Assessment of caecal parameters in layer hens fed on diets containing wheat distillers dried grains with solubles

G. A. WHITE, P. J. RICHARDS, S. WU, K. H. MELLITS, AND J. WISEMAN

Division of Animal Sciences, School of Biosciences, Sutton Bonington Campus, University of Nottingham, Loughborough, Leicestershire, LE12 5RD, UK, and Division of Food Sciences, School of Biosciences, Sutton Bonington Campus, University of Nottingham, Loughborough, Leicestershire, LE12 5RD, UK

Abstract

1. There is much interest in quantifying the nutritional value of UK wheat distillers dried grains with solubles (W-DDGS) for livestock species. A study was designed to evaluate caecal parameters (pH, short chain fatty acids (SCFAs) and bacterial diversity) in layer hens fed on balanced diets containing graded concentrations of W-DDGS.

2. A total of 32 layer hens (Bovans Brown strain at 27 weeks of age) were randomly allocated to one of 4 dietary treatments containing W-DDGS at 0, 60, 120 or 180 g/kg. Each treatment was fed to 8 replicate individually housed layer hens over a 5-d acclimatisation period, followed by a 4-week trial. Individual feed intakes were monitored and all eggs were collected daily for weeks 2, 3 and 4 of the trial, weighed and an assessment of eggshell “dirtiness” made. All hens were culled on d 29 and caecal pH and SCFAs measured. Polymerase chain reaction denaturing gradient gel electrophoresis of the bacterial 16 S rDNA gene was used to assess total bacterial diversity of luminal caecal content from hens fed the 0 and 180 g W-DDGS/kg diets. Unweighted pair group method with arithmetic mean (UPGMA) dendrograms were generated from DGGE banding patterns.

3. Increasing W-DDGS dietary concentrations resulted in a more acidic caecal environment. Caecal SCFAs were unaffected by diet aside from a quadratic effect for molar proportions of isobutyric acid. Diversity profiles of the bacterial 16S rRNA gene from luminal caecal contents were unaffected by W-DDGS inclusion.

4. The results of the current study suggest that W-DDGS can be successfully formulated into nutritionally balanced layer diets (supplemented with xylanase and phytase) at up to 180 g/kg with no detrimental effects to the caecal environment.

INTRODUCTION

With increasing global demand for the production of cleaner, renewable sources of energy, there is considerable interest in the production of ethanol from fermentation of cereal grains. This interest has led to significant expansion in the bioethanol industry over recent years, particularly in the US, although there has also been support for biofuel production from the European Union (Directive, 2003/30/EC). As a result of the greater production of bioethanol, there has also been a concurrent increase in the amount of co-products produced from the process that are entering the market. These co-products are generating much debate in terms of their potential nutritional value as a feed raw material for livestock. Wheat distillers dried grains with solubles (W-DDGS) is the main co-product produced from the UK bioethanol industry. Despite being potentially a rich source of nutrients, there are limitations regarding the general use of DDGS in animal diets; of particular note is the high probability of heat damage during the production process, with concomitant effects on lysine content and digestibility (Ergul et al., 2003; Fastinger et al., 2006).

Another limitation is the high fibre content of W-DDGS. This has traditionally limited W-DDGS
dietary inclusion mainly to ruminants. However, there is now significant interest in evaluating the nutritional potential of W-DDGS for use in non-ruminant diets. Evidence in the scientific literature suggests DDGS (of either wheat or maize origin) is typically formulated at 50–80 g/kg in starter diets for broilers and turkeys, and 100–150 g/kg in grower/finisher diets for broilers, turkeys and laying hens (Świątkiewicz and Koreleski, 2008). Much of the published data evaluating DDGS in poultry studies are from maize DDGS, due to its predominance from bioethanol production in the US. By contrast, there are few comparable poultry studies with W-DDGS.

Another consideration when formulating W-DDGS in poultry diet is the negative effect of non-starch polysaccharides (NSP) in the gastrointestinal tract. In wheat, water soluble arabinoxylans (pentosans) can result in increased viscosity within the intestinal lumen, resulting in reduced protein, fat and starch digestibility and low feed efficiency (Annisond and Choc, 1991; Khattak et al., 2006). With no endogenous enzymes to hydrolyse NSPs in the poultry digestive tract, these carbohydrates are typically fermented by the endogenous microbiota. The primary objective of the current study was to address concerns from the commercial poultry sector that the inclusion of W-DDGS at concentrations above 50 g/kg in layer diets would result in an increased level of fermentation (primarily of pentosans) within the avian caeca. It was postulated that any increased fermentation would be associated with concomitant changes to the avian caecal environment and bacterial diversity. This increased level of fermentation could result in the recognised problem of “dirty” (stained) eggs.

