Sudan University of Science and Technology

College of Graduate Studies

Effect of Inclusion Rate of Dried Distiller Grains (DDGS) and Enzymes on Growth Performance and Protein Profile in Broiler Chickens

تأثير إضافة ناتج تقطير الحبوب المجفف والإنزيمات علي معدل النمو والبروتين في فراخ اللاحم

Thesis submitted in fulfilment of the requirements for the Master's degree in Veterinary Medicine (Physiology)

By

Emad Gafar Salem Salih

B.V.Sc. (1994) Khartoum University

August 2018

Supervisor

Dr. Alaeldein Mahmoud Abudabos

Department of animal production,

College of Food and Agricultural Sciences, King Saud University.

Co- Supervisor

Dr. Shams Eldein Hasab Allah Ahmed

Department of Basic Medical Sciences

College of Veterinary Medicine (SUST)

الآية

بسم الله الرحمن الرحيم

قال تعالى:

﴿اقرأ باسم ربك الذي خلق ﴾﴿خلق الإنسن من علق﴾﴿اقرأ وربك الأكرم﴾﴿الذي علم بالقلم ﴾﴿علم الإنسن مالم يعلمْ﴾.

صدق الله العظيم

(سورة العلق الآيات من 1 الى 5)

Dedication

I dedicate my dissertation to my family and friends.
A special feeling of gratitude to my loving parents, Soul of my father Gafar May Allah mercy him and Lovely Mother Arafa. My Wife Suaad, My son Gafar ,My daughters Ghuida, Ghadeer and Ghuitha for their unconditioned Love and Support.
To Memory of my friend Prof. Hasab Alrasoul Hussain may Allah mercy him.

ACKNOWLEDGEMENTS

I would like to thank all of those who their encouragement and support was essential during the start and completing this research and study. I would like to thank the following individuals:

Prof. Dr. Alaeldein Mahmoud Abudabos, my supervisor for his tremendous support, advice, and supervision throughout the process of research and thesis writing.

My thanks are also due to Dr. Shams Eldein H. Ahmed, my Co-supervisor for his assistance on the scientific and technical part of the study.

Special thanks to Professor Hisham Seri for his unconditional support from the initial stage of this study up to the outcome, his generosity of knowledge sharing, and guidance.

Many thanks to Professor Galal Alazhri for his generous support & Guidance.

Above all, praise is to Allah, the Compassionate, and the most Merciful for giving me the health and strength to carry out this work.

ABSTRACT

The objective of the current study is to investigate the effect of corn distillers dried grains with solubles (DDGS) (which is the by-products of the process of Ethanol formation by fermentation of grains with the using of yeast and some enzymes) and enzyme supplementation on growth performance and carcass yield in broiler chickens. The experiment was a 5×3 factorial design with 450 broiler chicks and with diets containing 5 levels of DDGS (0, 6, 12, 18 and 24%) and 3 levels of enzyme (no supplementation, Rovabio® Excel enzyme and Tomoko® enzyme). Five pens with six chicks were fed the experimental diets from day one to 35 d of age. Diets containing 12, 18 and 24% DDGS decreased performance (P <0.05) at the start of the trial at 0-10 d. Inclusion of enzyme during 0-10 d improved body weight gain (BWG) and Production efficiency factor (PEF) (P <0.05). During the grower (11-24 d) and finisher (25-35 d) periods, chickens which had received 0, 6 or 12% DDGS converted feed to body weight more efficiently (P <0.05). Enzyme supplementation improved Feed conversion ratio (FCR) for the periods (11-24 and 25-35 d, respectively) (P <0.05). The cumulative performance results (0 to 35 d of age) showed that Tomoko enzyme improved FCR as compared to no enzyme while Rovabio was intermediate (P <0.05). Chickens which had received 0, 6 or 12% had better FCR (P <0.05) compared to 18 or 24% DDGS. Chick's performance was depressed at early age when the diet contained 12% DDGS but later they were able to tolerate higher levels of DDGS.

The study indicates that a maximum level of DDGS to use in the starter diets is 6% and it could be increased in the grower and finisher period to 12% and enzyme supplementation to diets containing DDGS can improve FCR and growth performance in broilers.

Keywords: distillers dried grains with solubles; enzymes; broiler; performance; carcass yield

المستخلص

الهدف من هذه الدراسة هو بيان تأثير مادة الذرة المقطرة مع الذوائب وهي عبارة عن نتاج ثانوي عن عملية تحويل الحبوب بواسطة الخميرة وبعض الانزيمات الى مركب الإيثانول) والاضافات الانزيمية على أداء النمو ومعدل التصافي في فراخ اللحم. كانت التجربة 5× 3عواملية صممت لتحتوي على 5 مستويات من الذرة المقطرة مع الذوائب المضافة (0 ,6 ,12 ,18 و 24 %) وثلاث مستويات من اضافات الانزيمات (بدون , انزيم روفابيو اكسل وانزيم توموكو). يحتوي كل قفص على 6 كتاكيت علفت حصرياً من علائق التجربة من عمر 0 – 35 يوم . الطيور التي علفت من العلائق المكونة من الذرة المقطرة مع الذوائب بنسب 12 ,18 و24 % أنخفض فيها الآداء (القيمة الاحتمالية أقل من 0.05) في بداية التجربة من عمر 0 – 10 أيام. أضافة الانزيمات خلال الفترة من 0 – 10 أيام حسَّنت من وزن الجسم و معامل فعالية الانتاج (القيمة الاحتمالية أقل من 0.05). في فترة العليقة النامية (11– 24 يوم) والعليقة الناهية (25 – 35 يوم) الطيور التي حصلت على علائق تحتوي على 0 ,6 و12 % من الذرة المقطرة مع الذوائب حولت العلف الى وزن جسم بفعالية (القيمة الاحتمالية أقل من 0.05). إضافة الانزيمات حسَّنت من معامل التحويل الغذائي خلال الفترات من 11 –24 و25– 35 يوم من أعمار الطيور(القيمة الاحتمالية أقل من 0.05). النتائج التراكمية للآداء (0 – 35 يوم من عمر الطيور) أظهرت أن الطيور التي تلقت انزيم التوموكو قد تحسَّن معامل التحويل الغذائي فيها عند مقارنتها بتلك التي لم تتلقى الانزيم في حين كان تأثير انزيم الروفابيو متوسطاً (القيمة الاحتمالية أكبرمن 0.05). الطيور التي تغذت على علائق بها نسبة مادة الذرة المقطرة مع الذوائب 0, 6 أو 12 % تحصلت على معدل تحويل غذائي أفضل من تلك التي تغذت على علائق ذات المحتوى 18 أو 24 % من نفس المادة (القيمة الاحتمالية أقل من 0.05). تدهور أداء الطيور التي تغذت على علائق تحتوي على 12% من مادة الذرة المقطرة مع الذوائب في العمر الصغير ولكن عندما كبرت صارت لديها مقدرة على تحمل المعدلات العالية من المادة.

توضح الدراسة أن الحد الأعلى الذي يجب استعماله من مادة الذرة المقطرة مع الذوائب في علائق البادئ هو 6 % ويمكن رفع النسبة في علائق النامي والناهي الى 12% , وأن إضافة الانزيمات للعلائق المحتوية على مادة الذرة المقطرة مع الذوائب يمكن أن يحَّسن معدل التحويل الغذائي ومعدل النمو في الدجاج اللاحم.

الكلمات المفتاحية:

الحبوب المقطرة , الانزيمات , الدجاج اللاحم , الأداء و معدل التصافي.

