However, again, this does not appear to be a universal benefit, in the absence of high levels of non-starch polysaccharides.

Egg and egg shell quality, in general, deteriorated with the age of the hens. Egg weight and shell weight increased although the increase in shell weight was not proportional to that of egg weight, resulting in a reduction in the percentage shell. Shell thickness decreased from 27 to 35 weeks of age and then increased up to 50 weeks. Haugh Units deteriorated with increasing hen age, and yolk colour increased overall. The age-related changes in egg and egg shell quality are similar to those reported previously (Roberts *et at.*, 1997). Inspection of Tables 21-26 suggests that the effects of wheat type and enzyme addition are generally greater in the younger aged birds. This may be related to the extent of development of the gastrointestinal tract and/or the duration of time that the birds have been consuming the experimental diets.

The diets based on the "normal" wheat resulted in consistently better shell quality, darker shell colour and higher Haugh Units than those based on the "pinched" wheat. The reason for this is unclear. The pinched wheat had a higher crude protein level which resulted in a higher crude protein level in the finished feed. Clearly wheat composition, other than levels of non-starch polysaccharides, can influence the value of wheat for layer diets. This aspect warrants further investigation. However, the difficulty in the commercial situation is that grains are not usually analysed prior to formulation and manufacture of diets. Rather, assumptions are usually made about the composition of grains, based on the appearance of the grain and knowledge of the growing conditions.

The effects of commercial feed enzymes found in this study differ from those reported for a previous study (Roberts and Choct, 1999; Roberts *et at.*, 1999). The improved shell breaking strength observed in the previous study in eggs from birds given enzymes was not found in the present study, except for Kemzyme. This may be due to differences in feed ingredients used in the two experiments. However, the decrease in shell colour in response to dietary enzyme inclusion, was consistent across the two studies with lighter coloured shells found in at least two of the enzymes. Percentage shell and shell thickness were improved only by Kemzyme in the present study. Yolk colour was slightly lower than the control for all diets containing enzymes. However, all treatment groups had yolk colour at very acceptable levels.

As was expected, the "keeping power" of eggs (the maintenance of high albumen height and Haugh Units) was greatly influenced by the storage conditions of the eggs. Consistently, albumen quality was highest in the fresh eggs, followed by eggs stored in the cool room for 4 weeks, with eggs stored at room temperature for 4 weeks having the lowest albumen height and Haugh Units. Because of the finding in an earlier study (Roberts and Choct, 1999; Roberts *et at.*, 1999) that the addition of feed enzymes resulted in lower Haugh Units, it was of interest to investigate any possible effects of feed enzyme supplementation on the keeping power of eggs. The only significant effect of enzymes was on Haugh Units at 45 weeks of age. However, there was no statistically significant interaction between enzyme and storage treatment.

Blood measurements, haematocrit and the plasma concentrations of sodium, potassium and ionised calcium, were not affected by the type of wheat on which the diet was based but were affected by the addition of commercial feed enzyme preparations. It appears that the addition of commercial enzyme preparations affects bird physiology either by affecting the availability of feed ingredients or by altering gastrointestinal function in the hens.

The results of Trial 1 indicate that the type of wheat on which the diet is based, and the addition of commercial enzyme preparations, have the potential to affect both egg internal quality and egg shell quality. There also appears to be an effect of age of bird, duration on the experimental diets and, possibly, an interaction between these two.

2. Wheat or wheat plus rye with or without enzymes: Birds 50-73 weeks of age (Trial 2)

2.1 Introduction

Because the wheats used in Trial 1 were very similar in levels of non-starch polysaccharides, it was not possible to compare the effect of adding commercial enzyme preparations to a wheat with low non-starch polysaccharide (NSP) levels and a wheat with high NSP levels. Therefore, for the second Trial of the project, it was decided to use a rye model to investigate the effect of high viscosity on bird performance and egg and egg shell quality, with and without the use of feed enzymes. The extract viscosity of cereal rye grain is high and it was anticipated that the addition of rye to wheat would increase the digesta viscosity of the birds. This treatment was intended to simulate at least the viscosity effects of high NSP/low AME wheat.

A supply of the same pinched wheat used in Trial 1 had been stored in a silo. This wheat was used for all diets in Trial 2. However, for half the diets, 20% of the wheat was substituted with cereal rye. Trial 2 also included an induced moult to see if the enzyme treatments influenced egg and egg shell quality, following a moult.

The hens which had received pinched wheat in Trial 1 continued to do so in Trial 2, to maintain continuity and determine any effects of hen age on response to enzyme supplementation. The birds that had received the normal wheat in Trial 1 received the wheat plus rye diets in Trial 2.

The measurements conducted in Trial 1 were also made in Trial 2. These included analyses of diets for extract viscosity and NSP levels, feed intake, excreta moisture, AME, egg and egg shell quality measurements, and keeping power of eggs. In addition, blood samples were taken at 72 weeks of age and, at the same age a subsample of birds (5 from each of the ten diets) was taken for measurement of digesta viscosity.

2.2 Methodology

2.2.1 Diets

Sufficient "pinched" wheat from Trial 1 was stored in a silo for use in Trial 2. In Trial 2, all diets were based on "pinched" wheat and formulated as shown in Table 3. For the birds that had previously received the "normal" wheat, 20% of the pinched wheat was substituted with cereal rye, in order to investigate the effect of increased digesta viscosity on bird performance with and without enzymes. The birds that had received the pinched wheat in Trial 1 continued to receive the same diets. Enzymes were added to the diets, as described in Chapter 1.

2.2.2 Bird Maintenance

Birds were maintained as described in Chapter 1. However, at 65 weeks of age, following the egg collection and measurement of AME, excreta moisture and feed intake, birds were placed into an induced moult. This was achieved by turning off all the lights and feeding the birds whole grain barley and shell grit. Full feed (same diets as prior to the moult) was returned at 68 weeks of age and birds were back in full production by 71 weeks of age. These diets were fed until the birds were 73 weeks of age.

2.2.3 Feed Intake, Apparent Metabolisable Energy and Excreta Moisture

Feed intake, excreta moisture and apparent metabolisable energy (AME) were measured at 55, 60, 65 and 73 weeks of age, as described in Chapter 1.

2.2.4 Egg and Egg Shell Quality Measurements

Eggs were collected at 55, 60, 65 and 73 weeks of age. Number of eggs collected and the analyses conducted are the same as those described in Chapter 1.

2.2.5 Keeping Power of Eggs

Eggs were collected for determination of keeping power at 55, 60 and 65 weeks of age. Measurements were performed in the same way as described in Chapter 1.

2.2.6 Digesta Viscosity

At the end of Trial 2, 5 birds from each diet from the larger flock (i.e. not the birds that were used for AME measurements) were killed by cervical dislocation. Each bird was weighed, the body cavity was opened and the contents of the jejunum (from duodenum to the Meckel's diverticulum), and ileum (from the Meckel's diverticulum to 4 cm above the ileocaecal junction) were collected. Digesta were kept on ice prior to centrifugation at 12,000*g*, for 10 min at 4^{o}C. Supernatant from each sample was removed, frozen and stored at –20^{o}C pending analyses.

The viscosity was determined on 0.5 ml of supernatants using a Brookfield DVIII Model viscometer at 25^{o}C with a CP 400 cone and shear rate of 5-500/s.

2.2.7 Plasma Electrolytes

Fifty birds were bled at 72 weeks of age for blood and plasma samples. As described in Chapter 1, a 2 ml blood sample was collected anaerobically in a 2 mL syringe via venipuncture of the cutaneous ulnar vein. Air was expelled and the syringe sealed immediately with a plastic cap. All samples were put in ice until analysed. Ionised calcium, sodium and potassium were analysed immediately after sample collection using an AVL Electrolyte 984 analyser (AVL Medical Instruments, Switzerland), allowing the analyser to take the centre point of the blood in the syringe. Duplicate haematocrit tubes were filled with blood, sealed and centrifuged in a Hawksley Microhaematocrit Centrifuge at 13,000 RPM for 3 minutes for the measurement of haematocrit.

Data were analysed by ANOVA with bird age, wheat type and enzyme treatment as independent variables. Differences between means were assessed by Fisher's (Protected) Least Significance Difference test. Significance was assumed at $P<0.05$.

2.3 Detailed Results

2.3.1 Diets

The extract viscosities of the pinched wheat and the diet based on the pinched wheat were similar to those in Trial 1 (Table 34). The extract viscosity of the cereal rye grain was very high. However, the extract viscosity of the diet in which 20% wheat was substituted with cereal rye was only about 3 times that of the diet based on wheat alone. When the levels of non-starch polysaccharides of the wheat and wheat plus rye diets were compared, the wheat plus rye diets were 10-15% higher for soluble, insoluble and total non-starch polysaccharides than were the wheat diets (Table 35).