Analysis of the intestinal microbiota based on laboratory culture is difficult as it has been reported that only 20% of human-associated gut bacterial species have been cultivated (Eckburg et al., 2005). These difficulties may in part be overcome through application of polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), a molecular approach through which regions of the universal bacterial 16 S rDNA gene at which the DNA sequence varies between species are specifically amplified from DNA isolated from intestinal content (or other sample of interest) using PCR and detected by DGGE, thus removing the requirement for culture (Muyzer et al., 1993; Hume et al., 2003; Ercolini, 2004). Different DNA sequences have dissimilar migratory properties when subjected to electrophoresis on DGGE gels and correspondingly discreet PCR amplicons visualised on the DGGE gel are representative of different bacterial species. Bacterial communities of different compositions therefore generate different banding patterns analogous to a community fingerprint. As such, DGGE was employed in the current study to compare bacterial diversity within the avian caecal environment.

The aim of the current study accordingly was to evaluate the potential of feeding graded concentrations of W-DDGS in layer hen diets. The study was designed to confirm whether W-DDGS could be included in balanced layer hen diets with no detrimental effects on the caecal environment.

**MATERIALS AND METHODS**

All animal protocols and procedures were conducted under both national and institutional guidelines as approved in advance of the programme by the Ethical Review Committee of the School of Biosciences, University of Nottingham, UK.

**Diets**

Two diets were originally formulated containing either 0 or 180 g W-DDGS/kg; subsequently termed D0 and D180 (W-DDGS supplied by Ensus Ltd, Teesside, UK). Table 1 shows analysed composition of the W-DDGS used, and Table 2 shows experimental formulations of diets D0 and D180. These two diets were then blended to generate two additional experimental diets, ultimately resulting in 4 trial diets containing 0, 60, 120 or 180 g W-DDGS/kg, respectively (termed D0, D60, D120 and D180; analysed composition of all diets given in Table 3). All dietary treatments (formulated by AB Vista, Marlborough, UK, manufactured by Target Feeds, Whitchurch, Shropshire,

| Table 1. Analysed composition of wheat distillers dried grains with solubles (g/kg as-fed unless otherwise stated) |
|---------------------------------------------------------------|
| **Dry matter** | **884** |
| **Crude protein** | **326** |
| **AME (MJ/kg)** | **10.04** |
| **Crude fibre** | **80** |
| **NDF** | **589** |
| **ADF** | **223** |
| **Total oil** | **72.5** |
| **Ash** | **46** |
| **Indispensable AA** | **Lysine** | **5.9** |
| | **Methionine** | **4.7** |
| | **Cysteine** | **11.8** |
| | **Methionine + cysteine** | **16.5** |
| | **Threonine** | **10.8** |
| | **Isoleucine** | **11.1** |
| | **Valine** | **14.5** |
| | **Leucine** | **23.2** |
| | **Histidine** | **6.5** |
| | **Phenylalanine** | **16.0** |
| | **Arginine** | **13.0** |

**AME**, apparent metabolisable energy; **NDF**, neutral detergent fibre; **ADF**, acid detergent fibre.
Determined analysis of experimental layer diets (g/kg as-fed)

| Diet | D0 1 | D180 2 |
|------|------|--------|
| Wheat DDGS | 0    | 180   |
| Wheat | 588  | 528   |
| Hipro soya bean meal | 143 | 54   |
| Maize gluten meal | 40   | 40    |
| Sunflower meal | 75   | 40    |
| Soy oil | 41   | 45    |
| Limestone | 91   | 93    |
| Sodium chloride | 2.0  | 1.0   |
| Sodium bicarbonate | 2.0 | 0.3   |
| DL methionine | 0.9  | 1.1   |
| Lysine HC3 | 1.6  | 4.0   |
| Dicalcium phosphate | 11.5 | 9.0   |
| Monosodium phosphate | 0.06 | –    |
| Vitamin/mineral premix 3 | 4.9 | 4.9 |
| Finase EC | 0.06 | 0.06 |
| Econase XT 25P | 0.075 | 0.075 |

DDGS, distillers dried grains with solubles
1Formulated to provide the following quantities (g) per kg complete diet: L-lysine, 7.7; L-methionine, 3.6; L-cysteine, 3.9; L-methionine + cysteine, 6.6; L-threonine, 5.7; L-tryptophan, 1.9; L-isoleucine, 6.4; L-leucine, 13.7; L-valine, 7.4; L-histidine, 4.9; L-arginine, 9.7.
2Formulated to provide the following quantities (g) per kg complete diet: L-lysine, 7.7; L-methionine, 3.6; L-cysteine, 3.1; L-methionine + cysteine, 6.7; L-threonine, 5.4; L-tryptophan, 1.8; L-isoleucine, 6.3; L-leucine, 13.3; L-valine, 7.4; L-histidine, 3.9; L-arginine, 7.6.
3Provided the following per kg of diet: retinol, 1.8 mg; cholecalciferol, 75 µg; niacin, 0.8 mg; pantothenic acid, 4 mg; thiamin, 0.8 µg; riboflavin, 0.8 mg; cyanocobalamin, 25 µg; folic acid, 0.3 mg; Fe, 10 mg; Mn, 79.6 mg; Ca, 5 mg; Cu, 0.25 mg. Diets were blended to produce two additional experimental dietary treatments giving 4 in total: D0, D60, D120 and D180 representing diets containing 0, 60, 120 and 180 g W-DDGS/kg, respectively.