List of Contents

List of Tables

List of Figures

List of Abbreviations

DDGS	Dried Distiller's Grains
NSPs	Non-Starch Polysaccharides
FCR	Feed Conversion Rate
PEF	Production Efficiency Factor
BWG	Body weight gain
Rovabio® Excel	Feed additive containing a combination of 19 active enzymes produced by only one non-genetically modified fungus (Penicillium funiculosum). Manufactured by (ADISSEO France)
Tomoko®	Commercial enzyme supplement that contains acidic protease, α-amylase, pectinase, phytase, glucoamylase, cellulase and Aspergillus awamori cells. Manufactured by (Biogenkoji Research Institute, 876-15, Kagoshima, Japan)
TSAA	Total Sulphur amino acids.

INTRODUCTION

Dried Distiller's Grains (DDGS) is a by-product of the newly-emerged ethanol industry, during the corn-to-ethanol production process (Figure 1). Production of DDGS has tripled in the past decade to an annual production of 12 million metric tons in 2006 (Renewable Fuels Association, 2007). The booming in ethanol production sector, leds to more available DDGS for livestock feed (Wood*et al*., 2011; Liu*et al*., 2011). On the other hand, feed cost remains to be the most determining factor in animal production without exception, and due to water scarcity in many areas, policy makers shifted from planting feed to more imported animal feed and by-products. From nutritional and technical economic point of views, DDGS due to its higher energy, phosphorus and amino acids content (table 1) when compared to corn, wheat and barley, DDGS are presented as a nutritional and cheap alternative feed in the animal's diet (Sandra Cruz.,2015).

Dried grains of cereal distillers are rich in protein, exogenous amino acids, B-group vitamins, biotin and mineral compounds, including phosphorus (Koreleski & Świątkiewicz 2006, Thacker & Widyaratne 2007, Min et al. 2008).

It also contributes to lower feed costs of broilers industry (Wang *et al*., 2007).

An increasingly growing interest worldwide in the use of DDGS, as alternative animals feed from both nutritional and economical points of view, has increased dramatically. However, the inclusion rate of DDGS in broiler feeds has been controversial and has varied by age. For example, young broilers are more sensitive to feed quality since their digestive systems are not fully developed until they are approximately two weeks old (Batal and Parsons, 2002). Therefore, due to high fibre content and low amino acid digestibility of DDGS, high level of

supplementation to broiler chickens during the two first weeks after hatching is not recommended.

Another point of view is considering health status of the studied animals as correlated to potential effects of DDGS with residual aflatoxin on lipid peroxidation in blood and liver as well as bacteria diversity in gastrointestinal tract.

The level of non-starch polysaccharides (NSPs) in DDGS can be two to three times higher than that of corn grain (Bennett and Richard, 1996; Cromwell et al., 1993). According to Swiatkiewicz and Koreleski (2007) corn DDGS contains high levels of total NSPs. Enzymes could reduce the negative effects of NSPs and improve the digestion and absorption of nutrients in poultry diets (Malkki, 2001). The use of enzymes may overcome the nutritional challenges associated with feeding high levels of DDGS to broilers because the high NSP content of DDGS provides substantial substrate for the xylanase and cellulase enzymes.

With the advance of the sequencing technology, the role of intestinal microbiota in health became apparent with the evidence of highly variable microbiota in poultry intestine that readily responds to many environmental changes (Stanley*et al.*, 2013). The diet change is the most influential variable in healthy subjects with humans responding to diet changes almost immediately (Turnbaugh*et al.*, 2009; Candela*et al.*, 2012). Inclusion of DDGS as well as enzymes has a capacity to change intestinal microbial community with beneficial, neutral or detrimental effects on poultry health (Stanley*et al.*, 2014). To date there were no studies that evaluated this effect DDGS may have on chicken health.

The objectives

The objectives of this study were to

1. Assess the effects of different levels of DDGs on feed intake, growth rate and performance of broiler

2. Measure Protein profile, and ALT and AST hepatic enzymes in relation to DDGs.
3. Assess the inclusion rate of DDGs on antioxidant biomarkers in plasma and tissues of broiler

CHAPTER ONE

LITERATURE REVIEW

1 I Dried Distiller's Grains (DDGS)

In recent years, increasing demand for ethanol as a fuel additive and decreasing dependency on fossil fuels have resulted in a dramatic increase in the amount of grains used for ethanol production. Dry-grind is the major process, resulting in distillers dried grains with solubles (DDGS) as a major co product. Like fuel ethanol, DDGS has quickly become a global commodity. However, high

compositional variation has been the main problem hindering its use as a feed ingredient. This review provides updated information on the chemical composition of distiller's grains in terms of nutrient levels, changes during dry-grind processing, and causes for large variation (figure 1). Fermentation causes major changes, but other processing steps are also responsible. The causes for varying DDGS composition are multiple, including differences in feedstock species and composition, process methods and parameters, the amount of condensed soluble added to distiller wet grains, the effect of fermentation yeast, and analytical methodology. Most of them can be attributed to the complexity of the dry-grind process itself. It is hoped that information provided in this review will improve the understanding of the dry-grind process and aid in the development of strategies to control the compositional variation in DDGS (Liu et al., 2011).

There were no significant differences between wheat and corn (4.61 vs 4.56, $P > 0.05$), but there were significant differences between wheat DDGS and corn DDGS (3.08 vs 2.21, $P < 0.05$) ,(table 1).

One Study indicated that bioethanol processing changes protein molecular structures, compared with original grains. Further study is

needed with a large set of the new bioethanol co products to quantify protein molecular structures (alpha-helix to beta-sheet ratio; amide I to II ratio) of the bioethanol co products in relation to nutrient supply and availability in animals (Yu et al., 2010).

Three points should be considered when feeding DDGS to broilers. First, variability in chemical composition of DDGS due to the processing method which includes fermentation method, time, and drying process which can reduce profitability of poultry operations because of increased feed costs and reduced production (Wen*et al*., 2010). Second, if mycotoxins are present within the corn grain prior to fermentation, the DDGS will contain three to four times the concentration of mycotoxins (Murthy*et al*., 2005; Ingledew, 2006). Third, the level ofnon-starch polysaccharides (NSPs) in DDGS will be two to three times as compared to corn grain (Cromwell*et al*., 1993;Bennett and Richard, 1996).

Good-quality DDGS is a potentially useful feed ingredient; an extensive compositional analysis of DDGS has been completed by several researchers. For example, the average composition of 118 samples of DDGS collected from 10 different dry grind facilities was about 30.2% protein, 10.9% crude fat, 8.8% crude fiber and 5.8% ash, the values for the coefficient of variation (CV) were 6.4, 7.8, 8.7 and 14.7, respectively (Spiehset al., 2002). In addition, composition of DDGS collected at one plant over a five-year period was about 31.3% protein, 11.9% crude fat, 10.2% crude fiber and 4.6% ash (Belyeaet al., 2004). Wenet al. (2010) also reported the results of a survey on the fiber composition and crude protein content in 20 corn-DDGS samples from China, the mean and CV values as follows: crude protein, 33.9% (8.7); acid detergent fiber, 19.8% (19.6); neutral detergent fiber, 31.8% (21.4) and hemicellulose, 12.0% (39.4).