Table 34: Feed and grain extract viscosities

Sample	Extract Viscosity cP
Feed based on pinched wheat	3.40
Pinched wheat	5.38
Feed based on pinched wheat + rye	10.60
Rye (grain alone)	347.47 (too high to measure accurately)

Table 35: Non starch polysaccharides (NSP) in grains and diets (g/kg)

Feed or Grain	**Sugar g/kg**	**Free Sugars g/kg**	**Insoluble NSP g/kg**	**Soluble NSP g/kg**	**Total NSP g/kg**
Feed based on "pinched" wheat	Rhamnose	0.00	0.30	0.08	0.39
	Fucose	0.00	0.00	0.05	0.05
	Ribose	0.00	0.40	0.03	0.43
	Arabinose	0.26	21.24	1.89	23.14
	Xylose	0.00	27.02	1.53	28.55
	Mannose	1.21	2.22	0.08	2.30
	Galactose	3.17	3.34	1.43	4.77
	Glucose	12.42	28.55	1.42	29.96
	TOTAL	17.07	73.80	5.79	79.59
"Pinched" Wheat (whole grain)	Rhamnose	0.00	0.51	0.05	0.57
	Fucose	0.00	0.00	0.04	0.04
	Ribose	0.00	0.054	0.02	0.56
	Arabinose	0.22	27.04	2.44	29.48
	Xylose	0.14	34.34	2.51	36.85
	Mannose	1.28	2.10	0.13	2.23
	Galactose	1.60	1.80	1.31	3.11
	Glucose	11.93	27.11	1.14	28.25
	TOTAL	15.17	82.63	6.77	89.40
Feed based on "pinched" Wheat + rye	Rhamnose	0.00	0.27	0.10	0.37
	Fucose	0.00	0.18	0.05	0.23
	Ribose	0.00	0.42	0.02	0.44
	Arabinose	0.32	22.73	2.77	25.51
	Xylose	0.11	30.95	2.82	33.77
	Mannose	1.47	2.71	0.15	2.87
	Galactose	3.17	3.61	1.42	5.03
	Glucose	13.98	32.49	1.46	33.95
	TOTAL	19.05	82.86	7.81	90.67
Rye (whole grain)	Rhamnose	0.00	0.00	0.06	0.06
	Fucose	0.00	0.19	0.05	0.24
	Ribose	0.00	0.00	0.16	0.16
	Arabinose	0.30	23.20	9.06	32.26
	Xylose	0.18	27.00	12.82	39.82
	Mannose	6.71	2.32	1.37	3.69
	Galactose	1.51	3.52	1.19	4.71
	Glucose	20.47	31.25	3.40	34.65
	TOTAL	29.17	77.72	24.86	102.58

2.3.2 Feed Intake

At 55, 60, 65 and 73 weeks of age, feed intake was not affected by either diet or enzyme treatment (Table 36). However, there was a significant effect of hen age.

Table 36: Feed intake of laying hens fed diets containing wheat or wheat + rye, with or without enzyme supplementation at 55 to 73 weeks of age.

Diets	**Feed intake g/d**			
	Pre-moult			Post-moult
	55 weeks	60 weeks	65 weeks	73 weeks
Wheat+Rye:				
Control	127.1	118.1	110.4	120.0
BioFeed Wheat	120.9	109.0	113.6	122.9
Avizyme	126.1	108.0	105.4	113.4
Roxazyme	118.7	100.7	107.9	114.9
Kemzyme	126.9	115.3	119.4	124.1
Wheat:				
Control	114.0	108.9[ab]	116.2[ab]	117.8
BioFeed Wheat	102.8	97.0[b]	89.9[c]	93.5
Avizyme	113.3	94.1[b]	96.5[bc]	112.7
Roxazyme	120.5	125.6[a]	112.3[abc]	129.2
Kemzyme	117.1	118.4[ab]	121.0[a]	135.9
Probability value in analysis of variance				
Diets		NS		
Enzyme		NS		
Age		<0.0001		

Mean ± SE. For a diet at a particular age, values with unlike superscript differ significantly $P<0.05$.
NS is not statistically significant.

2.3.3 Faecal Moisture Levels

Faecal moisture levels at 55, 60, 65 and 73 weeks of age were not significantly affected by diet, enzyme treatment or hen age (Table 37).

Table 37: Faecal moisture of laying hens fed diets containing wheat with or without enzyme supplementation at 55 to 73 weeks of age.

Diets	**Faecal moisture g/100 g**			
	Pre-moult			Post-moult
	55 weeks	60 weeks	65 weeks	73 weeks
Wheat+Rye:				
Control	69.06	70.86	68.18	64.13
BioFeed Wheat	75.02	72.61	74.45	70.07
Avizyme	69.10	70.27	72.63	78.42
Roxazyme	73.62	73.61	72.02	74.74
Kemzyme	71.12	70.05	69.22	68.15
Wheat:				
Control	71.36	68.51	69.71	66.76
BioFeed Wheat	73.59	75.42	73.99	74.61
Avizyme	70.57	70.37	68.25	66.56
Roxazyme	71.28	70.83	72.22	73.22
Kemzyme	71.68	71.14	71.02	74.61
	Probability value in analysis of variance			
Wheat Type	NS			
Enzyme	NS			
Age	NS			

Means ± SE. NS is not statistically significant.

2.3.4 Apparent Metabolisable Energy

The AME of the diets was not significantly affected by the type of grain on which the diet was based, nor was it affected by the addition of enzymes (Table 38). However, there was a significant effect of hen age.

Table 38: Apparent Metabolisable Energy (AME) of laying hens fed diets containing wheat with or without enzyme supplementation at 55 to 73 weeks of age.

Diets	**AME MJ/kg DM**			
	Pre-moult			Post-moult
	55 weeks	60 weeks	65 weeks	73 weeks
Wheat +Rye:				
Control	13.73	13.52	13.31	13.55
BioFeed Wheat	13.00	13.73	13.04	13.55
Avizyme	13.14	13.57	13.14	14.36
Roxazyme	13.46	13.38	13.16	13.40
Kemzyme	13.28	13.33	12.01	13.45
Wheat:				
Control	13.16	13.73	13.61	14.17
BioFeed Wheat	12.68	13.43	13.48	13.91
Avizyme	13.10	12.38	12.61	12.41
Roxazyme	13.69	14.18	13.78	14.75
Kemzyme	13.36	13.49	13.96	14.52
	Probability value in analysis of variance			
Wheat Type	NS			
Enzyme	NS			
Age	<0.0001			

Means ±SE.

2.3.5 Digesta Viscosity

Jejunal and ileal viscosity are shown in Table 39. Digesta viscosity in both the jejunum and ileum was higher in the diet containing rye. However, the addition of enzymes did not affect the digesta viscosity in either the jejunum or ileum.

Table 39: Jejunal and ileal viscosity of laying hens fed diets containing wheat and rye with or without enzyme supplementation.

	Jejunum	**Ileum**
Pinched Wheat		
Control	2.26 ± 0.22	5.50 ± 0.90
BioFeed Wheat	1.82 ± 0.23	2.19 ± 0.20
Avizyme	1.85 ± 0,12	3.39 ± 0.44
Roxazyme	1.99 ± 0.30	2.82 ± 0.40
Kemzyme	1.84 ± 0.17	3.65 ± 0.59
Pinched Wheat + Rye		
Control	2.75 ± 0.71	8.08 ± 2.66
BioFeed Wheat	2.24 ± 0.16	5.98 ± 1.21
Avizyme	2.51 ± 0.34	15.02 ± 7.08
Roxazyme	5.32 ± 1.95	8.03 ± 3.89
Kemzyme	3.04 ± 0.66	7.27 ± 2.04

Means ± SE

2.3.6 Production

Production prior to the induced moult is shown in Tables 40-42. Production declined between 55 and 60 weeks and increased again at 60-65 weeks (Table 40). Production was not affected by type of diet (Table 41). However, there was a significant effect of enzyme treatment on production towards the end of the laying life of the flock (Table 42). Production was highest for the Roxazyme group and lowest for Kemzyme and Avizyme.

Table 40: Effect of hen age on production at 55-65 weeks

Age of Hens (weeks)			**P Value**
50-55 wks	55-60 wks	60-65 wks	
88.69 ±0.51	81.21 ±0.66	84.31 ±0.62	<0.0001

Values are Means ± Standard Errors of the Means

Table 41: Effect of wheat type on production at 55-65 weeks of age

Type of Diet		**P Value**
Wheat	Wheat + Rye	
84.67 ±0.55	84.80 ±0.66	NS

Values are Means ± Standard Errors of the Means. NS is not statistically significant.

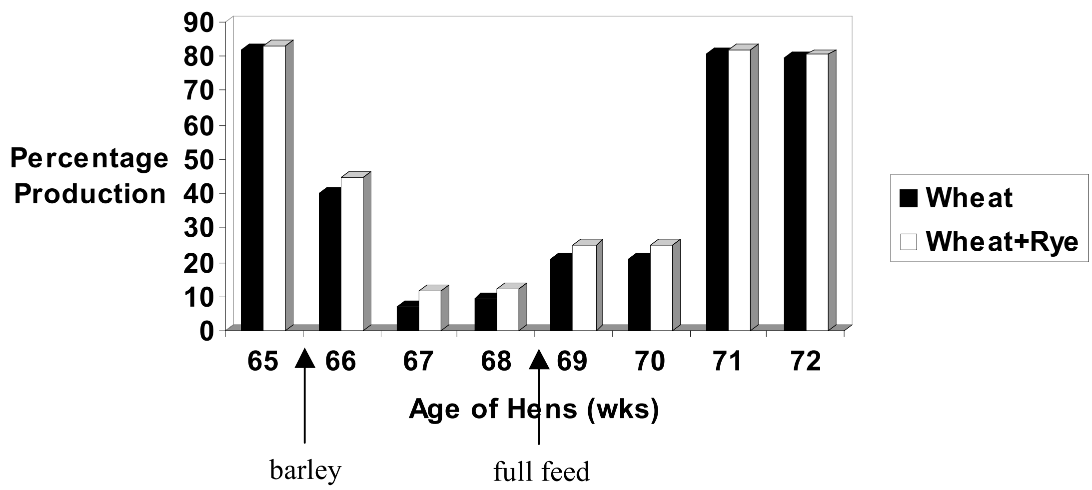
Table 42: Effect of enzyme treatment on production at 55-65 weeks of age.

