THE USE OF EXOGENOUS ENZYME SUPPLEMENTATION IN HULLESS BARLEY BASED DIETS FOR LAYING HENS

By

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ABSTRACT

Three experiments were conducted to study the performance response and fecal output of phosphorous and nitrogen of layers fed different cereal based diets either with of without exogenous enzyme supplementation.

In experiment 1, five diets formulated from wheat and four hulless barley (Silky, Falcon, Gainer and Dawn) cultivars were fed to two strains of hens (Hyline W36 and Dekalb Sigma) to measure production performance. Results showed that the hulless barley cultivars Silky, Gainer and Dawn can effectively replace wheat without any loss of egg mass, while all four hulless barley cultivars can replace wheat without any effect on feed efficiency or feed intake. The Hylines ate significantly less feed ($P>0.05$) than the Dekalbs, yet maintained equal production performance.

In experiment 2, each of the diets in trial one, were formulated either with or without an exogenous enzyme cocktail and fed to Shaver White laying hens. Results showed that enzyme supplementation resulted in significant improvements ($P<0.05$) in egg mass and feed efficiency, and small insignificant increases in egg production and egg weight over hens fed the unsupplemented diets. The variability that existed among the unsupplemented hulless barley based diets was also eliminated by enzyme supplementation.

For experiment 3, the dietary levels of some major nutrients (P, lysine and methionine) were lowered by up to 10% in the presence of appropriate exogenous enzymes and fed to laying hens. The results showed that, compared to a regular diet (without exogenous enzymes) feeding reduced levels of P, lysine and methionine in the presence of exogenous enzymes, did not result in any significant reduction ($P>0.05$) in egg production, egg mass and feed efficiency. In addition, fecal output of P and N was significantly reduced

($P<0.05$) by up to 12.5 and 25% respectively, when nutrient reduced and enzyme supplemented diets were fed to laying hens.

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FOREWARD

This thesis is written in manuscript style. The two manuscripts will be submitted to the Poultry Science Journal. The authors of these manuscripts are T. N. Nortey, W. Guenter and L. D. Campbell, Department of Animal Science, University of Manitoba, Winnipeg, Canada R3T 2N2.

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1. INTRODUCTION

Feed costs account for approximately 50-70% of total production cost in intensive poultry production. The major components of most poultry rations are cereals, and they provide an important source of energy. In western Canada, the most commonly used cereal is wheat, while less commonly used ones include barley, hulless barley and oat. A possible approach to reducing the relatively high feed cost component in commercial poultry production is the production and utilization of alternative feed grains such as barley and hulless barley. Hulless or naked barley (*Hordeum vulgare*) is different from regular barley in that the hull is weakly attached to the seed kernel and is easily removed during threshing (Bhatty, 1986; Thacker et. al., 1988). However the use of these grains in poultry feeds is limited due to the presence of the anti-nutritive factor (ANF) β-glucans (Hesselman and Aman, 1986; Campbell et.al., 1986b.) These non-starch polysaccharides (NSP's) are partially soluble and viscous in nature and depress the efficiency of nutrient utilization (Antoniou et. al., 1981; Classen et. al., 1985; Campbell et. al., 1986b; Hesselman and Aman, 1986) which results in depressed poultry performance. These soluble fibers also cause excessive moisture in the excreta resulting in dirty eggs for laying hens (Al Bustany and Elwinger, 1988), and an increase in the incidence of sticky droppings for young broilers.

The superiority of hulless barley over hulled barley, wheat and corn in supporting swine growth was demonstrated over 75 years ago in the United States of America (Bhatty, 1986). One major driving force behind replacing wheat with hulless barley is the similar nutrient composition (particularly protein) of the two feedstuffs (Hickling, 1995) and the cheaper price of hulless barley in Western Canada (Manitoba Agriculture

and Food). The high β-glucan content of hulless barley compared to regular (hulled) barley however, poses a major nutritional problem especially for young chicks. This is because young birds have poorly developed gastrointestinal tracts (Krogdahl, 1985), and do not have the full complement of endogenous enzymes to handle complex polysaccharides. One way of reducing the anti-nutritive effects posed by feeding barley-based diets to birds is through exogenous enzyme supplementation. Several published data have reported improvement in litter quality and a reduction in the frequency of sticky droppings following β-glucanase supplementation of barley based diets for broiler chicks. Improvements in growth rate, feed intake and feed efficiency have also been reported (Jensen et. al., 1957; Fry et. al., 1958; Moran and McGinnis, 1965; Hesselman et. al., 1981; Friesen et. al., 1992; Marquardt et. al., 1994).

It is well known that the use of exogenous enzymes is most effective during the first few weeks of a chick's life, due to the fact that young chicks have a less developed digestive tract and thus less digestive enzymes, (Krogdahl, 1985). However adult birds, although having a more developed digestive system, could still be limited in their ability to breakdown and utilize the NSP's (Campbell and Campbell, 1989). Therefore supplementing barley-based diets with the appropriate exogenous enzymes may reduce their anti-nutritive effects and increase their nutritive value. Generally, the use of an enzyme preparation having a broad range of activities is more effective than a purified enzyme (Nasi, 1988). In addition to improving the nutritive value of barley-based diets, the use of exogenous enzymes has been shown to result in concomitant reductions in excretory output of some essential minerals in poultry, (Ahmad et. al., 1999; Um et. al., 1999).

Limited research concerning the value of enzyme supplementation of barley-based diets for laying hens has been published. Results from different sources have been inconsistent and sometimes even contradictory. Berg and Bearse (1958), Berg (1959, 1961), Anderson et. al., (1960) and Arscott and Rose (1960) failed to obtain any improvement in egg production or feed efficiency with White Leghorn layers fed barley-based diets that had been supplemented with enzymes. In contrast, Nelson and Hutto (1958) obtained improved egg production, feed efficiency and percent hatch with both enzyme supplemented and water treated barley-based diets. Most of the published data involving barley-based diets for laying hens were conducted long ago. With the increasing need to explore alternative and less expensive, but yet high quality feedstuffs, research into the use of hulless barley as a replacement for wheat in poultry diets is of great interest.

The broad objectives of these studies were:

1) To document the effects of a crude exogenous fungal enzyme cocktail supplementation of hulless barley-based diets on laying hen performance and excretion of nutrients in feces.

2) To compare different hulless barley cultivars in supporting efficient production in laying hens.

Further details of specific objectives are presented in each manuscript that constitute this thesis.

2. Literature Review

2.1 Feeding Characteristics of Hulless Barley

The removal of the high fiber hull from hulled barley through development of hulless cultivars or through mechanical treatment has resulted in a product with a nutrient profile similar to that of wheat. Although it is expected that this would result in a feeding value superior to that of hulled barley (due to a lower fiber content and a corresponding increase in metabolisable energy), and similar to that of wheat, this has not always been the case (Anderson et. al., 1961; Coon et al., 1979). Other factors which have been suggested to explain the reduced feeding value of hulless barley over hulled barley are higher β-glucan contents (Burnett, 1966) and tannins (Gohl and Thomke, 1976).

2.1.1 Quality Characteristics of Hulless Barley

Hulless or naked barley has been in commercial production for a long time and its superiority over hulled barley, wheat or corn in supporting swine growth was demonstrated over 75 years ago in the United States (Bhatty, 1986). However hulless barley is a relatively new crop in Canada, and in Western Canada it has only recently been rediscovered as a potential feedstuff for livestock and poultry, (Bhatty, 1986).

The hull of hulless barley, which accounts for about 13% of the weight of the grain kernel, is composed mainly of fiber and is easily lost during threshing. As a result of the lower percentage of hulls, (≤5% kernels with hulls for feed grade hulless barley) hulless barley has lower fiber levels and increased starch and protein levels compared to

hulled barley. The energy content of hulless barley is much higher than in hulled barley. Hulless barley has a similar energy value as wheat and corn, and a similar protein content to wheat. However, due to its higher level of lysine, hulless barley has a more valuable essential amino acid profile than wheat (Hickling, 1995). Another advantage that hulless barley has over hulled barley is in transportation and storage. The higher density (80 kilogram/hectoliter vs. 62 kilogram/hectoliter) increases the amount of grain that can be transported by truck, rail car or ship, and the amount that can be put in a storage bin.

Absence of a fibrous hull should improve hulless barley as a feedstuff for poultry. However a greater concentration of the ANF, β-glucan found in the endosperm cell wall, can lead to unfavorable results when untreated hulless barley-based diets are fed to birds, particularly young broiler chicks, (Classen et. al., 1985; Campbell et. al., 1986a; Marquardt et. al., 1994).

Generally speaking, the composition of hulless barley is reasonably consistent (Campbell and Baidoo, 1997), but like hulled barley, climatic conditions may play a role in the endosperm cell wall concentration of soluble β-glucan and thus acid extract viscosiy (Aastrup, 1979).

2.1.2. General Compositions of Hulless and Hulled Barley

The composition of hulless barley relative to regular barley is presented in Table 1. Relative to hulled barley, hulless barley has a higher crude protein, lower acid detergent fiber, and lower crude fiber. The amount of fat in hulless barley is about 2.0% and this is slightly higher than that for hulled barley. The higher crude protein (CP) and

Table 1. Comparative nutrient profile of hulless barley and hulled barley (as fed basis)

Components	Barleys	
	Hulless	Hulled
Dry Matter %	88	88
Crude protein %	13.2	11.5
Crude fiber %	1.4	5
Acid detergent fiber %	2	6
Crude Fat %	2	1
Energy, AME kcal/kg	2800	2720
Minerals		
Calcium %	0.05	0.04
Phosphorous %(total)	0.35	0.34
Amino acids		
Lysine %	0.5	0.43
Methionine %	0.27	0.19
Cystine %	0.29	0.23
Tryptophan %	0.15	0.18
Threonine %	0.48	0.41
Beta-glucans %	4.5	4
Test weight kg/hl	80	62

Hickling (1995)

fat concentration in hulless barley can result in savings in cost of supplementing protein and fat for chicken diets (Hikling, 1995). This is especially true since the quality of protein in both hulled and hulless barley is reported to be relatively high (McIntosh et. al., 1995) with a Protein Efficiency Ratio (PER) value averaging 2.04 for ordinary cultivars. Amino acids such as lysine, methionine, cystine and threonine are present in greater concentrations in hulless than in hulled barley. The greater energy value of hulless barley over hulled barley for poultry (2800 versus 2720) is basically a result of the lower proportion of hulls and a resulting denser product.

Scarce data exists for the mineral and vitamin contents of hulless barley. However phosphorous and calcium levels show similar or slightly higher levels respectively, compared to hulled barley (Hickling, 1995).

2.1.3. Hulless Barley Amino Acid Profile and Digestibility

Hulless barley has an average protein content of 13-16%, compared to hulled barley (11-14%). Bhatty et. al., (1975) however, reported a protein content of 18.4% for "normal lysine" hulless barley. Generally speaking, hulless barley has a protein content 0.5-1.5% higher than hulled barley (Rossnagel et. al., 1983).

The amino acid profile of hulless barley is very well balanced and the amino acids are generally present in greater quantities than in hulled barley. The average amino acid composition of three hulless and three hulled barley cultivars are presented in Table 2 (Campbell, 1998). On average the essential amino acids lysine and methionine make up 0.44 vs. 0.41 and 0.29 vs. 0.26 % of hulless and hulled barley respectively.

Table 2. Amino acid content (as % dry matter) of three hulless and three hulled barley cultivars. (Averages and standard deviations)

	Hulless barley		Hulled barley	
Amino acid	Average	S.D	Average	S.D
Aspartic acid	0.84	*0.155*	0.81	*0.165*
Threonine	0.49	*0.097*	0.45	*0.098*
Serine	0.71	*0.134*	0.65	*0.128*
Glutamic acid	4.40	*1.114*	3.93	*1.033*
Proline	1.78	*0.451*	1.61	*0.387*
Glycine	0.56	*0.097*	0.53	*0.095*
Alanine	0.57	*0.103*	0.53	*0.102*
Valine	0.57	*0.109*	0.50	*0.099*
Methionine	0.29	*0.027*	0.26	*0.024*
Isoleucine	0.39	*0.079*	0.34	*0.072*
Leucine	0.94	*0.195*	0.84	*0.184*
Tyrosine	0.33	*0.080*	0.28	*0.081*
Phenylalanine	0.71	*0.200*	0.63	*0.179*
Histidine	0.32	*0.062*	0.29	*0.055*
Lysine	0.44	*0.085*	0.41	*0.084*
Arginine	0.59	*0.127*	0.54	*0.126*

Modified from Campbell, 1998.

Using Near Infrared Reflectance Spectroscopy (NIRS), Jaikaran et. al., (1998) analyzed 603 whole grain samples of hulless barley and 32 samples of hulled barley collected over a five-year period. Analysis for seventeen amino acids was performed and with the exception of aspartic acid, hulless barley had higher amino acid levels than hulled barley. The higher levels are likely a reflection of higher average crude protein in hulless barley, and may be due to the lack of hulls which account for about 10-15% of the weight of the kernel, (Bhatty, 1986; Campbell and Baidoo, 1977).

Table 3 shows the mean contents of seventeen amino acids as a percent of crude protein in hulled and hulless barley samples respectively, (Jaikaran et. al., 1998). Hulled barley protein was slightly higher in lysine than hulless barley for samples taken from Alberta, Canada.

Table 4 compares the total digestible amino acids of hulless and hulled barley determined by the modified TME technique (Sibbald, 1986). The essential amino acids lysine and methionine are 54.7 vs. 51.2% and 77.0 vs. 71.6% digestible in hulled and hulless barley respectively (Campbell, 1998). On average, hulled barley has a better amino acid digestibility than hulless barley (80.9 vs. 77.0). In estimating the nutritive value of any feedstuff for poultry or pigs, amino acid digestibility is an important factor. Classen et. al., (1985) suggested that hulless barley appears to have a lower nutritive value than hulled barley. Its poorer nutritive value is probably related to its high content of water-soluble β-glucan (Bhatty et. al., 1991) and high gut viscosities (Rotter et al., 1989, 1990) compared with hulled barley. However, the availability of amino acids to monogastric animals can also be reduced by other factors.

Table 3. Mean amino acid contents (as % of crude protein) of 17 samples each of hulled and hulless barley

Amino acid	Hulled Barley		Hulless Barley	
	Mean	*S.D*	Mean	*S.D*
Alanine	3.88	*0.25*	3.61	*0.24*
Arginine	4.58	*0.27*	4.60	*0.23*
Aspartic acid	5.90	*0.43*	4.94	*0.39*
Cystine	2.14	*0.17*	2.07	*0.22*
Glutamic acid	22.60	*2.53*	23.10	*1.69*
Glycine	4.01	*0.24*	3.83	*0.3*
Histidine	2.09	*0.18*	2.47	*0.15*
Isoleucine	3.28	*0.23*	3.52	*0.15*
Leucine	6.51	*0.4*	6.50	*0.25*
Lysine	3.42	*0.21*	3.24	*0.27*
Methionine	1.56	*0.1*	1.61	*0.1*
Phenylalanine	4.61	*0.52*	4.95	*0.26*
Proline	10.25	*1.18*	10.70	*0.91*
Serine	4.11	*0.23*	3.86	*0.13*
Threonine	3.24	*0.18*	3.11	*0.21*
Tyrosine	2.05	*0.26*	1.90	*0.19*
Valine	4.68	*0.26*	4.89	*0.22*

Modified from Jaikaran et. al. 1998.