The inclusion rate of DDGS in broiler's feeds has been controversial and varied by age. For example, young broiler chicks are sensitive to feed quality because their digestive systems are not fully developed until about two weeks (Batal and Parsons, 2002). Therefore, because of the high fiber content and low amino acid digestibility of DDGS, feeding high levels DDGS during the two first weeks after hatch is not recommended. In details, Lumpkinset al. (2004) fed 0 or 15% DDGS to broiler chickens during the starter period (d 1 to 18) and found no adverse effects on body weight gain or feed conversion. In a second part of the experiment, the birds were fed 0, 6, 12 and 18% DDGS from 1 to 42 d of age. No differences in performance or carcass characteristics were reported except when birds were fed diets that contained 18% DDGS during the starter period as a result to a marginal lysine deficiency. The group suggested that maximum amount of DDGS for starter, grower and finisher diets to be no more than 9, 12 and 15%, respectively. Junget al. (2011) reported that careful consideration should be given when 9% DDGS is fed to broilers for starter period (0 to 21 d) due to negative effects on feed efficiency. Wanget al. (2007a) fed diets containing three levels of DDGS to broilers to 42 d of age (0, 15 or 30%), no negative effects of feeding 15% DDGS were reported while, feeding 30% caused depression in performance due to an Arginine deficiency and caused lower arginine-to-lysine ratios.

Wanget al. (2007b) formulated diets based on digestible amino acid content to contain 0, 5, 10, 15, 20 or 25% DDGS from 1 to 49 d. Their results indicated that DDGS could be used at levels between 15 to 20% in broiler diets without negative effect on performance; however, dressing percentage or breast meat yield could be slightly lower. Liuet al. (2010) found that the inclusion of 20% DDGS led to

poorer FCR at 22-42 day. Wanget al. (2007c) found that broilers received a corn-SBM based diet with inclusion of DDGS from 5 and up to 25% had similar body weight gain at 14, 35 and 49 d of age, however, feed conversion ratio was poor in the diet containing 25% DDGS at 35 and 49 d. Parsons and Baker (1983) concluded that 20% of the SBM in broiler diets could be replaced by DDGS without the need to supplement lysine, and if 30% of the SBM was replaced, lysine must be supplemented. Waldroup et al. (1981) and Parson et al. (1983) showed that maize DDGS could replace 25-40 % of soybean meal in the mixture without deteriorating production results.

According to Świątkiewicz & Koreleski (2003), the optimal contribution of dried distillers maize in feed mixtures for slaughter chickens amounted to 2 % in the first and to 5 % in the second rearing period.

1 II Contamination with Moulds:

Moulds are unavoidable because they are naturally occurring compounds. They contaminate crops before harvest or invade feedstuffs of animals during processing, transport or storage (Yaling *et al*., 2008). Aflatoxins (AF) are a class of mycotoxins produced by fungal species of the genus *Aspergillus* (*flavus* and *parasiticus*). Aflatoxins are found in feed ingredients typically used for poultry diets. Aflatoxicosis was reported as a problem for poultry since the 1960s (Asplin and Carnaghan, 1961). Binder *et al.* (2007) conducted a survey of nearly 2,800 ingredients and feed samples in Asia and Europe and reported that20 and 32% of feed samples were contaminated with AFB_1 with a median contamination concentration of 0.013 and 0.015 mg/kg, respectively. Major forms of aflatoxins include B_1, B_2, G_1 and G_2, and the order of acute and chronic toxicity

of aflatoxin metabolites is AFB_1> AFG_1> AFB_2> AFG_2.Aflatoxin B_1 (AFB_1) was reported to be the most common and biologically active component (Busby and Wogan, 1981). Rodrigues (2008) showed that 99% of the 103 DDGS samples that were studied contained at least one detectable mycotoxin, with 8% containing detectable aflatoxin. Recently, Zhang *et al*. (2009) collected 20 DDGS samples from ethanol plants and measured the presence of aflatoxins, fumonisins, T-2 toxin,deoxynivalenol, and zearalenone, AFB_1 was detected in six DDGS samples, however, none contained aflatoxin or deoxynivalenol levels higher than the Food and Drug Administration (FDA) guidelines for use in animal nutrition and none of the other aflatoxin compounds, B_2, G_1 and G_2 were detected in any of the samples. Verrips *et al*. (2010) tested the prevalence of mycotoxins in 90 DDGS samples; the group reported 40% of the corn samples from the Midwestern region were positive and all DDGS samples tested positive for the mycotoxins.

The effect of aflatoxin ingestion on growth inhibition and body weight reduction in chicken is well documented at levels higher than 1 mg/kg diet. Miazzo *et al*. (2005) reported that aflatoxin B_1 at the level of 2.5 mg/kg significantly diminished body weight gain and growth rate in broilers during growing period. Zhao *et al*. (2010) found that birds fed 2 mg/kg of AFB_1 consumed less feed and gained 78% of the weight when compared to positive control birds (Magnoli *et al*., 2011). In poultry, the relative weight of the liver is increased by aflatoxin ingestion more than that of any other organ due to excessive build-up of hepatic lipids (Huff *et al*., 1986). Van Rensburg *et al*. (2006) reported that liver weight was 75% higher in chicks that received diets contaminated with 2 mg of AFB_1/kg of feed. Serum glutamyl transferase enzyme activity is a sensitive indicator of liver

disease, whether the disorder involves liver inflammation, lesions, or obstruction to the biliary tract (Kubena*et al*., 1990). In Van Rensburg study (2006), serum glutamyltransferase activity was decreased in birds due to consuming 2 mg of AFB_1/kg of diet. Stanley *et al*. (1993) reported a 17 to 42% drop in alanine aminotransferase activity due to AFB_1.

The presence and the negative effects of mycotoxins in feed have given rise to a demand for practical and economical detoxification procedures. Many procedures have been tried to eliminate the problem, detoxifying procedures such as heat application and various chemical addition to feedstuffs, their effectiveness, economics and practicality have been the main concerns most of the times (Bingham *et al*., 2003). Increased efforts are being undertaken to developa cost effective and safe procedures and products to effectively deal with contaminated feed ingredients. One method is the addition of mycotoxin binders to contaminated ingredients which is considered the most promising dietary approach to reduce the negative effects of mycotoxins (Ramos and Hernández, 1996; Galvano*et al*., 2001; Huwig *et al*., 2001; Avantaggiato *et al*., 2005). In order to examine the effectiveness of the binders, it is necessary to evaluate it with different inclusion rates, with different mycotoxins and under different environmental conditions (Garcia *et al*., 2003; Diaz *et al*., 2004).

1 III Presence of NSP:

It was reported that the presence of NSPs which is considered as anti-nutritional factor will affect negatively digestion in poultry (Pack and Bedford, 1997). Corn contains 9.7% NSP which represent an average of 80% of the cell wall (Bach Knudsen, 2001). While, SBM

contains 30.3% NSPs which constitutes 70–90% of the plant cell wall (Bach Knudsen, 2001). Corn DDGS contains more than 250 g/kg total NSP, 90% of that NSP is insoluble (Swiatkiewicz and Koreleski, 2007). Arabinoxylans are the predominant component of hemicellulose in DDGS (Cowieson, 2005). Ward and co-workers (2008) noted that arabinoxylans and cellulose were the predominant NSP; a value of 11.4% arabinoxylans was reported by the group. It was calculated that with a 15% inclusion rate of DDGS, the arabinoxylans content increased by 20% in final diet. Total available sugars (glucan and xylan) of DDGS were measured to be 29.4%, based on a total dry mass basis (Kim*et al*., 2008). Water soluble NSPs fed to young chick's decreased digestion and absorption of other nutrients by increasing the viscosity of digesta in the gut (Ward and Marquardt, 1983).