Table 3. Determined analysis of experimental layer diets (g/kg as-fed unless otherwise stated)

| Diet | D0 | D60 | D120 | D180 |
|------|----|-----|------|------|
| Dry matter | 897 | 896 | 897 | 900 |
| Crude protein | 166 | 169 | 171 | 171 |
| Crude fibre | 41 | 37 | 40 | 39 |
| ADF | 47 | 54 | 63 | 69 |
| NDF | 96 | 110 | 129 | 143 |
| Total oil | 62 | 65 | 67 | 73 |
| Ash | 144 | 127 | 126 | 133 |
| Calcium | 48 | 39 | 42 | 39 |
| Phosphorus | 5.0 | 4.8 | 5.0 | 4.3 |
| Starch | 364 | 357 | 349 | 357 |
| Finase EC (U/kg) | 600 | 635 | 674 | 696 |
| Econase XT 25P (U/kg) | 16800 | 15400 | 16300 | 17400 |

ADF, acid detergent fibre; NDF, neutral detergent fibre. D0, D60, D120 and D180 represent diets containing 0, 60, 120 and 180 g W-DDGS/kg, respectively.

UK, and analysed by Sciantec Analytical Services, North Yorkshire, UK) were formulated to be isoenergetic (apparent metabolisable energy 11.72 MJ/kg) and balanced for crude protein and standard ileal digestible amino acids (data reported in Masey O’Neill et al., 2014). All diets contained exogenous enzymes (ABVista Feed Ingredients, Marlborough, UK) to replicate commercial practice. Finase EC (5000 phytase units per g, fed at 0.06 g/kg of feed) provided 300 phytase units per kg of feed. One phytase unit is defined as the amount of enzyme required to release 1 μmol of inorganic P per min from sodium phytate at 37°C and pH 5.5. Econase XT 25P (160 000 XU per g, fed at 0.075 g/kg of feed) provided 12 000 XU of endo-1,4-β-xylanase activity (EC 3.2.1.8) per kg of feed. One unit of xylanase (XU) is defined as the amount of enzyme that liberates 1 nmol reducing sugars from birchwood xylan, measured as xylose equivalents, at pH 5.3 and 50°C. Exogenous enzyme inclusion was verified by analysis of all diets prior to commencement of the study (Enzyme Services and Consultancy, Ystrad Mynach, Wales) (see Table 3). Titanium dioxide was added to all diets (5 g/kg) as an indigestible marker.

Trial design
A total of 32 layer hens (Bovans Brown strain in early lay at 27 weeks of age) were obtained from a commercial supplier (Noble Foods Ltd, Tring, Hertfordshire, UK), housed individually and allocated to one of 4 dietary treatments in a completely randomised design. Environmental parameters were a lighting regimen of 15 h light:9 h dark, with a light intensity of 15 lux and environmental temperature maintained at 21°C throughout the study period. Hens were allocated to experimental diets for an initial period of 5 d (to allow acclimatisation to the new environmental surroundings) before the 4-week trial period commenced. At all times, feed and water were provided on an ad libitum basis. During the trial period, feed intakes were monitored and all eggs were collected daily, weighed and assessed for incidence of “dirty” eggs by a senior colleague (Noble Foods Ltd, Hertfordshire, UK) who was blinded to the dietary treatments.

During d 15–17 of the trial, excreta were collected for subsequent assessment of coefficient of apparent N metabolisability (CAMN). At d 29, all hens were killed by asphyxiation with carbon dioxide and cervical dislocation to confirm death. Within 1 min of death, the caeca were dissected out, and pH of caecal digesta was measured using a digital pH meter (Hanna Instruments, Bedfordshire, UK). Samples of caecal digesta were also collected and stored at −80°C prior to short chain fatty acids (SCFA) analysis. Additionally, caecal digesta samples from the hens on the two dietary extremes (D0 and D180 diets) were subjected to assessment of microbial diversity by PCR-DGGE.
Chemical analyses and calculations

All analyses were conducted in duplicate with repetition if variation was >5%. Diet and excreta samples were dried to a constant weight in a forced air convection oven at 100°C. Ground dried samples of diet and excreta (40–50 mg) were analysed in duplicate for N content using the Dumas method. Subsequently, the concentration of titanium dioxide, employed as an inert marker, was determined in diet and excreta samples using the method of Short et al. (1996). These chemical analyses allowed CAM<sub>N</sub> to be calculated using the following equation:

\[
\text{CAM}_N = 1 - \left( \frac{(N^E \times M^D)}{(M^E \times N^D)} \right)
\]

where \(N^E\) is the N concentration in excreta (g/kg DM), \(M^P\) is the marker concentration in the diet (g/kg DM), \(M^E\) is the marker concentration in excreta (g/kg DM) and \(N^D\) is the N concentration in the diet (g/kg DM).