Table 4. Total digestible amino acids (TDAA) of hulless and hulled barley [1]

	Hulless barley		Hulled barley	
Amino acid	Average	S.D	Average	S.D
Aspartic acid	75.30	*2.421*	79.90	*1.859*
Threonine	77.47	*4.296*	79.90	*1.941*
Serine	83.12	*3.494*	86.00	*3.040*
Glutamic acid	90.23	*1.991*	92.90	*0.533*
Proline	91.11	*2.724*	93.50	*1.495*
Glycine	76.04	*3.530*	81.10	*2.980*
Alanine	70.60	*2.787*	75.80	*0.872*
Valine	81.06	*3.985*	84.90	*2.461*
Methionine	71.57	*3.034*	77.00	*8.685*
Isoleucine	82.60	*3.920*	89.00	*6.244*
Leucine	82.88	*3.191*	87.90	*2.412*
Tyrosine	84.23	*3.458*	94.00	*3.747*
Phenylalanine	84.78	*3.312*	89.50	*0.396*
Histidine	75.79	*8.652*	72.60	*17.803*
Lysine	51.20	*6.708*	54.70	*23.377*
Arginine	53.27	*5.794*	56.20	*13.608*
	76.95	*4.846*	80.93	*5.716*

Modified from Campbell, 1998.

[1] Three hulled barley samples and five hulless barley samples

Some of the factors influencing the digestibility of crude protein and amino acids in monogastrics include fiber content, presence of tannins, lignins and pectins (Gohl and Thomke, 1976) and particle size (Sauer et. al., 1977; Sauer et. al., 1980; Sauer et. al., 1981).,

The source of dietary fiber has also been shown to affect protein digestibility. In a study to determine the type and degree of influence which various levels of fiber have on nutrient digestibility, Scheideler et. al., (1998) compared fiber from two sources (flaxseed or oats, or a combination of the two, with or without enzyme supplementation) to a control corn/soy type ration in two strains of Leghorn pullets. They determined that dietary flaxseed in pullet rations slowed growth and reduced essential nutrient (including protein) digestibility, rendering flaxseed a less desirable ingredient for pullet rations. According to Sauer and Thacker (1986), dietary fiber may reduce protein and amino acid digestibility because of its physical and chemical properties leading to a more rapid rate of passage. This reduction in intestinal transit time, associated with fiber-containing diets, would result in less time for digestion and absorption of dietary protein, (Eastwood, 1973).

The inclusion of fiber in the diet has been shown to increase the sloughing off of intestinal mucosal cells and to enhance the production of mucous, thereby increasing loss of endogenous amino acids (Schneeman et. al., 1982). In addition *in vitro* studies suggest that dietary fiber may adsorb trypsin and chymotrypsin, thereby decreasing the activity of these enzymes (Schneeman, 1978).

2.2. Antinutritional Factors of Hulless Barley and Their Effect on Nutrient Utilization

2.2.1. Non Starch Polysaccharides (NSP's)

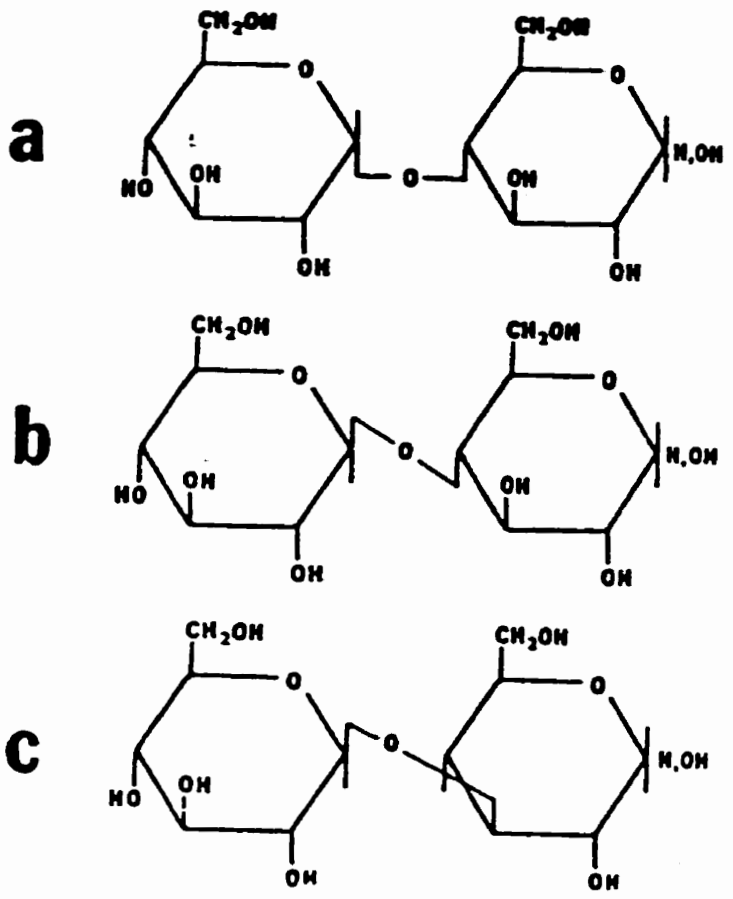
a) β-Glucan

β-glucans are found in the endosperm of cereals, particularly oats and barley, where they comprise about 75% of the endosperm cell walls, and 2 and 10% of the kernel weight of hulled and hulless barley respectively (Forrest and Wainwright, 1997). Unlike α-D-glucan or starch, which contains α (1→4) glucosyl bonds β-D glucans contain a mixture (30:70) of β (1→3) and β (1→4) glucosyl bonds, (Figure 1). Because of the mixture of glucosyl bonds, β-D glucans are less tightly folded and have reduced hydrogen bonding between polymers, thereby rendering them partially soluble in water. Various researchers (Bathgate et. al., 1974; Bathgate and Dalgliesh, 1978; Ballance and Manners, 1978) have shown that the 30:70 ratio of β(1→3) linkages to β(1→4) linkages is fairly consistent for all extracted β-glucan. However, according to Fleming and Kawakami (1977) the number of consecutive β(1→3) and β(1→4) linkages may vary. Generally β-glucans can be divided into two groups: water-soluble gum and water-insoluble hemicellulose.

i)Water-soluble gum

The water soluble fractions of β-glucans can comprise as much as 30% of the total NSP's of cereals (Henry, 1987), and about 44.7% of the total β-glucan content (Bhatty et. al., 1991). Water-soluble β-glucan contains different amounts of β(1→3) and

Figure 1. Glucosyl bonds in starch and β-D-glucans: a, α(1-4); b, β(1-4); c, β(1-3).



Scott et. al., (1999)

β(1→4) linkages at different temperatures. At 40°C soluble gum has two or three β(1→4) linkages that are separated by one β(1→3) linkage. However at 100°C water-soluble β-glucan has about five consecutive β(1→4) linkages, followed by one β(1→3) linkage, (Flemming and Kawakami, 1977). The larger the size of the soluble β-glucan, the more viscous it is, and it is believed that the larger sequences of β(1→4) and β(1→3) linkages permit polymer interactions like H-bonding and hence an increased viscosity (Palmer, 1975).

ii)Water Insoluble Hemicellulose

Not all of the cell wall β-glucan is extractable with water and more drastic extraction conditions are required to solubilize the rest of this component. This second group is classified as hemicellulose and their insolubility in water may arise from the fact that it may be derived from a larger carbohydrate polymer, or from a complex of polysaccharide with protein or other material (Ballance and Manners, 1978). This water-insoluble portion of β-glucans is generally soluble in alkaline solutions. Longer sections of β(1→3) or β(1→4) linkage sequences, association between β-glucan and cell wall proteins, and /or differences in molecular weight are the major differences between the water soluble and insoluble β-glucan (Balic and Stone, 1981). Ballance and Manners (1978) found that both water and alkali-extracted β-glucans contained similar ratios of β(1→3) to β(1→4) linkages namely 3 to 7. The cell walls, which had a protein content of approximately 5%, contained unidentified alkali labile linkages. β-glucans of similar molecular weight (about $33*10^6$ daltons) are extracted from endosperm cell walls by hot

water or alkali although alkali extraction (hemicellulose) results in a greater spread of molecular size compared to water extraction (gum) (Forrest and Wainwright, 1997). Ballance and Manners (1978) also showed no significant differences in molecular weight between the large water-soluble gum and the hemicellulose. The presence of firmly linked peptide sequences in the hemicellulose portion of the β-glucan is proven by the fact that treatment with a proteolytic enzyme results in a reduction in molecular weight (Forrest and Wainwright, 1997). According to Burnett (1966) the poor nutritional value of certain barleys is due to a component of barley, which under certain conditions gives rise to fairly stable highly viscous conditions in the alimentary canal. He suggests that the material responsible is β-glucan. However he notes that barley contains a relatively small percentage of primary β-glucan (gum) and therefore it seems unlikely that such a small amount of β-glucan could be the sole cause of the highly viscous conditions found in the small intestines of young chicks. He suggested that hemicelluloses (which form about 10% of the barley) were further solubilized in the alimentary canal, so giving rise to the release of additional quantities of gum. The relatively high pH conditions (7.5-8.0) in the small intestine associated with the presence of bicarbonates would probably render the hemicellulose more readily soluble than in water alone.

Varietal differences, soil type and climatic conditions, especially during the latter phase of growth can have a significant influence on the β-glucan content of barley (Hesselman and Thomke, 1982). Weather conditions like high temperature and/or low rainfall, which favor rapid maturity in barley, have been shown to result in increased NSP content of barley grain (Molina-Cano and Conde, 1982). The rapid maturity reduces the plants late ripening stage when the conversion of mixed linked β-glucans to their

insoluble form is proceeding at a rapid rate. The resulting grain has a higher than normal proportion of soluble β-glucan and extract viscosity. Extract viscosity of barley, caused by soluble β-glucans has been reported to decrease with latter stages of growth (Hesselman et. al., 1981). Work to support this has been done by Campbell et. al. (1991), who showed that maturity of barley affects feeding value, soluble β-glucan content and extract viscosity. The content of β-glucans increased with maturity indicating a lower solubility of the β-glucans with maturity. Barleys harvested at yellow stage ripeness (high moisture) have significantly higher extract viscosities than barleys harvested at combine ripeness (Hesselman and Thomke, 1982).

b) Arabinoxylans (Pentosans)

Another group of NSP's that occur in barley are arabinoxylans, which are found in the endosperm cell walls. They contain (1→4)-β-D-xylan chains with L-arabinofuranose residues attached through (1→2) or (1→3) linkages (McIntosh et. al., 1995). The arabinoxylans and β-glucans make up 20% and 70-75% respectively of the cell wall NSP's (Fincher 1975). Although viscosity related problems in animal nutrition have traditionally been associated with solubilization of β-glucan, and may be eliminated by β-glucanase supplementation (Burnett, 1966; Classen et. al., 1985), evidence also suggests that arabinoxylans may contribute to extract viscosity problems (Bhatty et. al., 1991). The total water extract and acid extract levels of arabinoxylans differ among different barley types. Total arabinoxylan least square means for hulless barley (either two- or six-rowed) was shown to be significantly less ($P<0.01$) than that of the six- or two- rowed covered (hulled) types. There is evidence in the literature that suggests that

the hulls contain up to 55% of the total arabinoxylan in barley. Therefore even small differences in hull content would affect total levels. Six rowed barley contains more hulls than two rowed barley cultivars, while hulless barley contains little to none. This is an explanation for the greater amounts of arabinoxylans in six- and two- rowed hulled barley than hulless barley (Fleury et. al., 1997).

A significant relationship has been observed to exist between the log acid-extract viscosity and acid-extracted levels of arabinoxylans and this relationship supports the observation that soluble arabinoxylans in barley may also contribute to viscosity-related problems in poultry (de Silva et. al., 1983).

2.2.2. Dietary Fiber

A factor limiting the use of hulled barley as a major feed ingredient for poultry is its comparatively high fiber content and resulting low metabolizable energy (ME) value (Classen et. al. al., 1985). Although removal of the high fiber hull through the development of hulless cultivars or by mechanical dehulling should overcome this problem, several reports indicate that the lack of a hull results in little or no improvement in feeding value for young chicks over hulled barley (Coon et. al., 1979). However for laying hens studies have shown that barley can satisfactorily substitute for other cereals (McNab and Shannon, 1975; Al Bustany and Elwinger, 1988).

The dietary fiber of hulless barley accounts for about 16% of the dry weight. Marlett (1991) analyzed hulless barley for soluble and insoluble dietary fiber content and composition, and found a total dietary fiber content of 15.7% of dry weight and a total β-

D-glucan content of 5.1% (Table 5). Working with two- and six- rowed hulled barley cultivars, Aalto et. al. (1988) determined total dietary fiber to be in the range of 15.0 to 24.1% of dry weight, water-insoluble dietary fiber between 11.1 to 19.2% and water-soluble dietary fiber between 3.3 to 5.9%. The two- and six- rowed cultivars of barley had one fourth and one sixth respectively of total fiber being water-soluble. Marlett (1991) reports that most of the glucose in the soluble fiber fraction of hulless barley samples was from β-glucan (Table 6).

2.2.3. Plant Phosphorous

a) Phytate

In most plant materials, a large proportion of phosphorous (P) is in the form of phytate (Ravindran et. al., 1994). Phytate is a complex salt of calcium and magnesium with myoinositol (1,2,3,4,5,-6 hexakis dihydrogen phosphate) and is regarded as the chief storage form of phosphorous and inositol in seeds and vegetative storage tissues. In mature seeds, phytate-P is present as a complex salt of calcium, magnesium and potassium, and/or with proteins (Ravindran et. al., 1995 Figure 2). In general the proportion of phytate P in seeds of cereals, grain legumes and oil-bearing plants varies from 60 to 80% of the total phosphorous in these materials. In barley, phytate P makes up about 0.27% of total dry matter and 64% of total P (Ravindran et. al., 1995). Research data indicates that phytate binds essential dietary minerals, making them unavailable or only partially available for uptake (Ravindran et. al., 1994,1995).

Table 5. Dietary fiber in hulles barley samples (%)

Fiber Fraction	Total neutral Sugars	β-glucans [1]	Uronic Acids[1]	Klasson Lignin[1]	Total fiber in fraction[1]	Total dietary Fiber	Pentosans [2]
Insoluble	8,5	1,4	0,3	0,6	10,8	**15.7**	**4.8**
Soluble	1,2	3,7	0	0	4,9		

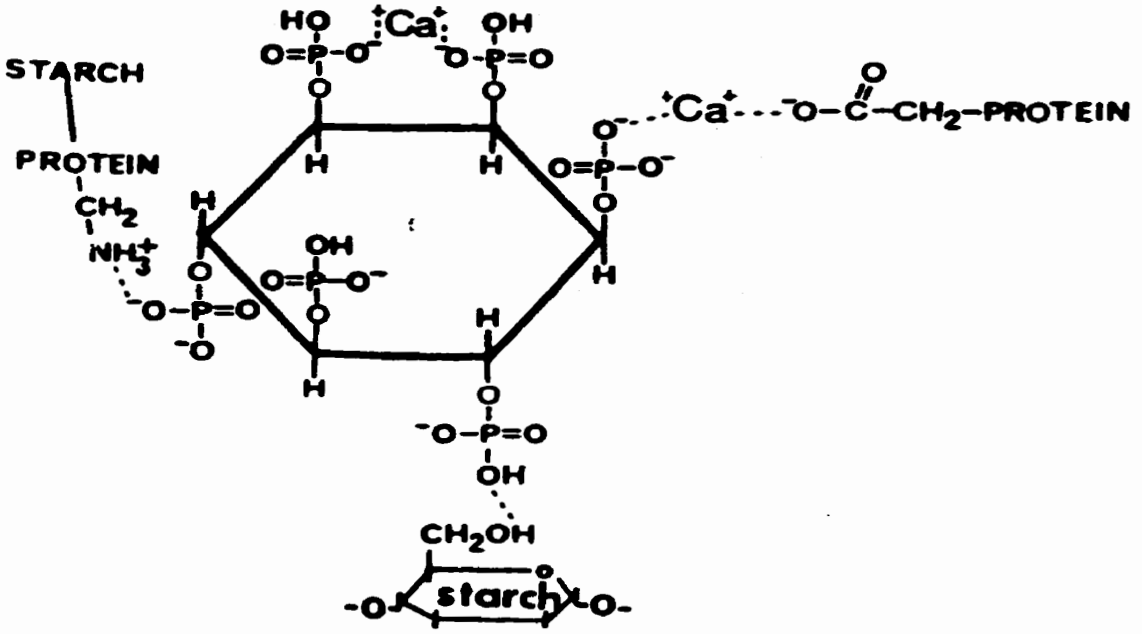
1 Modified from Marlett, 1991.
2 Andersson et. Al., 1999.