Enzyme Treatment					**P Value**
Control	Biofeed Wheat	Avizyme	Roxazyme	Kemzyme	
84.08 ±0.98	85.72 ±0.86	83.29 ±0.97	86.68 ±0.97	83.92 ±0.90	0.0136

Values are Means ± Standard Errors of the Means.

From the initiation of the induced moult at 65 weeks until full production was resumed at 71 weeks, production was reduced to less than 10% before increasing. The production on the different diets is shown in Figure 1. There was no effect of enzyme treatment in the pre-moult diets on production during the moult. However, there were significant effects of diet and stage of moult. Production declined more rapidly and reached lower levels in the birds receiving the wheat-based diet.

Figure 1: Production before, during and after the moult at 65-72 weeks of age.



2.3.7 Egg and Egg Shell Quality

Birds were moulted between 65 and 68 weeks of age and had returned to full production by 71 weeks of age. Egg and egg shell quality were significantly affected by bird age (Table 43). Egg weight increased as birds grew older. Shell reflectivity fluctuated. The induced moult improved shell breaking strength, deformation, shell weight, percentage shell, shell thickness, and albumen quality. Yolk colour showed significant fluctuations.

Table 43: Effect of hen age on egg and egg shell quality at 55-73 weeks of age.

Egg Quality Measurement	**Age of Hens**				P Value
	Pre-moult			Post-moult	
	55 wks	60 wks	65 wks	73 wks	
Egg Weight (g)	68.30 ±0.28	69.40 ±0.3	69.31 ±0.3	70.76 ±0.33	<.0001
Shell Reflectivity (%)	35.12 ±0.30	33.54 ±0.29	35.71 ±0.34	34.07 ±0.29	<.0001
Breaking Strength N	35.32 ±0.42	33.55 ±0.41	30.62 ±0.52	34.70 ±0.49	<.0001
Deformation (μm)	258.2 ±4.97	231.3 ±4.24	245.4 ±6.48	256.22 ±4.64	.0005
Shell Weight (g)	6.24 ±0.03	6.41 ±0.04	6.21 ±0.04	6.35 ±0.03	<.0001
Percentage Shell %	9.15 ±0.04	9.24 ±0.05	8.98 ±0.05	9.21 ±0.05	.0005
Shell Thickness (μm)	406.2 ±1.75	405.9 ±1.89	406.3 ±1.97	419.52 ±1.87	<.0001
Albumen Height (mm)	7.84 ±0.07	8.72 ±0.08	7.96 ±0.09	8.69 ±0.09	<.0001
Haugh Units	85.5 ±0.48	90.3 ±0.46	85.5 ±0.64	89.73 ±0.55	<.0001
Yolk Colour Score	12.01 ±0.04	11.68 ±0.04	11.46 ±0.04	11.28 ±0.05	<.0001

Values are Means ± Standard Error of the Mean. NS is not statistically significant
Hens were moulted at 65-68 weeks (following the 65 week egg collection).

There was a significant main effect of diet on a number of egg quality measures (Table 44). The wheat plus rye diet resulted in higher breaking strength, higher shell weight and better albumen quality. However, yolk colour was lighter for the wheat + rye diet.

Table 44: Effect of diet on egg and egg shell quality at 55-73 weeks of age.

Egg Quality Measurement	**Diet**		**P Value**
	Wheat	Wheat + Rye	
Egg Weight (g)	69.25 ±0.22	69.63 ±0.21	NS
Shell Reflectivity (%)	34.81 ±0.22	34.42 ±0.21	NS
Breaking Strength N	33.08 ±0.34	34.6 ±0.33	.0011
Deformation (μm)	249.57 ±3.87	246.28 ±3.43	NS
Shell Weight (g)	6.26 ±0.03	6.36 ±0.03	.0117
Percentage Shell %	9.06 ±0.03	9.15 ±0.04	NS (.0596)
Shell Thickness (μm)	408.43 ±1.31	410.51 ±1.37	NS
Albumen Height (mm)	8.17 ±0.07	8.43 ±0.06	.0018
Haugh Units	86.87 ±0.43	88.63 ±0.34	.0008
Yolk Colour Score	11.7 ±0.03	11.52 ±0.04	<.0001

Values are Means ± Standard Error of the Mean. NS is not statistically significant

There were significant effects of enzyme treatment on egg and egg shell quality (Table 45). Egg weight was higher for the control and lowest for Kemzyme. Shell colour was lighter in the eggs from birds receiving enzymes than it was for the control. Albumen height and Haugh Units were significantly lower for the Kemzyme group. Yolk colour varied among the enzyme treatment groups.

Table 45: Effect of enzyme treatment on egg and egg shell quality at 55-73 weeks of age.

Egg Quality Measurement	**Enzyme Treatment**					**P Value**
	Control	BF	AV	RX	KM	
Egg Weight (g)	69.65 ±0.39	69.53 ±0.33	69.37 ±0.33	69.52 ±0.32	69.13 ±0.34	<.0001
Shell Reflectivity (%)	33.9 ±0.35	34.26 ±0.33	34.15 ±0.34	34.78 ±0.34	35.97 ±0.35	.0001
Breaking Strength N	33.77 ±0.51	32.79 ±0.51	33.54 ±0.52	34.38 ±0.54	34.7 ±0.56	NS (.0764)
Deformation (µm)	248.57 ±6.21	246.5 ±5.83	249.88 ±5.4	248.41 ±5.73	246.26 ±5.75	NS
Shell Weight (g)	6.3 ±0.04	6.25 ±0.04	6.34 ±0.04	6.31 ±0.04	6.35 ±0.04	NS
Percentage Shell %	9.07 ±0.05	9.0 ±0.05	9.15 ±0.05	9.08 ±0.05	9.2 ±0.06	NS (.0839)
Shell Thickness (µm)	408.38 ±2.03	406.4 ±2.1	410.99 ±2.01	408.91 ±2.16	412.65 ±2.3	NS
Albumen Height (mm)	8.51 ±0.10	8.38 ±0.09	8.27 ±0.10	8.49 ±0.09	7.86 ±0.08	<.0001
Haugh Units	88.87 ±0.63	88.29 ±0.58	87.53 ±0.66	88.86 ±0.54	85.21 ±0.63	<.0001
Yolk Colour Score	11.6 ±0.05	11.48 ±0.06	11.80 ±0.05	11.59 ±0.05	11.57 ±0.06	<.0001

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

Tables 46-49 summarise the effects of type of diet and enzyme treatment on egg and egg shell quality at 55, 60, 65 and 73 weeks of age, respectively. Birds were placed into an induced moult immediately following the 65 week egg collection. They had returned to full production by 71 weeks of age. At 55 weeks of age, there were significant main effects of diet on shell weight and shell thickness (Table 46) with shell quality being higher for the wheat + rye diet than for the wheat diet. There were significant main effects of enzyme treatment on albumen height and Haugh units, with albumen quality being better when enzymes were added to the feed, particularly for Roxazyme.

Table 46: Egg and egg shell quality for different diets and enzyme treatments at 55 weeks of age.

