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Effect of wheat distillers dried grains with solubles and exogenous xylanase on laying hen performance and egg quality

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Metabolism and Nutrition

ABSTRACT

Despite the rapid increase in the use of wheat distillers dried grains with solubles (DDGS) within the poultry sector, little information is available on how the long-term feeding of this by-product will affect the performance of laying hens. This experiment examined the effect of wheat DDGS, with and without exogenous xylanase on dietary apparent metabolizable energy (AME), total tract dry matter retention (DMR), nitrogen retention (NR), fat digestibility (FD) coefficients, feed intake (FI), bodyweight gain (BWG), eggs laid, mean egg weight, egg mass output, and egg quality characteristics including Albumin height (AH), Haugh units (HU), yolk color, eggshell strength and thickness. A total of 320 Hy-Line brown laying hens were randomly allocated to 80 enriched layer colonies (groups of 4). Two control wheat-soybean meal based diets were formulated to contain 11.60 MJ/kg. One of the diets contained 300 g/kg wheat DDGS, while the other was DDGS free, with a respective crude protein content of 171.1 g/kg and 166.5 g/kg. Both diets were divided by two and half of them were supplemented with 2,500 U/kg of xylanase, resulting in four diets in total. Data was analysed as a 2x2 factorial arrangement of treatments with analysis of variance (ANOVA). Diets were fed *ad libitum* from 17 to 43 weeks of age and data was collected from 23 to 43 weeks. The inclusion of wheat DDGS reduced (P<0.001) DMR, FI, BWG, eggs laid, mean egg weight and egg mass. However, xylanase supplementation improved AME and NR in diets containing wheat DDGS and FD in diets without DDGS (DDGS x xylanase, P<0.05) and tended to improve (P<0.10) BWG and egg mass output. For egg quality measurements, the inclusion of DDGS improved (P=0.046) HU values, eggshell strength (P<0.001) and increased (P<0.001) yolk colour intensity. This experiment showed, xylanase can be used to improve the AME and NR of DDGS based diets. However, the long-term feeding of 300 g/kg wheat DDGS negatively impacts the productive performance of hens.

Key words: Wheat DDGS, laying hen, enzyme, productive performance, egg quality

INTRODUCTION

Due to the rapid and continual increase in bioethanol production, vast quantities of distillers dried grains with solubles (DDGS) are becoming more readily available for use within animal feeds. The use of DDGS in poultry feeds is relatively new to the European market, particularly for the UK where home-produced DDGS is primarily derived from wheat feedstock. Most published literature is based on corn DDGS with limited information available on wheat DDGS. A number of recent studies have focussed on determining the nutrient availability of DDGS for poultry (Pirgozliev *et al*., 2016; Whiting *et al*., 2017; Pirgozliev *et al*., 2018). However, when formulating diets based on the determined availability of nutrients it is not known whether in practice the productive performance will be as expected. A number of authors have stated the addition of exogenous enzymes may be an effective way to improve the nutritional quality of wheat-based diets for poultry (Bedford and Morgan, 1996: Adeola and Bedford, 2004; Pirgozliev *et al*., 2010). Research conducted using wheat derived DDGS as a feed ingredient for laying hens is lacking considerably, in particular what effect the long-term feeding may have on productive performance and egg quality. The main objectives of this study were to determine whether there are any differences between two diets formulated to the same nutrient specification when using a high wheat DDGS inclusion or no DDGS and to further examine whether xylanase supplementation will provide any additional benefit.

MATERIALS AND METHODS

Diet Formulation

A wheat-soybean meal control diet was prepared, containing 11.60 MJ/kg apparent metabolizable energy (AME) and 166.5 g/kg crude protein. A second wheat-soybean meal based diet was also prepared to contain 300 g/kg wheat DDGS. The DDGS diet was formulated to have the same AME as the control diet and a crude protein content of 171.1 g/kg (Table 1). The two diets were divided in to two equal parts and half of them were supplemented with 2,500 U/kg of xylanase, resulting in four diets in total (Table 2). Danisco Xylanase (EC 3.2.1.8) was developed by Danisco Animal Nutrition

(DuPont Industrial Biosciences, Marlborough, UK) and is a preparation of endo-1,4-ß-xylanase produced from a species of fungus called *Trichoderma reesei*. The diets did not contain any coccidiostat or antimicrobial growth promoters. The birds were fed the dietary treatments from the day of arrival at 17 weeks of age to 43 weeks of age.