Table 6. Neutral sugars in the insoluble and solube fiber fractions of hulless barley (%)

	Glucose	Xylose	Galactose/ Rhamose	Arabinose	Mannose
Insoluble fiber fractions	35	35	2	23	5
Soluble fiber fractions	85	7	2	4	2

Modified from Marlett, 1999.

Figure 2. Possible interaction of phytic acid with minerals, protein and starch



Thompson (1986)

b) Phosphorous Availability in Laying Hen Diets

Since a major portion of poultry diets consist of plant-derived ingredients, P from phytate assumes considerable nutritional significance. Under normal dietary conditions, phytate P is either unavailable to or poorly utilized by poultry due to insufficient quantities of endogenous avian phytase (Ravindran et al., 1995). As a result, expensive readily available inorganic P is used as a supplement in laying hen diets for optimum production. According to Simmons et. al., (1990) this results in high levels of total P contents in both feed and excreta. When the latter is used as fertilizer on crop farmlands, pollution of surface and underground water by P may occur. Therefore hydrolyzing phytate by phytase enzymes to release inorganic P and other nutrients could improve P availability in the diet. Phytase enzymes have been shown to increase P availability in both poultry and swine (Nelson et. al., 1971; Simmons et. al., 1990). Research data indicates that it is possible to feed low-P diets plus phytase enzymes to both broilers and layers, and achieve simultaneous improvements in P availability and reduction in P output in the droppings. In a layer experiment, Um et.al. (1999) fed a low P diet, 0.16% non-phytate P (NPP) in the presence of 250 Units phytase/kg diet. They reported improvements in egg production, feed conversion, an increase in P retention and a reduction in P output in the excreta.

Phytic acid has a tremendous chelating potential. It is a strong acid and forms a wide variety of insoluble salts with di-and tri- valent cations at neutral pH. These complexes are insoluble at physiological pH and render these elements such as calcium, magnesium, zinc, manganese, iron and copper unavailable for intestinal absorption.

Added phytase to the diets for chicken has been documented to increase the retention of these minerals for layers (Um et. al., 1999) and broiler chickens (Ahmad et. al., 2000).

Phytate in plant feedstuffs also bind dietary proteins and endogenous digestive protein enzymes thereby depressing protein availability (Carnoval et. al., 1988; Singh and Krikorian, 1982)

2.3. Barley in Laying Hen Rations

2.3.1. Responsiveness of Laying Hens to Barley Based Diets

Hulless barley differs from conventional barley in that the fibrous hull is less firmly attached to the kernel and consequently becomes detached during threshing. A considerable number of documented data exist on the use of barley based diets in young chick rations (Marquardt et. al., 1994; Classen et. al, 1985; Hesselman et. al., 1981). Comparative trials with maize and/or wheat have generally resulted in negative results. Friesen et. al. (1992) tested four different cereal-based diets in rations for young broiler chicks. They determined that the complete substitution of the test cereal (70%) for wheat yielded weight gains, feed intakes and feed: gain values that were inferior to those of the control wheat diet and the diets containing only 35% of the test grain.

Data on the use of barley based diets in laying hen rations have generally indicated that birds had similar or equal egg production performance as control birds on a wheat/corn diet (Classen et. al., 1988; Bhatty 1986; Anderson et. al., 1960). Berg, (1959) however found barley to be only 83% as efficient as corn in the production of eggs.

Generally it has been suggested that high proportions of barley should only be included in layer diets after 50 weeks of age to prevent any adverse effects on egg production parameters (Campbell, 1987 in Jeroch and Danicke, 1995).

2.4. Some Methods to Reduce the Antinutritive Effects Relating to Hulless Barley

A number of methods have been found to be effective in reducing the antinutritive effects of NSP's in barley. Some of them are water treatment, gamma irradiation and supplementing with exogenous enzymes.

2.4.1. Water Treatment

Water soaking for 8 hours at room temperature has been found to improve the feeding value of barley (Fry et. al., 1958). According to Burnett (1966) during the prolonged water treatment, it is likely that considerable solubilization of the hemicellulose in the barley occurs, and enzymes naturally present in the barley, or produced by the growth of bacteria, could degrade the material to less complex substances which have lower viscosities in solution, and hence better feeding values. The positive effect of water treatment of barley has been found to have a direct relationship to the conditions under which the barley was grown. Barley grown under hot and dry conditions, leading to an early harvest, respond more to water treatment than when grown under less dry conditions. This is because hot and dry growing conditions leading to an early harvest of barley, result in increased viscosity of acid extracts. Acid

extract viscosity is closely related to the amount of soluble β-glucan which interferes with the digestibility of nutrients (Aastrup, 1979).

2.4.2. Gamma Irradiation

Gamma irradiation has been used successfully to improve the feeding value of hulless barley. Feeding hulless barley diets containing irradiated grain resulted in higher broiler chick growth rate, and increased fat and starch absorption compared to birds fed the untreated control diet (Classen et. al., 1985). Campbell et. al. (1986a) also reported similar findings, where irradiated barley lead to an improvement in nutritional value.

This improvement may be attributed to disruption of the β-glucan fraction of the barley, and consequently lowering of the viscosity of the intestinal contents. Gamma irradiation had been found to have a marked effect on the capacity of carbohydrates to form viscous solutions. Hydrolysis of the β-glucans is indicated by the rise in reducing sugar level for irradiated hulless barley (Classen, et. al., 1985).

2.4.3. Supplementation of Exogenous Enzymes

a) Enzyme Applications in Hulless Barley Based Diets for Poultry

The presence of NSP's in the endosperm cell walls renders most cereals poorly digestible by monogastrics. In barley the major NSP's are β-glucans (Rotter et. al., 1990).

Non-starch polysaccharides cannot be hydrolysed by the endogenous enzymes of birds or if they are, only to a limited extent. White et. al., (1981) explains that the NSP's may prevent the endogenous enzymes from reaching important nutrients in the grain cells. It is also possible that some cell wall NSP's once in the digestive tract are able to form high molecular weight aggregates, which increase viscosity. Increased viscosity is known to slow transit time, increase gelling properties of the digesta and retard digestion and absorption, thereby causing depressed chick growth (Nasi, 1988; Antoniou et. al. 1981; Marquardt et. al., 1994).

Early published data has established that the nutritional value of barley could be improved by the addition of exogenous enzymes and this was attributed to ά-amylase or protease activity (Jensen et. al, 1957; Anderson et, al., 1961). Recent advances in biotechnology have made it possible to develop enzymes, which significantly reduce the antinutritive effects of NSP's and thereby improve the feeding value of barley-based diets. The enzymes that are most effective are β-glucanases and xylanases. Use of these enzymes has been shown to improve feed consumption, weight gain and feed conversion ratios for broilers (Hesselman et. al., 1981; Marquardt et. al., 1994; Friesen et. al., 1992), and laying hens (Classen et. al. 1988). It is generally believed that improvements in feeding value following enzyme addition to barley based diets, is due to viscosity reduction induced by β-glucanase, and not necessarily due to a complete hydrolysis of the polysaccharide and absorption of the released sugars (Hesselman et. al., 1981; Burnett, 1966).

b) Use of Phytase to Release Bound Phosphorous and Other Nutrients

The availability of P from plant sources is limited by the presence of a naturally occurring compound phytate. Salts of phytic acid (phytate) contain about two thirds of the P in cereal grain and oil seed meals, the major component of poultry feed (Ahmad et. al., 2000). Phosphorous and other minerals, when bound to the phytic acid become poorly available to both broilers and laying hens (Leske and Coon, 1999; Ahmad et. al., 2000; Carlos and Edwards, 1998). Also insoluble protein-phytate complexes are formed in the presence of phytate (Carnovale et. al., 1988; Singh and Krikorian, 1982).

The lower digestibility of protein in the presence of phytate has been reported by a number of researchers (Kies et. al., 1997; Um et. al., 1999). However this conclusion is not unanimous, and a few authors have reported that they did not find any significant improvements in ileal digestibility of crude protein (CP) and amino acid (AA) with phytase supplemented diets (Zhang et. al., 1999). The degradation of phytate in the digestive tract of poultry may be attributed to the action of phytases from one or more of three possible sources (Ravindran et. al., 1995). These sources include:

1/ intestinal phytase in digestive secretions,

2/phytase activity originating from microbes resident in the intestinal tract, and /or

3/ endogenous phytase activity present in some feedstuffs.

Birds have limited amounts of intestinal phytase enzymes, and therefore adding exogenous phytase to the diet will greatly increase phytic acid use, make P and other minerals/nutrients more available to the bird, and decrease the amounts of P appearing in the excreta. Vetesi et. al., (1997) concluded that exogenous phytase supplementation at a

dose of 600 phytase units/kg in diets for laying hens decreased the calcium (Ca) and P excretion by 18 and 15% respectively, while maintaining the strength of the tibias.

Um et. al., (1999) concluded that with exogenous enzyme supplementation the non-phytate P (NPP) concentration in the diet of Brown layers consuming about 130g/day of feed can be safely lowered from 0.26% (0.55% total P) to 0.16% (0.45 total P) without deleterious effects on performance. Addition of exogenous phytase (250 units of phytase/kg of diet) was also able to reduce the excretion of P, the retention of the minerals Ca, magnesium (Mg) and copper (Cu) were also improved by phytase enzyme addition.

Using male broiler chicks, Namkung and Leeson (1999) found that a diet with supplemental phytase had a higher (3.5%) nitrogen-corrected apparent metabolizable energy (AMEn) ($P \leq 0.01$), compared to a non-supplemented control diet. One reason for this observed increase may be the fact that phytase enzyme is able to release bound nutrients and make them readily available to the bird. Also chicks fed phytase had higher digestibilities for valine, isoleucine, non-essential amino acids ($P \leq 0.05$) and total amino acids ($P \leq 0.05$). Generally, research has shown that supplemental phytase improves P availability by about 20-40% (Denbow et. al., 1995; Sebastian et. al., 1996) and amino acid digestibility by 1-2%, (Yi et. al., 1996).

SUMMARY

Information on the use of hulless barley and exogenous enzymes in laying hen diets is limited and the results are inconsistent. Generally it has been assumed that the use of exogenous enzymes is of benefit to young chicks and that older birds, by virtue of their better digestive capabilities should be able to cope with the NSP's present in some cereal grains. Some studies have shown that, the incidence of dirty eggs rises and egg production decreases when hens are fed diets high in ANF's, particularly β-glucans and pentosans. The use of exogenous enzymes in laying hens diets that are high in β-glucans and other NSP's may therefore be one way of improving the nutritive value of certain cereals. In Western Canada, hulless barley when used in combination with exogenous enzymes in diets for layers may provide an inexpensive and high quality alternative for wheat.

3. Manuscript 1

Hulless Barley With or Without Exogenous Enzymes as a Replacement for Wheat in Laying Hen Diets

Abstract

Two trials were carried out to determine the replacement value of hulless barley for wheat in diets for laying hens. In the first trial, a five-month experiment was conducted to compare laying hen performance when 2 strains of hens were fed diets based on wheat and four hulless barley cultivars (Silky, Falcon, Gainer and Dawn). A total of 1280 Single Comb White Leghorn Layers consisting of 640 Dekalb Sigma and 640 Hyline W36 strains (with an average initial weight of 1.62 kg) were randomly assigned by strain to each of 5 dietary treatments. Feed intake, egg weight and feed efficiency were not significantly affected by diets. However there was a significant effect of diet on egg mass and egg production. Egg mass of hens fed the wheat control diet had a value of 52.5g/day. This was similar ($P>0.05$) to hens fed the Silky, Gainer and Dawn based diets, (52.8, 52.2 and 51.1 g/d respectively) but higher ($P<0.05$) than hens fed the Falcon based diet (50.4g/d). Hens being fed the control diet had significantly better ($P<0.05$) egg production than hens fed the Dawn and Falcon based diets (87.4 vs. 85.7 and 84.5% respectively), and similar ($P>0.05$) egg production to those fed the Silky and Gainer based diets (86.9 vs. 86.6%). There were no significant strain differences for feed efficiency, egg weight, egg mass and egg production. Feed intake of the Dekalb Sigma strain of birds, (101.4 g/h/d) was significantly higher ($P<0.05$) than that of the Hyline W36 strain, (99.2g/h/d). There was a significant strain*diet interaction for feed intake, feed efficiency, egg mass and egg production. It is concluded from the above data that certain hulless barley cultivars can be substituted for wheat without detrimental effects on egg production, egg mass and feed efficiency.

In the second experiment, a 9-month trial was carried out with 1280 Single Comb White Leghorn (Shaver White) hens. The purpose was to determine if feeding the 4 hulless barley cultivars either with or without exogenous enzymes, would result in equal or better performance compared to feeding a regular wheat based diet. Feed efficiency, egg mass and egg weight of hens fed Silky and Dawn diets were similar ($P>0.05$) to hens fed the Control diet which had values of 2.1, 47.6g/h/d and 57.1g respectively, but were significantly lower ($P<0.05$) than hens fed the Gainer based diet, 1.99, 50.21g/h/d and 58.7g respectively. Hens fed the Falcon based diet had the lowest egg production, 82.14%, and this was significantly lower ($P=0.061$) than that of hens fed the Gainer and Dawn based diets, 84.7 and 84.7% respectively, but similar to the Silky and Control diets, (83.3% and 84.3% respectively). Enzyme inclusion resulted in better feed efficiency, egg mass and egg weight for hens fed all the hulless barley diets, which were similar ($P>0.05$) to or better ($P<0.05$) than feeding the control diet, and better ($P<0.05$) than feeding the unsupplemented hulless barley diets. There is a positive response for egg mass and feed efficiency when the hulless barley based diets are supplemented with exogenous enzymes, and these are either similar ($P>0.05$) to, or better ($P<0.05$) than feeding the control diet. These results show that hulless barley, when used in combination with exogenous enzymes can effectively replace wheat in rations for laying hens.

INTRODUCTION

Cereal grains are a major source of energy in poultry diets. In Western Canada, wheat is the most commonly used feed ingredient in poultry diets. Less commonly used cereals include hulled barley, hulless barley, oats and to a very limited extent, rye. This is due to the presence of antinutritive factors such as β-glucan in barley and oats (Nasi, 1988; Campbell et. al., 1986b) and pentosans in rye (Fengler, 1987). In barley soluble β-glucans in the endosperm cell walls have the potential to gel in the digestive tract of chickens causing sticky droppings and reduced nutrient utilization and growth rate, especially in young poultry (Hesselman et. al, 1981; Rotter et. al., 1989). The use of suitable exogenous enzymes especially β-glucanases, xylanases, mannases and proteases, may be of practical importance in improving the feeding value of some feedstuffs, and also in breaking down the anti-nutritive substances found in certain feed raw materials. This will augment the digestive capacity of the animal, improve the availability of feed nutrients, and increase the release of nutrients in the upper part of the gastrointestinal tract (Nasi, 1988). This has been demonstrated extensively in young chicks, where dietary addition of suitable enzymes to barley-based diets have resulted in higher nitrogen corrected apparent metabolizable energy (AMEn) values of the feed, decreased extract viscosity, increased body mass gain and improved feed to gain ratio. In addition, dietary supplementation of suitable enzymes has been shown to increase the apparent protein and lipid digestibilities, and enhance nutrient digestion and absorption (Namkung and Leeson, 1999; Hesselman et. al., 1981; Marquardt et. al., 1994; Friesen et. al., 1992)

Mature laying hens have well developed digestive systems, compared to young chicks, and are better able to cope with high dietary levels of hulled barley or hulless barley (Anderson et. al., 1960; Aimonen and Nasi, 1991; Classen et. al., 1988). Although having more developed digestive capabilities, mature hens may still have limitations in their ability to cope with non starch polysaccharides (NSP's) (Petersen et. al., 1976) and can also benefit from dietary additions of enzymes (Campbell and Campbell, 1989).