Determination of caecal SCFAs

A standard solution of 1 ml/l formic, acetic acid \(^{13}\)C, acetic acid \(^{13}\)C, propionic, butyric, isobutyric and valeric acid was prepared and used as 5.5 ml aliquots in 20 ml headspace vials. All reagents were sourced from Sigma-Aldrich Co. Ltd., Dorset, UK. Samples were prepared by mixing ~0.2 g caecal content with 3.75 ml H<sub>2</sub>O and adding internal standard (acetic acid \(^{13}\)C) to a final concentration of 1 ml/l. The pH of the sample preparation was lowered to pH 2–3 through addition of dilute phosphoric acid. Sealed vials were incubated at 30°C for 5 min before headspace volatiles were sampled using a 50/30 \(\mu\)m DVB/Carboxen/PDMS StableFlex SPME (solid phase microextraction) fibre (Sigma-Aldrich) for a further 5 min at 30°C. Volatiles adhering to the SPME fibre were then transferred onto a ZB-FFAP column (30 mm × 0.25 mm ID, × 1 \(\mu\)m film thickness (Phenomenex, Cheshire, UK) and chromatogram with helium as the carrier gas at 18 psi. Gas chromatography starting temperature was 60°C, held for 1 min and increased to 180°C at 8°C/min. All compounds were detected with a DSQ Mass spectrometer (Thermo Fisher Scientific, Cheshire, UK) in scan mode, 20–150 m/z

Determination of caecal microbial diversity using PCR-DGGE

DNA was extracted from ~0.2 g aliquots of caecal content using a QIAamp DNA Stool Mini Kit (Qiagen Ltd., Manchester, UK) with the incorporation of a bead beating stage (0.2 g 0.1 mm glass beads for 1 min at 6000 rpm using a MagNalyser cell disruptor; Roche Diagnostics Ltd., West Sussex, UK). Extracted DNA was quantified and assessed for purity using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Leicestershire, UK) and stored at -20°C prior to PCR-DGGE analysis.

Extracted DNA was diluted to 15 ng/\(\mu\)l with nuclease-free water and used as a template for PCR amplification of the eubacterial 16S rRNA gene using universal primer pairs targeting either the V3 region; 341 f (5′-CCTACGGGAGGCAGCAG-3′) and 518 r (5′-ATTACCGCGGTCTGCTGG-3′) (Muyzer et al., 1993) or the V6–V8 region; 968 f (5′-AA CGC GAA GAA CCT TAC-3′) and 1401 r (5′-CGG TGT GTA CAA GAC CC3′). A 40-bp GC-rich sequence (GC-clamp) was added to the forward primer at its 5′ end as described by Muyzer et al. (1993). After visual confirmation of the PCR products by agarose gel (10 g/l) electrophoresis, both V3 and V6–V8 16S rDNA amplicons were analysed by DGGE using a Dcode vertical electrophoresis unit (Bio-Rad Laboratories, Hertfordshire, UK). Separation of the V3 amplicons was achieved using a 1× TAE (Tris-acetate EDTA) buffer/polyacrylamide gel (80 ml/l; 37.5:1 acrylamide:bisacrylamide; Severn Biotech Ltd., Worcester, UK) containing a linearly increasing 30%:55% urea-formamide denaturing gradient, in which the 100% denaturant stock solution contained 7 M urea (Severn Biotech Ltd) and 400 ml/l formamide (Severn Biotech Ltd). V6–V8 amplicons were separated using polyacrylamide gel (60 ml/l); all other conditions were unchanged. Electrophoresis was performed at 60°C in 1× TAE electrophoresis buffer for 10 min at 40 V followed by 6 h at 170 V. DNA amplicons were stained in 1:10 000 GelStar Nucleic Acid gel stain solution (Lonza, Maryland, USA) in 1× TAE for 10 min at 20°C prior to visualisation. Nucleic Acid gel stain solution (Lonza, Maryland, USA) in 1× TAE for 10 min at 20°C prior to visualisation.

Statistical analysis

Data were subjected to analysis of variance using a fully randomised design Genstat v14 (VSN, International Ltd, Hemel Hempstead, UK) with diet as the main factor, with linear and non-linear contrasts to account for the incremental increase in W-DDGS. Unweighted pair group method with arithmetic mean (UPGMA) dendrograms of DGGE banding patterns were generated by FPQuest Software Version 4.5 (Bio-Rad Laboratories) using the Dice coefficient. Analysis of molecular variance (AMOVA) was performed to compare the DGGE patterns of bacteria communities at a selected similarity level according to Excoffier et al. (1992) using GenAIEX v6.5 software as described by Pealkall and Smouse (2012). The Shannon–Wiener diversity index (\(H\)) was used to describe bacterial diversity as detected by DGGE (Shannon, 1948; Scanlan et al., 2006). This index was calculated by the following equation:
Shannon Wiener index = \( \sum_{i=1}^{s} (p_i)(\ln p_i) \)

where \( s \) is the number of species/DGGE bands in the sample, \( p_i \) is the proportion of species/DGGE bands for the \( i \)th species/DGGE band in the sample.