1 IV ENZYMES:

Enzymes could reduce the negative effects of NSPs and improve the digestion and absorption of nutrients in poultry diets (Malkki, 2001). Tomoko®, a commercial enzyme supplement that contains acidic protease, α-amylase, pectinase, phytase, glucoamylase, cellulose and *Aspergillus awamori* cells. Abudabos (2010) reported that body weight and ileal protein retention were positively affected by Tomoko enzyme supplementation to a corn-SBM diet at 42 d. Tomoko enzyme was able to restore the nutritional value in the low density corn-SBM diet. In diets with decreased caloric value and supplementing it with Tomoko enzyme when fed to broilers; performance values are improved to levels similar with birds fed diets of a higher caloric value. Similar result was reported by Saleh *et al*. (2006). Rovabio is a

multi-synergistic enzyme containing 19 enzyme activities that work on all major ingredients all produced by *Penicillium funiculosum*.

When considering incorporation of enzymes into broilers' diets, one approach is to change the nutrient density of the feed to reduce the cost per ton of feed and then, by adding enzymes, to restore the nutritional value of the feed. This results in performance better or at least similar to a normal feed density (Pack and Bedford, 1997). The use of enzymes may overcome the nutritional challenges associated with feeding high levels of DDGS to broilers since DDGS's high NSP content provides substantial substrate for the xylanase and cellulase enzymes.

Furthermore, Lumpkins *et al*. (2011) conducted a study to investigate the effects of Rovabio in a broiler diet that contained 12% DDGS, the result revealed that addition of the enzyme to the negative control feed which was formulated to contain 132 kcal/kg less energy, restored performance similar to the positive control. The inclusion of xylanase enzyme to broiler during the growing period increased positively feed intake by 4-5%, increased dry matter and hemicellulose digestibility by 5% and 20%, respectively while it did not affect FCR (Liu*et al*., 2010).

Recently, Jung *et al*. (2011) reported that enzymes supplementation to starter diet (0 to 21 d) which contained 9% DDGS inclusion level may overcome the negative effects of DDGS. Schwartz *et al*. (2010) used diets that contained 0 or 10% DDGS with two energy levels (low or high) and two different enzymes (a xylanase product and a broad array of enzyme activities) from d 1 to 49. They found that 14 d FCR improved by 11.4 and 4.7% with enzyme supplementation in the low- and high-energy diets, respectively.

Arce *et al*. (2010) tested the efficacy of protease and xylanase enzymes for broilers fed diets containing corn, soybean meal and DDGS. They found that the combination of protease and xylanase activities (RonozymeProAct + Ronozyme WX) gave the best performance. A work by Moran and Lehman (2008) using xylanase, amylase, protease and phytase supplementation to a corn-SBM with 10% DDGS diet for broilers raised to d 56 revealed a significant improvement in weight gain and feed efficiency. Adeola *et al*. (2010) evaluated the ileal digestible energy, ME, and MEn contents of corn DDG at 3 levels (0, 30 or 60 %) for broiler chickens from d 15 to 22 with and without carbohydrase premix supplementation (xylanase + amylase). Supplementation with carbohydrase improved ileal digestible energy, ME, and ME(n) of corn DDG in practical corn-SBM-based diets by 12, 5.7 and 6.2%, respectively.

Xylanase supplementation to the diet can break down NSP in DDGS and consequently improve utilization of diet components (Liuet al., 2011). On the other hand, Minet al. (2009) failed to show any significant differences due to enzymes supplementation (Allzyme SSF and Rovabio Excel) to corn- SBM-based diets or diets containing up to 30% DDGS even when the enzymes were used by four times of the recommended levels. Similarly, Westet al. (2007) reported that addition of the Rovabio Excel enzyme to corn- SBM diets which differ in amino acid and energy composition did not affect the performance.

Feed enzyme complex supplementation and extrusion both increased the nutritive value of triticale DDGS for broilers. Triticale DDGS can be fed at up to 10% of practical broiler diets without adverse effect on performance and breast muscle yield (Oryschak et al., 2010). The results of this study demonstrate that 20% DDGS

derived from ethanol production can be fed to laying hens, resulting in lower emissions of NH3 and H2S with no apparent adverse effects on hen performance (Wu-Haan et al., 2010).

1 V Antioxidation

A considerable amount of information has been generated on the feeding value and impact of corn (DDGS) on meat quality (Aldai*et al*., 2010a). DDG have potential to be a nutritionally important source of protein, oil and phenolic antioxidants (Inglett*et al*., 2010). Heincinger*et al*. (2011) found that DDGS inclusion increased the ether extract content of the diets which resulted in higher reduced glutathione (GSH) content and elevated glutathione peroxidase activity (GSHPx) in the liver. DDGS, even at a high inclusion level combined with Lys and Met supplementation, has no initiative effect on lipid peroxidation in the blood and liver of broiler chickens. Supplemental DDGS level up to 12% caused no lipid peroxidation to broiler meat (Schilling*et al*., 2010).DDGSis rich in vitamin E, a strong lipid antioxidant, interestingly during hot conditions in Japan, Supplemental DDGS in dairy cows reduced lipid peroxidation (Tanaka*et al* 2011).

Studies on camel antioxidant status indicated better and greater ability of withstand stress conditions (Mohamed 2007a,b; Mohamed*et al*., 2011). Antioxidant contents of meat are of health potentials to human; and therefore possible degree of oxidative stress may deter these potentials. Studies indicated species variations in terms of antioxidant contents of meat. For example, catalase and GSH-Px activities were much higher in camel than in chicken and cattle and higher in cattle than in chicken. Thiobarbituric Acid (TBA) value was lower in chicken than in camel (Gheisari, 2011).

Total polyunsaturated fatty acids concentrations were greater ($P < 0.05$) in DDGS steaks compared corn gluten feed steaks (Serger*et al*., 2011). DDGS in pig diets increases unsaturated fatty acids in pork (Boler*et al*. 2009).

Tocopherol serves as an antioxidant preventing free radical formation during lipid oxidation and found in great amount in DDGS (Botsoglou*et al*. 2003; Gibreel*et al*., 2011).

CHAPTER TWO

MATERIALS AND METHODS

2 I Animals and housing

Newly hatched broiler chicks (Ross-308) were obtained from a commercial hatchery. The chicks had been vaccinated against Newcastle and Infectious Bronchitis diseases at the hatchery. Chicks were sexed, weighed and were allocated to cages and received the experimental diets in electrically heated battery brooders with raised wire floors. The experiment was conducted in an environmentally controlled battery room at the Animal Production Department, College of Food Science and Agriculture Science, King Saud University, Riyadh, Saudi Arabia. The ambient environmental temperature in the first week of rearing was set at 33°C, and it was decreased to 22°C toward the end of the experiment. Ambient temperature and relative humidity were concurrently and continuously recorded at 4 hours interval using two data loggers (HOBO Pro Series Data Logger, Model H08-032-08, Onset Co., USA) placed inside the chamber.