Results of substituting barley for wheat have been inconsistent, and sometimes contradictory. Some published data report no significant changes or sometimes improved production performance (Classen et. al., 1988; Al Bustany and Elwinger, 1988). Al Bustany and Elwinger (1988) reported an increase in the incidence of dirty eggs resulting from feeding laying hens barley based diets. This may be due to a higher content of β-glucan in barley compared to either wheat or corn, causing an increase in intestinal viscosity and sticky droppings. Dietary enzyme supplementation of layer diets based on barley can be an effective way of enhancing the nutritive value of barley, although earlier work by Berg (1959) found no improvements in the rate of lay, feed efficiency, body weight gain or egg characteristics when a fungal and bacterial enzyme preparation was added to barley-based diets for White Leghorn hens. Using β-glucanase as the only dietary enzyme, Nasi (1988) found no improvements in rate of lay, feed intake and feed conversion between supplemented and unsupplemented barley based diets, although egg mass (g/h/d) decreased significantly with supplementation. However, supplementing the diets with a multi-enzyme premix, rate of lay, egg mass and feed conversion, were improved significantly. In his opinion, multi-enzyme products contain a variety of activities and their effect in degrading cell walls and liberating nutrients for utilization

may be synergistic. Supplementing barley based diets with an enzyme mixture (Avizyme SX, Finfeeds Int. Ltd.) resulted in hens gaining more body weight, while maintaining equal egg production early in the production cycle (Wyatt and Goodman, 1993).

From all the above, it can be concluded that hulled barley can be used in laying hen rations with little or no detrimental effects in performance. Limited data however exists on the use of hulless barley in laying hen diets. Therefore an experiment was conducted to study the performance response of layers fed wheat and hulless barley based diets with and without an exogenous crude enzyme cocktail supplementation.

MATERIALS AND METHODS

The hulless barley (*Hordeum vulgare*) cultivars (Silky, Falcon, Gainer and Dawn) that were used for both trials came from the same source (Glenlea Research Station). The crude protein of all the grains was determined using the Kjeldahl procedure as described by the Association of Official Analytical Chemists (AOAC, 1984). Amino acid analysis of the grains, feed, excreta and ileal contents was determined using the procedure as described by Andrews and Balder (1985). Analysis of methionine and cystine were conducted using performic acid oxidation by the method of Moore (1963). The diets were based on the amino acid digestibility of the grains (Table 7) and were formulated to be isonitrogenous and isocaloric to the wheat control diets. Feed and fresh water was provided *ad libitum* at all times and eggs were collected twice a day. Performance parameters recorded were egg production (hen-day %), feed intake (g/h/d), egg weight (g), egg mass (g/h/d) and feed efficiency (g/g egg).

Trial 1. Comparison of different hulless barley cultivars and wheat in laying hen diets

Five diets were formulated from one wheat and four hulless barley cultivars (Table 8) for each of the 2 strains of hens. A total of 640 Hyline W36 and 640 Dekalb Sigma Leghorn Layers at 32 weeks of age were each housed in 80 cage units (16 hens per replicate divided evenly among 4 adjacent cages, 8 replicates per treatment for each strain) and randomly assigned to one of five dietary treatments. The experiment lasted for

a total of 140 days, and was divided into five 28-day periods. All eggs laid on the final 3 days of each 28-day period were collected, weighed individually and an average weight for the period calculated. Feed intake, feed efficiency, egg weight, egg mass and egg production were calculated for each 28-day period and averaged for the 5-month trial. A 2*5 (strain and grain as main effects) factorial arrangement of a completely randomized design was used to analyze the data. The performance data collected were subjected to analysis of variance using General Linear Model (GLM) procedures of the Statistical Analysis System (SAS USER'S Guide, 1990).

Table 7: Total digestible amino acids (%) of hulless barleys used in trial

	Hulless barley					
Amino acid	**Silky**	**Falcon**	**Gainer**	**Dawn**	**Average**	**S.D** [1]
Aspartic acid	73.52	78.06	78.94	74.33	76.21	2.686
Threonine	**74.6**	**82.41**	**80.08**	**75.4**	**78.12**	3.743
Serine	83.89	86.16	86.78	79.3	84.03	3.390
Glutamic acid	89.89	92.37	91.65	88.43	90.58	1.774
Proline	91.79	93.43	93.8	88.11	91.78	2.599
Glycine	72.46	79.52	77.83	76.13	76.48	3.019
Alanine	68.48	73.76	73.54	69.57	71.33	2.708
Valine	77.76	85.49	81.16	79.94	81.08	3.254
Methionine	**73.97**	**72.58**	**73.4**	**68.16**	**72.03**	2.640
Isoleucine	80.01	87.11	81.75	80.68	82.38	3.228
Leucine	81.74	86.48	84.22	80.41	83.21	2.690
Tyrosine	82.67	88.19	83.7	81.82	84.09	2.836
Phenylalanine	82.42	88.57	86.88	83.36	85.31	2.900
Histidine	65.86	81.69	77.83	79.83	76.30	7.137
Lysine	**58.83**	**58.66**	**50.99**	**67.4**	**51.15**	6.705
Arginine	**47.09**	**58.58**	**52.69**	**54.14**	**53.13**	4.739

[1] S. D: Standard Deviation

Modified from Campbell, 1998

Table 8. Composition of one wheat, and four hulless barley based experimental diets

Ingredient	1 Control	2 Silky	3 Falcon	4 Gainer	5 Dawn
Wheat	71.2	0	0	0	0
Hulless barley	0	69.3	74.8	67.3	69.3
Canola [1]	10	10	10	10	10
Soybean meal	4.7	7.1	2.2	9	7.2
Limestone	8.7	8.6	8.6	8.6	8.6
Biophos	0.5	0.4	0.4	0.4	0.4
DL-Methionine	0.07	0.04	0.06	0.05	0.06
Lysine	0.18	0.06	0.2	0	0.08
Vitamin Layer [2]	1	1	1	1	1
Mineral Layer [3]	0.5	0.5	0.5	0.5	0.5
Tallow	1.4	1.5	1.1	1.6	1.5
Vegetable oil	1.3	1.5	1.1	1.6	1.5
Total	**100**	**100**	**100**	**100**	**100**
Calculated nutrient composition (%)					
Nutrient					
ME, kcal/kg	2800	2800	2800	2800	2800
Linoleic acid	1	1	1.1	1.1	1.1
Crude protein	16.4	16.4	16.4	16.4	16.4
Lysine	0.7	0.7	0.7	0.7	0.7
Methionine	0.35	0.35	0.35	0.35	0.35
Calcium	3.5	3.5	3.5	3.5	3.5
Avail. P.	0.25	0.25	0.25	0.25	0.25

[1] Contained 34.1% crude protein.

[2] Provided (per kg of diet): vitamin A, 8255IU; vitamin D_3, 1000IU; vitamin E, 5.46IU; vitamin B_{12}, 11.2ug; Methionine-DL, 500mg; Ethoxyquin, 186.6mg.

[3] Provided (per kg of diet):MnO, 165mg; ZnO, 55mg; Salt(iodized), 4.78g.

Trial 2 Hulless barley and wheat with or without exogenous enzymes in diets for laying hens.

Ten diets were formulated from one wheat and each of the four hulless-barley cultivars, (Table 9) either with or without an exogenous enzyme cocktail. The enzyme cocktail consisted of xylanase and ß-glucanase as the main enzymes for the wheat and hulless barley-based diets respectively, and was fortified with a broad spectrum of enzymes made up of amylase, protease and cellulase (Table 10). The enzyme premix was supplemented at a level of 0.2% of the diets. The diets were formulated to be isonitrogenous and isocaloric. Due to the lower nutrient (particularly amino acid) profile of the Gainer hulless barley variety, a proportionally larger amount of soybean meal was included in the ration to bring the total nutrient profile up to the recommended level (Table 9). A total of 1280 Single Comb White Leghorn (Shaver White) hens at 26 weeks of age were housed in eighty cage units (16 hens per replicate divided evenly among 4 adjacent battery cages, 8 replicates per treatment) and randomly assigned to one of the ten dietary treatments. The experiment, which lasted for a total of 252 days, was divided into nine 28-day periods. Production results were calculated for each 28-day period and averaged for the 9-month trial. All eggs laid on the final 3 days of each 28-day period were collected, weighed individually and an average egg weight for the period calculated. A 2*5 (grain and enzyme as main effects) factorial arrangement of a completely randomized design was used to analyze the data. The performance data collected were subjected to analysis of variance using General Linear Model (GLM) procedures of the Statistical Analysis System (SAS USER'S Guide, 1990).

Table 9. Composition of one wheat and four hulless barley based diets with or without exogenous enzymes

	1	2	3	4	5
Ingredient	**Control**	**Silky**	**Falcon**	**Gainer**	**Dawn**
Wheat	74.82	0	0	0	0
Hulless barley	0	75.47	77.36	64.27	76.14
Canola[1]	10	10	10	10	10
Soybean meal	2.02	1.7	0	11.46	1.1
Limestone	8.66	8.61	8.65	8.6	8.63
Biophos	0.52	0.42	0.42	0.38	0.41
DL-Methionine	0.04	0.04	0.05	0.05	0.03
Lysine	0.14	0.12	0.12	0	0.14
Vitamin [2]	1	1	1	1	1
Mineral Layer [3]	0.5	0.5	0.5	0.5	0.5
Tallow	1.3	1.14	0.9	2.74	1.05
Vegetable oil	1	1	1	1	1
Enzyme Premix[4]	0.2	0.2	0.2	0.2	0.2
Total	**100**	**100**	**100**	**100**	**100**
Calculated nutrient composition (%)					
Nutrient					
ME, kcal/kg	2800	2800	2800	2800	2800
Linoleic acid	1	1	1	1	1
Crude protein	17	17	17.7	17	17
Lysine	0.7	0.7	0.7	0.78	0.7
Methionine	0.35	0.35	0.35	0.35	0.35
Calcium	3.49	3.49	3.51	3.5	3.5
Avail. P.	0.25	0.25	0.252	0.25	0.25

[1] Contained 34.1% crude protein.

[2] Provided (per kg of diet): vitamin A, 8255IU; vitamin D_3, 1000IU; vitamin E, 5.46IU; vitamin B12, 11.2ug; Methionine-DL, 500mg; Ethoxyquin, 186.6mg.

[3] Provided (per kg of diet):MnO, 165mg; ZnO, 55mg; Salt(iodized), 4.78g.

[4] Exogenous enzymes were mixed in 0.2% of canola for the "+ enzyme" diets

Table 10. Composition of the enzyme blends supplemented in diets

(The enzyme blend supplied per kg of diet:)

Enzyme	Wheat ration	Barley ration
Xylanase	500 units	200 units
Glucanase	200 units	500 units
Amylase	1740 units	1740 units
Invertase	0.005 g	0.005 g
Protease	800 units	800 units
Cellulase	0.005 g	0.005 g

RESULTS AND DISCUSSION

Trial 1 Comparison of different hulless barley cultivars and wheat in laying hen diets

The production parameters and P-values for main effects of grain and their interactions for the different performance traits are shown in Table 11. Egg production results show that birds fed the Control wheat-based diet had a hen-day production (87.4%) that was similar ($P>0.05$) to both the Silky and Gainer based diets (86.9 and 85.6% respectively) but significantly better ($P<0.05$) than the Falcon and Dawn based diets (84.5 and 85.7% respectively). Hens fed the Silky based diets showed a trend towards having the best egg weights.

Egg mass showed a similar trend to hen-day production in that hens fed the Falcon based diet had the lowest value (50.4g/h/d) and this was significantly lower ($P>0.05$) than egg mass of the hens fed the control diet (52.6g/h/d). Although hens fed the Dawn based diet had a low egg mass (51.1g/h/d) this value was similar ($P>0.05$) to that of the birds fed the wheat based diet. The consistently lower production values of hens fed the Falcon based diets, compared to the other diets may be partly explained by its NSP profile. Table 12 shows the NSP contents of the various diets. Arabinose and xylose together reflect total arabinoxylan, while glucose represents β-glucan and cellulose. Total NSP of the Falcon based diet is 90.8mg/g of the diet, and this is made up

Table 11. Production parameters and P-values of 2 strains of hens fed different cereal based diets (Trial one)

Factor	Parameter					
	Feed Intake (g/h/d)	Hen Day Production (%)	Egg Weight (g)	Egg Mass (g/h/d)	Feed Efficiency (g/g/egg)	Mortality[2] (%)
Strain						
Dekalb Sigma	101.4 [a1]	86.4	59.9	52.1	1.94	0.00
Hyline W36	99.2 [b]	86.1	59.8	51.5	1.91	0.70
Pooled SEM	*0.27*	*0.34*	*0.15*	*0.29*	*0.012*	
Grain						
Wheat	100.9	87.4 [a]	59.8	52.6 [ab]	1.93	0.23
Silky	99.9	86.9 [ab]	60.1	52.8 [a]	1.91	0.00
Falcon	99.7	84.5 [c]	59.9	50.4 [c]	1.97	0.08
Gainer	100.2	86.6 [ab]	59.9	52.2 [ab]	1.93	0.16
Dawn	100.8	85.7 [bc]	59.2	51.1 [bc]	1.96	0.23
Pooled SEM	*0.43*	*0.55*	*0.23*	*0.46*	*0.019*	
Anova Summary						
Sources of variation			**P-Values**			
Strain	0.0001	0.556	0.451	0.171	0.983	0.003
Grain	0.195	0.0029	0.126	0.021	0.229	0.433
Strain*Grain	0.487	*0.0001*	0.321	*0.011*	*0.019*	

[1 a,b]. Means within a column with different superscripts are significantly different ($P<0.05$)

[2] Determined using Analysis of frequency and count data (SAS, 1989)

Table 12. Neutral sugars in the fiber fraction of the experimental diets (mg/g)

Diet	Fiber fraction					Total NSP (GC Method)
	Arabinose	Xylose	Mannose	Galactose	Glucose	
Control	16	20.6	1.8	5.8	28.1	72.3
Silky	16.2	19	2.4	6.2	39.6	83.4
Falcon	18	19.4	2.5	5.5	45.5	90.8
Gainer	15	17.1	2.1	6	43.4	83.6
Dawn	15	18.3	2.1	3.9	45	84.3