Student’s \( t \)-test was performed on the species richness and Shannon–Wiener index of each group using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, USA, www.graphpad.com).

**RESULTS**

Hens took longer than expected to acclimatise to the trial environment which was reflected in a low laying percentage over the first week of the study. Therefore, egg parameters presented are from weeks 2 to 4.

**Caecal pH and SCFA**

Mean caecal data (pH and SCFA values) are shown in Table 4. Caecal pH was significantly affected by diet \( (P < 0.001) \) with values ranging from 7.3 for hens on the D0 diet to 6.1 for those on the D180 treatment. This linear effect was highly significant \( (P < 0.001) \) with increasing W-DDGS dietary concentrations associated with a more acidic caecal environment. SCFA analysis revealed a significant quadratic effect for molar proportions of isobutyric \( (P < 0.05) \), but no other significant dietary effects were observed for molar proportions of acetic, propionic, butyric, valeric acid or total SCFAs within the caecum.

**Caecal microbial diversity analysis by PCR-DGGE**

Total bacterial diversity of luminal caecal content from hens on diets with and without W-DDGS (180 g/kg) was compared by PCR-DGGE. Surveys were made of both the V3 and V6–V8 regions of the universal bacterial 16S rRNA gene with visualisation of the amplicons allowing the determination of different bacterial community structures. To determine whether specific changes in diversity were promoted through addition of W-DDGS, UPGMA analysis was performed to determine similarities in bacterial community fingerprints. UPGMA analysis of profiles of the V3 16S rRNA gene region revealed that community fingerprints were distributed in two significant clusters \( (P < 0.05, \text{AMOVA; Figure (a))} \); however, clustering did not relate to dietary inclusion of W-DDGS. DGGE banding patterns for the V6–V8 16S rRNA gene region were distributed into three significant clusters \( (P < 0.05, \text{AMOVA; Figure (b))} \) that were again independent of W-DDGS inclusion.

Species richness was resolved by enumeration of bands in each DGGE profile. In profiles generated from surveys of either region of the 16S rRNA gene (V3 or V6–V8), species richness was not influenced by W-DDGS inclusion \( (P < 0.05, \text{Student’s } t \text{-test, Table 5).} \) The Shannon–Wiener index is a measure of species diversity in a community that considers both the

---

**Table 4. Effect of increasing level of wheat distillers dried grains with solubles on caecal and performance parameters of layer hens (from 27 to 31 weeks of age)**

| Diet | Caecal pH | SCFA (mmol/l) | Acetic | Propionic | Butyric | Iso-butyric | Valeric | Total SCFA |
|------|-----------|---------------|--------|-----------|---------|-------------|---------|------------|
| D0   | 7.3       | 78.0          | 3.8    | 9.6       | 14.0    | 3.8         | 9.6     | 135.4      |
| D60  | 7.0       | 88.2          | 2.7    | 9.1       | 19.0    | 2.7         | 9.1     | 152.8      |
| D120 | 6.3       | 88.0          | 3.2    | 11.6      | 20.0    | 3.2         | 11.6    | 157.8      |
| D180 | 6.1       | 89.0          | 2.9    | 10.2      | 23.0    | 2.9         | 10.2    | 161.2      |

**Performance parameters**

| Diet | Egg production | CAMN |
|------|----------------|------|
| D0   | 49.0           | 0.396|
| D60  | 49.9           | 0.390|
| D120 | 49.7           | 0.395|
| D180 | 49.4           | 0.391|

**SED, standard error of the difference.**

1 Data collected from birds at slaughter (at 31 weeks of age).
2 Data from weeks 2, 3 and 4 of the trial.
3 Coefficient of apparent metabolisability of nitrogen.

D0, D60, D120 and D180 represent diets containing 0, 60, 120 and 180 g W-DDGS/kg, respectively.
number and evenness of species. Shannon–Wiener index values calculated for hens with and without W-DDGS from profiles of the V3 and V6–V8 16S rRNA gene regions were not significantly affected (P < 0.05, Student’s t-test, Table 5).

