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EFFECT OF DIFFERENT LEVELS OF WHEAT DARK GRAINS AND ENZYMES SUPPLEMENTATION ON FATTY ACIDS OF BROILER CHICKEN'S BREAST MUSCLE

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ABSTRACT

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An experiment was conducted to determine the effects of diets containing different levels of wheat distillers dried grains with solubles (wDDGS) and enzyme inclusion on the composition of categories of fatty acids of ether extract from broiler breast meat. One hundred and sixty eight (168) day-old broiler chickens were fed diets in a completely randomized design in 3 x 2 factorial arrangements (i.e. 3DDGS levels; 0, 15, 30 and 2 enzyme levels -, +) was used. The experiment lasted for six (6) weeks. The chicks were individually weighed at day-old and assigned to 6 groups (treatments) of 28 chicks each. Each group was further divided into 4 sub-groups (replicates) of 7 birds per replicate. Birds of each replicate were housed in floor pens containing wood shavings as litter material. Each pen was equipped with a plastic chick feeder and plastic drinker. Six iso nitrogenous diets were formulated and manufactured with and without enzyme inclusion (endo-1, 4-beta-xylanase 9200 U/g, alpha-amylase 1600 U/g and subtilisin as protease 16000 U/g). The diets and clean drinking water were provided *ad libitum* throughout the experiment. Results revealed that enzyme supplementation significantly ($P < 0.05$) increased the concentrations of polyunsaturated fatty acids (PUFA) and significantly reduced that of saturated fatty acids (SFA) and mono-unsaturated fatty acids (MUFA). It is therefore worthwhile to note that from the aspect of the fatty acid composition, inclusion of 15% wDDGS in the diets of broilers has no detrimental effect on the quality of broiler breast meat. Similarly, enzyme supplementation had to some extent some positive effect in improving the quality of broiler breast meat.

INTRODUCTION

Wheat distillers dried grains with soluble (wDDGS) are a by-product from bioenergy production and available in increasing quantities for use in livestock/poultry feed. Zhou *et al.* (2012) reported that dietary manipulations of poultry diets have been extensively studied with a view to improve the quality of poultry meat. Similarly, literature It is therefore worthy to investigate if wDDGS-based diets being fibrous could influence the increased concentration of the fatty acids in

indicated that several studies have been conducted to evaluate fatty acids in various animal products but none of these researches has been concerned with broiler breast meat. Further, breast meat being the most popular segment of poultry meat, its lipids are known to contain considerable amounts of essential fatty acids which are a primary area of consumer concern for health implications. edible boiler breast tissues/meat. With increasing consumer awareness of meat quality and the relationship between dietary fat and the

incidence of cardiovascular related disease. Therefore, in the present study, 3 inclusion levels of wDDGS i.e. 0, 15, and 30% were compared with and without an enzyme mixture of XAP incorporated at 0, 0.25g/kg.

MATERIALS AND METHODS

Experimental Site

The study was conducted at Cockle Park farm, Newcastle University, under the ethical guidelines for non-regulated procedure of Newcastle University, Newcastle Upon Tyne, UK. Newcastle upon Tyne commonly known as Newcastle, is a city in Tyne and Wear, North East England, 103 miles (166 km) south of Edinburgh and 277 miles (446 km) north of London on the northern bank of the River Tyne, 8.5 mi (13.7 km) from the North Sea. The ground beneath the city is formed from Carboniferous strata of the Middle Pennine Coal Measures Group—a suite of sandstones, mudstones and coal seams which generally dip moderately eastwards. To the west of the city are the Upper Pennine Coal Measures and further west again the sandstones and mudstones of the Stainmore Formation, the local equivalent of the Millstone Grit (Bedrock Geology UK North).

Experimental enzymes, wDDGS and Diets

Experimental enzymes:

The enzyme used for the study was obtained from Danisco Animal Nutrition, UK. The enzyme was a mixture of xylanase, amylase and protease, marketed to increase the digestion efficiency of wheat-based broiler, turkey and duck feeds. It is specifically developed for use in mixing-grains in poultry diets, containing vegetable and other protein sources, and ingredients of high fibre content. The enzymes were derived from *Bacillus subtilis* and *Trichoderma reesei* fermentation processes. The enzymes mixtures contains wheat flour, calcium propionate as carriers and binders. Each g of enzyme contain 9200 U of endo-1, 4-beta-xylanase, 16000 U subtilisin as protease and 1600 U of alpha – amylase (Bedford, 2000).