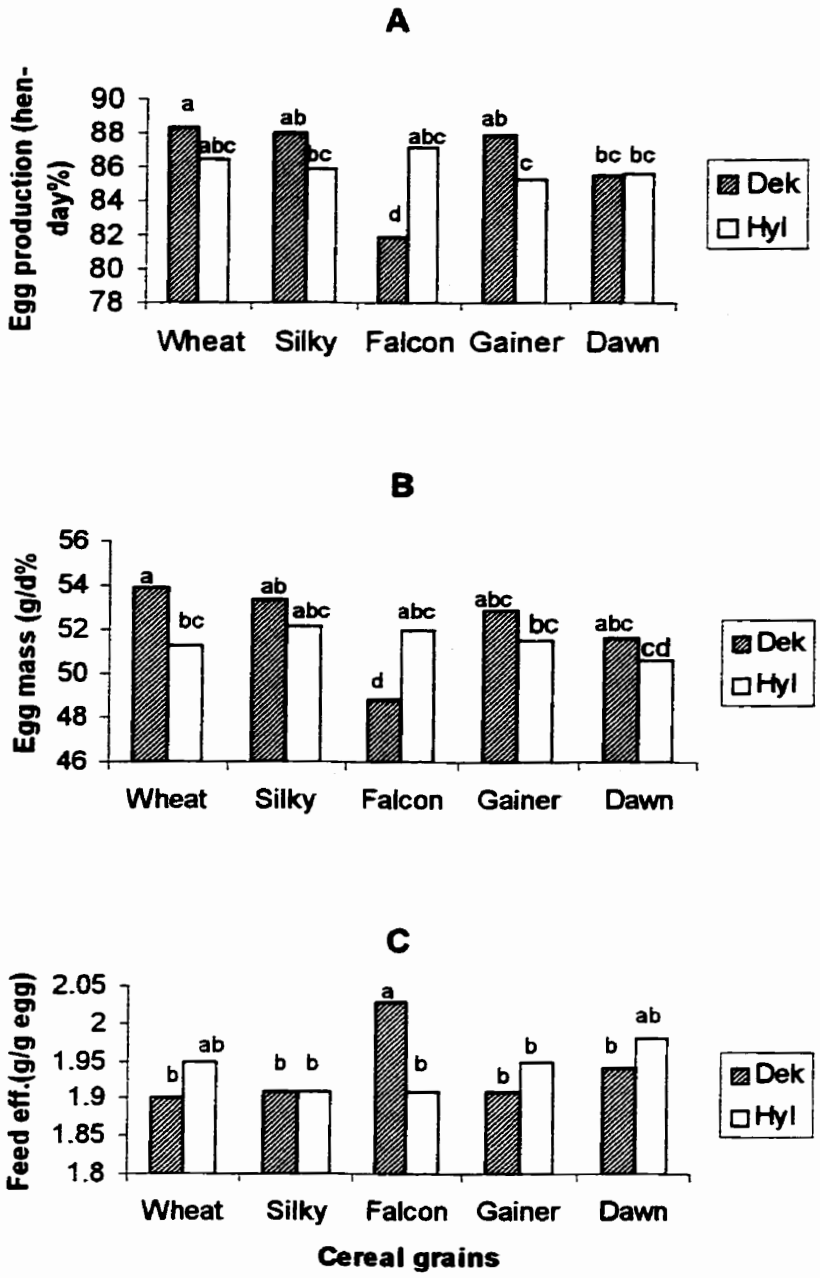
of 37.4mg/g and 45.5mg/g arabinoxylan and glucose respectively. These values are the highest for the five diets while the wheat control diet has the lowest NSP level. From a nutritional point of view, higher levels of NSP's are indicators of poorer quality feed. It is an established fact that the presence of arabinoxylans and β-glucans in feed for monogastrics results in a lowering of their nutritive value, and a reduced performance in both young chicks (Anderson et. al., 1961; Classen et. al., 1985; Campbell et. al., 1986a;) and laying hens (Nasi 1988; Bhatty et. al., 1991). It can be deduced from the above that, the Falcon based diet, having the greatest concentration of NSP's would be expected to produce the most unfavorable results when fed to the experimental birds. The differences between the chemical characteristics of the various hulless barley cultivars may be due to genotype. Andersson et. al., (1999a) indicated that differences in chemical characteristics of different barleys are due to genotype and environment and/or a combination of the two. Genotypic differences may cause barleys to have different concentrations of either starch in their kernels (Andersson et. al., 1999c) or β-glucans in their cell walls (Andersson et. al., 1999b), all of which can influence productive performance when fed to birds. In the present trial, all the barleys were grown under the same environmental conditions, and therefore genotype may be the main reason for the differences.

Grain effects on egg weight, feed intake and feed efficiency were not significant. However, grain effects were significant for hen day production ($P<0.005$) and egg mass ($P<0.05$). These results would suggest inherent differences in the physical and chemical properties of the grains. The 2 grains used in this trial, wheat and hulless barley have starch as the dominant constituent. Compared with wheat, hulless barley contains similar levels of crude protein (13.5 vs. 13.2% respectively), but lower amounts of crude fiber

(2.6 vs. 1.4% respectively) (Hickling 1995). However the β-glucan level in hulless barley and wheat are 4.5% (Hickling, 1995) and 0.67% (Henry 1985) respectively. Also within hulless barleys, cultivar differences exist in chemical compositions and nutrient availabilities (Jeroch and Danicke, 1995). These differences could have an impact on the feeding value of the grains, and are a likely explanation for the observed differences.

Feed intake was influenced by strain of bird ($P=0.0001$), and although there were no significant strain effects for egg production, egg weight and feed efficiency, a trend ($P=0.171$) towards increased egg mass is apparent. The Dekalbs had significantly higher mortalities than the Hylines ($P<0.05$). However among the five diets there were no significant differences in mortality. There was a significant grain*strain interaction effect for egg production egg mass and feed efficiency (Figure 3) ($P=0.0001$, 0.011 and 0.019 respectively). This indicates that the two strains of layers react differently to the different diets. Mean comparisons of the various diets fed to the 2 strains of birds are shown in Table 13. Feed intake for the Dekalb strain was consistently higher than the Hyline strain for all the diets. This may be due to the higher body weights, and thus higher maintenance requirement needs of the Dekalb strain. An interesting observation that was made was that the Dekalb strains were less able to cope with the Falcon based diet, and showed significantly poorer ($P>0.05$) results for egg production, egg mass and feed efficiency (81.8 vs. 87.1%; 48.8 vs 52.0g/h/d; 2.03 vs 1.91 respectively). Egg weight was also slightly better for the Hylines, who also ate less feed than the Dekalb strain (60.0 vs. 59.7g and 99.2 vs. 100.2g/h/d respectively).

Figure 3. Overall egg production (A), egg mass (B) and feed efficiency (C) of two strains of hens fed different cereal based diets



Dek = Dekalb Sigma

Hyl = Hyline W36

Table 13 Performance traits of 2 strains of layers fed different grain based diets

Performance Traits	Control		Silky		Falcon		Gainer		Dawn		S.E
	Dekalb[1]	Hyline	Dekalb	Hyline	Dekalb	Hyline	Dekalb	Hyline	Dekalb	Hyline	
Feed int. (g/h/d)	102,5[a2]	99,4[dc]	101,3[abc]	98,5[d]	100,2[bcd]	99,2[d]	101,2[abc]	99,1[d]	101,9[ab]	99,7[cd]	0,62
Hen day egg prod. (%)	88,4[a]	86,4[abc]	87,9[ab]	85,9[bc]	81,8[d]	87,1[abc]	87,9[ab]	85,3[c]	85,8[bc]	85,7[bc]	0,77
Egg wt. (g)	60,2[a]	59,4[ab]	60,1[a]	60,2[a]	59,7[ab]	60,0[a]	59,8[ab]	60,1[a]	59,6[ab]	58,9[b]	0,33
Egg mass (g/h/d)	53,9[a]	51,2[bc]	53,4[ab]	52,2[abc]	48,8[d]	52,0[abc]	52,9[abc]	51,5[bc]	51,7[abc]	50,6[dc]	0,66
Feed efficiency (g/g egg)	1,91[b]	1,95[ab]	1,91[b]	1,91[b]	2,03[a]	1,91[b]	1,91[b]	1,94[b]	1,94[b]	1,98[ab]	0,03

[1] Dekalb Sigma and Hyline W36 strains

[2] [abc] Means within a row with different superscripts are significantly different

The Dekalb strain produced significantly more ($P<0.05$) eggs than the Hyline strain (87.9 vs. 85.3%) and also consumed significantly more ($P<0.05$) feed (101.2 vs. 99.1g/h/d) respectively.

The average combined performance of the 2 strains fed the hulless barley diets relative to the Control is shown in Figure 4. None of these differences were significant. The Hylines, which were a lighter strain of hens compared to the Dekalbs, gained significantly more ($P<0.05$) weight than the Dekalbs over the course of the 5-month trial (40gms vs. 20gms) (Table 14). Except for the hens that were on the Dawn based diet which had no weight change, all the hens that were on the other diets gained between 30 and 50 gms over the course of the experiment, and these weight gains were significantly higher ($P<0.05$) than for hens fed the Dawn based diet.

Results of this trial have shown that certain hulless barley cultivars can effectively replace wheat in diets for laying hens. Under the conditions of this experiment Silky and Gainer hulless barley cultivars can replace wheat without any loss in egg production and egg mass. Silky, Gainer and Dawn can effectively replace wheat without loss of egg mass, while all the cultivars used can replace wheat without any effect on feed efficiency or feed intake. In this trial the β-glucan induced sticky feces due to feeding barley based diets (Gohl and Thomke, 1976) as evidenced by an increase in the incidence of dirty eggs, did not pose any problem.

Figure 4. Performance of hens fed hulless barley based diets relative to control

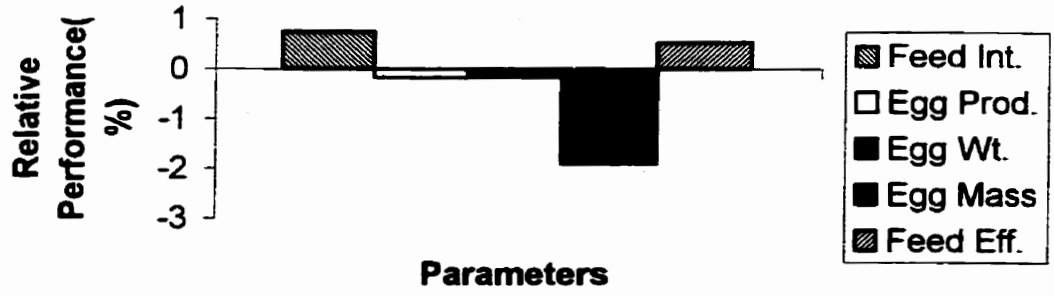


Table 14. Weight change of 2 strains of hens fed Various diets

Strain	Weight increase (kg)
Hyline	0.04 [a]
Dekalb	0.02 [b]
Pooled SEM [1]	0.006
Grain	
Wheat	0.04 [a]
Silky	0.03 [a]
Falcon	0.05 [a]
Gainer	0.04 [a]
Dawn	0.00 [b]
Pooled SEM	0.0104

Anova Summary

Sources of Variation	P-values
Strain	0.027
Grain	0.033
Strain*Grain	0.886

[1]: **Standard error of the mean**

[ab]: **Means within a column with different superscripts are significantly different (P<0.05)**

Trial 2 Hulless barley and wheat with or without exogenous enzymes in diets for laying hens

The performance data, P-values of main effects of cereal grains and enzyme supplementation, and their interactions for the different performance traits are shown in Table 15. There was no significant enzyme*grain interaction for all the parameters studied and therefore only the main effects will be discussed. Enzyme effects for feed intake, hen-day production and egg weight, were not significant (P=0.286, 0.221 and 0.115 respectively). The combined positive trends of feed intake, egg production and egg weight however resulted in a significant enzyme effect for egg mass and feed efficiency (P=0.02 and P<0.01 respectively). There was a significant grain effect for egg weight, egg mass and feed efficiency (P<0.05, P<0.005 and P<0.001 respectively) and a strong positive trend for egg production (P=0.06). The results would suggest that inherent differences exist among the grain samples that cause differences in their physical and chemical properties when they are ingested. Similar results were obtained in Trial 1 (Table 11), where significant grain effects were obtained for egg production (P=0.0029) and egg mass (0.021). It was concluded from Trial 1 that differences in NSP profile (Table 12) of the different diets (mainly due to the cereal grains) could be the cause of the significant grain effects that were observed in bird performance. In Trial 2, the same wheat and hulless barley cultivars that were used in Trial 1, were used for feed formulation. Therefore the significant grain effects for egg weight, egg mass and feed efficiency may be due to genetic differences.

Table 15. Production parameters and P-values of hens fed different cereal based diets with or without exogenos enzymes

Factor	Parameter					
	Feed Intake (g/h/d)	Hen Day Production (%)	Egg Weight (g)	Egg Mass (g/h/d)	Feed Efficiency (g/g/egg)	Mortality[2] (%)
Enzymes						
Without	99.2	83.4	57.1	47.2 b	2.14 [a]	1.41
With	98.7	84.3	57.7	48.5 a	2.08 [b]	1.17
Pooled SEM	*0.34*	*0.47*	*0.26*	*0.38*	*0.01*	
Grain						
Wheat	99.3	84.3 [ab]	57.1 [b]	47.7 [b]	2.10 [b]	0.63
Silky	98.9	83.3 [ab]	57.4 [b]	47.7 [b]	2.15 [ab]	1.09
Falcon	99.1	82.1 [b]	57.3 [b]	46.5 [b]	2.18 [a]	0.39
Gainer	98.7	84.8 [a]	58.7 [a]	50.2 [a]	1.99 [c]	0.39
Dawn	98.7	84.8 [a]	58.5 [a]	47.3 [b]	2.18 [ab]	0.39
Pooled SEM	*0.55*	*0.74*	*0.43*	*0.59*	*0.02*	
Anova Summary						
Sources of variation			**P-Values**			
Enzyme	0.286	0.221	0.115	0.021	0.009	0.59
Grain	0.937	0.061	0.008	0.001	0.0001	0.07
Enzyme*Grain	0.804	*0.415*	0.761	*0.479*	*0.379*	

[1] [a,b]. Means within a column with different superscripts are significantly different (P<0.05)

[2] Determined using Analysis of frequency and count data (SAS, 1989)

Wyatt and Goodman (1993) also suggested that barley could be used in laying hen rations with no detrimental effects on performance, but barley cultivars may cause different reactions. They also suggested that some factors probably more than just total β-glucan in the barley might be interacting to alter digestion and dietary energy utilization.

The performance of hens fed the different grains irrespective of enzyme treatment shows that hens fed the Gainer-based diet performed well in all the parameters studied, and this was either equal to ($P>0.05$) or better than ($P<0.05$) the rest of the diets. This may be due to the fact that a larger amount of soybean meal was included in the ration to compensate for the lower nutrient density of the Gainer hulless barley variety. For the remaining hulless barley based diets, performance of the birds was similar to ($P>0.05$) the control wheat diet. Birds being fed the Falcon-based diet, however, had a feed efficiency, which was significantly poorer ($P>0.05$) than the control diet (2.18 vs. 2.10 respectively).

Performance of layers fed the various cereal grains either with or without exogenous enzyme supplementation shows no significant enzyme effects for egg production, egg weight and feed intake (83.44 vs. 84.26; 57.11 vs. 57.69; 99.19 vs. 98.66) respectively. Enzyme supplementation resulted in significant ($P<0.05$) improvements in egg mass (47.2 vs. 48.5) and feed efficiency (2.14 vs. 2.08) respectively.

Mean comparisons of the ten different diets are shown in appendix Table 1A. In general, enzyme supplementation of the individual diets resulted in positive but insignificant trends in egg production and egg weight. Feed intake also reduced upon supplementation with enzymes. However on the Falcon-based diet hens responded significantly ($P<0.05$) in egg mass and feed efficiency when the diets were supplemented with exogenous enzymes. An explanation for this may be the fact that, generally diets that are relatively poorer (higher in NSP's) may show greater improvements in feeding value for

birds when NSP degrading enzymes are added. Although the NSP contents of the "without enzyme" diets in Trial 2 were not directly measured, it can be seen that of the 4 hulless barley cultivars used, Falcon was used in greatest concentrations for both Trials 1 and 2. Since the same variety was used, it is therefore possible that the "without enzyme" Falcon-based diet in Trial 2 would be the poorest, and therefore enzyme supplementation would result in relatively larger improvements in hen performance. The without-enzyme egg mass of 44.96g/d was significantly ($P<0.05$) improved to 47.98g/d with supplementation, and this was equal to both the unsupplemented and supplemented wheat control diet (47.56 vs. 47.75g/d respectively). Feed efficiency of hens fed the unsupplemented Falcon-based diet was 2.24, and this improved significantly ($P<0.05$) to 2.11 upon supplementation with enzymes. This improved value was similar ($P>0.05$) to both the unsupplemented and supplemented control wheat diets (2.10 vs. 2.09 respectively). Although improvements in performance upon enzyme addition to the individual diets were not significant for egg production and egg weight, it is interesting to note that in both these parameters, and also for egg mass and feed efficiency, the variability that existed between the unsupplemented hulless barley based diets, was reduced upon supplementation with enzymes. Hens fed the unsupplemented Falcon based diet had an egg production of 81.1%. This was significantly lower ($P>0.05$) than egg production of hens fed the Gainer and Dawn based diets (84.5 and 84.8% respectively). Upon addition of enzymes to these diets, however, these differences that existed in egg production for hens fed the Falcon, Gainer and Dawn based diets was removed and the values (83.2 vs. 85.1 vs. 84..8 % respectively) were no longer significantly different ($P>0.05$). The same trend is also noted for egg weight, egg mass and feed efficiency where enzyme supplementation reduced the differences in the production figures for the unsupplemented hulless barley based diets. In similar trials, Wyatt and Goodman

(1993) and Wyatt et., al.,(1991) fed Wanabet hulled barley-based diets without exogenous enzymes to Single Comb White Leghorn layers. This resulted in significant decreases in body weight gain, daily feed intake, total egg production and feed conversion compared with a regular corn soybean diet. However supplementing the Wanabet barley-based diet with β-glucanase resulted in an increase in performance equal to that observed in the regular corn soybean diet. Improved performance of laying hens fed diets supplemented with exogenous enzymes have been reported by only a few other researchers (Nasi, 1988; Um et. al., 1998; Ely 1963). Petersen and Sauter, (1968), reported that significant ($P<0.05$) improvements in egg production were noted when barley based diets were supplemented with exogenous enzymes at the 0.25% inclusion level, but not at the 0.5% inclusion level.