**Performance parameters**

Although the primary objective of the study was an assessment of the influence of increasing concentrations of dietary W-DDGS on caecal parameters, the protocol adopted allowed a preliminary evaluation of influences on general
Table 5. Species richness and Shannon–Wiener index determined from 16S rRNA genes amplified from luminal caecal content of layer hens on diets with or without wheat DDGS (W-DDGS)

| 16S rRNA gene target | V3 | V6–V8 |
|----------------------|----|-------|
|                      | D0 | D180 | SED | P     | D0 | D180 | SED | P     |
| Richness indexes (S) | 13.5 | 13 | 1.5 | >0.05 | 9.9 | 10.5 | 0.8 | >0.05 |
| Shannon–Wiener index (H) | 2.3 | 2.3 | 0.1 | >0.05 | 2.2 | 2.3 | 0.1 | >0.05 |

egg production (g egg/hen/d) and eggshell cleanliness. Over the 4-week trial period, there was no evidence of any “dirty” eggs. In total, \( n = 13/615 \) eggs were soft-shelled and \( 5/615 \) eggs were broken in cage over weeks 2–4 inclusive of the trial. Dietary effects on mean feed intake and CAM\( _{N} \) were also recorded and are shown in Table 4. Feed intakes and CAM\( _{N} \) were unaffected by dietary treatment.

DISCUSSION

Data evaluating the caecal environment revealed a highly significant dietary effect \((P < 0.001)\) for caecal pH, with more acidic luminal contents associated with increasing W-DDGS dietary inclusion. This observed difference in caecal acidity could be explained by the reasoning that an increasing rate of inclusion of W-DDGS across the 4 dietary formulations would be associated with an accompanying increase in dietary fibre concentration (evident in Table 3). This increased fibre would result in differing levels of NSP fermentation by the avian caecal microbiota, given that the predominant fermentation chambers within the avian gastrointestinal tract are the caeca (Józeńiać et al., 2004). An increased level of caecal fermentation would probably result in increased molar proportions of SCFAs and a more acidic caecal environment. Although not statistically significant, molar proportions of total SCFAs did increase as inclusion rate of W-DDGS increased which could explain the increasing caecal acidity. Similarly, the indicative increase in molar proportions of butyric acid would probably contribute to the increased caecal acidity (as well as suggesting some degree of change to the caecal microbiota). The authors also postulate that the variation in caecal pH between dietary treatments could be at least partly due to an intrinsic property of the experimental diets themselves, such as pH levels. This would seem a reasonable assumption given that DDGS is an acidic material with a pH value typically between 3.6 and 5.0 (Shurson and Alghandi, 2008), although it might be expected that other variables (gizzard activity, intestinal buffers, etc.) would have more of an influence on caecal acidity in poultry.

It was postulated that changes in caecal fermentation levels across treatments would be associated with differences in the diversity of bacterial species. However, assessment of bacterial diversity within the caeca, detected using PCR-DGGE, revealed no significant changes in bacterial population structure between the hens on the D0 and D180 diets. Although clustering was apparent for both the V3 and V6–V8 regions of the 16S rRNA gene, it was not linked to diet (as evidenced by the even distribution of D0 and D180 diets within clusters). PCR-based 16 S rDNA techniques have been applied successfully to detect changes in poultry microbial populations (Hume et al., 2003; Amit-Romach et al., 2004; Waters et al., 2005) with 16 S rDNA gene V3 and V6–V8 hypervariable regions shown to be appropriate for fingerprinting the diversity of intestinal bacteria (Yu and Morrison, 2004). The chicken caecum is colonised by a highly numerous and species-rich bacterial community (Barnes, 1979; Bjerrum et al., 2006). A diverse microbial population is associated with several host benefits. Aside from contributing to feed conversion by generation of substrates (SCFAs) through fermentation of host indigestible carbohydrates (Van Der Wielen et al., 2000), competition for resources between the bacterial community of the gastrointestinal tract can exclude pathogens such as Salmonella (Impye et al., 1987; Nava et al., 2005). Additionally, the presence of SCFAs in the avian caeca has been reported to have bacteriostatic effects (Van Der Wielen et al., 2000).

It is somewhat difficult to draw firm conclusions from the caecal environment data as the significant changes observed in caecal pH (along with indicative changes in molar proportions of butyric and total SCFAs) would suggest differing levels of caecal fermentation across diets which would have been expected to be reflected in changes to the caecal microbiota. However, the lack of a shift in caecal bacterial diversity measured in the current study, detected using PCR-DGGE, suggests that W-DDGS at 180 g/kg does not cause a gross change in bacterial population structure within the avian caeca. Given this, it may be beneficial to also employ the use of other techniques (next-generation DNA sequencing, etc.) to aid in the interpretation of any future work of a similar nature.

There is growing interest in evaluating the nutritional value of feeding W-DDGS to layers, as reflected by the generation of prediction equations of energy values of W-DDGS for poultry (Cozzanet et al., 2010). The analysed composition of the W-DDGS used in the current study (Table 1) appears typical of that reported
elsewhere; nutrient profiles from a range of W-DDGS samples from European ethanol plants by Cozannet et al. (2010) included DM (dry matter) ranging between 890 and 940 g/kg, CP (crude protein) (326–389 g/kg), CF (crude fibre) (62–109 g/kg) and ash (45–67 g/kg).

