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The temperature of storage of a batch of wheat distillers dried grains with solubles samples on their nutritive value for broilers

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Short title: **Storage of DDGS for broilers**

ABSTRACT 1. A batch of wheat distillers dried grains with solubles (DDGS) was obtained immediately after production and was separated into 5 equal parts and placed in woven polypropylene sacks. The samples were stored under five different temperature conditions for one year as follows: kept at a constant -20ºC; kept at -20ºC for 24 h period and after that kept at a constant +4ºC; kept at a constant +4ºC only; kept at a constant +15ºC; stored at ambient temperature (range of weekly mean temperatures was from +4 to +22ºC).

2. Each of the 5 wheat DDGS samples was included (200 g/kg) in a nutritionally complete diet and fed to broiler chickens from 7 to 21 d of age. The chemical composition of the DDGS samples was determined at the beginning and at the end of the one year storage period.

3. The nitrogen corrected apparent metabolisable energy (AMEn) and the nutrient availability of each sample was measured using a total collection technique. The growth performance of birds was also determined.

4. The DDGS samples kept at a constant -20ºC had higher dry matter, lower oxidation value and lower antioxidant contents. The DDGS sample that was stored at ambient temperatures had a higher (P<005) AMEn than the rest of the DDGS samples.

5. The results of this experiment have shown that there can be changes in the AMEn of wheat DDGS during storage at ambient temperatures. In general, there were no serious effects of storage of DDGS on its feeding value to broiler chickens.

Key words: storage, wheat DDGS, broilers, metabolisable energy

INTRODUCTION

Wheat distillers dried grains with solubles (DDGS), by-product of bioethanol production, is rich in fat and protein and is used in poultry diet formulations (Whiting et al., 2016). Differences in seasonal demand results in some batches of DDGS being stored for long periods at ambient temperatures before they are incorporated in poultry diets. DDGS have a relatively high content of unsaturated fatty acids so there is a potential that the batch may deteriorate during the storage. Lipid oxidation has been implicated as a primary factor in the deterioration in distiller's grains and brewer's spent grain products (Rasco, 1988). However,

research assessing the effect of storage on feeding value of wheat DDGS for poultry is lacking.

The aim of the study was to investigate the effects of five different storage temperature regimens on N-corrected dietary apparent metabolisable energy (AMEn), total tract nutrient retention coefficients, and growth performance when fed to chickens (from 7 to 21d of age).

MATERIALS AND METHODS

Storage of DDGS samples

The wheat DDGS sample used in this experiment was obtained directly from the manufacturer (Ensus UK Limited, Yarm, UK) immediately after production and was separated into 5 equal parts and placed in woven polypropylene sacks. The sacs held approximately 25kg and were not stacked, so there was little compaction. The samples were stored under five different temperature conditions for approximately one year. The storage conditions were as follows: kept at a constant -20ºC; kept at -20ºC for 24 h period and after that kept at a constant +4ºC; kept at a constant +4ºC only; kept at a constant +15ºC; stored at ambient temperature (range of weekly mean temperatures was from +4 to +22ºC). The -20ºC for 24h period, followed by + 4ºC was included to evaluate the possibility that freezing alone could affect the nutrient availability of DDGS. The ambient temperature changes during this period were measured with a digital data logger (Electronic Temperature Instruments Ltd, Worthing, UK) installed in one of the ambient stored bags. The ambient stored DDGS sample did not experience any freezing temperatures during the storage (Figure 1). The DDGS samples were visually inspected at the end of storage and all appeared free from fungal contamination.

Husbandry and sample collection

Nutrient availability and growth performance were examined in a broiler chicken experiment from 7 to 21 d age. Each of the five DDGS samples were incorporated into a nutritionally complete diet in meal form at 200 g/kg (800 g of the basal feed +200 g of each DDGS sample) (Table 1). The nutrient specification of the diets met the breeder's recommendation (Aviagen Ltd.). A sixth dietary treatment was also fed that was the basal feed only. Male Ross 308 broiler chickens were obtained from a commercial hatchery at day old and were

placed in a single floor pen and fed on a proprietary broiler starter feed until 5 d of age. Then 2 birds were randomly allocated to one of 60 pens (n=120 birds in total) with 0.16 m^2 solid floor area to accustom them to a pen environment but fed then proprietary feed until 7 d of age. On the first day of the experimental feeding period the chicks were weighed and the experimental diets were randomly allocated to the pens. Access to the feed and the water was *ad libitum*. There were 10 replicates for each diet. The temperature was 30ºC at 7 d and was gradually reduced to 20ºC at the end of the 14 d feeding period (21 d age). A standard lighting program for broilers was used, decreasing from 23:1 h (light:dark) from 1 d old to 18:6 h at 7 d of age, which was maintained until the end of the study.