The average performance of hens fed the various hulless barley based diets relative to the control (all with exogenous enzyme supplementation) is shown in Figure 5. Egg production of the hulless barley diets was 0.73 % better than the control wheat diet. Feed efficiency, egg production and egg weight also had improvements of 0.48, 1.99 and 0.73% respectively, although these improvements were not significantly different ($P>0.05$). Feed intake also decreased by 0.45%.

Several factors have been attributed to the enhanced performance of laying hens that are fed enzyme supplemented barley based diets. Wyatt and Goodman (1993) suggested that enzyme supplementation of barley based diets may have a positive response on energy bioavailability and laying hen performance. They concluded in their experiments that enzyme supplementation resulted in a 4% increase in TME over unsupplemented diets. This is in line with observations made with young chicks (Petersen et. al., 1976; Rotter et. al., 1990; Friesen et. al., 1992; Marquardt et. al., 1994). Another important explanation for the improved performance of barley based layer diets plus enzymes is a reduction in the

antinutritive effect of NSP's (Nasi, 1988). He concluded that the addition to the diet of enzymes that degrade gel-forming viscous and structural polysaccharides is a potential method of improving the nutritive value of some feed ingredients. These enzymes can break down anti-nutritional substances present in barley and improve availability of the nutrients in the feed. However, addition of β-glucanase alone to barley based diets resulted in no improvements in performance. Multi enzyme preparations are more useful since they have a variety of activities, and their effects in degrading cell walls and liberating nutrients for utilization may be synergistic.

There is a lot of published data involving young chicks that have shown results that are in line with this observation (Rotter et. al., 1989; Burnett, 1966; Hesselman et. al., 1981). The degradation of cell wall NSP's results in a release of encapsulated nutrients within the cell, and a lowering of digesta viscosity, thus improving the rate of diffusion among substrates, enzymes and digestion end-products (Campbell and Bedford, 1992; Bedford and Schulze, 1998). Barley-based diets, when fed to laying hens, have been shown to result in an increase in the incidence of dirty egg. This is a result of the soluble β-glucans in barley which increase intestinal viscosity and cause sticky droppings (Al Bustany and Elwinger, 1988). In the present study the incidence of dirty eggs was not a problem. The initial and final weights, and weight change of hens fed the individual diets, are shown in Table 16. The weight change for the 'with' and 'without' enzymes diets for each of the cereals were not significantly different ($P>0.05$), except for the Silky based diet. Hens on the 'without' enzyme diet lost 6.2 g at the end of the experiment, while

Figure 5. Performance of hens fed hulless barley based diets relative to control (+enzyme)

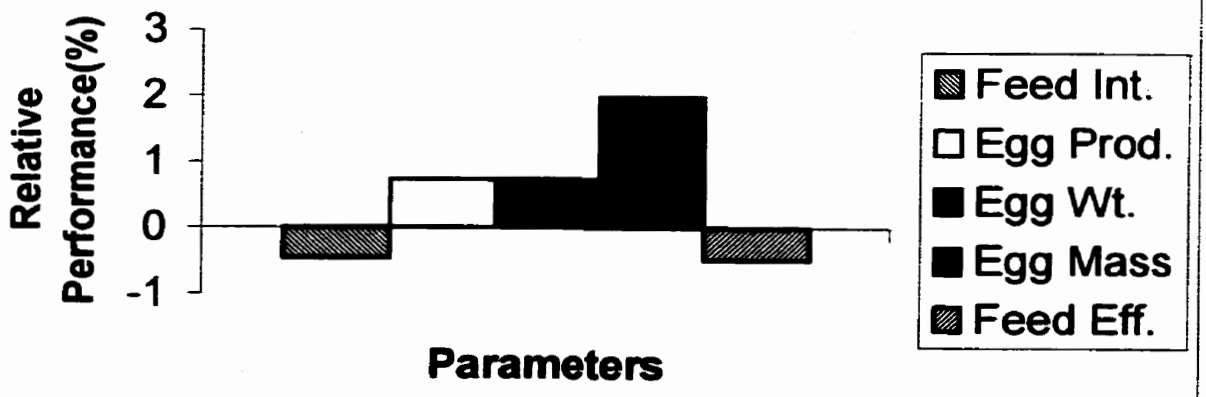


Table 16. Body weight change of hens fed different cereal based diets with (+) and without (-) exogenous enzymes over 9 28-day periods (252 days)

Diet	Initial body Wt. (g)	Final body Wt. (g)	Weight gain (g)
Control -	1505	1506	1[b]
Control +	1505	1526	21[ab]
Silky -	1518	1511	-6[b]
Silky +	1515	1608	93[a]
Falcon -	1525	1520	-5.0[b]
Falcon +	1529	1525	-4[b]
Gainer -	1530	1551	21[ab]
Gainer +	1540	1574	34[ab]
Dawn -	1540	1544	4[b]
Dawn +	1519	1544	25.0[ab]

[ab]: Means within the same column with different superscripts are significantly different ($P<0.05$).

those on the 'with' enzyme diet gained an average of 92.5g, and these were significantly different from each other. This observed increase in weight suggests that there is an increase in energy availability when the Silky-based diet is supplemented with exogenous enzymes.

In the present study, enzyme application resulted in significant improvements in egg mass (47.2 vs. 48.5g/d), and in feed efficiency (2.14 vs. 2.08). Small but insignificant improvements were also noted for egg production (83.4 vs 84.3%) and egg weight (57.1 vs. 57.7g), while feed intake reduced slightly (99.2 vs. 98.7 g/h/d). These results are contrary to reports published in the literature that have either found no improvements in performance when enzymes are included in the diets for laying hens (Berg, 1959, 1961), to little improvement with older birds in general (Classen et. al., 1988). Some reasons for this lack of response have been the fact that older birds have greater digestive capabilities, and therefore do not benefit from the advantages that younger birds have, when enzymes are added to their diets (Krogdahl, 1985). Results of this study have however demonstrated that laying hens aged 26 to 62 weeks did benefit from the addition of exogenous enzyme to the diet. This trial has also demonstrated that feeding hulless barley cultivars with exogenous enzymes removes some of the variability in feeding quality that exists among these hulless barley cultivars.

4. MANUSCRIPT 2

Effects of exogenous enzymes on production traits, nitrogen and phosphorous outputs in laying hens fed diets based on hulless barley

ABSTRACT

Diets using an equal proportion mix of 3 different hulless barley cultivars (Silky, Gainer and Dawn), were fed to laying hens at 32 weeks of age to assess production performance and output of nutrients, particularly P and N. Diet one was formulated without exogenous enzymes to meet the minimum nutrient requirements as suggested by NRC (1994). The nutrient profiles of three dietary ingredients (canola, soybean and mixed barleys) were uplifted by 5% and 10% respectively (for phosphorous, lysine and methionine) and diets 2 and 3, were formulated based on these uplifted profiles, to be similar in nutrient composition to diet 1. At 44 weeks old, a balance trial using the same diets was initiated to determine the effect of dietary addition of enzymes on nutrient retention and excreta output of nutrients, particularly phosphorous and nitrogen. A 2*3 factorial arrangement of treatments (2 strains * 3 diets) in a completely random design was used to analyze the data. Birds that were fed the 5 and 10% uplifted diets, had egg production, feed efficiency and egg mass values that were similar ($P<0.05$) to values obtained for hens that were fed the regular diet (88.6 vs. 88.6 vs. 88.5%; 1.97 vs. 2.00 vs. 1.98; and 51.2 vs. 50.9 vs. 51.9 g/h/d, respectively). The egg weight of hens fed the regular diet (58.61) was significantly higher ($P<0.05$) than the egg weights of hens fed both the 5 and 10% uplifted diets (57.8 vs. 57.2g respectively). Feed intake of birds fed the regular diet (102.6g/h/d) was also higher ($P<0.05$) than that of birds fed the 5% uplifted (95.3g/h/d) and the 10% uplifted (96.3%) diets. Between the 2 strains of layers, (Shaver White and Hyline W36) the Shavers had significantly higher ($P<0.05$) egg production, feed efficiency and egg mass than the Hylines (89.3 vs. 87.2%; 1.95 vs. 2.01; 52.0 vs. 50.6 g/h/d). The Hylines also ate more ($P<0.05$)

feed than the Shavers (102.1 vs. 94.2g/h/d). Phosphorous output of the hens fed the 5% and 10% uplifted diets were significantly lower ($P>0.05$) than the output of hens fed the unsupplemented diet (0.3 and 0.3 vs. 0.4g/h/d respectively). Nitrogen excretion was also reduced significantly ($P>0.05$) from 1.6g/h/d (for the hens fed the unsupplemented diets) to 1.5 and 1.4 g/h/d (for hens fed the 5% and 10% uplifted diets respectively). Digestibility of most of the individual amino acids also improved significantly ($P<0.05$) with the uplifted diets.

INTRODUCTION

Previous results (manuscript 1) have demonstrated a positive response in egg mass and feed efficiency when hulless barley based diets plus exogenous enzymes are substituted for wheat in laying hen rations. These results have also shown that mature hens, although having more developed digestive capabilities compared to young chicks, can also benefit from dietary additions of enzymes. This is in agreement with the observations of Petersen and Sauter (1968), who found significant ($P<0.05$) improvements in egg production and feed efficiency when western barley based diets supplemented with exogenous enzymes were fed to laying hens. Addition of a multi enzyme preparation to barley based diets for layers also resulted in a significant improvement ($P<0.05$) in feed efficiency (Nasi, 1988).

In addition to improving performance, dietary addition of enzymes particularly phytase, either as the sole enzyme or as part of a multienzyme premix, has been shown to improve digestibility of dietary nutrients (amino acids and some minerals) and also result in reductions in excreta output of phosphorous in both young chicks and laying hens. Working with 3-week-old broilers fed a corn-soy diet, Sohail and Roland (1999) demonstrated that supplementing phytase in grower diets containing reduced levels of non-phytin phosphorous (NPP) and Ca, significantly improved performance and bone strength. In studies with male broiler chicks consuming a diet containing 600 phytase units (FTU) per kilogram of diet and with reduced lysine, total sulfur amino acids and metabolizeable energy, Zhang et. al. (1999) showed that body weight gain, feed intake and feed conversion was comparable to chicks fed a standard diet. Namkung and Leeson (1999) fed phytase

enzymes to day-old male broilers in diets low in Ca and P. In addition to performances that were comparable to the control diets, chicks fed the experimental diets had higher digestibilities for valine, isoleucine, non essential and total amino acids. Ibrahim et. al. (1999) also showed that with phytase supplementation of diets for 3-week-old birds, it was possible to reduce the recommended level of total phosphorous in the diet from 0.72 to 0.56%. Broilers on such a diet excreted one third less P than those on a control diet. Ravindran et. al. (1999) demonstrated that addition of either xylanase or phytase alone to wheat based diets for broilers did not improve performance, however when both enzymes were fed as a cocktail, weight gain and feed efficiency were improved. They suggested that the xylanase, by reducing digesta viscosity, might facilitate the action of phytase on phytic acid complexes and the absorption of liberated nutrients. Work with laying hens (Um et. al., 1999), showed that retention of Ca, P, Mg and Cu were greater in hens fed phytase supplemented, low P diets than those fed unsupplemented control diets. Excretions of P with phytase supplemented diets were significantly ($P<0.05$) less than the unsupplemented control diets, although N excretions were not significantly reduced. The ash contents in the tibias were not significantly affected, but contents of Ca, P, Mg and Zn in ash was significantly increased by phytase addition. There were also concomitant improvements in egg production, egg weight and feed conversion.

Carlos and Edwards (1998) demonstrated that phytase, and to a lesser extent 1,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2D_3$) can also be used to increase the utilization of phytate P in laying hens.

From all of the above, it can be seen that the use of phytase either alone or in combination with other enzymes in diets for both young chicks and laying hens can result in improved performance and reductions in nutrient output. Very little data exists on the use

of hulless barley in diets for laying hens. Therefore this experiment was conducted mainly to study the effect of feeding low levels of dietary nutrients (P, lysine and methionine) in combination with an enzyme cocktail containing β-glucanase and phytase on output of nutrients. Production performance was also evaluated.

MATERIALS AND METHODS

Performance trial

The purpose of this experiment was to determine the efficacy of an exogenous enzyme cocktail in enhancing nutrient availability in diets for laying hens that had been formulated to be 5 and 10% limited in some of the major nutrients.

Three hulless barley (*Hordeum vulgare*) cultivars (Silky, Gainer and Dawn) were mixed in equal proportions and used to formulate three diets for laying hens. Diet formulation was based on the average amino acid data of the individual grains (Table 7, Manuscript 1). Diet one was formulated without exogenous enzymes to meet the minimum nutrient requirements as suggested by NRC (1994) (Table 17). The resulting nutrient profile was uplifted by 5% (i.e. the phosphorous, lysine and methionine values were increased by 5%) in the computer feed formulation matrix (for soybean, canola meal and mixed barleys) and a new diet (diet 2) was formulated with enzymes to meet this uplifted profile. Diet 3 was also formulated in a similar way, but with a 10% uplifted nutrient profile of diet 1. Thus the soybean, canola and mixed barleys of diets 2 and 3 supplied 95 and 90% respectively of the P, lysine and methionine supplied by diet 1. The enzyme cocktail consisted mainly of β-glucanase, xylanase, amylase and phytase fortified with a broad spectrum of other enzymes (Table 18). A total of 576 Single Comb White Leghorn Layers (288 each of Hyline W36, and Shaver White strains) at 32 weeks of age, were randomly assigned by strain to each of the 3 dietary treatments in a 5-month trial. Each dietary treatment had 6 replicates of 16 hens per strain.

Table 17. Experimental diets of the control and uplifted diets

	Diets		
	1	2	3
Ingredient	**Control**	**5% uplifted**	**10% uplifted**
Barley Mix[1]	67.3	69.1	70.5
Canola[2]	10	9.8	9.8
Soybean Meal	9	7.5	6.2
Limestone	8.57	8.57	8.65
Biophos	0.4	0.35	0.3
DL-Methionine	0.05	0.04	0.03
Vitamin[3]	1	1	1
Mineral Layer[4]	0.5	0.5	0.5
Tallow	1.58	1.43	1.36
Vegetable Oil	1.6	1.5	1.4
Enzyme Premix[5]		*0.2*	*0.2*
Total	**100**	**100**	**100**
Calculated Composition (%)			
Nutrient			
ME, Kcal/kg	2800	2800	2800
Linoleic acid	1.09	1.08	1.07
Crude Protein	16.7	16.3	15.9
Lysine	0.68	0.64	0.61
Methionine	0.36	0.34	0.32
Calcium	3.5	3.5	3.5
Available P	0.25	0.237	0.225

[1] Contained equal proportions of Silky, Gainer and Dawn cultivars.

[2] Contained 34.1% crude protein.

[3] Provided (per kg of diet): vitamin A, 8255IU; vitamin D_3, 1000IU; vitamin E, 5.46IU; vitamin B_{12}, 0.0112mg; vitamin B12, 11.2ug; Methionine-DL, 500mg; Ethoxyquin, 186.6mg.

[4] Provided (per kg of diet):MnO, 165mg; ZnO, 55mg; Salt(iodized), 4.78g.