The concept of wDDGS and its nutrient content

Wheat DDGS is a by-product, or simply a dried residue remaining after extracting the starch portion located in the endosperm of the cereal grain (wheat), it is fermented in the ethanol production process to produce ethanol and

carbon dioxide. The majority of the starch is removed and it gives an aroma of alcohol. It is formed after the removal of the predominant nutrient in the wheat, and other nutrients in wheat grain the resulting by-product is wDDGS. It increases the concentration of nutrients up to three times more than in the original grain (Widyaratne and Zijlstra, 2007; Min *et al.*, 2009). The wDDGS contains the remaining grain nutrients (protein, fibre and oil). After the completed fermentation, the alcohol is removed by distillation and the remaining fermentation residues are then dried. The continued increase in ethanol demand and its production, especially in the US, Europe, and some countries, has been reported to increase the production of enormous amounts of DDGS flooding into the animal feed industry (Kleinschmit *et al.*, 2007). Meanwhile, the major constraint associated with the use of DDGS for poultry is the high variability of the nutrient content and availability, as well as the poor digestibility of some amino acids such as lysine, tryptophan and arginine (Choct, 2006), in addition to the presence of high fibre, possible mycotoxins and some anti-nutrients e.g. phytate. However, using enzymes to improve the nutrient availability of wDDGS may increase the protein quality and the release of metabolisable energy (ME) thereby resulting in an improved product for poultry. Being a by-product produced by means of dry mill ethanol production, wDDGS is predominantly characterized by a dark brown colour.

Experimental design and treatments

Six isonitrogenous diets, with metabolisable energy and fibre content allowed to vary, were formulated for the study by substituting wDDGS for coarsely ground wheat and soybean meal. The inclusion of wDDGS and enzymes in the experimental diets is summarized in Table 1. Enzyme addition was done by mixing with required amount in a bucket with about 2 kg of coarsely ground wheat which was then compounded mixed alongside other ingredients and the premix was added in the diets. Diets were allotted in a completely randomized design in a 3 x 2 factorial arrangement using 3 wDDGS levels of 0, 15, 30% and 2 enzyme levels of -E, +E designated as diets A, B, C, D, E & F as presented in Table 1.

Table 1. Description of diets containing different amounts of wDDGS and enzyme

Diet	wDDGS inclusion (%)	Enzyme inclusion
A	0	No
B	0	Yes
C	15	No
D	15	Yes
E	30	No
F	30	Yes

Table 2. Composition of experimental finisher diets containing wDDGS and enzymes

Ingredients	Diets (wDDGS %)					
	0	15	30	0	15	30
Diet ID	No Enzyme A	Enzyme B	No Enzyme C	Enzyme D	No Enzyme E	Enzyme F
wDDGS	0	0	150	150	300	300
Wheat	649	648.8	575	574	498	497.8
Soybean meal	294	294	218	218.8	143	143
Soybean oil	10	10	10	10	12	12
Limestone	20	20	20	20	20	20
MCP	10	10	10	10	10	10
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Premix*	12.5	12.5	12.5	12.5	12.5	12.5
Methionine	1	1	1	1	1	1
Lysine	1	1	1	1	1	1
Enzyme	0	0.25	0	0.25	0	0.25
Total	1000	1000	1000	1000	1000	1000
Calculated nutrient composition (g/kg) (unless otherwise stated)						
Crude Protein	225	225	223	223	221	221
ME (MJ/kg)	13.7	13.7	13.3	13.3	13.0	13.0
Starch	380.6	380.6	339.4	338.8	296.4	296.4
Crude Fibre	34	34	76	76	118	118
Ether Extract	75	75	67	67	61	61
Calcium	11	11	10	10	10	10
Phosphorus	5	5	6	6	7	7
Amino Acids						
Arginine	14.7	14.7	13.6	13.6	12.5	12.5
Histidine	6.1	6.1	5.9	6.0	5.8	5.8
Isoleucine	9.7	9.7	9.4	9.4	9.2	9.2
Leucine	17.3	17.3	17.1	17.2	17.1	17.1
Lysine	11.5	11.5	10.0	10.0	8.4	8.4
Methionine	4.4	4.4	4.3	4.3	4.1	4.1
Phenylalanine	11.6	11.6	11.6	11.6	11.5	11.5
Threonine	8.2	8.2	8.1	8.1	7.9	7.9
Tryptophan	3.1	3.1	2.9	2.9	2.7	2.7
Valine	10.7	10.7	10.8	10.8	10.8	10.8
Total AA	97.3	97.3	93.7	93.9	90	90

