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# Behaviour of Hens Fed a Glycanase Enzyme in a Wheat and Triticale Diet

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**Abstract:** Caged laying hens (26-38 weeks-of-age) were fed a wheat and triticale diet with and without a commercial glycanase enzyme. Various pecking and behavioural activities of hens were monitored at 28 and 36 weeks, including the measurement of excreta pH, dry matter and moisture content. There was little influence of enzyme supplementation on hen behaviour, although there was a significant reduction ($P<0.05$) of trampling and increase ($P<0.05$) in stereotype head flicking observed in hens fed enzymes. A stepwise procedure indicated 38% of the variation associated with excreta pH was explained by excreta dry matter, excreta moisture, light intensity, receiving tail and body pecks. Receiving body pecks, giving vent pecks, excreta moisture, trampling, excreta pH, receiving tail pecks and head flicks were the variables most closely associated with light intensity. This study only identified a few changes in hen behaviour from feeding enzymes. More comprehensive investigations are required.

**Key Words:** Laying hens, enzymes, behaviour, excreta pH, excreta moisture

## Introduction

At high dietary concentrations, the feeding value of cereals, particularly those with low AME (Apparent Metabolisable Energy) are reduced and result in poor bird performance (Choct *et al*., 1996). This is largely attributed to the physio-chemical properties of the non-starch polysaccharides (NSP), which are believed to interfere with the digestion and absorption processes of the small intestine depressing the availability of nutrients. Diets rich in rye and wheat (Bedford and Classen, 1992; Choct and Annison, 1990) and those with added soluble NSP (Fengler and Marquardt, 1988) show high intestinal viscosity, which has a depressing effect on bird performance. The viscous nature of the soluble NSP increases bulkiness and viscosity of digesta reduces the rate of diffusion of substrates (Fengler and Marquardt, 1988) and slows digesta flow rate (Salih *et al*., 1991) and increases microbial populations in the small intestine (Feighner and Dashkevicz, 1988; Choct *et al*., 1996) with potential implications for the bird's welfare.

There are strong possibilities that the concentrated supply of NSP and its fermentation may lead to an elevation in gut acidity, which may have detrimental implications to the health and welfare of the bird. In humans and dogs, diet problems variously known as "food allergies" and "food intolerances" are common and several studies have associated the malabsorption of carbohydrates with osmotic diarrhoea and low pH of faeces (Holtug *et al*., 1992). Hindgut fermentation and gut acidity could be a key factor in health and welfare problems in animals (Pluske *et al*., 1997). Acidosis in poultry was reviewed by Taylor (2002). It is possible that some of the behavioural vices in birds, such as, vent pecking might also be related to physiological and metabolic changes associated with acidosis in the hindgut. For example, European laying strains with a 50% inclusion rate of wheat in their diets had poorer plumage than hens fed 25% wheat due to feather pecking (Abrahamsson *et al*., 1996). The use of enzymes in the bird's diet has been successful in reducing the viscosity of the digesta and has lead to an improvement in feed conversion efficiency (Bedford, 1997), but little is known on the influence of enzymes on bird behaviour. Hartini *et al*. (2001) however, reported mortality from cannibalism was similar in birds fed high soluble fibre diets with or without enzyme supplementation. The objective of this study was to test the hypothesis that a commercial glycanase enzyme could be used in a layer diet to modify hen behaviour and ameliorate acidosis.

## Materials and Methods

**Rationale:** The rationale for the trial was to feed a glycanase enzyme in a wheat and triticale diet to laying hens during the peak egg production period and compare behaviour and excreta variables with birds not provided enzyme supplementation. Observations were made on hens when activities such as egg collection, manure removal and manual feeding of birds was occurring. During these periods, the light intensity in the shed was increased above 10 lux so that staff could carry out these activities. Behavioural interactions between birds were more likely to occur during these disturbances in the shed and role of enzymes on behaviour could be more clearly demonstrated. Fear, aggression, stereotype and other pecking activities were monitored. Excreta pH, dry matter (DM) and moisture content was measured as indicators of digestive processes.