[5] Canola meal was used as a carrier for the enzyme premix

Table 18. Composition of enzyme blend supplemented to uplifted hulless barley based diets

(The enzyme supplied per kg of diet;)

Enzyme	Concentration
Xylanase	200 units
Glucanase	500 units
Amylase	4350 units
Invertase	0.01g
Protease	1200 units
Cellulase	0.02 g
Phytase	400 units

Feed and water was given *ad-libitum* and eggs were collected and enumerated twice daily. All eggs laid on the final 3 days of each 28-day period were collected, group weighed and an average weight calculated. Feed intake, feed efficiency, egg weight, egg mass and egg production were calculated for each 28-day period and averaged for the 5-month trial.

Balance trial

Twelve weeks into the trial at 44 weeks of age, a balance trial was initiated with 96 birds to determine the effect of dietary addition of enzymes on nutrient retention and excreta output of P and N. The trial lasted 14 days and was made up of a 10-day adaptation period followed by a 4-day excreta collection period. Four birds were randomly chosen by cage from 4 selected replicates (for each strain) and used for the balance trial. Enough of each diet was set aside and chromic oxide (Cr_2O_3), an insoluble marker was added at a level of 0.3%. The feeding trough for each selected group of birds was partitioned off from the rest of the group that formed a replicate in the performance trial, and the appropriate Cr_2O_3-treated feed was fed *ad-libitum*. Water was also given *ad-libitum*. Following the adaptation period, stainless steel collection trays were carefully placed under the appropriate cages, and the droppings collected. Excreta samples were collected at the same time every day for 4 days, after first removing any foreign material (feed and feathers). The sample was then bagged and sealed in bags, weighed, frozen and freeze dried. After every collection, the trays were cleaned thoroughly and repositioned under the cages. The 4-day excreta collections were pooled for each replicate to give a total of 24 samples (4 replicates per dietary treatment per strain). At the termination of the collection period, 4 birds per treatment were sacrificed by cervical dislocation. The contents of the small intestines from

the Meckel's diverticulum to 1.5 cm prior to the ileal-cecal junction were emptied into sterile plastic bags frozen and freeze dried. The left tibias were also removed, scrapped clean of any adhering tissue placed in plastic bags and weighed. The pooled excreta samples were finely ground through a 1 mm sieve using a Cyclotech 1093 sample mill and analyzed for chromic oxide (internal marker) (Williams et. al., 1962) gross energy (GE) nitrogen (Leco NS-2000 analyzer), and P. The left tibias were dried at 60^0C for 4 days in a Coldstream Fleming Pedlar oven and analyzed for ash, P and Ca. The ileal contents were analyzed for chromic oxide (Williams et. al., 1962) and amino acids (Moore, S. 1963). Apparent dry matter digestibility, nutrient digestibility and metabolizable energy (AMEn) values were calculated as follows (Hill et. al., 1960):

Diet digestibility (%) = $[1-(Cr_2O_{3\ \%feed}/Cr_2O_{3\ \%excreta})] \times 100$

Nutrient digestibility (%) = $[1-(\text{nutrient}_{\%excreta}/\text{nutrient}_{\%\ feed}) \times (Cr_2O_{3\ \%feed}/Cr_2O_{3\ \%excreta})] \times 100$

AMEn (kcal/kg) = $GE_{kcal/kg\ feed} - [GE_{kcal/kg\ excreta} \times (Cr_2O_{3\ \%\ feed}/Cr_2O_{3\ \%excreta})] - 8.22 \times \{N_{kg/kg\ feed} - [N_{kg/kg\ excreta} \times (Cr_2O_{3\ \%feed}/Cr_2O_{3\%excreta})]\}$

Statistical analysis, using a 2*3 factorial arrangement of treatments (2 strains*3 diets) in a completely randomized design was used to analyze the data. The data collected were subject to analysis of variance using General Linear Model (GLM) procedures of the Statistical Analysis System (SAS USER'S Guide, 1990).

RESULTS

Performance Trial

The performance and P-values of main effects of diet and strain of bird, and their interaction for the different performance traits are shown in Table 19. Egg weights of hens fed the standard diet was 58.6g and this was significantly higher ($P<0.05$) than egg weights of hens fed both diets 2 and 3 (57.8 vs. 57.5g respectively). For the diets 1, 2 and 3, egg production (88.5, 88.5 and 88.6 %), egg mass (51.9, 51.2 and 50.9 g/h/d) and feed efficiency (1.98, 1.97 and 2.00 g/g egg) were not significantly different ($P>0.05$) from each other. Of the two strains of birds, the Hylines ate significantly more ($P<0.05$) feed than the Shavers (102.1 vs. 94.2 g/h/d, $P=0.0001$). The Hylines however, had significantly lower egg production (87.2 vs. 89.9%, $P=0.002$) egg mass (50.7 vs. 52.0 g/h/d, $P=0.03$) and poorer feed efficiency (2.01 vs, 1.95 g/g egg, $P=0.0004$ respectively). For this trial, initial vs. final body weights of the Hylines and Shavers were 1.61 vs. 1.66 and 1.64 vs. 1.66 kg respectively. Although the Shavers had heavier initial body weights, they gained significantly lower ($P>0.05$) weights than the Hylines throughout the course of the trial (0.02 vs. 0.05kg. respectively) (Table 20). When fed the three diets, independent of strains, hens ate 102.7 g/h/d of diet 1, and this was significantly higher ($P<0.05$) than the amount they ate of both diets 2 and 3 (95.5 and 96.3 g/h/d respectively).

Table 19. Production parameters and P-values of 2 strains of hens fed the control and uplifted diets

Factor	Parameter					
	Feed Intake (g/h/d)	Hen Day Production (%)	Egg Weight (g)	Egg Mass (g/h/d)	Feed Efficiency (g/g/egg)	Mortality[2] (%)
Strain						
Hyline W36	102.1 [a1]	87.2 [b]	58.2	50.7 [b]	2.01 [a]	0.35
Shaver White	94.2 [b]	89.9 [a]	57.8	52.0 [a]	1.95 [b]	0.52
Pooled SEM	*0.46*	*0.57*	*0.22*	*0.39*	*0.01*	
Diet						
Control	102.7 [a]	88.5	58.6 [a]	51.9	1.98	0.00
5% uplift	95.5 [b]	88.5	57.8 [b]	51.2	1.97	0.35
10% uplift	96.3 [b]	88.6	57.5 [b]	50.9	2.00	0.52
Pooled SEM	*0.56*	*0.7*	*0.27*	*0.48*	*0.01*	
Anova Summary	**P-Values**					
Sources of variation						
Strain	0.0001	0.002	0.31	0.03	0.0004	0.65
Diet	0.0001	0.99	0.023	0.329	0.311	0.24
Strain*Diet	0.0001	0.48	0.33	0.66	0.944	

[1] Means within a column with different superscripts are significantly different ($P<0.05$)

[2] Determined using analysis of frequency and count data (SAS, 1989)

Table 20. Weight change of 2 strains of hens fed the wheat and hulless barley based diets.

Strain	Weight increase (kg)
Hyline	0.05[a1]
Shaver	0.02[b]
Pooled SEM	0.008
Diet	
Control	0.02
5% Uplift	0.04
10% uplift	0.04

Anova Summary

Sources of Variation	P-values
Strain	0.01
Diet	0.65
Strain*Diet	0.08

[1] Means within a column with different superscripts are significantly different ($P<0.05$)

Mortality between the two strains and among the three diets was not significantly different (P>0.05), (Table 19). There is a highly significant (P=0.001) strain*diet interaction for feed intake (Table 19, Figure 6).

Balance trial

The effect of diet on apparent excreta amino acid digestibility is shown in Table 21. The average amino acid digestibilities of diets 1, 2, and 3 are 81.4, 82.3 and 83.8% respectively. Birds on diet 3 in all cases had the best digestibility for the individual amino acids and this was always significantly better (P<0.05) than for birds on diet 1, and sometimes equal to or better than birds on diet 2. This is true for all the essential amino acids for poultry except for methionine, where the digestibility of diets 1 and 3 were not significantly different (P>0.05) from each other (82.4 vs. 83.9% respectively). For the amino acids threonine, cystine, arginine and lysine, significant improvements (P<0.05) in digestibility of 3.7, 6.3, 1.6 and 2.7 percentage units respectively were achieved in diet 3 over diet 1. Between the two strains of birds, there were no significant differences (P>0.05) in amino acid digestibility. Apparent ileal digestibility of the various amino acids were not significantly different (P>0.05) for all the diets (Table 22). Generally, the ileal digestibilities of amino acids were higher than the excreta digestibilities. The exceptions are for threonine, proline and leucine where the excreta digestibilities were either slightly higher than or equal to the ileal digestibilities (78.9 vs. 78.2%; 91.3 vs. 90.0%; 86.7 vs. 86.7% respectively).

Figure 6. Overall feed intake of 2 strains of hens fed regular and nutrient reduced diets

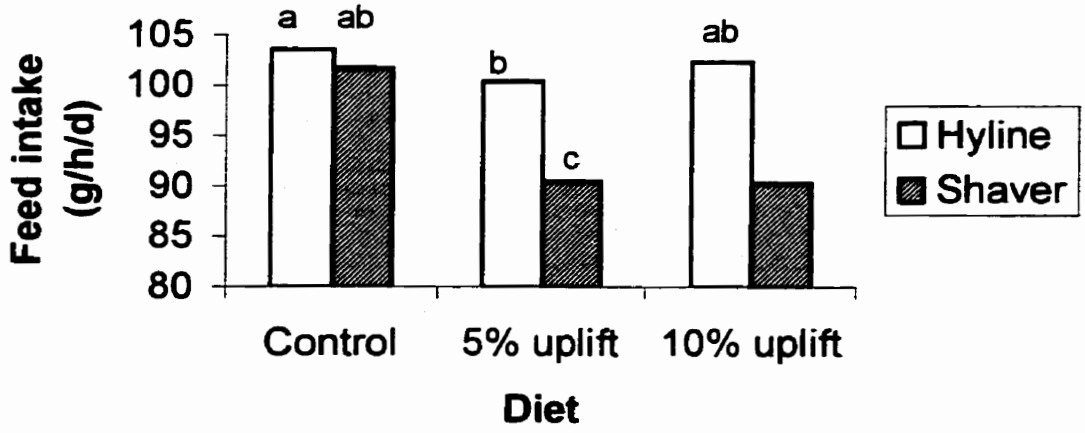


Table 21. Effect of diet on apparent excreta amino acid digestibility (%)

	Treatment				Strain		
Amino acid	Diet 1	Diet 2	Diet 3	*SEM*[3]	Hyl	Sha	*SEM*
VAL[1]	**79.3** [b2]	**78.9** [b]	**82.3** [a]	***0.74***	**80.4**	**79.9**	***0.6***
THR	**76.1** [b]	**76.6** [b]	**78.9** [a]	***0.51***	**76.9**	**77.5**	***0.42***
LEU	**84.3** [b]	**84.6** [b]	**86.7** [a]	***0.35***	**85.3**	**85.2**	***0.28***
ILE	**80.2** [c]	**81.8** [b]	**84.1** [a]	***0.56***	**82.5**	**81.6**	***0.46***
PHE	**84.6** [b]	**85.3** [ab]	**86.5** [a]	***0.52***	**85.3**	**85.6**	***0.42***
HIS	**80.6** [b]	**81.1** [ab]	**82.3** [a]	***0.46***	**81.3**	**81.4**	***0.38***
LYS	**78.8** [b]	**79.2** [b]	**80.9** [a]	***0.58***	**79.7**	**79.6**	***0.47***
ARG	**84.9**	**85.6**	**86.3**	***0.51***	**85.1**	**86.1**	***0.41***
MET	**82.4** [ab]	**80.8** [b]	**83.9** [a]	***0.8***	**81.4**	**83.3**	***0.65***
TYR	78.0[b]	81.8[a]	83.7[a]	*0.87*	81.8	80.6	*0.71*
CYS	78.2[c]	81.2[b]	83.1[a]	*0.46*	80.8	80.9	*0.37*
ASP	80.8[ab]	80.2[b]	81.5[a]	*0.39*	81.1	80.6	*0.32*
SER	83.4[ab]	82.9[b]	84.2[a]	*0.35*	83.4	83.6	*0.28*
GLU	90.3[b]	91.0[a]	91.6[a]	*0.19*	90.9	90.9	*0.16*
PRO	90.2[b]	91.3[a]	91.3[a]	*0.27*	90.6	91.3	*0.23*
GLY	70.4	70.9	74.8	*1.8*	73.2	70.9	*1.47*
ALA	76.5[ab]	75.9[b]	78.1[a]	*0.56*	77.1	76.5	*0.46*
Average	**81.4**	**82.3**	**83.8**	**0.58**	**82.2**	**82.1**	**0.48**

[1] : Means in bold are essential amino acids for poultry

[2] : Means in a row with no common superscripts are significantly different ($P<0.05$)

[3] SEM: Standard error of means

Table 22. Effect of diet on apparent ileal amino acid digestibility (%)

Amino acid	Diet 1	Treatment Diet 2	Diet 3	*SEM*	Strain Hyl	Sha	*SEM*
VAL[1]	**85.8**	**85.1**	**83.4**	***2.86***	**83.9**	**85.7**	***2.33***
ILE	**88.2**	**88.9**	**84.7**	***3.46***	**86.5**	**88**	***2.83***
LEU	**89.4**	**89.5**	**86.7**	***2.66***	**88.1**	**88.9**	***2.17***
THR	**83.1**	**82.3**	**78.2**	***3.89***	**80.7**	**81.7**	***3.18***
PHE	**91.5**	**90.9**	**88.5**	***2.21***	**89.9**	**90.6**	***1.8***
HIS	**89.6**	**89.5**	**86.6**	***2.38***	**88.1**	**89**	***1.95***
LYS	**87.9**	**86.4**	**84.9**	***3.07***	**86**	**86.8**	***2.51***
ARG	**91.9**	**91**	**87.3**	***2.56***	**89.7**	**90.4**	***2.09***
MET	**89.9**	**91.6**	**93.4**	***2.29***	**91.7**	**91.6**	***1.87***
CYS	81.8	84.6	83.8	*2.86*	84.1	82.7	*2.34*
SER	86.2	85.8	81.7	*3.01*	83.9	85.1	*2.46*
GLU	93	93.2	91.1	*1.77*	91.8	93	*1.44*
PRO	92.6	92.7	90	*1.65*	90.9	92.6	*1.35*
TYR	91.4	88.9	85.5	*2.89*	88.7	88.5	*2.36*
ASP	86.5	84.9	82.5	*3.09*	84.2	85	*2.53*
GLY	86.2	85.8	83.2	*2.86*	84.2	85.9	*2.33*
ALA	87.7	87.3	84.2	*2.85*	85.8	87	*2.33*
Average	**88.4**	**88.1**	**85.6**	**2.73**	**87.0**	**87.8**	**2.23**

[1] : Means in bold are essential amino acids for poultry

Table 23 shows the effect of diet on AMEn, and on the digestibility of fat and dry matter. Diet 2 had an AMEn of 2980 kcal/kg and this represented a significant increase ($P<0.05$) of 2.3% over the AMEn of diet 1 (2914 kcal/kg). Diet 3 (3034 kcal/kg) was also significantly higher ($P<0.05$) by 4.4% than diet 1. Fat digestibility was 91.1% for diet 3, and this was significantly higher ($P<0.05$) than diets 1 and 2 (89.8 vs. 89.7% respectively). Dry matter digestibility was also highest for diet 3 (75.2%) and this was significantly better ($P<0.05$) than diets 1 and 2 (72.5 and 73.3% respectively).

Between the two strains, the Hylines demonstrated better ($P<0.05$) dry matter digestibility and were better able to utilize the diets as shown by higher AMEn values. Table 24 shows the excreta content of N and P from the hens fed the 3 different diets. The P output of the of hens on diet 1 was 0.4g/h/d and this was significantly higher ($P<0.05$) than P output of hens fed diets 2 and 3 (0.3 and 0.3g/h/d respectively). This represents a 25% reduction in P content in the excreta. Results of N output from this study showed improvement with enzyme supplemented diets. Hens fed the unsupplemented diets excreted 1.6 g/h/day of nitrogen, and this was significantly higher ($P<0.05$) than the output of hens fed both the 5% and 10% uplifted diets (1.5 vs. 1.4 g/h/d respectively). The ash, P and Ca contents of the tibias are shown in Table 25. There were no dietary or strain effects on any of these values.