Egg Quality Measurement	**Diet Type**	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	**Mean**	D	E	D*E
Egg Weight (g)	W	66.8	67.6	68.2	68.7	68.6	**68.0**	NS	NS	NS
	W+R	68.6	69.3	67.9	69.0	68.3	**68.6**			
	Mean	**67.7**	**68.4**	**68.0**	**68.9**	**68.5**				
Shell Reflectivity (%)	W	34.5	35.1	33.9	36.5	35.5	**35.1**	NS	NS	NS
	W+R	33.7	34.8	36.9	34.5	35.8	**35.1**			
	Mean	**34.1**	**35.0**	**35.4**	**35.5**	**35.7**				
Breaking Strength (Newtons)	W	33.6	35.6	35.8	35.0	34.8	**34.9**	NS	NS	NS
	W+R	36.3	34.7	33.8	36.8	36.8	**35.7**			
	Mean	**35.0**	**35.2**	**34.8**	**35.9**	**35.8**				
Deformation (μm)	W	225.7	260.7	277.3	255.3	279.7	**259.7**	NS	NS	NS
	W+R	249.3	258.3	267.0	245.0	263.7	**256.7**			
	Mean	**237.5**	**259.5**	**272.2**	**250.2**	**271.7**				
Shell Weight (g)	W	6.18	6.02	6.26	6.11	6.27	**6.17**	0.025	NS	NS
	W+R	6.41	6.29	6.14	6.36	6.39	**6.32**			
	Mean	**6.29**	**6.16**	**6.20**	**6.24**	**6.33**				
Percentage Shell (%)	W	9.26	8.93	9.19	8.91	9.14	**9.09**	NS	NS	NS
	W+R	9.36	9.10	9.04	9.22	9.37	**9.22**			
	Mean	**9.31**	**9.01**	**9.11**	**9.07**	**9.26**				
Shell Thickness (μm)	W	403.7	394.5	409.6	401.1	404.01	**402.6**	0.041	NS	NS
	W+R	416.8	402.9	405.6	407.7	415.7	**409.7**			
	Mean	**410.2**	**398.7**	**407.6**	**404.4**	**409.9**				
Albumen Height (mm)	W	7.21	7.81	7.87	8.06	7.65	**7.72**	NS	0.025	NS
	W+R	7.85	8.04	7.78	8.43	7.65	**7.95**			
	Mean	**7.53**	**7.93**	**7.82**	**8.25**	**7.65**				
Haugh Units	W	81.4	85.7	85.3	86.9	83.9	**84.6**	NS	0.031	NS
	W+R	85.7	86.9	85.5	89.2	84.8	**86.4**	0.06		
	v	**83.6**	**86.3**	**85.4**	**88.0**	**84.4**				
Yolk Colour Score	W	11.90	11.93	12.03	11.97	11.90	**11.95**	NS	NS	NS
	W+R	11.87	12.03	12.43	12.00	12.03	**12.07**		0.07	
	Mean	**11.88**	**11.98**	**12.23**	**11.98**	**11.97**				

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
Diet W is wheat-based; Diet W+R is wheat-based with 20% wheat substituted with cereal rye.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
D is diet; E is enzymes; D*E is diet-enzyme interaction.

At 60 weeks of age, there were significant effects of diet on shell breaking strength, shell weight, percentage shell, Haugh Units and yolk colour (Table 47). Egg shell quality and Haugh Units were better for the wheat + rye diet than for the wheat diet. However, yolk colour was higher for the wheat diet. The only statistically significant effects of enyzme treatment were on shell breaking strength and percentage shell, which were better for the Roxazyme and Kemzyme groups and worst for the Control and Biofeed Wheat groups, with Avizyme intermediate.

Table 47: Egg and egg shell quality for different diets and enzyme treatments at 60 weeks of age.

Egg Quality Measurement	Diet Type	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	**Mean**	D	E	D*E
Egg Weight (g)	W	71.1	69.8	68.9	68.3	68.7	**69.3**	NS	NS	NS
	W+R	69.9	67.6	69.9	70.0	69.8	**69.5**			
	Mean	**70.6**	**68.7**	**69.4**	**69.2**	**69.3**				
Shell Reflectivity (%)	W	34.2	33.3	33.3	34.0	35.7	**34.1**	NS	NS	NS
	W+R	32.4	32.3	32.9	32.6	34.8	**33.0**	(.056)	(.057)	
	Mean	**33.3**	**32.8**	**33.1**	**33.3**	**35.2**				
Breaking Strength (Newtons)	W	30.3	30.8	33.5	35.1	33.5	**32.6**	.0261	.0474	NS
	W+R	34.0	33.4	33.9	35.3	35.7	**34.5**			
	Mean	**32.1**	**32.1**	**33.7**	**35.2**	**34.6**				
Deformation (μm)	W	237.3	206.7	233.0	226.3	221.3	**224.9**	NS	NS	NS
	W+R	238.0	231.4	226.0	262.7	230.7	**237.8**			
	Mean	**237.7**	**218.8**	**229.5**	**244.5**	**225.9**				
Shell Weight (g)	W	6.27	6.36	6.33	6.37	6.32	**6.33**	0.025	NS	NS
	W+R	6.46	6.14	6.55	6.59	6.66	**6.48**			
	Mean	**6.36**	**6.25**	**6.44**	**6.48**	**6.49**				
Percentage Shell (%)	W	8.82	9.12	9.20	9.35	9.23	**9.14**	.0361	.0479	NS
	W+R	9.25	9.08	9.39	9.41	9.59	**9.34**			
	Mean	**9.04**	**9.10**	**9.29**	**9.38**	**9.41**				
Shell Thickness (μm)	W	400.2	406.7	401.0	410.8	402.7	**404.3**	NS	NS	NS
	W+R	407.4	402.2	409.8	405.8	411.9	**407.4**			
	Mean	**403.8**	**404.5**	**405.4**	**408.3**	**407.3**				
Albumen Height (mm)	W	8.76	8.56	8.96	8.86	7.71	**8.57**	NS	NS	.0052
	W+R	9.10	9.18	8.32	8.88	8.88	**8.87**	(.055)	(.063)	
	Mean	**8.93**	**8.87**	**8.64**	**8.87**	**8.30**				
Haugh Units	W	89.9	89.4	91.6	90.9	84.9	**89.3**	.0354	NS	.0063
	W+R	92.5	93.4	88.0	91.2	91.0	**91.2**		(.075)	
	Mean	**91.2**	**91.4**	**89.8**	**91.1**	**88.0**				
Yolk Colour Score	W	11.80	11.63	11.80	11.80	11.97	**11.80**	.0044	NS	NS
	W+R	11.60	11.53	11.73	11.47	11.47	**11.56**			
	Mean	**11.70**	**11.58**	**11.77**	**11.63**	**11.72**				

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
Diet W is wheat-based; Diet W+R is wheat-based with 20% wheat substituted with cereal rye.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
D is diet; E is enzymes; D*E is diet-enzyme interaction.

At 65 weeks of age, just prior to the induced moult, there were significant effects of diet on albumen height, Haugh Units and yolk colour (Table 48). Albumen height and Haugh Units were higher but yolk colour lower, for the wheat + rye diet. Albumen height and Haugh Units for all the enzyme treatments tended to be lower than the control.

Table 48: Egg and egg shell quality for different diets and enzyme treatments at 65 weeks of age.

Egg Quality Measurement	**Diet Type**	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	**Mean**	D	E	D*E
Egg Weight (g)	W	69.9	71.0	69.6	67.9	68.6	**69.4**	NS	NS	NS
	W+R	69.6	69.4	68.4	70.9	67.9	**69.2**			
	Mean	**69.7**	**70.2**	**69.0**	**69.4**	**68.2**				
Shell Reflectivity (%)	W	35.2	34.6	36.5	36.9	36.6	**36.0**	NS	NS	NS
	W+R	34.4	35.6	33.9	35.1	38.1	**35.4**			
	Mean	**34.8**	**35.1**	**35.2**	**36.0**	**37.4**				
Breaking Strength (Newtons)	W	31.5	28.6	29.5	31.0	28.2	**29.7**	NS (.088)	NS	NS
	W+R	30.4	28.7	33.1	32.7	32.8	**31.5**			
	Mean	**30.9**	**28.6**	**31.3**	**31.8**	**30.5**				
Deformation (μm)	W	276.8	251.7	259.0	221.0	243.7	**250.1**	NS	NS	NS
	W+R	274.1	221.0	224.1	245.2	238.6	**240.6**			
	Mean	**275.4**	**236.6**	**241.9**	**232.9**	**241.2**				
Shell Weight (g)	W	6.14	6.30	6.18	6.07	6.21	**6.18**	NS	NS	NS
	W+R	6.23	6.24	6.36	6.25	6.15	**6.25**			
	Mean	**6.18**	**6.27**	**6.27**	**6.16**	**6.18**				
Percentage Shell (%)	W	8.78	8.87	8.91	8.93	9.08	**8.91**	NS	NS	NS
	W+R	9.00	9.02	9.35	8.80	9.07	**9.04**			
	Mean	**8.88**	**8.95**	**9.13**	**8.87**	**9.08**				
Shell Thickness (μm)	W	397.8	404.6	406.8	409.7	412.3	**406.2**	NS	NS	NS
	W+R	406.2	407.4	410.9	400.1	407.5	**406.4**			
	Mean	**402.0**	**406.0**	**408.9**	**404.9**	**409.9**				
Albumen Height (mm)	W	8.09	7.90	7.59	7.50	7.15	**7.65**	.0004	.0003	NS
	W+R	8.86	8.62	8.04	8.42	7.40	**8.27**			
	Mean	**8.48**	**8.26**	**7.82**	**7.96**	**7.28**				
Haugh Units	W	85.6	85.0	82.6	83.4	79.8	**83.3**	.0003	.0024	NS
	W+R	91.3	90.0	86.6	88.1	82.6	**87.7**			
	Mean	**88.4**	**87.5**	**84.6**	**85.7**	**81.2**				
Yolk Colour Score	W	11.70	11.50	11.67	11.53	11.67	**11.61**	.0003	NS	NS
	W+R	11.30	11.43	11.43	11.30	11.10	**11.31**			
	Mean	**11.50**	**11.47**	**11.55**	**11.42**	**11.38**				

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
Diet W is wheat-based; Diet W+R is wheat-based with 20% wheat substituted with cereal rye.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
D is diet; E is enzymes; D*E is diet-enzyme interaction.

At 73 weeks of age, when the birds had returned to full production following the induced moult, the only difference between the two diets was that yolk colour was higher for the wheat than the wheat + rye (Table 49). Shell reflectivity and yolk colour varied among enzyme treatments but albumen height and Haugh Units tended to be lower than the control. Shell colour was darkest for Avizyme and lightest for Kemzyme. Haugh Units were highest in the control birds and lowest for Kemzyme. Yolk colour was lowest for Biofeed Wheat.