Chemical Analyses

Chemical analyses of the diets and excreta were carried out in duplicate. Dry matter (DM) content of the diets and excreta was determined by drying samples in a forced air oven for 48 hours at 60°C until a constant weight (AOAC Official Method 934.01, 2006). Ash content was determined after combustion of the diets and DDGS at 500°C for 24 hours (AOAC Official Method 942.05, 2005). Crude protein (CP) content (N x 6.25) of the diets and excreta was determined by the combustion method (AOAC Official Method 990.03, 2006) using Leco (FP-528 N; Leco Corp., St. Joseph, MI) with ethylenediaminetetraacetic acid (EDTA) as a standard (Sweeney, 1989). Oil content (as ether extract) of the diets and excreta was extracted with petroleum ether using a Soxtec Avanti 2050, Foss UK Ltd (AOAC Official Method 945.16, 2005). Gross energy (GE) content of the diets and excreta was measured using an adiabatic bomb calorimeter (Parr 6200 Instrument Company, Moline, IL, 61265, USA) (FOA, 2003). Neutral detergent fibre (NDF) content in the diets was analysed according to Van Soest *et al*. (1991) using an FT 122 Fibertec™ hot extraction unit (200-230V). Amino acid content in the diets were determined by SSNIFF Spezialdiäten GmbH (Soest, Germany) according to the EC directives 2000/45/EC for tryptophan (OJEC, 2000), and EC/98/64 (L 257/16) for the rest of the amino acids (OJEC, 1998).

Birds and Housing

The study was approved by the Animal Experimental Committee of Harper Adams University. A total of 320, seventeen week old, Hy-Line brown laying hens were obtained from a commercial supplier (Country Fresh Pullets Ltd, Oswestry, Shropshire). The birds were randomly allocated to 80 enriched layer coops (Hellmann Poultry GmbH & Co. K.G) in groups of four, with 20 replications per treatment. Bird housing was equipped with a nest box, scratching area, perch, two nipple drinkers inside and a separate feeder at the front. Housing dimensions were 1205 cm x 50 cm x 67 cm and consisted of a wire mesh flooring which contained no bedding material. Room temperature was maintained at 21°C and relative humidity was between 50 and 70%. The birds had *ad libitum* access to feed and water. Lighting was set to 10 hours day length upon arrival and at 20 weeks of age lighting was increased by 30 minutes each week until 16 hours light was given each 24 hours.

To ensure all birds were healthy and environmental conditions were adequate, birds were observed at least twice a day. The study started when the birds had reached 50% egg production at 23 weeks of age. During the final 96 hours of the study excreta were quantitatively collected daily and dried immediately at 60°C, until a constant weight.

Productive Performance

Hens were weighed using a BW-2050 balance (Weltech International Limited, England), on a per coop basis, at the beginning and end of the experimental study period. Total feed intake of each coop was determined by subtracting the residual feed from total feed at the end of the study. Egg numbers were recorded daily and egg weight was recorded from one day's production each week. Egg mass output was calculated as egg number x egg weight.

Egg Quality Measurements

All egg quality measurements were determined monthly at 28, 32, 36, 40 and 43 weeks of age. Eggs were selected at random and analysed within two hours of collection. Prior to analysis, eggs were subject to candling to ensure they were not cracked or damaged. Any eggs that were damaged were replaced with intact eggs from the same cage. The albumen height (AH) and Haugh units (HU) were recorded using Technical Services and Supplies (TSS) Egg Ware (Chessingham Park, Dunnington, York, YO19 5SE, England). Yolk colour was visually measured using a Roche colour fan (DSM YolkFan™) which uses a 15 scale colour index to distinguish the yolk colour density. The same egg was used to determine AH, HU and yolk colour. Eggshell breaking strength was measured by quasi-static compression, using an Instron testing machine (Model 5540, Norwood, MA 02062, USA). The same egg was then broken in half around the equator, the contents were discarded and the shell was carefully rinsed and dabbed dry. Eggshell thickness was then measured by averaging three measures taken along the equator of the eggshell using a TSS QCT shell thickness micrometer. The shell membranes were included in this measurement.