As a general indication of the production level of the birds, data from the current study suggest that W-DDGS can be included at concentrations of up to 180 g/kg in layer diets containing exogenous enzymes, with no detrimental effects to egg production. These results are in good agreement with a similar, larger study (Niemiec et al., 2012) where inclusion concentrations of W-DDGS of up to 200 g/kg were successfully fed in balanced diets with no dietary effect on laying performance.

An assessment of eggshell cleanliness was undertaken in the current trial but no dirty eggs were observed. The supplementation of layer diets with exogenous enzymes can overcome the negative effects of NSPs by decreasing intestinal viscosity and reducing the incidence of stained/dirty eggs (Lazaro et al., 2003; Khattak et al., 2006). Diet formulations in the current study included phytase (at 600–700 U/kg) and XU (15 400–17 400 U/kg). These exogenous enzymes were formulated in the diets to reflect commercial practice. The lack of difference in CAM values is expected, given that the 4 diets were formulated to be both iso-energetic and balanced for crude protein and standard ileal digestible amino acids.

The results of the current study provide valuable evidence that W-DDGS can be formulated into nutritionally balanced layer diets containing NSP enzymes and phytase at inclusion levels of up to 180 g/kg with no detrimental effects to the microbial diversity of the caecal microbiota. These results, taken together with the preliminary egg performance data, should instil a greater degree of confidence in the use of W-DDGS in layer diets.

ACKNOWLEDGEMENTS

Technical input from Dr Robert Linforth (Division of Food Sciences), Neil Saunders (Division of Animal Sciences) and the Bio Support Unit (University of Nottingham) is gratefully acknowledged. The authors also wish to thank Dr Oluyinka Olukosi (SRUC) for useful advice and Dr Lorraine Salmon (Premier Nutrition) and Dr Helen Masey O’Neill (AB Vista Feed Ingredients) for diet formulation and supply of enzymes used in this study.

FUNDING

This research was carried out as part of the Environmental and Nutritional Benefits of Bioethanol Co-products (ENBBIO) project, supported by ABAgri Ltd, AB Vista Feed Ingredients, ADAS Ltd, Aurin, Agriculture and Horticultural Development Board-BPEx, EBLEX, Dairy-Co and HGCA divisions, Noble foods, Ensus PLC, Evonik Industries, Glencore Grain UK Ltd, Hook2Sisters, Marks and Spencer PLC, NESPIC, Premier Nutrition, Sciantec Analytical Services Ltd, Syngenta Seeds UK, Scotch Whisky Research Institute and Tulip Ltd, and sponsored by the UK Department for Environment Food and Rural Affairs, through the Sustainable Livestock Production LINK Programme.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

REFERENCES

Amiri-Romach, E., Sklan, D. & Uni, Z. (2004) Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. Poultry Science, 83: 1093–1098. doi:10.1093/ps/85.7.1093

Annison, G. & Choc, M. (1991) Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. World’s Poultry Science Journal, 47: 232–242.

Barlow, E.M. (1979) The intestinal microflora of poult and game birds during life and after storage. Journal of Applied Bacteriology, 46: 407–419. doi:10.1111/j.1365-2672.1979.tb01809.x

Bjerre, L., Engberg, R., Lesk, T., Jensen, B., Fenster, K. & Pedersen, K. (2006) Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. Poultry Science, 85: 1151–1164. doi:10.1093/ps/85.7.1151

Cozannet, P., Lessire, M., Gay, C., Metayer, J.P., Predot, Y., Skria, F. & Nolet, J. (2010) Energy value of wheat dried distillers grains with solubles in roosters, broilers, layers, and turkeys. Poultry Science, 89: 2290–2241. doi:10.3382/ps.2010-00833

DIRECTIVE (2003/30/EC) Directive 2003/30/EC of the European Parliament and of the Council of 8 May 2003 on the promotion of the use of biofuels or other renewable fuels for transport. Official Journal of the European Union, L 125: 42–46.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nislow, K.E. & Relman, D.A. (2005) Diversity of the human intestinal microfloral flora. Science, 308: 1635–1638. doi:10.1126/science.1110591

Ersolm, D. (2004) PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. Journal of Microbiological Methods, 56: 297–314. doi:10.1016/j.Fucknit.2003.11.006

Erez, T., Martinez-Amuzua, C., Parsons, C., Walters, B., Brannon, J. & Noll, S. (2003) Amino acid digestibility in corn distillers dried grains with solubles. Poultry Science, 82: 70.

Escoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetica, 131: 479–491.