**Birds and management:** The study was conducted in the cage layer facility at the poultry unit at the Pig and Poultry Production Institute located at Roseworthy Campus, University of Adelaide, 60 km north of Adelaide and 10 km east of Gawler in South Australia. The facility comprised two rows of single tier cages, with a row of incandescent globes fixed at roof level above each row of cages. The globes were 100 watt placed 2.5 m apart and 3 m above cages. At 18 weeks-of-age 480 laying birds from a commercial Hyline strain were housed five per cage in 96 Harrison 'Welfare' back-to-back single tier cages (each 500 mm wide by 545 mm deep; 545 $cm^2$/bird) in a fan ventilated insulated laying shed with louvered windows. A 16 h light program was provided to birds with lights on at 0400 h and off at 2000 h. Light intensity was kept at 10 lux (measured at feed trough level in centre of shed) and increased to 20-30 lux when staff conducted routine activities such as feeding, egg collection and operation of the manure belt. Birds had *ad libitum* access to mash feed in hoppers and water from nipple drinkers.

**Diet formulation:** The wheat and triticale diets (with and without a commercial glycanase enzyme) were formulated to provide similar energy (11.4 MJ/kg) to nutrient ratios for protein (16.5%),

Table 1: Effect of diet on excreta characteristics and behaviour of hens at 28 weeks-of-age

| Variable | Treatments | | | | |
|---|---|---|---|---|---|
| | Wheat | Wheat + E1 | Triticale | Triticale + E2 | LSD. |
| Trampled | 0.167 | 0.0833 | 0 | 0.208 | NS |
| Trampling | 0.125 | 0 | 0 | 0.125 | NS |
| Head pecks received | 0.667 | 0.417 | 0.542 | 0.667 | NS |
| Head pecks given | 0.542 | 1.167 | 0.208 | 0.708 | NS |
| Body pecks received | 0.292 | 0.0375 | 0.042 | 0.125 | NS |
| Body pecks given | 0.458 | 0.167 | 0.333 | 0.125 | NS |
| Tail pecks received | 0 | 0.080 | 0 | 0 | NS |
| Tail pecks given | 0.041 | 0 | 0 | 0.040 | NS |
| Vent pecks received | 0 | 0 | 0.041 | 0 | NS |
| Vent pecks given | 0 | 0.125 | 0 | 0 | NS |
| Cage pecking | 2.125 | 0.0958 | 0.792 | 2.083 | NS |
| Toe pecks | 0 | 0.333 | 0 | 0.083 | NS |
| Air pecks | 0.125 | 0.042 | 0.500 | 0.042 | NS |
| Head flicking | 1.542b | 5.330a | 0.292b | 1.750b | 3.407 |
| Light intensity (lux) | 15.77b | 28.066a | 18.875ab | 24.45ab | 10.23 |
| Excreta DM | 93.43b | 93.75ab | 93.84ab | 94.20a | 0.53 |
| Excreta moisture (%) | 67.19 | 64.51 | 65.93 | 66.18 | NS |
| Excreta pH | 6.05 | 6.11 | 6.05 | 6.11 | NS |

E1=glycanase enzyme 80 ppm, E2=glycanase enzyme 100 ppm, LSD=least significant difference, NS=not significant. Means within rows and comparisons followed by the same letter are not significantly different at P<0.05. Means in rows for behaviour variables are means of incidences measured over 10 min.

linoleic acid (1.13%), essential amino acids (methionine 0.38%; lysine 0.77%), calcium (3.77%), total (0.62%) and available (0.43%) phosphorus, sodium (0.15%) and chloride (0.16%).

**Wheat Diet composition:** The ingredient composition for the wheat diet was wheat (75.75%), soybean meal (6%), meat and bone meal (8.5%) limestone powder (7.0%) limestone chips (1%), tallow (0.5%), sunflower oil (0.5%), sodium bicarbonate (0.15%), L-lysine (0.17%), DL-methionine (0.13%), mill run (0.156%), vitamin mineral premix (0.10%) choline chloride (0.04%) and yolk colourant (0.004%). Commercial glycanase (0.008%) was added to the treatment diet.