Statistical Analysis

The experiment was conducted using a randomized block design. Digestibility and production performance data for this study was analysed as a 2x2 factorial arrangement of treatments with analysis of variance (ANOVA) using Genstat 17th edition (Lawes Agricultural Trust, VSN International Ltd, Oxford, UK). Egg quality data were analysed using a repeated measures ANOVA. Duncan's multiple range test was used to determine differences between treatment groups if there was a statistically significant diet and enzyme interaction. In all instances, differences were reported significant at $P<0.05$.

RESULTS

Apparent Metabolizable Energy (AME) and Nutrient Retention

The effect of the different treatments on energy utilisation are presented in Table 3. The DDGS diet reduced ($P<0.001$) DMR. Interactions ($P<0.05$) between DDGS and xylanase were observed for

AME, NR and FD (Table 3). The interactions for AME and NR showed no improvements with the addition of xylanase to the control diet, however the addition of xylanase to diets containing DDGS improved AME and NR compared with DDGS diets containing no xylanase. Lysine was analysed to be low (Table 1) in the DDGS diet despite being formulated to standardized ileal amino acid composition as recommended in the Hy-Line International breeder specifications. It is not clear why this is but may be due to only one representative sample being taken for analysis. The interaction for FD showed digestibility of fat was highest in DDGS diets and the addition of xylanase did not increase FD further. However, an improvement in FD was reported by the addition of xylanase to diets containing no DDGS.

Data on bird performance and production parameters measured throughout the entire experimental period are shown in Table 4. The inclusion of DDGS reduced (P<0.001) daily feed intake, body weight gain (BWG), egg production, mean egg weight and egg mass output. The addition of xylanase had no effect (P>0.05) on performance parameters, but tended (P<0.10) to improve BWG and egg mass. No interactions (P>0.05) between DDGS and xylanase were observed.

Egg Quality

Results for egg quality parameters are presented in Table 5. The inclusion of DDGS improved (P<0.05) HU values, eggshell strength and increased yolk colour intensity. Age at which measurements were taken had an effect (P<0.05) on AH, HU values, yolk colour, eggshell thickness and eggshell strength. The only interaction (P<0.001) observed was between age and DDGS for yolk colour (data not shown in table). The interaction appears to be due the control diet having a more intense pigment at 28 weeks compared with subsequent measures (control diet; 2.50 at 28 wks, 1.73 at 32 wks, 1.48 at 36 wks, 1.33 at 40 wks and 1.28 at 43 wks). Whereas the DDGS diet had the lowest pigment intensity at 28 weeks compared with subsequent measurements (DDGS diet; 3.68 at 28 wks, 4.15 at 32 wks, 4.23 at 36 wks, 4.55 at 40 wks and 4.25 at 43 wks SEM 0.090).

DISCUSSION

This experiment examined the long-term productive performance of laying hens fed diets containing a high level of wheat DDGS with and without the addition of xylanase. The effect these diets had on energy utilisation, nutrient availability and egg quality were also determined.

Apparent Metabolizable Energy and Nutrient Retention

The actual AME of the diets was only 0.2 MJ/kg DM lower than the calculated values and the determined values for protein were as expected, therefore the diets should have performed the same. However, the DDGS diet reduced DM and NR. The interaction showed that the addition of the xylanase only had an effect on AME and NR when the diet contained DDGS and in this case it increased AME by 0.83 MJ and NR by 13%, compared with the DDGS diet. The interaction for FD shows that xylanase does not improve the digestibility of fat for hens fed diets containing 300 (g/kg) wheat DDGS, but does however, improve the digestibility of fat in diets containing no DDGS, this is likely due to the higher inclusion of highly digestible soy oil in the DDGS as well as the fat in DDGS being relatively well digested. The effectiveness of exogenous enzymes to improve the nutritive value of bioethanol co-products has been determined primarily for DDGS produced from corn origin (Adeola and Ileleji, 2009; Liu *et al*., 2011). However, compared with corn, wheat contains more NSP, therefore it may be assumed that using exogenous NSPases in diets containing DDGS from wheat based origin may derive greater benefits. It is well established that one of the main limitations of feeding DDGS to poultry is the high fiber content. Exogenous xylanase is commonly used in poultry diets to ameliorate the negative effects caused by highly fibrous feed ingredients. Apparent metabolizable energy has been negatively correlated with NSP, particularly soluble NSP, as this fraction relates to viscosity (Bedford and Morgan, 1996). Although viscosity was not