Table 49: Egg and egg shell quality for different diets and enzyme treatments at 73 weeks of age.

Egg Quality Measure	Diet Type	**Treatment Group**						**Statistical Analysis** P Values		
		C	BF	AV	RX	KM	**Mean**	D	E	D*E
Egg Weight (g)	W	71.3	70.84	70.85	68.86	69.66	**70.30**	NS	NS	NS
	W+R	70.02	70.71	71.30	72.48	71.55	**71.21**			
	Mean	**70.66**	**70.78**	**71.08**	**70.67**	**70.61**				
Shell Reflectivity (%)	W	34.17	33.73	32.17	34.00	36.27	**34.07**	NS	.0405	NS
	W+R	32.57	34.63	33.6	34.63	34.9	**34.07**			
	Mean	**33.37**	**34.18**	**32.88**	**34.32**	**35.58**				
Breaking Strength Newtons	W	36.46	33.94	34.28	33.83	36.29	**34.96**	NS (0.08)	NS (0.09)	NS
	W+R	37.38	36.52	34.47	35.28	39.51	**36.63**			
	Mean	**36.92**	**35.21**	**34.38**	**34.56**	**37.9**				
Deformation (μm)	W	245.67	266	280.67	283.00	242.33	**263.53**	NS	NS	NS
	W+R	244.33	275.86	231.00	248.67	249.33	**249.66**			
	Mean	**245**	**270.85**	**255.83**	**265.83**	**245.83**				
Shell Weight (g)	W	6.31	6.39	6.51	6.39	6.29	**6.38**	NS	NS	NS
	W+R	6.43	6.28	6.36	6.32	6.51	**6.38**			
	Mean	**6.37**	**6.33**	**6.44**	**6.35**	**6.4**				
Percent Shell (%)	W	8.89	9.02	9.21	9.31	9.03	**9.09**	NS	NS	.0119
	W+R	9.24	8.91	8.93	8.73	9.13	**8.99**			
	Mean	**9.07**	**8.97**	**9.08**	**9.02**	**9.08**				
Shell Thickness (μm)	W	408.6	419.93	428.17	429.57	416.57	**420.57**	NS	NS	.0015
	W+R	426.47	412.93	416.1	406.47	430.43	**418.48**			
	Mean	**417.53**	**416.43**	**422.13**	**418.02**	**423.5**				
Albumen Height (mm)	W	9.5	8.56	9.07	8.71	7.94	**8.76**	NS	.0172	. NS
	W+R	8.73	8.37	8.55	9.04	8.49	**8.63**			
	Mean	**9.11**	**8.46**	**8.81**	**8.88**	**8.21**				
Haugh Units	W	94.4	88.87	92.37	90.03	85.67	**90.27**	NS	.0229	NS
	W+R	90.27	87.1	88.33	91.3	88.97	**89.19**			
	Mean	**92.33**	**87.98**	**90.35**	**90.67**	**87.31**				
Yolk Colour Score	W	11.63	10.87	11.77	11.4	11.5	**11.43**	.0016	<.0001	NS
	W+R	11	10.9	11.57	11.23	10.93	**11.13**			
	Mean	**11.32**	**10.88**	**11.67**	**11.32**	**11.22**				

Values are Means ± Standard Errors of the Mean. NS is not statistically significant.
Diet W is wheat-based; Diet W+R is wheat-based with 20% wheat substituted with cereal rye.
C is control (without enzyme), BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme
D is diet; E is enzymes; D*E is diet-enzyme interaction.

2.3.8 Keeping Power of Eggs

Tables 50-58 summarise the effect of storage conditions on egg internal quality at 55, 60 and 65 weeks of age. At all ages, albumen quality was highest in the fresh eggs and lowest in eggs which had been stored at room temperature for 4 weeks, with eggs that had been stored in a cool room for 4 weeks being of intermediate internal quality (Tables 50, 53, 56). Type of diet affected only albumen height at 65 weeks of age, with the wheat + rye diet resulting in higher Haugh Units, an effect that was becoming apparent at 60 weeks (Tables 54,57). There were, however, effects of enzyme treatment on albumen quality at 60 and 65 weeks, with the Kemzyme group having the lowest Haugh Units (Tables 55, 58).

Table 50: Effect of egg storage treatment on egg internal quality at 55 weeks of age.

Egg Quality Measurement	**Treatment Temperature**			**P Value**
	Fresh	4 wks cold	4 wks room	
Egg Weight (g)	68.3 ±0.2	67.0 ±0.4	59.9 ±0.4	<.0001
Albumen Height (mm)	7.8 ±0.07	6.2 ±0.09	3.4 ±0.06	<.0001
Haugh Units	85.5 ±0.5	74.6 ±0.8	49.5 ±0.7	<.0001

Values are Means Standard Error of the mean. NS is not statistically significant

Table 51: Effect of diet on egg internal quality at 55 weeks of age.

Egg Quality Measurement	**Diet**		**P Value**
	Wheat	Wheat + Rye	
Egg Weight (g)	65.6 ±0.3	66.1 ±0.4	NS
Albumen Height (mm)	6.3 ±0.1	6.3 ±0.1	NS
Haugh Units	73.6 ±1.0	74.0 ±1.0	NS

Values are Means Standard Error of the mean. NS is not statistically significant

Table 52: Effect of enzyme treatment on egg internal quality at 55 weeks of age.

Egg Quality Measurement	**Enzyme Treatment**					**P Value**
	Control	BF	AV	RX	KM	
Egg Weight (g)	65.8 ±0.6	65.3 ±0.6	65.7 ±0.5	66.2 ±0.5	66.3 ±0.5	NS
Albumen Height (mm)	6.2 ±0.2	6.3 ±0.2	6.2 ±0.2	6.5 ±0.2	6.3 ±0.2	NS
Haugh Units	73.2 ±1.5	73.8 ±1.6	73.1 ±1.5	74.9 ±1.7	74.0 ±1.5	NS

NS is not statistically significant
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

Table 53: Effect of egg storage treatment on egg internal quality at 60 weeks of age.

Egg Quality Measurement	Treatment Temperature			P Value
	Fresh	4 wks cold	4 wks room	
Egg Weight (g)	69.4 ±0.3	67.1 ±0.4	62.8 ±0.4	<.0001
Albumen Height (mm)	8.7 ±0.08	6.3 ±0.10	3.6 ±0.04	<.0001
Haugh Units	90.3 ±0.5	75.3 ±0.8	51.0 ±0.5	<.0001

Values are Means Standard Error of the mean.

Table 54: Effect of diet on egg internal quality at 60 weeks of age.

Egg Quality Measurement	Diet		P Value
	Wheat	Wheat + Rye	
Egg Weight (g)	66.9 ±0.3	67.5 ±0.3	NS (0.0741)
Albumen Height (mm)	6.7 ±0.1	6.9 ±0.1	NS
Haugh Units	76.2 ±1.0	77.3 ±1.0	NS

Values are Means Standard Error of the mean. NS is not statistically significant

Table 55: Effect of enzyme treatment on egg internal quality at 60 weeks of age.

Egg Quality Measurement	Enzyme Treatment					P Value
	Control	BF	AV	RX	KM	
Egg Weight (g)	67.5 ±0.6	66.8 ±0.5	67.2 ±0.5	66.9 ±0.5	67.5 ±0.5	NS
Albumen Height (mm)	6.9 ±0.2	6.9 ±0.2	6.8 ±0.2	7.1 ±0.2	6.5 ±0.2	0.0348
Haugh Units	76.6 ±1.7	77.2 ±1.7	76.4 ±1.6	78.7 ±1.6	74.9 ±1.6	0.0057

NS is not statistically significant
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

Table 56: Effect of egg storage treatment on egg internal quality at 65 weeks of age.

Egg Quality Measurement	**Treatment Temperature**			**P Value**
	Fresh	4 wks cold	4 wks room	
Egg Weight (g)	69.3 ±0.3	68.1 ±0.4	64.0 ±0.4	<.0001
Albumen Height (mm)	8.0 ±0.01	6.4 ±0.09	3.3 ±0.04	<.0001
Haugh Units	85.5 ±0.6	76.2 ±0.7	46.1 ±0.6	<.0001

Values are Means Standard Error of the mean.

Table 57: Effect of diet on egg internal quality at 65 weeks of age.

Egg Quality Measurement	**Diet**		**P Value**
	Wheat	Wheat + Rye	
Egg Weight (g)	67.5 ±0.3	67.8 ±0.3	NS
Albumen Height (mm)	6.3 ±0.1	6.6 ±0.1	0.0371
Haugh Units	72.4 ±1.1	74.3 ±1.1	NS

Values are Means Standard Error of the mean. NS is not statistically significant

Table 58: Effect of enzyme treatment on egg internal quality at 65 weeks of age.