At 17 d of age, the solid floor of each pen was replaced with a wire mesh floor, and the total droppings were collected for four days until the end of the study. This change did not have an effect on bird behaviour and daily feed intakes (FI). Feed intake for the same period was recorded for the determination of dietary AMEn and total tract nutrient retention coefficients. The total feeding period was 14 d. All birds were weighed at the end of the study, and the weight gain (WG) and feed conversion efficiency (FCE) were determined. The droppings (egesta and excreta with visible feather, skin and regurgitated feed removed) were collected for the last 96 h of the feeding period (collected every 24 h to avoid fermentation losses) and the excreta samples were dried at 60ºC.

On the last day of the experiment, at 21d old, one bird from each pen was selected at random and killed by cervical dislocation. The liver of each bird was collected and stored at –70°C for antioxidant status analysis.

The Animal Experimental Committee of Harper Adams University approved all procedures.

Chemical Analysis

Dry droppings samples were weighed and milled to pass through a 0.75-mm mesh. Gross energy concentrations of the control feed, DDGS and droppings were measured using an adiabatic bomb calorimeter (Model: 1261 Isoperibol Bomb Calorimeter, Parr Instrument Company, Moline, IL, USA). Nitrogen was determined using a Leco nitrogen analyser (Leco FP-528, Leco Corporation, St Joseph, MI, USA) according to AOAC method 968.06 (AOAC, 2000). Ether extract was determined according to AOAC methods 920.39 and 942.05, respectively (AOAC, 2000). The colour score of the stored DDGS samples was carried out using a Chroma Meter CR-400 from Konica Minolta (Sunderland, UK) to determine luminance and chromaticity scores using CIELAB scoring. The peroxide values (PV) in the

stored DDGS samples were determined according to AOAC method 965.33, by dissolving the oil sample in a solvent and potassium iodide and then titrating with sodium thiosulfate and using starch as an indicator (AOAC, 2000). The PV reveals the current level of oxidative rancidity, measured as milli equivalents of peroxide per kilogram (meq/kg).

Non-starch polysaccharides (NSP) and total starch (TS) contents in the DDGS samples were determined following the methods of Englyst (1994) and Englyst (2000), respectively.

The GE, DM, nitrogen and fat of each dried droppings sample and the experimental diets were determined as described for the wheat samples. The AMEn of the diets was calculated as described by Hill and Anderson (1958). The coefficients of total tract nutrient retention were determined as the difference between intake and voiding of the nutrient, divided by their respective intake.

The concentration of antioxidants, including total vitamin E, vitamin A and coenzyme Q_{10} in DDGS samples and liver was determined using an HPLC system as previously described (Surai et al., 2001; Karadas et al., 2009).

Statistical procedure

The observational unit was the cage with two birds. Statistical analyses were performed using the GenStat statistical software package (GenStat 17 release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The AMEn and the nutrient retention coefficients of all diets, including the basal diet and diets including DDGS samples stored under different conditions were determined. Then the AMEn and the nutrient retention coefficients of the DDGS samples were obtained using the substitution method (Finney, 1978) using the data from the basal only diet. The results of all DDGS samples were statistically compared using a randomised block analysis of variance. Duncan's multiple range test was used to determine significant differences between DDGS treatment groups. In all instances, differences were reported as significant at $P \leq 0.05$.

RESULTS

There were differences in dry matter contents of the stored wheat DDGS samples: The sample stored at ambient temperature had the lowest dry matter content of 819 g/kg, and the sample stored at -20ºC had the highest dry matter content of 883 g/kg, respectively (Table 2). The average colour score of the stored DDGS samples was 42.6, varying from 40.0 for the