ME = Metabolisable Energy, MCP= Monocalcium Phosphate, AA = Amino Acids,

*Premix of 12.5kg contained Vitamin A12,500iu, Vitamin D3 3,000iu, Vitamin E-150g, Vitamin K 3g, Vitamin B1 2g, Vitamin B2 8g, Vitamin B6 5g, Vitamin B12 30mg, Nicotinamide 50g, D-cal Pan 10g, Folic Acid 2g, Biotin 150mg, Vitamin C 4g, Choline 300g, Copper (Copper Sulphate pentahydrate) 10g, Manganese (Manganese Sulphate monohydrate) 80g, Zinc (Zinc Oxide) 60g, Iron (Ferrous Sulphate monohydrate) 30g, Iodine (Calcium Iodate) 2g, Selenium (Sodium Selenite 0.25mg, Selenium rich yeast 0.08mg), Molybdenum (Sodium molybdate) 0.20mg, Calcium (30%), Sodium Chloride (8%)

Determination of fatty acids composition of broiler breast muscles

Fatty acid composition of ether extract from broiler breast muscles was determined according to a modified version of the method described by Sukhija and Palmquist (1988). The ether extracts collected from the representative samples of breast meat of birds from each replicate were esterified for their fatty acid analysis by using a Gas Chromatograph (Shimadzu, GC – 2014, Japan) equipped with a Varian CP – SIL 88 fused silica capillary column (100m x 0.25mm ID x 0.2µm film thickness). Individual peaks were identified by comparing sample peaks with the standard peaks from known 52 FAME standards. Gas chromatographic analyses were carried out where fatty acids were quantified by comparing their peaks with the relevant peak areas of the corresponding standard fatty acids. Each fatty acid was then expressed as a percentage of the total fatty acids quantified.

Different categories of fatty acids (Saturated or SFA, monounsaturated or MUFA and polyunsaturated or PUFA) were also determined by summation of respective fatty acids. The fatty acids that have no double bonds refers as SFA and those that have 1 double bond refers as MUFA while those that have more than 1 double bond refers as PUFA.

Statistical Analysis

Data collected were statistically analysed using ANOVA by using general linear model (GLM) in the Minitab 16 statistical package. Normal distribution of the data was determined by analysis of the residuals using the Anderson Darling test. As some of the data for fatty acids were not normally distributed, a log transformation was carried out before the statistical analysis was performed on this data set. Tukey's post-hoc test was used if there were more than two means to compare for significance at $P < 0.05$.

RESULTS AND DISCUSSION

The effects of different levels of wDDGS and enzyme inclusion on fatty acids composition of lipids from broiler breast meat are presented in 3 categories as presented in Table 3. The highest values of SFA and PUFA

were observed at 30% wDDGS level while 0% wDDGS had the highest MUFA. Similarly, enzyme supplementation significantly ($P < 0.05$) increased PUFA and also significantly reduced SFA and MUFA.

The interaction of wDDGS levels and enzyme supplementation is presented in Table 4. The results indicated that enzyme supplementation had significantly ($P < 0.05$) changed the SFA and PUFA proportions more at lower wDDGS levels than at 30% wDDGS.