Egg Quality Measurement	**Enzyme Treatment**					**P Value**
	Control	BF	AV	RX	KM	
Egg Weight (g)	68.1 ±0.5	68.3 ±0.5	67.5 ±0.5	67.4 ±0.5	67.1 ±0.5	NS
Albumen Height (mm)	6.7 ±0.2	6.6 ±0.2	6.3 ±0.2	6.5 ±0.2	6.0 ±0.2	0.0085
Haugh Units	74.9 ±1.8	74.8 ±1.7	72.5 ±1.8	74.2 ±1.7	70.3 ±1.7	0.0097

NS is not statistically significant
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

2.3.9 Blood Electrolytes

At 72 weeks of age, there was a significant effect of enzyme treatment on haematocrit, which was significantly lower for Roxazyme than for all other groups (Table 59). When each wheat was considered separately, this effect was statistically significant for the wheat diet (P=0.0033) but not for the wheat+rye diet (P=0.0665). At 72 weeks of age, there was a tendency for plasma sodium to be higher for the wheat plus rye diet (P=0.058).

Table 59: Effect of enzyme supplementation on haematocrit and plasma electrolyte concentrations in laying hens at 72 weeks of age.

Diets	Haematocrit (%)	Na (mmol/L)	K (mmol/L)	Ionised Ca (mmol/L)
Wheat +Rye:				
Control	30.1±0.8	151.9±1.8	5.6±0.3	1.61±0.09
Biofeed Wheat	30.6±0.9	150.9±1.9	5.3±0.1	1.67±0.06
Avizyme	30.6±1.0	153.0±2.5	5.8±0.2	1.81±0.08
Roxazyme	28.8±0.6	152.0±1.7	5.4±0.1	1.68±0.02
Kemzyme	32.6±0.9	152.8±1.2	5.6±0.2	1.71±0.04
Wheat:				
Control	30.6±0.8[a]	150.5±0.5	5.4±0.2	1.65±0.05
Biofeed Wheat	31.6±0,7[a]	150.3±1.5	5.6±0.3	1.75±0.11
Avizyme	29.6±0.7[a]	149.2±0.5	5.6±0.2	1.62±0.03
Roxazyme	27.6±0.4[b]	150.5±0.7	5.6±0.2	1.69±0.04
Kemzyme	30.1±0.5[a]	151.0±1.2	6.2±0.2	1.65±0.05
Statistical Analysis				
Wheat Type	NS	NS (0.058)	NS	NS
Enzyme	0.0012	NS	NS	NS
Wheat*Enzyme	NS	NS	NS	NS

Means ± SE. [a,b] Means within columns, for a particular wheat type, with no common superscript differ significantly (P<0.05)

2.4 Discussion of Results

As expected, the extract viscosity of the cereal rye grain was very high. However, the extract viscosity of the diet which had 20% of the wheat substituted with rye was only about 3 times that of the diet based on wheat only. Some of this is attributable to the dilution of the rye with the other feed ingredients. However, there may also have been an effect of endogenous enzymes in the other feed ingredients. Similarly, the levels of soluble, insoluble and total non-starch polysaccharide were only 10-15% higher in the wheat+rye diets, as compared with the wheat diets.

Feed intake, excreta moisture and AME were similar for the wheat and wheat+rye diets and were not significantly affected by the addition of feed enzymes. However, the digesta viscosity was higher in both the jejunum and ileum for the wheat+ rye diets than for the wheat diets. Feed enzymes did not reduce digesta viscosity.

Production was not different between the wheat and wheat+rye diets. However, there were significant overall effects of the addition of feed enzymes. Production was higher than the control for Biofeed Wheat and Roxazyme and lower than the control for Kemzyme and Avizyme. During the induced moult, production declined more rapidly and reached lower levels for the wheat diet than the wheat+rye diets. However, enzyme addition in pre-moult diets did not affect production during the induced moult.

Egg and egg shell quality were significantly affected by hen age and were generally improved following the induced moult. The wheat+rye diet improved egg internal quality and egg shell quality, in comparison to the wheat diet, although yolk colour was greater for the wheat diet. The addition of enzymes to the diets had significant effects on egg and egg shell quality. Shell colour was generally lighter for the groups receiving enzymes and albumen quality was reduced by all the enzymes except Roxazyme. This reduction in Haugh Units is similar to that observed in an earlier study (Roberts and Choct, 1999; Roberts *et at.,* 1999). It is possible that the potentially negative effects of enzymes on shell colour and Haugh Units are more apparent towards the later stages of the commercial laying cycle. The variation in yolk colour, while not commercially significant, is of interest in terms of possible modes of action of the enzyme supplements.

The keeping power of eggs was not significantly affected by either diet or enzymes at 55 weeks of age. However, at 65 weeks of age, there was a significant effect of diet on albumen height and a significant interaction between diet and storage treatment. Albumen height was better maintained after 4 weeks in the cold room for the wheat diet than for the wheat+rye diet. Enzyme treatment had significant effects on albumen height and Haugh units at 60 and 65 weeks of age. However, there were no significant interactions between enzyme treatment and storage treatment. This indicated that the effects of enzymes were primary and not affected by storage treatment.

There were some effects of enzyme treatment on haematocrit with Roxazyme resulting in significantly lower haematocrit than the other treatment groups. The reason for this is not clear.

Overall, the addition of cereal rye to a wheat-based diet did not negatively affect bird production and egg quality, even though digesta viscosity was increased. It may be that the increase in digesta viscosity was not sufficient to cause negative effects. Alternatively, laying hens, being mature birds, may be less susceptible to negative effects of increased digesta viscosity than is the case in broilers.

3. One type of wheat with or without enzymes: Birds 73-87 weeks of age (Trial 3)

3.1 Introduction

The hens were returned to full production, following the induced moult, on the same diets that they had received from 50 weeks of age. However, following the return to full production, at 73 weeks of age, birds were placed from the 10 diets on to 5 diets. These diets were based on one type of wheat, which was newly sourced and contained the same enzymes as the birds had received during Trials 1 and 2. This final experiment, Trial 3, was conducted to see if there were any residual effects from the previous treatments.

3.2 Methodology

Birds received a wheat-based diet containing the same enzymes that they had been receiving throughout the project. The wheat used was different from those used in Trials 1 and 2 and was newly sourced (a "new season" wheat). However, this meant that there were only 5 diets for the final trial. The basal diet was formulated and the enzymes were included at the levels outlined in Chapter 1.

Production was monitored continuously and eggs were collected for analysis at 82 and 87 weeks of age. Detailed measurements of egg internal quality and egg shell quality were made, as described in Chapter 1. Keeping power was measured at 82 weeks of age.

AME, excreta moisture and feed intake were not measured.

3.3 Detailed Results

3.3.1 Feed Analysis

The levels of non-starch polysaccharides in the diet are shown in Table 60. The wheat on which the diet was based was a "new season wheat". However, the total NSP level was similar to that of the wheat-based diets in Trials 1 and 2.

Table 60 Non-Starch Polysaccharide Levels in Diet Based on New Season Wheat

Sugar g/kg	Free Sugars g/kg	Insoluble NSP g/kg	Soluble NSP g/kg	Total NSP g/kg
Rhamnose	0.00	0.00	0.09	0.09
Fucose	0.00	0.00	0.08	0.08
Ribose	0.00	0.00	0.16	0.16
Arabinose	0.26	19.84	3.40	23.24
Xylose	0.00	28.38	3.03	31.41
Mannose	1.74	2.72	0.70	3.42
Galactose	3.09	3.65	1.84	5.49
Glucose	11.75	25.00	1.85	26.85
TOTAL	16.69	70.67	9.89	80.56

3.3.2 Production

Production in the period following the induced moult is summarised in Tables 61-62. Production declined with hen age (Table 61). However, there was no significant effect of enzyme treatment on production (Table 62).

Table 61: Effect of hen age on production at 73-87 weeks

Age of Hens (weeks)			P Value
73-76 wks	77-81 wks	82-87 wks	
83.07 ±0.89	77.14 ±0.95	71.69 ±1.31	<0.0001

Values are Means ± Standard Errors of the Means.

Table 62: Effect of enzyme treatment on production at 73-87 weeks of age.

Enzyme Treatment					P Value
Control	Biofeed Wheat	Avizyme	Roxazyme	Kemzyme	
77.01 ±1.85	76.46 ±2.27	77.62 ±1.48	79.89 ±1.38	78.08 ±0.92	NS

Values are Means ± Standard Errors of the Means. NS is not statistically significant.

3.3.3 Egg and Egg Shell Quality

For the egg collections at 82 and 87 weeks of age, there were significant differences between the two ages for shell reflectivity, shell weight, percentage shell, albumen height, Haugh Units and yolk colour (Table 63). For all of these measurements, egg quality was lower at 87 weeks than at 82 weeks.

The only significant effect of enzyme treatment at 82 and 87 weeks of age was on shell colour and yolk colour (Table 64). Shell colour was lighter than the control for Biofeed Wheat, Avizyme and Kemzyme, but not for Roxazyme. Yolk colour was generally higher for the enzyme-supplemented groups.

When looked at separately at 82 and 87 weeks of age, the only effect of enzyme treatment was on yolk colour (Tables 65, 66), as mentioned above.

Table 63: Effect of hen age on egg and egg shell quality at 82-87 weeks of age.