146 sample stored at ambient temperature to 45.1 for the sample stored at a constant +4ºC. The
147 DDGS sample stored at -20ºC had a peroxide value of 0.0 mEq/kg, as there were few
148 differences in the peroxide values of the rest of the samples and the average was 17.7
149 mEq/kg. The mean total vitamin A, vitamin E and coenzyme Q_{10} concentrations (presented as
150 µg/g DM), of stored wheat DDGS samples were 0.0718, 37.3, and 2.846 (µg), respectively.
151 For vitamin E and coenzyme Q_{10}, the sample stored at -20ºC had the lowest contents, and the
152 sample stored at constant +15ºC had the highest contents, respectively (Table 2).
153 The starch content of DDGS samples stored at ambient and +15 ºC was lower compared to
154 those stored at -20ºC and +4ºC. Interestingly, the starch content of the DDGS sample stored
155 at changeable (-20ºC for 24h and after that constant +4ºC) temperature was intermediate
156 (Table 2).
157 Birds remained healthy throughout the study period and there were no mortalities. Results on
158 bird growth performance, energy and retention coefficients are summarised on Table 3. There
159 were no differences (P>0.05) in FI, WG, FCE, liver weight and retention coefficients. The
160 growth performance of the birds was somewhat lower than the breeder's standards (Aviagen
161 Ltd, Edinburgh, UK) but the diets were fed in mash form. The AMEn of DDGS samples
162 stored at ambient temperature did not differ (P>0.05) from those stored at changeable (-20ºC
163 for 24h and after that constant +4ºC) temperature, but was higher (P<0.05) compared to the
164 rest of the DDGS samples.
165 There were no differences (P>0.05) between hepatic antioxidant contents of broilers fed
166 differently stored DDGS samples (Table 4).
167
168 DISCUSSION
169
170 The study evaluated the effect of storage of one batch of wheat DDGS on dietary AMEn,
171 nutrient utilisation and growth performance of broilers. The proximate nutrient and GE
172 contents of the experimental wheat DDGS sample was similar to published reports
173 (Bolarinwa and Adeola, 2012; Whiting et al., 2016) although there is large variability
174 between different DDGS batches. The colour score of the wheat DDGS samples was in the
175 expected range (Cozanett et al., 2011) and did not indicate major changes due to storage
176 temperatures. Differences in nutrient retention coefficients was similar to those reported by
177 Whiting et al. (2016) when broilers were fed diets containing 150 g/kg wheat DDGS. The
178 starch content of the DDGS samples was expectedly low because most available starch would

be removed during distillation. The small reduction in total amounts in the + 15ºC and the ambient temperature stored samples was unexpected but probably not nutritionally important. The original hypothesis of this experiment was that high storage temperatures could negatively affect the nutritional value of DDGS batches. Differences in seasonal demand for DDGS can result in batches being stored for variable lengths of time. The results of this experiment indicated that there was no evidence of a detrimental effect of storage and, in fact, storage at ambient temperature gave an improved nutrient availability. There was no effect on growth performance, but the experimental diets had a high nutrient specification so small differences in nutrient availability were unlikely to give statistically significant differences in growth performance.

Fats are susceptible to breakdown by oxidation to form peroxides, which are unstable compounds, and can become rancid. Apart of the DDGS sample stored at -20ºC, the rest of the samples had 17.7 average PV value that was similar to those reported for stored rice bran (Atapattu et al., 2013). In general fresh oils have a peroxide values well below 10 mEq/Kg while peroxide values in the 30-40 mEq/Kg range are generally associated with a rancid taste. Although the oxidation of oils is influenced by many factors, the storage temperature and light are two of the main factors that influence the rate of autoxidation of feed (Berger, 1994). All samples were stored in dark. The relatively small differences between the PV in DDGS samples suggest that there were no large changes in the oxidative rancidity of the fat under different temperature storage conditions. The moisture in the DDGS samples were also low suggesting that there would have been little microbial activity causing hydrolytic fat degradation (Allison & Treseder, 2008). The lack of response to feed intake and weight gain to different DDGS samples suggest no changes in dietary palatability. The lack of differences in hepatic antioxidant content and the good health of the birds also suggests that there was no production of harmful toxic products during DDGS storage.

There was some variation in vitamin E and A contents in the DDGS samples, as those stored at constant positive temperatures had higher values. However, this variation was small relative to the amounts of these vitamins that would have been supplied in the vitamin and mineral premix. The hepatic antioxidants content was in agreement with previous research and did indicate good health of the birds (Karadas et al., 2014).