Table 23. Effect of diet on apparent excreta digestibility (%) of selected dietary components and on nitrogen corrected apparent metabolizable energy values

Dietary	Treatment				Strain		
Component	Diet 1	Diet 2	Diet 3	*SEM*[2]	Hyl	Sha	*SEM*
Fat	89.8 [b1]	89.7 [b]	91.1 [a]	*0.36*	89.9	90.4	*0.29*
Dry Matter	72.5 [b]	73.3 [b]	75.2 [a]	*0.32*	74.1 [a]	73.2 [b]	*0.27*
Energy (kcal/kg)							
AMEn	2914 [c]	2980. [b]	3033 [a]	*9.45*	2995 [a]	2955 [b]	*7.73*

[1]: Means within a row with different superscripts are significantly different (P<0.05)

[2] SEM: Standard error of the means

Table 24. Excretion of N and P from layers fed experimental diets

Parameters	Treatment				Strain		
	Diet 1	Diet 2	Diet 3	*SEM*[2]	Hyl	Sha	*SEM*
		(g/h/d)					
Nitrogen	1.6 [a1]	1.5 [b]	1.4 [c]	*0.03*	1.6 [a]	1.5 [b]	*0.02*
Phosphorous	0.4 [a]	0.3 [b]	0.3 [b]	*0.01*	0.3	0.4	*0.01*

[1] Means within a row with different superscripts are significantly different (P<0.05)

[2] SEM: Standard error of the means

Table 25. Content of ash, P and Ca in tibia of layers fed the Experimental diets

Nutrients	Treatment				Strain		
	Diet 1	Diet 2	Diet 3	*SEM*	Hyl	Sha	*SEM*[1]
Ash (% DM)	59.1	61.3	60.2	*0.76*	60.4	59.9	*0.62*
P (% of DM)	7.5	7.3	7.4	*0.23*	7.3	7.5	*0.19*
Ca (% of DM)	34.7	34.7	34.1	*0.61*	34.1	34.8	*0.5*

[1] SEM: Standard error of the means

Discussion

The level of phosphorus in laying hen diets is of great economic and environmental concern. The NRC (1994) recommended daily level of NPP for layers consuming 100g of feed per day is 0.25g. In practical layer diets however, much higher levels (0.4g) have been recommended (Leeson and Summers, 1997). Results of the present performance trial indicate that the dietary level of NPP for hens 32 to 52 weeks of age can be lowered from 0.25 to 0.22 % in the presence of exogenous enzymes (which includes 100 phytase units/kg diet) without any detrimental effects on performance. In this experiment, the laying performance of hens fed diet 3 (10% uplift, 0.225% NPP + enzyme) was equal to that of hens fed diet 2 (5% uplift, 0.237% NPP + enzyme). This result implies that diet 2 provides more than sufficient NPP, and that 0.225% of NPP plus exogenous enzymes (diet 3) is adequate to maintain egg production, egg weight, egg mass and feed efficiency. Based on the feed intakes and NPP alone, hens on diets 2 and 3 were provided with 0.226 and 0.216 g NPP/h/day respectively. Additional available P might have been made available to the birds by supplementing enzymes. These results are in agreement with the conclusions drawn by Um et. al. (1999) who stated that dietary reductions in NPP levels from 0.26 to 0.16% and in the presence of exogenous enzymes do not affect laying performance. Results with broilers (Sebastian et. al., 1996) also showed that phytase supplementation of low NPP diets increased ($P<0.05$) the relative retention of total P by 12.2 percentage units compared to a regular diet.

The graded decreases in P levels in this experiment have also indicated that the level of dietary P influences its retention. Excretion of P was reduced by enzyme

supplementation. Compared to the control diet, the reductions in dietary NPP level of diets 2 and 3 were 5% and 10%, while the reductions of P excretion (Table 24) were 25% for both. The differences seem to reflect an improvement in P digestibility by enzyme supplementation.

Results of this trial have also shown that the levels of lysine and methionine in the diets can be reduced to 0.61 and 0.32% respectively, without any adverse effects on production. These reductions are 12.8 and 13.5% lower than the suggested levels for practical layer diets for lysine and methionine respectively (0.70 and 0.37) (Leeson and Summers, 1997).

In this trial supplementing the diets with exogenous enzymes also resulted in reductions in N output. This is in agreement with work done with broilers (Yi et. al., 1996). Um et. al., (1999) however did not find any reductions in N output when P-reduced diets were supplemented with phytase.

Results of this trial showed that there were improvements in apparent excreta amino acid digestibility of most of the essential amino acids (Table 21) for diets 2 and 3, and especially of the amino acids lysine and methionine, to levels either equal to, or above diet 1. Apparent ileal amino acid digestibilities of the different diets indicate that no differences exist between the unsupplemented standard diet (diet 1) and the two uplifted diets (2 and 3). Overall ileal digestibility of diet 3 was slightly lower than that of diet 2. This may be explained by the scarce ileal contents of most of the birds on diet 3 (due to better digestibility) resulting in comparatively more endogenous material being collected and analyzed.

The differences within the excreta amino acid digestibilities may be due to the fact that removal of β-glucan, an energy source for bacteria in the lower intestinal tract,

lowers the amount of endogenous amino acid synthesis, which would overestimate excreta losses and underestimate digestibility. However for the apparent ileal amino acid digestibilities, there is no effect of colon and cecal bacterial synthesis. The differences in excreta amino acid digestibility may also indicate differences in the ability of the birds to digest the different amino acids. In general ileal amino acid digestibilities were higher than the excreta amino acid digestibilities. This difference demonstrates that amino acid metabolism by hindgut microflora in chickens may be substantial and that digestibilities measured at the terminal ileum are more accurate measures of amino acid availability than those measured in the excreta.

There is limited amino acid digestibility data reported in the literature derived from laying hens (Um et al., 1999). It is however likely that the same mechanisms involved with broilers (i.e. cleavage of phytate-protein bonds by phytase enzymes) are also involved in the case of laying hens.

The differences in production observed when hens were fed the three different diets (Table 19) is in general agreement with results obtained by several researchers. Petersen and Sauter (1968) concluded that there was a significant decrease ($P>0.05$) in feed intake when a control diet was supplemented with an exogenous enzyme at a level of 0.2%. Working with layers, Nasi (1988) reported that feed intake of laying hens reduced from 114.8 g/h/d to 113.6 g/h/d, when the experimental diets were supplemented with exogenous enzymes. In a bioassay to determine the effect of phytase on nutrient retention in layers, Leske and Coon (1999) reported a reduction in intake of soybean meal and corn when phytase was added to the feed. In this experiment, diets 2 and 3 were supplemented with an exogenous enzyme cocktail. It is generally a known fact that enzyme supplementation results in significant improvements in ME value for young

chicks (Marquardt et. al., 1994; Rotter et. al., 1990; Friesen et. al., 1992; and Villamide et. al., 1997) and to a lesser extent, laying hens (Aimonen and Nasi, 1991). It is also well known that layers are able to adapt their feed intakes to variations in the dietary energy content (Jeroch and Danicke, 1995; Leeson and Summers, 1997). The reduction in feed intake and resulting comparable performances observed in this trial when diets 2 and 3 were fed (Table 19) can be explained partly by the composition of the diets and partly by improvements in digestibility of certain nutrients and an increase in the AMEn values (Table 23). Several studies have been conducted with broilers in which enzyme supplementation to diets have resulted in higher AMEn (Namkung and Leeson, 1999; Villamide et. al., 1997; Friesen et. al., 1992) improved fat digestibilities (Classen et. al., 1985; Friesen et. al., 1992) and phosphorous digestibilities (Ahmad et. al., 2000; Sebastian et. al., 1996).

Generally it is acknowledged that the increase in the nutritive value following the addition of enzymes containing β-glucanase and phytase to diets for poultry is a direct result of a reduction in viscosity due to ß-glucanase and a cleavage of the phytate-nutrient complexes by phytase enzyme. The net result is that digestive enzymes have easier access to ingested nutrients and also end products of digestion are better assimilated.

A reason for the absence of any significant differences in P, Ca and particularly ash contents of the tibias (Table 25) may be explained by the observations of Um et. al. (1999), who stated that, graded decreases in P level and phytase supplementation may not significantly influence ash contents of tibias in laying hens because bone size may already have been established at the time test feeds are introduced.

The differences that were observed (Table 19) in production performance between the 2 strains of birds can best be explained by their genetic make-up. In an experiment to study the factors influencing laying hen response to enzyme supplementation, Ely (1963) concluded that one of the factors that govern the degree of response of hens to enzyme supplementation was the genetic background of the hen. It is therefore possible in this experiment that genetic differences allow the Shavers to eat less feed and yet produce better than the Hylines. For this trial, initial vs. final body weights of the Hylines and Shavers were 1.61 vs. 1.66 and 1.64 vs. 1.66 kg respectively. The Hyline birds had lower initial body weights at the start of the experiment their weight increase over the course of the trial was significantly ($P<0.05$) greater than that of the Shavers, resulting in equal final mature body weights at the end of the trial.

In summary results of the present experiment indicate that the dietary levels of some of the major nutrients (particularly P, lysine and methionine) for hens 32-52 weeks of age, can be lowered by 10% from 0.25, 0.68 and 0.36% respectively to 0.23, 0.61 and 0.32% respectively in the presence of the appropriate enzymes, without any significant reductions in egg production, egg mass and feed efficiency. Simultaneous reductions in the output of N and P can also be achieved by feeding N and P-reduced diets and exogenous enzymes to layers. Energy contents of the diets were also improved with enzyme supplementation.

5. GENERAL DISCUSSION

Exogenous enzymes are widely used in feed formulation as dietary supplements to help eliminate or reduce the antinutritional factors in cereal-based diets. In poultry, several benefits have been reported in the literature through supplementing exogenous enzymes to diets for laying hens and broilers. Some of these benefits include improvements in growth rate, feed intake and feed efficiency for broilers (Friesen et. al., 1992) and improved egg production, feed efficiency and percent hatch for layers (Nelson and Hutto, 1958). In addition, Um et. al., (1999) reported reductions in excretory output of some essential minerals in laying hens. It is well known that the use of exogenous enzymes is most effective during the first few weeks of a chicks life due to the fact that young chicks have a less developed digestive tract and thus less digestive enzymes (Krogdahl, 1985). However adult birds, although having a more developed digestive system, could still be limited in their ability to breakdown NSP's (Campbell and Campbell, 1989). Limited research concerning the value of enzyme supplementation to diets particularly, barley-based diets for laying hens has been published. What literature there is, is sometimes contradictory. While some published data report no improvement (Berg and Bearse, 1958) others like Nelson and Hutto (1958) reported improvements when barley based diets were supplemented with exogenous enzymes and fed to laying hens. Also most of the published data involving barley-based diets for laying hens were published long age. There is an increasing need to explore alternative and inexpensive, but yet high quality feedstuffs as a replacement for wheat in laying hen rations. These experiments were therefore conducted to find the replacement value of hulless barley either with or without exogenous enzymes in laying

hens diets, and to determine if enzyme supplementation would lead to a reduction in the excretion of nutrients in the feces. In the first experiment five diets were formulated without enzymes from one wheat and four hulless barley (Silky, Falcon, Gainer and Dawn) cultivars and fed to 2 strains of hens (Hyline W36 and Dekalb Sigma). Results showed that certain hulless barley cultivars can effectively replace wheat in diets for laying hens. Hulless barley cultivars Silky, Gainer, and Dawn can effectively replace wheat without any loss of egg mass, while all the cultivars can replace wheat without any effect on feed efficiency or feed intake. Between the 2 strains of hens, the Hylines ate significantly less feed than the Dekalbs, while maintaining equal production as the Dekalbs.

When each of these diets were formulated either with or without enzymes and fed to laying hens (Shaver White) (trial 2), results showed that enzyme supplementation resulted in significant improvements in egg mass and feed efficiency, and small but insignificant increases in egg production and egg weight over the unsupplemented diets. Feed intake was also reduced upon supplementation with exogenous enzymes.

The results of trial 1 suggested inherent differences in physical and chemical properties of the hulless barleys, resulting in different production performances when fed to the birds. These are in agreement with results of studies conducted by Andersson et. al. (1999a) who concluded that, genotypic differences may cause barleys to have different concentration of either starch in their kernels, or β-glucans in their cell walls, all of which can influence production performance when fed to birds. Jeroch and Danicke (1995) concluded that cultivar differences exist in chemical compositions and nutrient availabilities in barleys, and these may have an impact on feeding values when fed to poultry. The results have also demonstrated that quality variations in the hulless barley cultivars can be reduced through exogenous enzyme application.

Results of manuscript 2 have also demonstrated that the dietary levels of some major nutrients (P, lysine and methionine) for laying hens, can be lowered by up to 10% in the presence of the appropriate enzymes without any significant reductions in egg production, egg mass and feed efficiency. In addition, by feeding N and P-reduced diets and exogenous enzymes, excreta output of these nutrients can be reduced by up to 12.5 and 25% respectively. This result agrees with work done with layers by Um et. al., (1999). They fed reduced levels of NPP to laying hens, in the presence of enzymes and achieved concomitant reductions in excreta output of nutrients, and improved performances.

6. CONCLUSIONS

1. Certain hulless barley cultivars effectively replaced wheat in diets for laying hens without compromising egg production, egg mass and feed efficiency.
2. Enzyme application in hulless barley-based diets for hens removed some of the variability in feeding quality that existed among some hulless barley cultivars.
3. Exogenous enzyme application resulted in significant improvements in egg mass and feed efficiency compared to the unsupplemented diets but insignificant improvements in egg production and egg weight when fed to laying hens.
4. The dietary levels of P, lysine and methionine for hens was lowered by up to 10% in the presence of exogenous enzymes without any significant reductions in egg production, egg mass and feed efficiency.
5. Excreta digestibility of the essential amino acid lysine was improved from 78.8% to 80.9% when the dietary lysine level was reduced by 10% in the presence of exogenous enzymes.
6. Excreta digestibility of methionine was not affected when the dietary level was reduced by 10%, in the presence of the appropriate exogenous enzymes.
7. Reductions in the output of N and P by 12.5 and 25% respectively were obtained when NPP levels in the diets were reduced from 0.27 to 0.25% and 0.25 to 0.225% respectively in the presence of exogenous enzymes.

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APPENDICES

APPENDIX

Table 1A Performance traits of layers fed different grain based diets with (+) or without (-) exogenous enzyme supplementation

Performance Trait	Control		Silky		Falcon		Gainer		Dawn		S.E
	-	+	-	+	-	+	-	+	-	+	
Egg prod. h.d %	84.8^{a}	83.8^{ab}	82.0^{ab}	84.5^{a}	81.1^{b}	83.2^{ab}	84.5^{a}	85.1^{a}	84.8^{a}	84.8^{a}	1.05
Egg wt. (g)	56.9^{bcd}	57.4^{abcd}	57.3^{abcd}	57.4^{abcd}	56.6^{cd}	58.1^{abc}	58.7^{ab}	58.8^{a}	56.1^{d}	56.9^{bcd}	0.57
Egg mass (g/d)	47.6^{bcd}	47.8^{bc}	46.8^{cd}	48.6^{abc}	44.9^{d}	47.9^{abc}	49.9^{ab}	50.5^{a}	46.8^{cd}	47.7^{bc}	0.84
Feed int.(g/h/d)	99.9	98.6	98.7	98.9	99.1	99.1	99.3	98.1	98.9	98.5	0.77
Feed efficiency (g/g egg)	2.10^{bc}	2.09^{bc}	2.17^{ab}	2.14^{b}	2.24^{a}	2.11^{bc}	2.03^{cd}	1.96^{d}	2.14^{b}	2.09^{bc}	0.03

^{abc} Means within a row with different superscripts are significantly different