Egg Quality Measurement	**Age of Hens wks**		**P Value**
	82	87	
Egg Weight (g)	69.44 ±0.33	69.33 ±0.34	NS
Shell Reflectivity (%)	35.71 ±0.33	37.29 ±0.33	.0006
Breaking Strength N	31.626 ±0.48	31.977 ±0.51	NS
Deformation (µm)	243.31 ±5.46	258.18 ±6.7	NS .0879
Shell Weight (g)	6.28 ±0.04	6.15 ±0.04	.0239
Percentage Shell %	9.06 ±0.05	8.89 ±0.05	.0134
Shell Thickness (µm)	407.41 ±1.95	402.94 ±2.09	NS
Albumen Height (mm)	7.84 ±0.09	7.42 ±0.09	.0012
Haugh Units	84.89 ±0.59	81.95 ±0.69	.0013
Yolk Colour Score	11.21 ±0.06	10.95 ±0.05	.0007

Values are Means ± Standard Error of the Mean. NS is not statistically significant

Table 64: Effect of enzyme treatment on egg and egg shell quality at 82-87 weeks of age.

Egg Quality Measurement	**Enzyme Treatment**					**P Value**
	C	BF	AV	RX	KM	
Egg Weight (g)	69.18 ±0.53	69.22 ±0.55	69.19 ±0.51	70.06 ±0.51	69.25 ±0.53	NS
Shell Reflectivity (%)	35.45 ±0.49	36.74 ±0.50	37.38 ±0.48	35.84 ±0.59	37.07 ±0.52	.0382
Breaking Strength N	31.6 ±0.77	31.51 ±0.72	31.85 ±0.79	31.79 ±0.87	32.25 ±0.779	NS
Deformation (µm)	250.08 ±9.22	255.13 ±10.27	234.00 ±6.73	267.27 ±11.17	247.71 ±10.38	NS
Shell Weight (g)	6.18 ±0.06	6.16 ±0.06	6.21 ±0.07	6.27 ±0.07	6.25 ±0.06	NS
Percentage Shell %	8.95 ±0.07	8.93 ±0.08	8.99 ±0.09	8.96 ±0.08	9.04 ±0.08	NS
Shell Thickness (µm)	409.89 ±2.78	402.68 ±3.19	408.56 ±3.43	402.23 ±3.36	402.53 ±3.18	NS
Albumen Height (mm)	7.64 ±0.14	7.47 ±0.17	7.82 ±0.14	7.70 ±0.13	7.51 ±0.14	NS
Haugh Units	83.68 ±0.99	81.83 ±1.26	84.71 ±0.96	84.08 ±0.86	82.79 ±1.0	NS
Yolk Colour Score	10.7 ±0.07	10.89 ±0.81	11.38 ±0.08	11.42 ±0.07	11.03 ±0.12	<.0001

Values are Means ± Standard Error of the Mean. NS is not statistically significant
C is control group, without enzymes.
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

Table 65: Egg and egg shell quality for different diets and enzyme treatments at 82 weeks of age.

Egg Quality Measurement	**Enzyme Treatment Group**						**P Value**
	C	BF	AV	RX	KM	Mean	
Egg Weight (g)	69.32 ±0.75	69.04 ±0.80	69.44 ±0.66	69.64 ±0.7	69.74 ±0.74	69.44 ±0.33	NS
Shell Reflectivity (%)	34.71 ±0.74	35.6 ±0.64	36.58 ±0.69	35.05 ±0.87	36.58 ±0.71	35.71 ±0.32	NS
Breaking Strength (Newtons)	31.36 ±1.04	32.41 ±0.85	30.83 ±1.07	30.55 ±1.24	33.02 ±1.10	31.63 ±0.48	NS
Deformation (μm)	255.83 ±14.88	233.9 ±8.41	223.83 ±7.35	254.92 ±12.67	248.28 ±15.44	243.31 ±5.46	NS
Shell Weight (g)	6.22 ±0.08	6.26 ±0.07	6.3 ±0.08	6.3 ±0.10	6.32 ±0.08	6.28 ±0.04	NS
Percentage Shell (%)	8.99 ±0.10	9.1 ±0.09	9.08 ±.011	9.05 ±0.12	9.09 ±0.11	9.06 ±0.05	NS
Shell Thickness (μm)	411.33 ±4.08	406.22 ±4.22	409.82 ±4.1	405.5 ±4.9	404.2 ±4.51	407.41 ±2.09	NS
Albumen Height (mm)	7.88 ±0.18	7.74 ±0.22	8.06 ±0.2	7.85 ±0.19	7.64 ±0.18	7.84 ±0.09	NS
Haugh Units	85.433 ±1.16	83.93 ±1.61	86.22 ±1.35	85.03 ±1.21	83.82 ±1.21	84.89 ±0.59	NS
Yolk Colour Score	10.82 ±0.1	10.85 ±0.13	11.52 ±0.13	11.57 ±0.11	11.32 ±0.20	11.21 ±0.06	<.0001

Values are Means ± Standard Error of the Mean. NS is not statistically significant
C is control group, without enzymes.
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

Table 66: Egg and egg shell quality for different diets and enzyme treatments at 87 weeks of age.

Egg Quality Measurement	**Enzyme Treatment Group**						**P Value**
	C	BF	AV	RX	KM	Mean	
Egg Weight (g)	69.05 ±0.76	69.4 ±0.75	68.93 ±0.78	70.48 ±0.75	68.77 ±0.77	69.33 ±0.34	NS
Shell Reflectivity (%)	36.18 ±0.64	37.88 ±0.75	38.18 ±0.65	36.63 ±0.77	37.57 ±0.77	37.29 ±0.32	NS
Breaking Strength (Newtons)	31.84 ±1.15	30.63 ±1.15	32.88 ±1.14	33.06 ±1.18	31.51 ±1.12	31.98 ±0.51	NS
Deformation (µm)	244.24 ±10.89	276 ±18.3	244.17 ±11.18	279.83 ±18.46	247.17 ±14.07	258.18 ±6.7	NS
Shell Weight (g)	6.14 ±0.09	6.07 ±0.09	6.13 ±0.11	6.24 ±0.09	6.18 ±0.09	6.15 ±0.04	NS
Percentage Shell (%)	8.91 ±0.1	8.76 ±0.02	8.9 ±0.13	8.86 ±0.11	9.00 ±0.12	8.89 ±0.05	NS
Shell Thickness (µm)	408.45 ±3.81	399.13 ±4.78	407.3 ±5.53	398.95 ±4.62	400.85 ±4.5	402.94 ±2.09	NS
Albumen Height (mm)	7.40 ±0.21	7.19 ±0.24	7.58 ±0.2	7.56 ±0.18	7.37 ±0.21	7.42 ±0.09	NS
Haugh Units	81.93 ±1.57	79.72 ±1.92	83.2 ±1.32	83.12 ±1.22	81.77 ±1.6	81.95 ±0.69	NS
Yolk Colour Score	10.58 ±0.11	10.93 ±0.1	11.25 ±0.09	11.27 ±0.09	10.73 ±0.1	10.95 ±0.05	<.0001

Values are Means ± Standard Error of the Mean. NS is not statistically significant
C is control group, without enzymes.
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

3.3.4 Keeping Power of Eggs

Storage conditions had a highly significant effect on egg weight, albumen height and Haugh units with the values being highest in the fresh eggs, lowest in the eggs stored at room temperature with eggs stored in the cool room being intermediate (Table 67). There were significant effects of enzyme treatment on egg weight and albumen height, although Haugh Units were not different (Table 68).

Table 67: Effect of egg storage treatment on egg internal quality at 82 weeks of age.

Egg Quality Measurement	**Treatment Temperature**			**P Value**
	Fresh	4 wks cold	4 wks room	
Egg Weight (g)	70.1 ±0.2	68.5 ±0.4	65.8 ±0.3	<.0001
Albumen Height (mm)	8.3 ±0.07	6.3 ±0.06	3.7 ±0.04	<.0001
Haugh Units	87.3 ±0.4	75.2 ±0.5	50.9 ±0.5	<.0001

Values are Means ± Standard Error of the Mean.

Table 68: Effect of enzyme treatment on egg internal at 82 weeks of age.

Egg Quality Measurement	**Enzyme Treatment**					**P Value**
	Control	BF	AV	RX	KM	
Egg Weight (g)	68.7 ±0.4	68.1 ±0.4	68.6 ±0.4	69.4 ±0.4	68.3 ±0.4	0.0283
Albumen Height (mm)	6.8 ±0.2	6.5 ±0.2	6.8 ±0.2	6.7 ±0.2	6.4 ±0.1	0.0407
Haugh Units	76.5 ±1.1	74.2 ±1.2	75.9 ±1.2	75.3 ±1.2	74.2 ±1.0	NS

Values are Means ± Standard Error of the Mean.
NS is not statistically significant
BF is BioFeed Wheat, AV is Avizyme, RX is Roxazyme, KM is Kemzyme

3.4 Discussion of Results

In Trial 3, another unsuccessful attempt was made to source new season wheat that was high in non-starch polysaccharide levels (low AME wheat). Once again, it was not possible to obtain such wheat. This reinforces the finding from Trial 1 that new season wheat is not necessarily high NSP/low AME wheat and that the new season wheat phenomenon is something that occurs only occasionally. The levels of soluble, insoluble and total non-starch polysaccharides in the wheat and the diet prepared from it were similar to those reported for Trials 1 and 2. Extract viscosity was not measured for this wheat.