**Triticale diet composition:** The ingredient composition for the triticale diet was triticale (77.85%), soybean meal (4.2%), meat and bone meal (8%) limestone powder (7.1%) limestone chips (1%), tallow (0.3%), sunflower oil (0.7%), sodium bicarbonate (0.20%), L-lysine (0.20%), DL-methionine (0.13%), mill run (0.146%), vitamin mineral premix (0.10%) choline chloride (0.04%) and yolk colourant (0.004%). Commercial glycanase (0.010%) was added to the treatment diet.

**Experimental Design and Analysis:** All birds received a standard layer mash diet from housing at 18 weeks until the start of the experiment at 26 weeks. For the experimental phase (26-38 weeks), 4 diets were fed, comprising the wheat control, wheat with glycanase enzyme, triticale control and triticale with glycanase enzyme. A randomised block design was used for allocation of treatments with 12 replicates per treatment. Each replicate comprised 10 birds housed 5/cage in two adjacent cages. Base SAS software (SAS Institute, 1988) was used to perform an analysis of variance (by GLM procedure) to determine the effects of diet on behaviour and excreta variables. Duncan's Multiple Range Test was used to separate treatment means. The 'proc corr' procedure in SAS was used to determine the correlation between behaviour variables and light intensity. The SAS 'step-wise' regression was used to determine the strength of the relationships between selected excreta variables, behaviour and light intensity.

**Behaviour:** Behaviour of hens was monitored at 28 and 36 week-of-age using the procedure of Lehner (1996). From each replicate, 2 birds were randomly selected and each bird was observed for 10 min. The observer slowly approached the cage, and measured the light intensity (Gossen Panlux Light Meter) at feed trough level and 1 min later started the observations and recording. Observations were conducted from a distance of 0.75-1.0 m depending on the width of the aisle. All the observations were carried out over four days while activities such as egg collection, manual feeding of birds and operation of the manure belt were being carried out. All trampling, stereotype pecks and head, body, tail and vent pecks received or delivered were recorded.

**Excreta measurements:** Fresh excreta was collected for pH, moisture content and dry matter (DM) analysis. Excreta was collected with a clean scaping tool over a 4 h period after the disposal of the day's droppings, from a 400cm$^2$ section on the manure belt under the centre of replicate cages. Samples were weighed and stored in an icebox and transferred to an oven set at 60 °C for overnight drying to determine fresh excreta %. Dried samples were then ground and 2 g duplicate samples were oven dried at 100 °C to determine the DM percentage (AOAC, 1980). Excreta pH analysis was determined by diluting 10 g excreta samples in deionised water, adding a glass bead and mixing by vortex. The pH was measure using a glass calomel pH probe on a Selby pH meter (AOAC, 1980).

## Results

**Light intensity:** The light intensity at feed trough level for the birds on the wheat diet with enzyme was significantly higher (P<0.05) compared to wheat and triticale diets at 28 weeks (Table 1) but treatments were not different at 36 weeks of age (Table 2).

**Diet and behaviour:** At 28 week-of-age birds provided the wheat diet with enzyme (Table 1) had a higher incidence (P<0.05) of head flicking but there was no significant difference observed at 36 weeks of age. Trampling was significantly lower (P<0.05) for

Table 2: Effects of diet on excreta characteristics and behaviour of hens at 36 weeks-of-age

| Variable | Treatments | | | | |
|---|---|---|---|---|---|
| | Wheat | Wheat + E1 | Triticale | Triticale + E2 | LSD. |
| Trampled | 0.042 | 0.083 | 0.292 | 0.25 | NS |
| Trampling | 0.083ab | 0a | 0.25b | 0.0417a | 0.192 |
| Head pecks received | 1.042 | 0.625 | 0.25 | 1.125 | NS |
| Head pecks given | 0.625 | 1.583 | 1.083 | 0.75 | NS |
| Body pecks received | 1.25 | 1.792 | 1.667 | 2.458 | NS |
| Body pecks given | 1.5 | 1.458 | 2.667 | 0.75 | NS |
| Tail pecks received | 0.583 | 0.542 | 0.583 | 1.1667 | NS |
| Tail pecks given | 0.50 | 0.417 | 0.75 | 0.667 | NS |
| Vent pecks received | 0.167 | 0.083 | 0.208 | 0.25 | NS |
| Vent pecks given | 0 | 0 | 0 | 0 | NS |
| Cage pecking | 2.542 | 1.292 | 1.917 | 1.25 | NS |
| Toe pecks | 0.125 | 0 | 0 | 0.25 | NS |
| Air pecks | 1.292 | 0.50 | 0.708 | 0.333 | NS |
| Head flicking | 1.375 | 2.333 | 1.583 | 1.958 | NS |
| Light intensity (lux) | 21.146 | 25.542 | 23.104 | 25.562 | NS |
| Excreta DM | 96.378 | 96.467 | 96.257 | 96.312 | NS |
| Excreta moisture | 66.433 | 65.842 | 67.767 | 67.8 | NS |
| Excreta pH | 6.17 | 6.21 | 6.12 | 6.2 | NS |