The DDGS sample that was stored at ambient temperatures had a higher AMEn than the rest of the DDGS samples. Research on wheat storage also showed that wheat stored at ambient temperature had a greater metabolisable energy than those stored at constant -20ºC (Pirgozliev et al., 2006). Similar effects have been observed in storage experiments with

whole grain wheat (Choct et al., 1995; Choct and Hughes, 1997), showing that the metabolisable energy of stored wheat is affected by changes that occur during ambient storage. Interestingly, the AMEn of the ambient temperature stored DDGS was higher that the sample stored at constant 15ºC. The overall ambient temperature for the storage was 12.6ºC, but for 33% of the time it was above 15ºC. There is a possibility that some temperature dependent changes occurred in this DDGS sample. Walters and Choct (1998) suggested that degradation of some non-starch polysaccharides during storage may be the cause of the increased in AME of stored cereal samples. Hesselman et al. (1981) observed a reduction of beta glucan content in stored barley. Endogenous enzyme activity has been suggested as the mechanism for these effects. However, it's unlikely that DDGS would have any residual enzyme activity, although during the saccharification phase of DDGS production cycle, alpha- and gluco- amylase enzymes are added to the mash in order to remove any residual glucose residue (Smith et al., 2006).

In conclusion, the results of this experiment have shown that there can be changes in the metabolisable energy of wheat DDGS during storage at ambient temperatures. The study has been performed in a relatively cool climate with no extremes of temperature but these conditions evidently can give a small improvement in the energy availability of DDGS. In general, there were no serious effects of storage of DDGS on its feeding value to broiler chickens. However, there is a large variability between different batches of DDGS thus further studies that use multiple batches of DDGS may allow more definite conclusions.

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Figure. Average weekly temperature of the storage period of wheat DDGS (16th January 2014 to 9th February 2015).

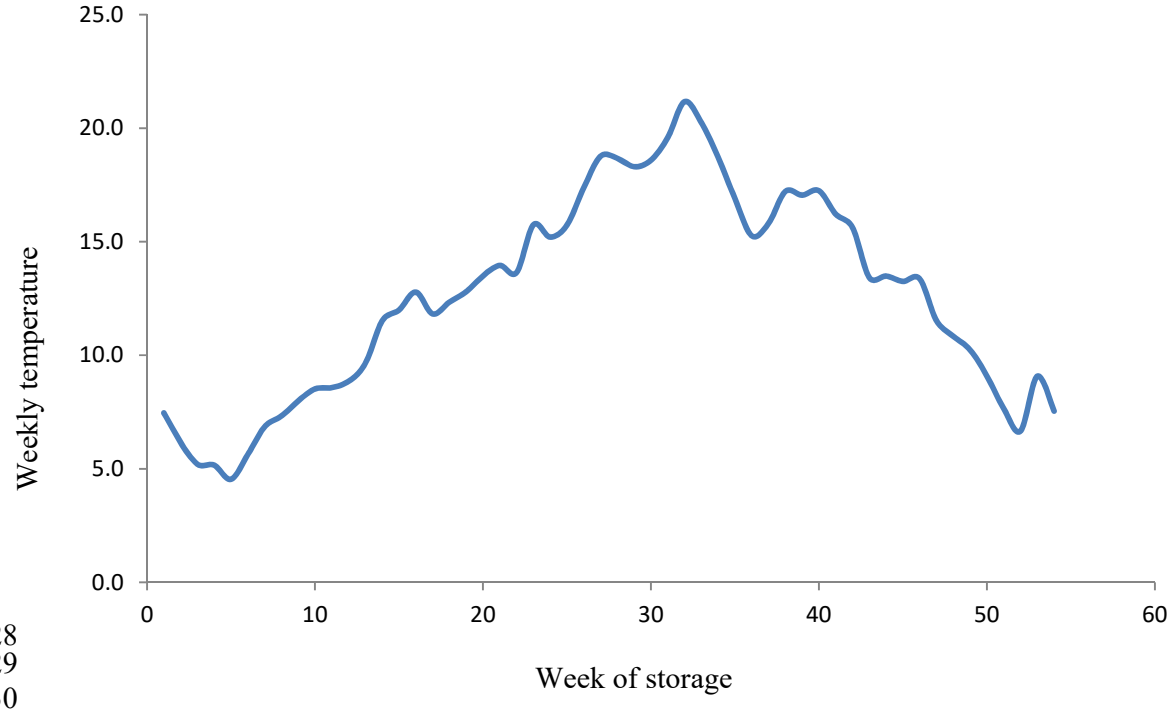

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Table 1. *Experimental diet formulation*

Ingredient	Basal diet g/kg	DDGS containing diet g/kg
DDGS	-	200
Wheat	670	536
Soya bean meal (CP=48%)	172	137.5
Full fat Soya meal	99	79
Soya oil	20	16
Lysine	3	2.4
Methionine	4.1	3.1
Monocalcium phosphate	11.4	9.4
Limestone	13.5	11
Salt	3	2.4
Vitamin/mineral premix[1]	4	3.2
Calculated composition		
ME (MJ/kg)	12.95	12.90
Protein (g/kg)	204.0	241.2
Fat (g/kg)	50.0	53.1
Lysine (g/kg)	13.0	10.4
Met + Cys (g/kg)	9.4	7.5
Calcium (g/kg)	8.5	7.6
Phosphorus av (g/kg)	4.0	4.7
Sodium (g/kg)	1.7	1.3