Fasting, N., Latham, J. & Maran, D. (2006) Amino acid availability and true metabolizable energy content of corn distillers dried grains with solubles in adult cecectomized roosters. Poultry Science, 85: 1212–1216. doi:10.1093/ps/85.7.1212

Hume, M., Kurena, L., Edrington, T., Donskay, C., Moore, R., Ricke, S. & Nisbet, D. (2005) Poultry digestive microflora
biodiversity as indicated by denaturing gradient gel electrophoresis. Poultry Science, 82: 1100–1107. doi:10.1093/ps/82.7.1100

Infus, C.S., Mead, G.C. & Henton, M. (1987) Influence of continuous challenge via the feed on competitive exclusion of salmonellae from broiler chicks. Journal of Applied Bacteriology, 63: 139–146. doi:10.1111/j.1365-2672.1987.tb03589.x

Jozefak, D., Rutkowski, A. & Martin, S.A. (2004) Carbohydrate fermentation in the avian ceca: a review. Animal Feed Science and Technology, 113: 1–15. doi:10.1016/j.anifeedsci.2003.09.007

Khattak, F.M., Pasha, T.N., Havat, Z. & Mahmud, A. (2006) Enzymes in poultry nutrition. Journal of Animal and Plant Sciences, 16: 1–7.

Lazaro, R., Garcia, M., Aranibar, M.J. & Mateos, G.G. (2003) Effect of enzyme addition to wheat-, barley- and rye-based diets on nutrient digestibility and performance of laying hens. British Poultry Science, 44: 256–265. doi:10.1080/0007166031000085616

Massy O’Neill, H.V., White, G.A., Li, D., Bedford, M.R., Htoo, J.K. & Wiseman, J. (2014) Influence of the in vivo method and basal dietary ingredients employed in the determination of the amino acid digestibility of wheat distillers dried grains with solubles in broilers. Poultry Science, 93: 1178–1185. doi:10.3382/ps.2013-05578

Muñzer, G., De Waal, E.C. & Utterlinde, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology, 59: 695–700.

Nava, G.M., Biele, I.R., Callaway, T.R. & Castañeda, M.P. (2005) Probiotic alternatives to reduce gastrointestinal infections: the poultry experience. Animal Health Research Reviews, 6: 105–118. doi:10.1079/AHR2005103

Niebic, J., Riedel, J., Szulc, T. & Stepniak, M. (2012) Feeding wheat distillers dried grains with solubles (DDGS) to laying hens and its effect on performance and egg quality. Annals of Animal Science, 12: 105–115. doi:10.2478/v10229-012-0009-3

Peakall, R. & Smouse, P.E. (2012) GenALEx 6.5: genetic analysis in excel. Population genetic software for teaching and research—an update. Bioinformatics, 28: 2537–2539. doi:10.1093/bioinformatics/bts460

Scanlan, P.D., Shanahan, F., O’Mahony, C. & Marchesi, J.R. (2006) Culture-independent analyses of temporal variation of the dominant fecal microbiota and targeted bacterial subgroups in Crohn’s disease. Journal of Clinical Microbiology, 44: 3980–3988. doi:10.1128/JCM.00312-06

Shannon, C.E. (1948) A mathematical theory of communication. The Bell System Technical Journal, 27: 379–423 and 623–656. doi:10.1002/bltj.1948.27.issue-3

Short, F.J., Gorton, P., Wiseman, J. & Boorman, K.N. (1996) Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology, 59: 215–221. doi:10.1016/0377-8401(95)00916-7

Sirson, J. & Alghandi, A.S. (2008) Quality and new technologies to create corn co-products from ethanol production. In: Barcock, B.A., Hayes, D.J. & Lawrence, J.D. (Eds), Using Distillers Grains in the U.S. and International Livestock and Poultry Industries, Chapter 10 (Iowa, Iowa State University, Midwest Agribusiness Trade Research and Information Center at the Center for Agricultural and Rural Development). Available: http://www.mattri.istsate.edu/ DGbook/distillers_grain_book.pdf

Świątkiewicz, S. & Korleski, J. (2008) The use of distillers dried grains with solubles (DDGS) in poultry nutrition. World’s Poultry Science Journal, 64: 257–265. doi:10.1017/S0043933908000044

Van der Wielen, P.W.J.J., Besterveld, S., Noteram, S., Hofstra, H., Urrungco, B.A.P. & Van Knapen, F. (2000) Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Applied and Environmental Microbiology, 66: 2536–2540. doi:10.1128/AEM.66.6.2536-2540.2000

Waters, S.M., Duffy, C.F. & Power, R.F.G. (2005) PCR-DGGE analysis of caecal microflora of NatustatTM-supplemented Turkeys challenged with histomonas meleagridis. International Journal of Poultry Science, 4: 620–627. doi:10.3923/ijps.2005.620.627

Yu, Z. & Morrison, M. (2004) Comparisons of different hypervariable regions of rrs genes for use in fingerprinting of microbial communities by PCR-denaturing gradient gel electrophoresis. Applied and Environmental Microbiology, 70: 4800–4806. doi:10.1128/AEM.70.8.4800-4806.2004