E1=glycanase enzyme 80 ppm, E2=glycanase enzyme 100 ppm, LSD=least significant difference, NS=non significant. Means within the same rows and comparisons followed by the same letter are not significantly different (P>0.05). Means in rows for behaviour variables are means of incidences measured over 10 min.

the birds fed enzyme treated diets than control fed birds during week 36 (Table 2). For the other behaviour activities, no significant differences were found.

**Diet and excreta:** Excreta DM of the wheat diet was significantly higher ($p<0.05$) than the triticale diet with enzyme at 28 weeks of age (Table 1) but no treatment effects were observed at 36 weeks (Table 2). Enzyme diets showed a small increase ($p>0.05$) in pH relative to control diets. Excreta moisture content was not significantly different between the diets.

**Correlations:** Because of the lack of significant change in behaviour and excreta variables associated with the dietary treatments it was decided to pool the data and conduct a correlation analysis between the incidences of behaviours and light intensity (Table 3). A correlation was found between tail pecks received and given; cage pecking and air pecking; body and vent pecks received; trampling and trampled and between some pecking activities and light intensity including body pecks received and vent pecks given (Table 3).

**Stepwise regression:** Because of the significant correlations found between behaviour variables the data was pooled and a stepwise procedure conducted to find which of all the other independent behaviour and excreta characteristics could be included in models for light intensity and excreta pH. The following 6 variables, received body peck ($R^2$=0.12), give vent peck ($R^2$=0.22), excreta moisture ($R^2$ = 0.31), trampled ($R^2$ = 0.34), excreta pH ($R^2$=0.34), receive tail peck (R =0.38) and head flick ($R^2$=0.41) were selected in order of importance for their association with light intensity and collectively explained 41% of the variation associated with light intensity. None of the other variables made any substantial improvement in the explained variation.

Likewise for the stepwise procedure for the variable excreta pH, the 5 variables selected were excreta dry matter ($R^2$=0.17), excreta moisture ($R^2$=0.26), light intensity (R =0.31), receive tail peck ($R^2$=0.34) and receive body peck ($R^2$=0.38). Collectively these variables explained 38% of the variation associated with excreta pH. None of the other variables made any further contribution to the explained variation.

## Discussion

**Light Intensity:** The results obtained for light intensity raises some interesting questions. It was shown that despite the randomization process used to allocate the experimental diets, the light intensity observed was significantly higher for the wheat diet at 28 weeks but not at 36 weeks. The question must be asked why there was a difference in the result obtained at 28 and 36 week-of-age? The shed had 2 small louvered windows (1 x 0.6 m) at both ends of the facility to allow airflow through the shed when the positive ventilation system turned on or off depending on thermistor control. When the fans were running, the flaps on the window would open slightly to allow ventilation through the shed. As this occurred light intensity for birds within the vicinity of the windows would increase. The amount of light coming into the shed depended on the whether it was a cloudy day or clear day. In addition when the dimmer control switch was adjusted by staff there may have been a small variation in the final light intensity achieved depending on the setting of the dimmer. Together these factors probably resulted in the variation in light intensity observed at 28 and 36 weeks in this study. The key issue, however which is raised by the study, is the need for ensuring light intensity for each replicate is similar. This would be difficult to achieve for any experiment unless special light fittings and light deflectors are installed. The finding on light intensity in this study raises the possibility that for many studies may be flawed because variations in light intensity are known to influence laying hens in a number of ways (Appleby, *et al.*, 1992). It is recommended that light intensity of individual replicates is measured in all experiments and adjustment made to the lighting system to remove this variation in experiments. In the commercial industry attempts should also be made to keep the light intensity the same for all birds. Our experiment has shown that small variations in light intensity can account for an increase in pecking activity which could lead to cannibalism (Glatz, 2001).