Poultry meat quality is related to the growth of chicken, which thereby affects the muscular development of broilers (Zhang *et al.*, 2012). Suchy *et al.* (2002) found that the overall quality of the meat that is produced from broiler chickens is related to the quality of their muscles. Snezana *et al.* (2010) reported that the protein and lipid contents of muscles in broiler chickens can be used as an indicator of overall meat quality. Fatty acid composition is very important for consumers for quality meat. Higher inclusion of wDDGS had higher SFA and lower PUFA values which indicated that this higher wDDGS (30%) level had detrimental effect on the quality of the meat. On the other hand 15% inclusion level had similar composition for type of fatty acids. Moreover, 15% wDDGS level reduced palmitic acid of breast meat which in turn has been associated with increased risk of coronary heart disease and some tumours (Fattore and Fanelli, 2013). Though SFA was significantly ($P < 0.05$) higher at 30% inclusion of wDDGS than other two inclusion levels, stearic acid was found higher at 15% inclusion which had less hazard to healthy condition of consumers than palmitic acid (Nick, 2011). Inclusion of 15% wDDGS also increased many types of MUFA compared to others. However, essential fatty acid linoleic acid was significantly ($P < 0.05$) lower in 15% inclusion of wDDGS than 0% inclusion of wDDGS. This indicated that higher inclusion of wDDGS influenced higher SFA and PUFA content of breast meat.

Since the dietary concentrations of nutrients are translated in the edible tissues, and since the experimental diets contained an increased level of wDDGS (Table 3), this elevated composition of PUFA could be expected due to these differences.

Therefore, feeding wDDGS based diets to broiler chickens can positively affect breast meat by increasing the long chain PUFA composition which consumers are encouraged to increase their daily intake of it for health benefits (Rymer and Givens, 2005). The higher PUFA values obtained in this study confirmed the findings of different researchers (Bonoli *et al.*, 2007; Corzo *et al.*, 2009; Nkukwana *et al.*, 2014) who described these as nutritionally undesirable to the consumers as these render the meat more susceptible to oxidation than SFA and consequently negatively affects meat flavour. Thus, the keeping quality especially in tropical countries where humidity is usually high remains a serious concern over long term keeping quality of meat which is essential as not everybody can afford facilities such as freezers to preserve meat. Enzymes supplementation increase the meat keeping quality of breast muscle of broiler by increasing PUFA and decreasing SFA content. Enzyme supplementation was reported to reduce palmitic acid concentration which has a detrimental effect in human health (Fattore and Fanelli, 2013). It is therefore worthwhile to

note that enzyme supplementation had to some extent some positive effect in improving the quality of broiler breast meat.

Conclusion

Based on this study, it appeared that inclusion of 15% wDDGS in the diets supported reduction in values and concentrations of SFA and PUFA. From the aspect of the fatty acid composition, it could be proclaimed that wDDGS could be used in broiler diet up to 15% level of inclusion without showing any detrimental effect or with minimum effect of quality of broiler breast meat. Similarly, enzyme supplementation significantly ($P < 0.05$) increased the concentrations of polyunsaturated fatty acids (PUFA) and significantly reduced that of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Enzyme supplementation therefore, had to some extent some positive effect in improving the quality of broiler breast meat for healthy condition of consumers and thus increasing the keeping quality of meat.

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Table 3. Mean for the main effects of wDDGS and enzyme on the main categories of fatty acids of breast meat from broiler chickens

Fatty acid (%)	wDDGS (%)				P value	NE	Enzyme Inclusion		
	0	15	30	SEM			E	SEM	P value
SFA	33 ^b	34 ^b	38 ^a	0.9	0.001	35 ^a	34 ^b	0.8	0.023
MUFA	37 ^b	36 ^a	27 ^b	0.7	0.001	35 ^a	31 ^b	0.6	0.001
PUFA	31 ^b	32 ^b	36 ^a	0.8	0.001	30 ^b	35 ^a	0.6	0.025

a, b, Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, NE = No Enzyme, E= Enzyme, SFA= Saturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, PUFA= Polyunsaturated Fatty Acids

Table 4. Interaction effect of wDDGS x enzyme on categories of fatty acids of breast meat from broiler chickens

Fatty acid (%)	Enzyme Inclusion							SEM	P value
	No enzyme			+ Enzyme					
	wDDGS (%)								
	0NE	15NE	30NE	0E	15E	30E			
SFA		34 ^c	34 ^c	37 ^a	31 ^c	33 ^{bc}	38 ^a	1.3	0.001
MUFA		38	38	29	35	33	25	1.0	0.067
PUFA		28 ^{cd}	29 ^c	34 ^c	34 ^c	34 ^c	38 ^a	1.1	0.014

a,b,c Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, NE = No Enzyme, E= Enzyme, SFA= Saturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, PUFA= Polyunsaturated Fatty Acids.