[1]The premix provided (units/kg diet): 12,000 IU retinol, 5,000 IU cholecalciferol, 34 mg α-tocopherol, 3 mg menadione, 2 mg thiamine, 7 mg riboflavin, 5 mg pyridoxine, 15 µg cobalamin, 50 mg nicotinic acid, 15 mg pantothenic acid, 1 mg folic acid, 200 µg biotin, 80 mg Fe as iron sulfate (30%), 10 mg Cu as a copper sulfate (25%), 100 mg Mn as manganous oxide (62%), 80 mg Zn as zinc oxide (72%), 1 mg I as calcium iodate (52%), 0.2 mg Se as sodium selenite (4.5%), and 0.5 mg Mo as sodium molybdate (40%).

Table 2. *Dry matter (DM), colour score (CS), peroxide value (PV), vitamin E, vitamin A, coenzyme Q_{10}, and total starch (TS) contents of the DDGS samples stored under different temperature conditions.*

Storage conditions of DDGS samples	DM (g/kg)	CS	PV (mEq/kg)	vit E (µg/g)	vit A (µg/g)	Q_{10} (µg/g)	TS (g/kg DM)
Before storage	896	38.6	*	*	*	*	37.0
-20ºC	883	43.5	0.0	33.4	0.066	2.43	18.1
+4ºC	882	45.1	17.6	39.0	0.087	2.92	18.7
-20ºC for 24h then +4ºC	880	40.6	18.2	37.6	0.084	2.77	11.9
+15ºC	878	43.9	16.9	41.0	0.075	3.19	9.1
Ambient temperature	819	40.0	18.2	35.3	0.047	2.92	9.8

*Not determined before storage.

Table 3. *The effect of dietary DDGS samples stored at different temperature on daily feed intake (FI) presented on dry matter, weight gain (WG), feed conversion efficiency (FCE), liver weight, dietary N corrected apparent metabolisable energy (AMEn), total tract dry matter retention (DMR), fat digestibility (FD) coefficients.*

Storage conditions of DDGS samples	FI (g/b/d)	WG (g/b/d)	FCE (g:g)	Liver weight (g)	AMEn (MJ/kg DM)	DMR	FD
-20ºC	42.0	28.4	0.678	17.2	10.71[a]	0.441	0.754
+4ºC	43.2	29.5	0.688	20.6	10.76[a]	0.458	0.709
-20ºC for 24h then +4ºC	44.6	29.6	0.666	17.8	11.51[ab]	0.499	0.813
+15ºC	44.4	29.9	0.673	17.4	10.89[a]	0.452	0.721
Ambient temperature	45.6	30.6	0.673	19.0	12.13[b]	0.448	0.795
SEM	1.622	1.217	0.0134	1.01	0.350	0.0224	0.0379
P	0.560	0.783	0.810	0.117	0.030	0.400	0.242

The values for FI, WG and FCE are based on the average for 14 days feeding period from 7 to 21d age. Dietary AME, DMR and FD were determined between 17 and 21 d of age. The values of AMEn, DMR and FD are for the DDGS samples as derived by substitution method explained in text.

There is a statistically significant difference between treatments when $P \leq 0.05$.

411 **Table 4**. *The effect of dietary DDGS samples stored at different temperature on*
412 *concentration (µg/g) and total (µg) hepatic vitamin A, vitamin E and coenzyme Q_{10} when fed*
413 *to broilers for 14 days.*
414

Storage conditions of DDGS samples	vit A (µg/g)	vit A (µg)	vit E (µg/g)	vit E (µg)	Q_{10} (µg/g)	Q_{10} (µg)
-20ºC	1.4	24.4	9.9	164	16.3	277
+4ºC	1.2	26.4	7.43	157	15.8	323
-20ºC for 24h then +4ºC	1.4	25.2	10.4	187	15.6	279
+15ºC	2.0	34.4	11.2	208	16.5	288
Ambient temperature	1.7	32.1	13.5	248	17.8	333
SEM	0.30	5.51	1.87	38.3	1.31	29
P	0.480	0.628	0.267	0.466	0.779	0.522

415 There is a statistically significant difference between treatments when $P \leq 0.05$.