2 II Experimental design and diets

The experiment was performed from 0 to 35 d of age; chicks were given *ad libitum* access to feed and water, with continuous lighting and controlled ventilation. The birds were distributed into 75 experimental pens (five replicates per treatment with six chicks (male: female, 3: 3) per replicate and 15 treatments). A typical isocaloric and isonitrogenous starter (0 to 10 d), grower (11 to 24 d) and finisher (25 to 35 d) diets based on DDGs-corn-soybean meal were formulated in mashed form which met the recommendations of the Ross 308 breed Management Guide (Tables 1-3). Diets were formulated based on digestible amino acids and contained the same levels of lysine, total sulphur amino acids (TSAA) and threonine. Five DDGS-corn-SBM

diets were formulated to contain different concentrations of DDGS (0, 6, 12, 18 and 24%). Each diet was supplemented with or without enzyme in a factorial arrangement (5 levels of DDGS and 3 enzymes (no supplementation, the supplementation of Rovabio® Excel and Tomoko® enzyme), resulting in a total of 15 experimental diets as follows: T1 = 0% DDGS without enzyme, T2 = 0% DDGS with Rovabio, T3 = 0% DDGS with Tomoko, T4 = 6% DDGS without enzyme, T5 = 6% DDGS with Rovabio, T6 = 6% DDGS with Tomoko, T7 = 12% DDGS without enzyme, T8 = 12% DDGS with Rovabio, T9 = 12% DDGS with Tomoko, T10 = 18% DDGS without enzyme, T11 = 18% DDGS with Rovabio, T12 = 18% DDGS with Tomoko, T13 = 24% DDGS without enzyme, T14 = 24% DDGS with Rovabio and T15 = 24% DDGS with Tomoko

2 III **Measurement**

Body weight and feed intake were measured weekly and there were no mortality of birds during the experiment. Feed conversion ratio (FCR) was computed for each group and production efficiency factor (PEF) was determined as follows by the method described by Abudabos et al. (2016):

PEF= (Livability × Live weigh (kg) ÷ (Age in days × FCR) ×100.

At 28 d of age, two birds were placed in metabolism cages according to the current treatment and were fed the experimental diets. The excreta were collected and stored at -20°C until the time of the nutrient retention analysis by the total collection method. Feed and excreta were analyzed to determine the nutrient retention. Gross

energy determinations of feed and excreta samples were performed using a bomb calorimeter (Parr Instruments Co., Moline, IL). The AMEn of the diets was calculated using Cr as the digestive marker. Nitrogen and ether extract contents of feed and excreta samples were also determined (AOAC 1984).

Blood samples were collected weekly and prior to slaughtering. At 35 d, three males from each treatment were sampled, after Islamic slaughtering, liver and thigh samples were collected. the skin, feathers, head, neck, and shanks were removed. The remaining carcasses were dissected into breasts, thighs, drumsticks and abdominal fat and were weighed. The percentages of eviscerated carcass and the yield of each part were calculated based on dressed weight.

A Malondialdehyde (MDA) concentration was determined using the direct chemical-extraction method. The total antioxidant capacity (TAC) was assayed in the plasma and liver using colorimetric method at a wavelength of 570 nm (Cayman chemical company kits, USA). Plasma DNA damage was measured in plasma and liver samples using (Cayman chemical company kits, USA).

Blood samples were collected from one bird/pen weekly, and plasma was harvested and stored at -20°C until completion of the biochemical analyses, which included assessments of the total protein, albumin, and globulin concentrations. The hepatic glutamic-pyruvic

transaminase and glutamic oxaloacetic transaminase enzyme concentrations were determined using a commercially available kit (UV/Kinetic method), according to the manufacturer's recommended procedures.

2 IV Ethical approval

This experiment was approved by the Departmental Board of Studies on Ethics, Methodology and Welfare, King Saud University, Kingdom of Saudi Arabia.

2 V Statistical analysis

Data were analyzed by the general linear model procedure for a randomized complete block design with 5 x 3 factorial treatment arrangements. Data were examined to determine significance of main effect (DDGS and enzyme) and interactions (DDGS x enzyme). The overall level for statistical significance was set at $P < 0.05$. All values were expressed as means ± standard error of the mean (SEM).

The following model was used for the experiment:

$Yijk=\mu+\alpha i+\beta j+(\alpha\beta)ij+eijk$

where αi = the effect of DDGS level (i = 1, 5) , βj = the effect of enzyme (j = 1, 3), and $+(\alpha\beta)ijk$ = the interaction between DDGS level and enzyme (k =1, ..., 15). It was assumed that $eijk \sim N(0, \sigma 2)$, i.e. independently and identically distributed with the normal distribution.

CHAPTER THREE

RESULTS and Discussion

3 I Feed consumption and efficiency

No significant two-way interaction (Enzyme x DDGS) was observed for FI, BWG, FCR or PEF (P > 0.05) for the period from 0 to 10 d. However, DDGS level in the diet affected all parameters significantly (Table 4; *P* <0.05). Chicks which had received 18 and 24% DDGS consumed less amount of feed compared to those which had received 0, 6 or 12% (*P* <0.05). On the other hand, chicks which had received 0 or 6% DDGS diets gained more weight as compared to those which had received 18% or 24% DDGS diets. A linear decrease in BWG occurred as a result of DDGS inclusion from 12 to 24%. A significant difference in FCR was observed due to DDGS inclusion (*P* <0.001), chicks which had received 0 or 6% DDGS converted feed to body weight more efficiently compared to those which had received 18 or 24%. A significant drop in PEF occurred when the level of DDGS was more than 6% (*P* <0.05).

Diets containing 6% DDGS did not affect performance parameters during the starter period. However, diets containing 12, 18 and 24% DDGS decreased performance at the same period of life. This result agreed with Lumpkins *et al.* (2004) who reported that a diet containing 6% DDGS did not affect BWG, while 12% and 18% DDGS reduced BWG and deteriorated FCR in starter broilers. Jung *et al.* (2011) recommended that careful attention should be given when 9% DDGS is fed to broilers for the starter period (0 to 21 d) due to the negative effects on feed efficiency.

On the other hand, enzyme supplementation had no effect on FI or FCR for the period (0-10 d) because the digestive system is not

fully developed. However, enzyme supplementation increased BWG and PEF (P <0.05) as compared to un-supplemented diet for the same period of life.

This finding is matching with, Lumpkins *et al*. (2011) who conducted a study to investigate the effects of Rovabio in a broiler diet that contained 12% DDGS, the result revealed that addition of the enzyme to the negative control feed which was formulated to contain 132 kcal/kg less energy, restored performance like the positive control.

And agree with the result of a study states that inclusion of xylanase enzyme to broiler during the growing period increased positively feed intake by 4-5%, increased dry matter and hemicellulose digestibility by 5% and 20%, respectively while it did not affect FCR (Liu*et al*., 2010).

Recently, Jung*et al*. (2011) reported that enzymes supplementation to starter diet (0 to 21 d) which contained 9% DDGS inclusion level may overcome the negative effects of DDGS. Schwartz*et al*. (2010) used diets that contained 0 or 10% DDGS with two energy levels (low or high) and two different enzymes (a xylanase product and a broad array of enzyme activities) from d 1 to 49. They found that 14 d FCR improved by 11.4 and 4.7% with enzyme supplementation in the low- and high-energy diets, respectively.

This result indicated that NSP from the high DDGS diet most likely limited the growth performance of broilers at early ages. Corn DDGS contains more than 25% total NSP, and 90% of that NSP is insoluble (Swiatkiewicz and Koreleski, 2007). Arabinoxylans are the predominant component of hemicellulose with level of 11.4% in DDGS (Cowieson, 2005). It was estimated

that by using 15% DDGS, the arabinoxylan content was increased by 20% in the final diet.

The performance results for the grower period from 11 to 24 d of age are presented in Table (5). No significant two-way interaction (Enzyme x DDGS). However, DDGS affected all performance parameters significantly (P <0.05). Chicks which had received 0 or 6% DDGS consumed more feed and gained more weight (P <0.05) as compared to all other treatments. Chicks which had received 0, 6 or 12% DDGS converted feed to body weight more efficiently and had higher PEF as compared to those which had received 18 or 24%. Enzyme supplementation had no effect on FI or BWG for the period (11-24 d). However, enzyme improved (P <0.05) FCR and PEF.