194 directly measured in this experiment, the improvement observed in AME by the addition of
195 xylanase may suggest the enzyme inhibited the formation of viscous solutions in the gut. There
196 is evidence that exposure to water-soluble arabinoxylans in the gut of poultry increase mucus
197 layer thickness, thus restricting the transport of nutrients (Johnson & Gee, 1986), which may
198 be counteracted by the use of exogenous xylanase (Adeola and Bedford, 2004). Although, it
199 would be expected that viscosity would compromise the digestibility of fat more than other
200 nutrients. However, this may be more the case with young birds compared with older birds, as
201 a result of the low bile acid concentration in the digesta, thus limiting the absorption of lipids.
202 The interaction between DDGS and xylanase improved NR. Pirgozliev *et al*. (2010) found
203 similar improvements when feeding wheat based diets supplemented with xylanase to laying
204 hens.

205 ***Productive Performance***

206 The performance standards for Hy-Line Brown laying hens, as outlined by Hy-Line
207 International (2016), state that average daily feed consumption, should be between 108 and
208 114 g/b/d. Birds fed the control diet had a daily feed consumption of 107 g/b/d, while birds
209 receiving the DDGS diet consumed a much lower amount of 98.2 g/b/d. For each of the
210 variables measured, birds fed the control diet performed much better than those fed the DDGS
211 diet. It is not clear why there is a reduction in feed consumption for birds fed the DDGS diet,
212 as energy levels appear to be very similar to birds fed the control diet. A number of researchers
213 have reported a reduction in egg production, egg weight, egg mass output and body weight
214 when feed intake is restricted (Gerry and Muir 1976; Cerniglia *et al*., 1984; Cunningham and
215 Polte 1984; Scott *et al*., 1999; Moreira *et al*., 2012). Therefore, it may be suggested that the
216 reduction in feed consumption of birds fed the DDGS diet is likely of significant detriment to
217 productive performance. Feeding behaviour can be affected by a concept known as the ileal
218 brake, which is a mechanism initiated by nutrient sensing receptors in the ileum. In the presence

of fat, protein and carbohydrates, these receptors respond by releasing bioactive molecules that slow gut motility and increase digestive secretions in the proximal part of the small intestine (Van Citters and Lin, 1999). The basic concept is that unabsorbed nutrients present in the ileum may delay gastric emptying and have marked effects on satiety, consequently affecting feed intake. The high DDGS diet contained protein which was likely more slowly digested. Protein and added fat act as a gastric brake as discussed and the fibre will be fermented in the caeca into volatile fatty acids (VFA's) which also act to signal an ileal brake. The VFA effect would be maximised when the xylanase is added to the DDGS diet which might explain the improved AME and NR in the DDGS diets. The fact that the xylanase was unable to increase intake in the DDGS diet lends further support to this hypothesis in that it would accelerate fat digestibility somewhat in the small intestine, reducing the brake effect here but increasing the VFA brake in the caeca by accelerating fibre digestion. Liu *et al*. (2017; 2016) reported diets containing a higher concentration of lipids cause a reduction in feed intake. The authors also noted that the high lipid diets contained higher levels of fiber, therefore suggesting, high levels of fiber in high lipid diets also reduce feed intake when fed to broilers. There may also be a possibility for mycotoxins to be present in grains used in the production of biofuel. Mycotoxins can remain stable during the fermentation and distillation processes and as a result, may be concentrated by up to three-fold in DDGS compared with the original grain (Bothast *et al*. 1992; Wu and Munkvold 2008). Mycotoxins in animal feeds can negatively affect animal health, reduce performance and feed intake. However, in the current experiment mycotoxins were not measured.

Egg Quality Measurements

There was a significant increase in Haugh unit values for birds fed the DDGS diets but in general Haugh unit values across all treatments were high. The Haugh unit (Haugh, 1937) is a measure of egg quality based on albumen height which is adjusted according to the weight of the egg. Despite being a commonly used method, the validity of the test has been criticized in the past (Silversides and Villeneuve, 1994; Silversides and Scott, 2001) because the weight of an egg is influenced by factors such as the age and breed of a bird. Therefore, the high Haugh unit values seen in the present study may be a result of the adjustment for egg weight. Diets containing wheat DDGS had a significant effect on yolk colour. In contrast, most studies using wheat DDGS in laying hen diets have found no effect on yolk colour (Wall *et al*., 2010; Niemiec *et al*., 2012). Egg yolk colouring comes from ingested xanthophyll carotenoids (lutein and zeaxanthin), derived from plant material, such as wheat. Although, wheat only contains approximately 2mg/kg of xanthophylls (Graham and Rosser, 2000), processing effects during bioethanol production can increase nutrient concentration by up to three fold, which may explain the significant increase in yolk colour intensity in the DDGS diets in the present study. Although it should be noted that the DDGS diets contained more digestible fat diets, and it is the fat which transports the colours to the egg. Considering the birds fed the DDGS diets laid fewer eggs it may suggest there were more fat soluble nutrients to add per egg that the control diets where 20% more egg mass was produced