Table 3: Simple correlation coefficients (r) between behavioural activities measured at 28 and 36 weeks-of-age

| Variable | Trampled | Trampling | Head pecks | | Body pecks | | Tail pecks | | Vent pecks | | Cage pecking | Toe pecks | Air pecking | Head flicking | Light intensity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | received | given | received | given | received | given | received | given | | | | | |
| Trampled | | 0.312** | -0.034 | 0.11 | 0.108 | -0.039 | -0.083 | -0.022 | 0.06 | -0.066 | 0.099 | 0.151 | -0.09 | -0.034 | -0.159 |
| Trampling | | | -0.023 | -0.096 | 0.0126 | 0.128 | -0.095 | -0.001 | -0.077 | -0.049 | 0.049 | -0.78 | -0.047 | 0.088 | -0.045 |
| Head pecks received | | | | 0.087 | 0.125 | 0.088 | -0.102 | -0.05 | -0.053 | -0.045 | 0.033 | -0.081 | 0.119 | -0.061 | 0.041 |
| Head pecks given | | | | | 0.048 | 0.210* | -0.086 | 0.015 | 0.005 | 0.034 | 0.072 | -0.063 | 0.035 | -0.113 | 0.098 |
| Body pecks received | | | | | | 0.010 | 0.227** | 0.188 | 0.239* | -0.074 | 0.092 | 0.046 | 0.119 | -0.034 | 0.357*** |
| Body pecks given | | | | | | | 0.198 | 0.065 | 0.004 | -0.006 | -0.017 | -0.094 | 0.112 | -0.071 | 0.190 |
| Tail pecks received | | | | | | | | 0.366*** | 0.268** | -0.051 | 0.022 | 0.059 | 0.241* | 0.031 | 0.139 |
| Tail pecks given | | | | | | | | | 0.203** | -0.045 | 0.159 | -0.072 | 0.182 | 0.035 | 0.095 |
| Vent pecks received | | | | | | | | | | -0.03 | 0.149 | 0.114 | 0.210* | -0.086 | -0.034 |
| Vent pecks given | | | | | | | | | | | 0.054 | -0.031 | -0.057 | -0.082 | 0.284** |
| Cage pecking | | | | | | | | | | | | -0.111 | 0.342*** | -0.106 | -0.085 |
| Toe pecks | | | | | | | | | | | | | -0.091 | -0.076 | 0.00 |
| Air pecks | | | | | | | | | | | | | | -0.098 | 0.041 |
| Head flicking | | | | | | | | | | | | | | | 0.085 |

* = P<0.05, ** = P<0.005, *** = P<0.001

**Pecking:** Our hypothesis that enzymes reduce pecking activities was not supported by the result. The trial didn't show any differences in pecking activities between enzyme treated diets and control. In this trial, while adequate number of hens and replications were used observations were only carried out for 10 min (at 26 and 38 weeks-of-age). This is a tiny fraction of a bird's daily activity, probably reducing the sensitivity that was needed to detect significant results. It is known light intensity increases pecking activities (Appleby *et al*., 1992) and in this trial a correlation was observed between body pecks received and light intensity. The variation in light intensity accounted for changes in body pecking, vent pecking, excreta moisture and pH observed between birds.

Negative correlations have been established between underlying fearfulness and production (Jones and Hughes, 1986). Considering the anti-nutritive activity of a high grain diet is partly mediated by microbial interaction which enzymes can reverse (Choct *et al*., 1996), it is possible that enzymes ameliorate the stressful internal stimuli. It is believed the reduction of fear is reflected in adaptive or displacement behaviour. In this study, head flicking was the only significant stereotypic behaviour found in enzyme treated wheat (P<0.05). Moreover, its correlation with almost all the pecking activities was negative, though small. Head flicking has been characterised as a coping mechanism and as a symptom of being "better off" (Duncan, 1970; Mauldin and Siegel, 1979) and is by far the most prominent behaviour in White-Leghorns in battery cages (Webster and Hurnik, 1990). The significant (P<0.05) reduction in trampling observed during the second period supports this view, as trampling is associated with panic and fear (Mills and Faure, 1990).