Neither the two-way interaction nor enzyme supplementation had an influence on FI, BWG or PEF for the finisher period (25 to 35 d of age) (Table 6; P > 0.05). However, Tomoko enzyme improved FCR (P <0.05) when compared to no supplementation. DDGS inclusion affected all parameters for the finisher period (P <0.05). FI and BWG were decreased (P <0.05) when the DDGS level increased over 6%. However, FCR decreased when the DDGS was included at a rate higher than 12%.

The cumulative performance results (0 to 35 d of age) are presented in Table (7). A significant two-way interaction (Enzyme x DDGS) was observed for FCR (P <0.05).

Chicks which had received Tomoko enzyme had slightly better FCR at 0, 6, 12 and 18% DDGS as compared to Rovabio but at 24% DDGS, chicks which had received Rovabio had slightly better FCR. Tomoko, in general improved FCR as compared to no enzyme while Rovabio was intermediate (P <0.05). Enzyme

supplementation improved BWG numerically for the cumulative period and had no effect on FI ($P > 0.05$). Abudabos (2012) reported that body weight and ileal protein retention were positively affected by the Tomoko enzyme supplementation of a corn-SBM diet at 42 d, Tomoko enzyme was able to restore the nutritional value of the low-density corn-SBM diet.

Chicks received 0 or 6% DDGS consumed more feed and gained more weight as compared to those which had received 12, 18 or 24%. However, chicks received 0, 6 or 12% had better FCR as compared to 18 or 24% DDGS. Furthermore, Lumpkins *et al.* (2011) conducted a study to investigate the effects of Rovabio in a broiler diet that contained 12% DDGS. The results revealed that the addition of the enzyme to the negative control feed, which was formulated to contain 132 kcal/kg less energy, restored performance like that of the positive control because the inclusion of xylanase enzymes to broiler feed during the growing period increased feed intake positively by 4-5% and increased dry matter and hemicellulose digestibility by 5 and 20%, respectively.

For grower and finisher broilers and based on FCR, it can be concluded that the inclusion rate of DDGS in the diet can be increased up to 12% without compromising the performance. Lumpkins et al. (2004) suggested that the maximum amount of DDGS used for starter, grower and finisher diets should be no more than 9, 12 and 15%, respectively. Wang et al. (2007b) fed three levels of DDGS to broilers 42 d of age (0, 15 or 30%). No negative effects of feeding broilers 15% DDGS were reported; however, feeding broilers 30% DDGS caused a decrease in performance due to an arginine deficiency, which also caused lower arginine-to-lysine ratios. Wang et al. (2007a) found that

broilers that received a corn-SBM-based diet with DDGS ranging from 5 to 25% had similar body weight gains at 35 and 49 d of age; however, the FCR was poor in the diet containing 25% DDGS at 35 and 49 days of age.

Neither two-way interaction (Enzyme X DDGS) nor enzyme supplementation had an influence on the parts yield or dressing percentages at 35 d ($P > 0.05$). On the other hand, breast percentage was higher when the diets contained 0 and 6% DDGS as compared to 18 and 24% ($P < 0.05$). On the contrary, thigh percentages and thigh meat were lower when the diets contained 0 and 6% DDGS as compared to 18 and 24% ($P < 0.01$, $P < 0.001$, respectively). Wang et al. (2007c) formulated diets based on digestible amino acid content to contain 0, 5, 10, 15, 20 or 25% DDGS. Their results indicated that DDGS could be used at levels between 15 to 20% in broiler diets without a negative effect on performance; however, the dressing percentage, or breast meat yield, was slightly lower compared to those without DDGS.

3 II Protein profile and Liver Enzymes:

As shown in table (10) the total blood protein, albumin, Globulin and Liver enzymes ALT and AST are not affected with the different increment rates nor the supplementation of Rovabio and Tomoko enzymes as compare with the blood references range of the broiler chicken parameters (Bahman et al 2010 and Silva et al 2007). This result indicates that the DDGS used in this experiment is good is that has no mycotoxinal effect on birds although there was no mycotoxin binder in the diet.

3 III Antioxidation:

A considerable amount of information has been generated on the feeding value of the DDGS, even at a high inclusion level

combined with lysine and Methionine supplementation, has no initiative effect on lipid peroxidation in the blood and liver of broiler chickens (Table 11).

This result match with the result states that supplemental DDGS level up to 212% caused no lipid peroxidation to broiler meat (Schilling et al.,2010).

DDGS is rich with Vitamin E, which is a strong lipid antioxidant, interestingly during hot conditions in Japan, Supplemental of DDGS in dairy cows reduced lipid peroxidation (Tanaka et al.,2011).

Table 1. Nutritional composition of DDGS, barley and wheat —adapted from different authors.

Nutrients	**un.**	**Lescano, 2013**	**NRC, 2012**		
		DDGS	**corn**	**barley**	**wheat**
Dry matter	%	89.72	88.31	89.90	88.67
Crude protein	%	29.94	8.24	11.33	14.46
Crude fibre	%	7.87	1.98	3.90	2.57
Ether extract	%	8.34	3.48	2.11	1.82
NDF	%	33.92	9.11	18.29	10.60
ADF	%	13.94	2.88	5.78	3.55
Total amino acids					
Lysine	%	0.820	0.250	0.400	0.390
Digestible lysine	%	0.530	0.185	0.300	0.320
Methionine	%	0.610	0.180	0.200	0.220
Digestible methionine	%	0.510	0.149	0.164	0.194
Threonine	%	1.080	0.280	0.360	0.400
Digestible threonine	%	0.800	0.216	0.274	0.336
Tryptophan	%	0.210	0.060	0.130	0.170
Digestible tryptophan	%	0.150	0.048	0.107	0.150
Valine	%	1.490	0.380	0.520	0.580
Digestible valine	%	1.150	0.312	0.416	0.510
Minerals					
Calcium	%	0.18	0.02	0.06	0.06
Total phosphorus	%	0.84	0.26	0.35	0.39
Phytic phosphorus	%	0.26	0.21	0.22	0.22
Apparent digestibility coefficient	%	45.27	26.0	39.0	46.0
True digestibility coefficient	%	48.85	34.0	45.0	56.0
Available phosphorus	%	0.58	0.05	0.13	0.17
Energy					
Gross energy	kcal/kg	4943.25	3933	3939	3788
Digestible energy	kcal/kg	3647.25	3451	3150	3313
Metabolizable energy	kcal/kg	3507.75	3395	3073	3215
Net energy	kcal/kg	2339.00	2672	2327	2472

Table 2*:* Composition of dietary treatments fed to broilers during starter period

Ingredients	0%DDGS[¥]	6%DDGS [¥]	12% DDGS [¥]	18% DDGS [¥]	24% DDGS [¥]
Corn, yellow ground	59.19	56.76	54.40	51.55	48.98
Soybean meal (48)	33.70	28.90	25.15	21.65	18.20
DDGS	0.00	6.00	12.00	18.00	24.00
Corn Oil	3.70	3.90	3.99	4.28	4.29
Dicalcium phosphate	2.12	1.90	1.70	1.44	1.20
Limestone	0.54	0.72	0.85	1.10	1.30
Salt	0.46	0.43	0.41	0.38	0.35
L-Lysine	0.27	0.35	0.44	0.53	0.60
L-Threonine	0.14	0.16	0.18	0.20	0.22
DL-Methionine	0.33	0.33	0.33	0.32	0.31
Choline Chloride	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral premix [1]	0.50	0.50	0.50	0.50	0.50
Contents by calculation					
TME_n, kcal/kg	3000	3000	3000	3000	3000
Protein, %	22	22	22	22	22
Lysine, %	1.28	1.28	1.28	1.28	1.28
Methionine + Cystine, %	0.95	0.95	0.95	0.95	0.95
Calcium, %	0.96	0.96	0.96	0.96	0.96
Available Phosphorus, %	0.48	0.48	0.48	0.48	0.48

[1]Vitamin-mineral premix contains in the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg.