CONCLUSION

Due to the improvements seen by the addition of xylanase to the DDGS diet on energy utilisation and nutrient availability, it would be expected that this treatment would also have the greatest effect for the bird's productive performance. However, the addition of xylanase did not improve overall laying hen performance. It may be concluded that the reduction in feed consumption of birds fed the DDGS diet is primarily responsible for the overall poor productive performance. The fact xylanase failed to restore feed intake means it cannot be used to compensate performance losses seen for the inclusion of DDGS in laying hen diets. This experiment demonstrates that despite birds showing improvements in energy utilisation and NR with the addition of xylanase to DDGS diets, the long-term feeding of this by-product at an inclusion 300 g/kg has a detrimental impact on the productive performance of hens.

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442 **Table 1.** Experimental diet formulation (g/kg)

Ingredients	Control diet	DDGS diet
Wheat	675.9	494.6
Wheat DDGS	0.0	300.0
Hipro Soya bean meal	180.0	50.0
Soya oil	20.0	34.6
Lysine HCl	1.0	3.8
Methionine	2.5	2.2
Tryptophan	0.3	0.3
Limestone	95.0	102.0
DiCal Phosphate	20.0	10.0
Salt	5.0	1.5
Vit/Min Premix[1]	0.01	0.01
Calculated composition (as fed basis)		
ME (MJ/kg)	11.60	11.60
CP	166.5	171.1
Ca	42.1	42.3
Avail P	4.9	4.5
Analyzed values (DM basis)		
DM	894.0	882.0
GE (MJ/kg)	16.45	17.85
CP	173.9	192.5
Fat	32.3	64.4
NDF	52.0	107.4
Analyzed amino acid composition (DM basis)		
Alanine	4.51	4.85
Arginine	8.14	6.43
Aspartic acid	9.98	7.18
Cysteine	1.41	1.51
Glutamic acid	24.25	27.30
Glycine	4.69	4.63
Histidine	3.18	3.11
Isoleucine	4.53	4.13
Leucine	8.20	8.65

Lysine	6.54	5.86
Methionine	2.73	2.73
Phenylalanine	5.64	5.73
Proline	8.40	10.53
Serine	5.55	5.40
Threonine	4.06	3.99
Tyrosine	3.60	3.62
Valine	5.10	5.09

[1]Vitamin and mineral premix provided (units per kg/feed): retinol, 2160 µg; cholecalciferol, 75 µg; α-tocopherol, 25 mg; menadione, 1.5 mg; riboflavin, 5 mg; pantotenic acid, 8 mg; cyanocobalamin, 0.01 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; and zinc, 80 mg.

448 **Table 2.** Treatment identification

Treatment ID	Description	Exogenous xylanase activity	Enzyme recovery
Diet 1	Control (C)	0	Not analyzed
Diet 2	C + xylanase	2500 U/kg	2841 U/kg
Diet 3	300 g/kg DDGS	0	Not analyzed
Diet 4	300 g/kg DDGS + xylanase	2500 U/kg	2681 U/kg

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450 **Table 3.** The effect of xylanase supplementation to wheat distillers dried grains with solubles (DDGS) on apparent metabolizable energy (AME), dry matter (DM) and nitrogen

451 retention and fat digestibility determined on excreta when fed to laying hens from 42 to 43 weeks of age

Treatment factor	AME	Retention coefficients		
	(MJ/kg DM)	DM	Nitrogen	Fat
Diet				
Control	12.70	0.687	0.406	0.707
DDGS	12.90	0.639	0.337	0.828
Xylanase				
-	12.63	0.659	0.364	0.760
+	12.97	0.666	0.380	0.776
SEM	0.107	0.008	0.009	0.007
Diet*xylanase				
Control - xylanase	12.79^{a}	0.690	0.414^{c}	0.688^{a}
Control + xylanase	12.62^{a}	0.684	0.399^{bc}	0.726^{b}
DDGS - xylanase	12.48^{a}	0.629	0.314^{a}	0.832^{c}
DDGS + xylanase	13.31^{b}	0.648	0.361^{b}	0.825^{c}
SEM	0.151	0.012	0.014	0.010
Probabilities of statistical differences				
DDGS	NS	<0.001	<0.001	<0.001
Xylanase	0.038	NS	NS	NS
Diet*xylanase	0.003	NS	0.038	0.043

452 There is a statistically significant difference when $P<0.05$ and statistical tendency when $P<0.10$; SEM – standard error of means.