Correlation analysis showed toe pecking was negatively correlated to pecking activities similar to head shaking while cage pecking is positively correlated, which could probably explain the difference. Air pecking is another stereotypic behaviour strongly correlated to cage pecking. In confined birds, pecks are generally categorised as agonistic or stereotypic (Duncan and Wood-Gush, 1972; Shea, *et al*., 1990) and their relative margin of occurrence in the presence of stress might reflect a coping mechanism to improve their welfare (Mauldin and Siegel, 1979) or it could simply be a mild response to stress.

The aetiology of feather pecking amongst domestic fowl is not fully understood, in spite of the fact that various possible causative factors have been investigated. Together with many environmental and management factors, diet; especially deficiency of certain nutrients, have been associated with feather pecking, including fibre (Hartini *et al*., 2001) methionine (Neal, 1956), and arginine (Siren, 1963). Shea *et al*. (1990) reported a significant reduction of feather pecking (P<0.05) in birds fed a higher tryptophan diet. Most of these studies however, were based on an earlier hypothesis attributing feather pecking to the need for some specific nutritional substance rather than a physiological influence. Although much has been known for quite some time about the anti-nutritive physiological activity of high cereal grain diet and NSP, there has not been much investigation done about its possible relationship with feather pecking and poultry welfare and the possible benefit of using enzymes in diets.

One of the widely investigated major effect of feather pecking is its impact on plumage condition (Hughes and Michie, 1982; Hughes, 1985) and plumage condition could therefore be taken as common denominator for comparing the incidences of feather pecking in enzyme treatments. Studies by Al Bustany and Elwinger, (1988) however, reported no significant differences in plumage condition between enzyme treated and high grain control diets.

**Excreta variables:** Our hypothesis of enzymes reducing pecking activities was based on the assumption that there would be a significant change in acidosis. However, no significant effect was found for all the variables measured. This result disagrees with the findings of Choct *et al*. (1996) who demonstrated a significant reduction in excreta moisture content. However, Choct *et al*. (1996) also noted, while enzymes decreased fermentation in the small

intestine, they also lead to a corresponding increase in the caecal fermentation and production of VFA at the same time. Although his study involved the use of extract NSP, it raises the question whether enzymes can reduce intestinal gut acidity along the whole gut tract. Many of the disease conditions and behavioural abnormalities seen in association with high grain diet could be related to any part of the tract. In this trial, only 38 % of the variation in excreta pH could be explained by the pecking and other excreta variables.

The pathogenesis of disease conditions or behavioural changes, however, may not be limited to gut acidity alone. Gut acidity affects the composition of gut micro flora (Hungate *et al*., 1952) and it is possible endotoxin from dead microbes may enter the circulatory system. Moore *et al*. (1979) reported a significant increase in intercaceal endotoxin level in horses corresponding to a dramatic pH drop. Dougherty *et al*. (1975) detected endotoxin flow in the circulatory system of sheep and cattle after feeding a high grain diet. Although the latter's study was preliminary to suggest any causative relationship for the many disease conditions associated with high grain consumption, it is evident that there is some connection between endotoxin and the pathogenesis of certain of the behavioural changes. Studies by Godfrey *et al*. (1995) have shown that virginiamycin can effectively control acidosis. The successful management of acidosis using antibiotics or probiotics is a common practice and clear indication that reducing the proliferation of lactic acid producing microbes is more important.

This study has shown some potential benefits for hen behaviour from feeding enzymes, which need further investigation. Although much has been known about the anti-nutritive activity of NSP, less is known on its possible implication of high grain diets on bird behaviour. Taylor (2002) reported small changes in lactic acidosis that could lead to negative effects in the gut which could impact on production responses. The difficulty faced is grains vary widely in protein and energy contents, depending on variety and season. Likewise there is a vast array of commercial enzyme products available, which vary in ability to enhance nutritional value of grains. Further research has to ascertain the possible mechanism and interaction of diet and enzymes and effects of gut microbes and hindgut function on bird behaviour. This may require extensive experimentation with a range of enzymes and diets and detailed behavioral observations on hens extending for the full laying period.

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