[¥]For starter period, each diet was supplemented with 0.05% Rovabio or Tomoko enzymes.

Rovabio® Excel (ADISSEO France S.A.S) is a feed additive containing a combination of 19 active enzymes produced by only one non-genetically modified fungus (*Penicillium funiculosum*).

Tomoko® (Biogenkoji Research Institute, 876-15, Kagoshima, Japan) is a commercial enzyme supplement that contains acidic protease, α-amylase, pectinase, phytase, glucoamylase, cellulase and *Aspergillus awamori* cells.

Table 3: Composition of dietary treatments fed to broilers during growing period

Ingredients	0%DDGS[¥]	6% DDGS [¥]	12% DDGS [¥]	18% DDGS [¥]	24% DDGS [¥]
Corn, yellow ground	57.85	55.12	52.31	50.00	47.33
Soybean meal (48)	34.50	31.00	27.60	23.86	20.40
DDGS	0.00	6.00	12.00	18.00	24.00
Corn Oil	2.70	2.90	3.10	3.35	1.42
Dicalcium Phosphate	2.40	2.15	1.90	1.65	1.35
Limestone	0.55	0.76	0.96	0.96	1.45
Common Salt	0.46	0.43	0.41	0.38	0.35
L-Lysine	0.38	0.46	0.54	0.61	0.70
L-Threonine	0.21	0.23	0.24	0.26	0.27
DL-Methionine	0.40	0.40	0.39	0.38	0.37
Choline Chloride	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral premix [1]	0.50	0.50	0.50	0.50	0.50
Contents by calculation					
TME_n, kcal/kg	3100	3100	3100	3100	3100
Crude Protein, %	21	21	21	21	21
Lysine, %	1.15	1.15	1.15	1.15	1.15
Methionine + Cystine, %	0.87	0.87	0.87	0.87	0.87
Calcium, %	0.87	0.87	0.87	0.87	0.87
Available Phosphorus, %	0.44	0.44	0.44	0.44	0.44

[1]Vitamin-mineral premix contains in the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg.

[¥]For grower period, each diet was supplemented with 0.05% Rovabio or Tomoko enzymes.

Table 4: Composition of dietary treatments fed to broilers during finishing period

Ingredients	0%DDGS[¥]	6% DDGS [¥]	12% DDGS [¥]	18% DDGS [¥]	24% DDGS [¥]
Corn, yellow ground	65.036	62.767	59.45	57.208	54.515
Soybean meal (48)	27.60	23.70	20.69	16.80	13.20
DDGS	0.00	6.00	12.00	18.00	24.00
Corn Oil	3.40	3.55	3.95	3.95	4.22
Dicalcium Phosphate	1.86	1.62	1.39	1.18	0.94
Limestone	0.53	0.71	0.88	1.08	1.29
Common Salt	0.33	0.30	0.28	0.25	0.22
L-Lysine	0.26	0.358	0.42	0.51	0.295
L-Threonine	0.124	0.145	0.10	0.184	0.19
DL-Methionine	0.31	0.30	0.29	0.288	0.28
Choline Chloride	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral premix [1]	0.50	0.50	0.50	0.50	0.50
Contents by calculation					
TME_n, kcal/kg	3150	3150	3150	3150	3150
Crude Protein, %	19	19	19	19	19
Lysine, %	1.03	1.03	1.03	1.03	1.03
Methionine + Cystine, %	0.80	0.80	0.80	0.80	0.80
Calcium, %	0.80	0.80	0.80	0.80	0.80
Available Phosphorus, %	0.395	0.395	0.395	0.395	0.395

[1]Vitamin-mineral premix contains in the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg.

[¥]For finisher period, each diet was supplemented with 0.05% Rovabio and Tomoko enzymes

Table 5: Effect of DDGS and enzyme supplementation on broiler growth performance at 10 d of age

Treatment	DDGS (%)	Enzyme	Performance			
			FI (g)	BWG (g)	FCR (g: g)	PEF
1	0	No	230.4	206.5	1.12	222.6
2	0	Rovabio	227.9	203.6	1.12	219.4
3	0	Tomoko	223.8	205.6	1.09	227.4
4	6	No	217.4	188.5	1.16	198.2
5	6	Rovabio	237.5	211.8	1.12	223.7
6	6	Tomoko	235.5	210.9	1.12	225.2
7	12	No	228.4	194.4	1.18	199.2
8	12	Rovabio	222.7	196.0	1.14	202.0
9	12	Tomoko	228.1	197.1	1.16	201.0
10	18	No	199.4	162.4	1.23	164.3
11	18	Rovabio	219.8	177.1	1.24	175.2
12	18	Tomoko	215.5	180.2	1.20	184.5
13	24	No	200.1	155.8	1.29	152.7
14	24	Rovabio	209.6	164.2	1.28	160.2
15	24	Tomoko	202.4	158.5	1.28	155.6
SEM±			5.44	5.20	0.017	5.98
DDGS average						
0			227.4^{a}	205.2^{a}	1.10^{d}	223.1^{a}
6			230.1^{a}	203.7^{ab}	1.13^{cd}	215.7^{a}
12			226.4^{a}	195.8^{b}	1.16^{c}	200.7^{b}
18			211.6^{b}	173.2^{c}	1.22^{b}	174.7^{c}
24			204.0^{b}	159.5^{d}	1.28^{a}	154.2^{d}
SEM±			3.14	3.00	0.010	3.45
Enzyme average						
0			215.2	181.5^{b}	1.19	187.4^{b}
R			223.5	190.5^{a}	1.17	196.1^{a}
T			221.1	190.4^{a}	1.17	198.7^{a}
SEM±			2.43	2.32	0.007	2.67
P-values						
DDGS			<0.0001	<0.0001	<0.0001	<0.0001
Enzyme			0.0503	0.0095	0.0859	0.0099
Enzyme x DDGS			0.1339	0.2053	0.6398	0.2469

FI, BWG, FCR and PEF were corrected by mortalities.

$^{a-d}$Means within a column with different superscript letters differ ($P < 0.05$).

Table 6: Effect of DDGS and enzyme supplementation on broiler growth performance at 24 d of age

Treatment	DDGS (g)	Enzyme	Performance			
			FI (g)	BWG (g)	FCR (g: g)	PEF
1	0	No	1145.2	800.3	1.43	277.1
2	0	Rovabio	1118.6	797.8	1.40	311.4
3	0	Tomoko	1117.7	788.7	1.42	300.8
4	6	No	1104.8	775.7	1.42	291.5
5	6	Rovabio	1111.2	794.3	1.40	298.2
6	6	Tomoko	1117.5	788.7	1.42	307.3
7	12	No	1085.1	729.3	1.48	272.9
8	12	Rovabio	1071.9	766.2	1.40	289.7
9	12	Tomoko	1040.2	775.1	1.35	313.2
10	18	No	984.0	639.5	1.54	232.9
11	18	Rovabio	1014.4	651.1	1.56	227.4
12	18	Tomoko	1001.4	657.5	1.53	242.8
13	24	No	926.6	569.4	1.63	202.0
14	24	Rovabio	939.6	600.6	1.57	201.8
15	24	Tomoko	936.6	613.1	1.53	203.7
SEM±			25.64	17.99	0.025	8.03
DDGS average						
0			1127.2[a]	795.6[a]	1.42[b]	296.4[a]
6			1111.2[a]	786.3[a]	1.41[b]	299.0[a]
12			1065.7[b]	756.9[b]	1.41[b]	291.9[a]
18			1000.0[c]	649.4[c]	1.54[a]	234.4[b]
24			935.3[d]	594.4[d]	1.58[a]	202.5[b]
SEM±			14.81	10.39	0.014	3.66
Enzyme average						
0			1049.7	702.8	1.50[a]	262.6[b]
R			1051.1	722.0	1.47[b]	265.3[a]
T			1042.7	724.6	1.45[b]	275.9[a]
SEM±			11.69	8.20	0.011	3.40
P-value						
DDGS			<0.0001	<0.0001	<0.0001	<0.0001
Enzyme			0.8538	0.1246	0.0021	0.0004
Enzyme x DDGS			0.9325	0.8771	0.0782	0.0263

FI, BWG, FCR and PEF were corrected by mortalities.