Production declined with hen age but was not affected by the addition of feed enzymes. In Trial 3, as was found in Trial 2, the addition of some of the feed enzymes resulted in lighter coloured egg shells. This finding provides support for the suggestion made in Chapter 2, that the negative effect on shell colour which was reported from an earlier study (Roberts and Choct, 1999; Roberts *et at.,* 1999) seems to occur in older birds. The extent of this colour change is unlikely to be of significant concern commercially. However, it warrants monitoring in the commercial situation. The variability in yolk colour among the enzyme groups was found once more in Trial 3. The mechanism of this effect is not clear and the differences in yolk colour observed are of interest in terms of possible modes of action of the feed enzymes. The differences are small and unlikely to be of commercial significance.

The keeping power measurements in Trial 3 found that enzymes affected egg weight and albumen height but not Haugh Units at 82 weeks of age. However, as there was no statistically significant interaction between enzyme treatment and egg storage treatment, this would appear to be a primary effect of the feed enzymes, rather than an effect of feed enzymes on the keeping power of eggs. However, the finding of lower Haugh units for some enzyme groups at 82 weeks of age supports the observation made in a previous study (Roberts and Choct, 1999; Roberts *et at.,* 1999).

4. Overall Conclusions and Recommendations

The aim of this project was to investigate the efficacy of adding commercial feed enzyme preparations to wheat-based diets in laying hens. Most of the studies on the use of feed enzymes have been conducted in broilers. Relatively little is known about the situation in layers.

Experimental diets, based on one of two types of wheat, “normal” wheat and “pinched” wheat, were fed to the birds in Trial 1 from 25 to 50 weeks of age. The Apparent Metabolisable Energy (AME) of the two diets produced from the “normal” and “pinched” wheat were very similar when measured at 35, 40, 45 and 50 weeks of age. The AME of the diets in Trial 1 was not significantly affected by either the type of wheat on which the diet was based, nor by the inclusion of enzymes. In addition, feed intake and excreta moisture were not significantly affected by either the type of wheat on which the diets were based nor the use of enzyme preparations. These findings suggest that enzymes do not improve AME and litter quality in layers, in the absence of high levels of NSP.

For Trial 1, production changed with the age of the birds and was slightly better for the diet based on “pinched” wheat but there was no effect of enzyme supplementation. Egg internal quality and egg shell quality were generally better for birds receiving the “normal” wheat. The reason for this is not clear although it is, presumably, due to factors other than the levels of NSP in the diets. Enzyme supplementation of the diets resulted in some effects on egg internal quality and egg shell quality. In general, the effects of diet and enzymes were greatest when the birds were younger. This response may be due to the age of the birds and the maturity of their gastrointestinal systems or it may reflect the amount of time that they have been consuming the diets, or both of these factors. When averaged over all the egg collections made during Trial 1, at 27, 30, 35, 40, 45 and 50 weeks of age, the diets based on “normal” wheat resulted in darker shell colour, better egg shell breaking strength, heavier and thicker egg shells and better albumen quality. Over this same time period, the addition of commercial enzyme preparations was found to affect shell colour, shell breaking strength, percentage shell, shell thickness and yolk colour. Shell colour was slightly lighter for some of the enzymes, particularly Roxazyme and Kemzyme. This is probably not of commercial significance. However, it is an effect which should be monitored when enzymes are used in layers. Shell breaking strength was not consistently improved by the addition of feed enzymes, although there were some beneficial effects with Kemzyme. The percentage shell (ratio of shell weight to egg weight, expressed as a percentage) and shell thickness were best for Kemzyme. Yolk colour varied, being generally lower for the enzyme groups. The reason for this is not clear. The effect is slight and not of commercial significance as all yolk colours were very acceptable. However, it is interesting in terms of the mode of action of the enzymes.

The keeping power of eggs (maintenance of albumen height and Haugh Units during egg storage) was measured at 40 and 45 weeks of age. As would be expected, albumen height and Haugh Units were highest in the fresh eggs, followed by the eggs stored at cool room temperature and, lowest of all, the eggs stored at room temperature. Although there were some effects of diet and enzyme on albumen height and Haugh Units, these “primary” effects were not influenced by the storage treatment itself.

At 45 weeks of age, blood samples were taken from the same birds that were used for the AME measurements. The haematocrit (proportion of red blood cells to the volume of whole blood) and the concentrations of sodium, potassium and ionised calcium (the portion of the calcium in blood that is available for biological activities such as bone and egg shell formation) were measured. There were some interesting effects with haematocrit being higher for Biofeed Wheat than for the other treatment groups and ionised calcium higher for Avizyme. The significance of these findings is not clear but it appears that the feed enzymes have some effects on the physiology of the birds, either directly or indirectly.

In Trial 2, although the cereal rye grain had a very high extract viscosity, the diets which were based on wheat plus rye had an extract viscosity only 3 times that of the diets containing wheat only. This finding was surprising and may be due to the presence of endogenous enzymes in other feed ingredients used in the diets. When the levels of non-starch polysaccharides of the wheat and wheat plus rye diets were compared, the wheat plus rye diets were 10-15% higher for soluble, insoluble and total non-starch polysaccharides than were the wheat diets.

As found in Trial 1, feed intake, excreta moisture and the apparent metabolisable energy of the diets were not significantly affected by the type of diet or the inclusion of feed enzymes. Digesta viscosity was higher in both the jejunum and ileum for the wheat plus rye diets as compared with the wheat diets. However, the addition of feed enzymes did not reduce the digesta viscosity in either part of the gut. This finding is surprising, given the higher extract viscosity of the wheat plus rye diets and raises questions about the ability of feed enzymes to reduce digesta viscosity in laying hens.

In Trial 2, production at 55-65 weeks was affected by dietary enzyme supplementation, with production being slightly higher than the control for Biofeed Wheat and Roxazyme and slightly lower for Avizyme and Kemzyme. However, during the period of the induced moult, production was affected by diet but not by the addition of enzymes. For the wheat diets, production dropped more rapidly and to lower levels than for the wheat plus rye diets.

There were some significant effects of the grains on which the diets were based on egg internal quality and egg shell quality. The wheat plus rye diets resulted in higher shell breaking strength and better albumen quality than the wheat diets. There were also significant effects on egg internal quality and egg shell quality of the feed enzymes. Egg weight was higher for the control and lowest for Kemzyme. Shell colour was lighter in the eggs from birds receiving enzymes than it was for the control. Albumen height and Haugh Units were significantly lower for the Kemzyme group. These effects on shell colour and albumen quality are similar to those reported in a previous study (Roberts and Choct, 1999; Roberts *et at.*, 1999). Yolk colour varied among the enzyme treatment groups.

As was found in Trial 1, there were some effects on egg keeping power of the diets and the enzymes. However, again, these were primary effects and the egg storage treatment did not modify them further. The only effect on blood parameters was that haematocrit was lower for the group receiving Roxazyme in the wheat diet.

In Trial 3, production declined as the birds aged but was not affected by enzymes. The only effect of enzymes on egg internal quality and egg shell quality was that shell colour was lighter than the control for Biofeed Wheat, Avizyme and Kemzyme (but not Roxazyme) and yolk colour was generally darker for the enzyme groups. The effects of enzymes on the keeping power of eggs were, again, primary effects which were not further modified by the egg storage treatment.

4.1 Implications

There are several main conclusions that result from this study:

1. New season wheats are not necessarily high in non-starch polysaccharides

2. The "new season wheat" phenomenon appears to occur occasionally, rather than regularly

3. Enzymes do not necessarily reduce litter moisture

4. Enzymes do not necessarily increase apparent metabolisable energy

5. Different wheats produced different levels of egg internal quality and egg shell quality which appeared to be independent of the levels of non-starch polysaccharides and the levels of crude protein

6. Enzymes do not necessarily improve egg shell quality, although they appear to do so under some circumstances

7. The effects of diets and enzymes varied with the age of the bird (and possibly also with the length of time that the birds had been receiving the diets)

8. Enzymes did not alter the keeping power of eggs, beyond any primary effects that they had on albumen quality

9. A moderate elevation in the digesta viscosity resulting from the inclusion of rye in the diets did not have negative effects on bird performance

10. The addition of feed enzymes had some effects on blood parameters, presumably reflecting effects on the birds' physiology

11. Enzymes did not reduce digesta viscosity, at least where digesta viscosity was moderately elevated

12. Enzymes did not affect the performance of hens during an induced moult

4.2 Recommendations

This project, in conjunction with earlier studies, indicates that the addition of commercial enzyme preparations has the potential to improve egg shell quality. This is particularly likely to be the case if the grain on which the diets are based is high in levels of non-starch polysaccharides. However, because it seems that the "new season" wheat phenomenon is an occasional rather than a regular occurrence, the use of feed enzymes may be mainly a form of insurance in layer feeds.

The potential negative effects of commercial feed enzymes on egg internal quality and shell colour, while relatively small and possibly not of commercial significance, need to be monitored when feed enzymes are incorporated into layer feeds.

Because the addition of commercial feed enzymes to layer feed represents a significant cost to the producer (even if only a small cost in relation to the entire operation), individual producers need to conduct their own cost-benefit analysis before using feed enzymes on a regular basis.

5. References

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