453 **Table 4.** The effect of experimental diets on daily feed intake (FI), body weight gain (BWG), eggs laid, mean egg weight and egg mass output from 23 to 43 weeks of age

Treatment factor	FI (g/b/d DM)	BWG (g/b/d)	Egg production %	Mean egg weight (g)	Egg mass output (g/b/d)
Diet					
Control	107.0	2.86	93.2	62.8	59.0
DDGS	98.2	1.40	85.6	57.9	50.1
Xylanase					
-	101.3	1.98	88.3	60.5	53.7
+	103.8	2.27	91.0	60.2	55.4
SEM	1.160	0.112	0.920	0.260	0.71
Diet*xylanase					
Control - xylanase	105.0	2.77	93.0	62.8	58.1
Control + xylanase	108.7	2.94	94.0	62.8	60.0
DDGS - xylanase	97.5	1.19	84.2	58.3	49.4
DDGS + xylanase	99.0	1.60	87.1	57.6	50.9
SEM	1.640	0.159	1.30	0.370	1.00
Probabilities of statistical differences					
DDGS	<0.001	<0.001	<0.001	<0.001	<0.001
Xylanase	NS	0.075	NS	NS	0.093
Diet*xylanase	NS	NS	NS	NS	NS

454 There is a statistically significant difference when $P<0.05$ and statistical tendency when $P<0.10$; SEM – standard error of means.

455 **Table 5.** The effect of experimental diets on albumen height (AH), Haugh units (HU), yolk colour, eggshell

456 breaking strength and eggshell thickness from 28 to 43 weeks of age

Treatment factor	Albumen height (mm)	Haugh unit values	Yolk colour scores	Eggshell strength (N)	Eggshell thickness (mm)
Diet					
Control	9.65	96.8	1.49	30.6	0.339
DDGS	9.92	98.4	4.17	32.8	0.342
Xylanase					
-	9.72	97.3	2.77	31.9	0.343
+	9.85	98.0	2.89	31.3	0.338
SEM	0.120	0.556	0.042	0.418	0.003
Age					
28 weeks	10.5	101.1	2.65	35.9	0.359
32 weeks	9.74	97.8	2.94	30.6	0.364
36 weeks	10.18	99.6	2.85	27.4	0.294
40 weeks	9.33	95.0	2.94	33.5	0.368
43 weeks	9.21	94.6	2.76	30.6	0.317
SEM	0.114	0.532	0.063	0.613	0.004
Age*DDGS					
28 weeks					
Control	10.38	100.6	2.50	33.4	0.352
DDGS	10.53	101.6	3.68	38.3	0.366
32 weeks					
Control	9.59	96.9	1.73	29.6	0.357
DDGS	9.88	98.7	4.15	31.6	0.370
36 weeks					
Control	9.82	97.8	1.48	27.1	0.298
DDGS	10.54	101.5	4.23	27.9	0.290
40 weeks					
Control	9.36	94.9	1.33	32.9	0.367
DDGS	9.30	95.0	4.55	34.6	0.369
43 weeks					
Control	9.10	93.9	1.28	29.3	0.320
DDGS	9.32	95.4	4.25	31.9	0.315
SEM	0.188	0.873	0.090	0.881	0.005
Probabilities of statistical differences					
DDGS	0.125	0.046	<0.001	<0.001	0.455
Xylanase	0.429	0.384	0.060	0.324	0.143
Age	<0.001	<0.001	0.007	<0.001	<0.001
DDGS*xylanase	0.352	0.325	0.934	0.888	0.914
Age*DDGS	0.193	0.177	<0.001	0.154	0.117
Age*xylanase	0.775	0.788	0.533	0.901	0.904
Age*DDGS*xylanase	0.942	0.818	0.796	0.405	0.905

457 There is a statistically significant difference when $P<0.05$ and statistical tendency when $P<0.10$; SEM – standard

458 error of means.