[a-d]Means within a column with different superscript letters differ (P <0.05)

Table 7: Effect of DDGS and enzyme supplementation on broiler growth performance at 35 d of age

Treatment	DDGS (g)	Enzyme	Performance			
			FI (g)	BWG (g)	FCR (g: g)	PEF
1	0	No	1564.4	868.3	1.80	302.3
2	0	Rovabio	1467.9	826.6	1.78	300.1
3	0	Tomoko	1486.9	854.2	1.74	304.0
4	6	No	1428.6	804.3	1.78	288.7
5	6	Rovabio	1461.2	807.0	1.81	282.8
6	6	Tomoko	1483.1	843.4	1.76	298.3
7	12	No	1352.7	740.2	1.82	256.8
8	12	Rovabio	1411.6	789.6	1.79	279.5
9	12	Tomoko	1381.1	794.8	1.74	290.5
10	18	No	1254.7	672.8	1.87	225.0
11	18	Rovabio	1268.2	676.8	1.88	220.1
12	18	Tomoko	1245.8	706.6	1.77	239.8
13	24	No	1211.8	564.0	2.15	173.7
14	24	Rovabio	1126.8	567.5	1.99	181.5
15	24	Tomoko	1239.4	600.4	2.07	175.6
SEM±			39.86	24.69	0.038	7.88
DDGS average						
0			1506.4[a]	849.7[a]	1.77[c]	302.1[a]
6			1457.6[a]	819.2[a]	1.78[bc]	289.9[ab]
12			1381.8[b]	774.9[b]	1.79[bc]	278.6[b]
18			1256.2[c]	685.4[c]	1.84[b]	228.3[c]
24			1192.7[c]	577.3[d]	2.07[a]	176.9[d]
SEM±			23.01	14.26	0.022	4.55
Enzyme average						
0			1362.4	729.9	1.89[a]	251.1
R			1347.1	733.5	1.85[ab]	252.8
T			1367.3	760.5	1.82[b]	261.6
SEM±			18.17	11.26	0.017	3.60
P-value						
DDGS			<0.0001	<0.0001	<0.0001	<0.0001
Enzyme			0.7061	0.1062	0.0169	0.0812
Enzyme x DDGS			0.3539	0.8388	0.2353	0.6755

FI, BWG, FCR and PEF were corrected by mortalities.

[a-d]Means within a column with different superscript letters differ (P <0.05)

Table 8. Effect of DDGS and enzyme supplementation on cumulative broiler growth performance from 0 to 35 d of age

Treatment	DDGS (%)	Enzyme	Performance		
			FI (g)	BWG (g)	FCR (g: g)
1	0	No	2940.0	1875.1	1.57
2	0	Rovabio	2814.5	1828.0	1.54
3	0	Tomoko	2828.4	1848.5	1.53
4	6	No	2750.4	1767.9	1.56
5	6	Rovabio	2809.8	1813.2	1.55
6	6	Tomoko	2833.9	1843.3	1.54
7	12	No	2666.2	1663.8	1.60
8	12	Rovabio	2706.2	1751.2	1.54
9	12	Tomoko	2649.4	1767.0	1.50
10	18	No	2438.1	1474.7	1.65
11	18	Rovabio	2502.4	1504.9	1.66
12	18	Tomoko	2462.7	1544.3	1.60
13	24	No	2341.5	1289.2	1.82
14	24	Rovabio	2276.0	1332.3	1.71
15	24	Tomoko	2378.4	1371.9	1.74
SEM±			59.71	38.80	0.017
DDGS average					
0			2860.1[a]	1850.5[a]	1.55[c]
6			2798.0[a]	1808.1[a]	1.55[c]
12			2673.9[b]	1727.5[b]	1.55[c]
18			2467.7[c]	1507.9[c]	1.64[b]
24			2332.0[d]	1331.1[d]	1.75[a]
SEM±			33.89	22.40	0.010
Enzyme average					
0			2627.3	1614.1	1.64[a]
R			2621.8	1646.0	1.60[b]
T			2630.6	1675.0	1.58[c]
SEM±			26.23	17.70	0.010
P-value					
DDGS			<0.0001	<0.0001	<0.0001
Enzyme			0.9723	0.0528	<0.0001
Enzyme x DDGS			0.6332	0.8048	0.0169

FI, BWG, FCR and PEF were corrected by mortalities.

[a-d]Means within a column with different superscript letters differ (P <0.05)

Table 9: Effect of DDGS and enzyme supplementation on parts yield as percentages of broiler dressed weight at 35 d

Treatment	DDGS (%)	Enzyme	Dressing (%)	Breast (%)	Drumstick (%)	Thigh (%)	Fat (%)	Liver (%)	Heart (%)	Thigh meat (%)	Drumstick meat (%)
1	0	No	76.8	29.3	13.2	15.7	2.6	2.2	0.9	11.7	9.3
2	0	Rovabio	77.1	26.1	13.9	15.6	3.3	2.2	0.7	11.9	10.3
3	0	Tomoko	76.8	31.1	13.0	15.6	2.3	2.2	1.1	12.8	9.7
4	6	No	76.0	30.8	12.5	14.2	3.0	2.6	0.7	11.4	8.9
5	6	Rovabio	76.0	30.3	13.9	16.2	2.4	2.2	0.8	13.1	9.7
6	6	Tomoko	75.7	26.7	12.9	16.0	3.8	2.2	0.7	13.4	9.3
7	12	No	74.1	29.0	14.9	16.8	2.4	2.0	1.0	13.8	10.0
8	12	Rovabio	78.0	25.7	13.9	16.5	3.4	2.2	0.9	13.4	9.9
9	12	Tomoko	74.7	28.4	13.2	17.2	2.2	2.2	0.9	13.5	9.1
10	18	No	74.2	26.1	14.5	17.3	2.5	2.7	1.2	13.8	9.5
11	18	Rovabio	72.3	21.3	14.4	16.9	3.6	2.4	0.9	13.2	10.0
12	18	Tomoko	74.3	26.6	14.1	16.9	3.3	2.3	0.9	13.5	10.0
13	24	No	73.0	26.0	14.5	16.9	2.6	2.5	1.2	13.6	9.9
14	24	Rovabio	72.8	21.9	14.6	16.5	2.7	2.4	0.9	13.5	10.0
15	24	Tomoko	73.6	22.7	14.3	16.6	4.1	2.5	0.9	13.5	9.9
SEM±			2.11	2.39	0.76	0.55	0.53	0.25	0.14	0.46	0.57
DDGS											
0			76.9	28.8^{a}	13.4	15.6^{b}	2.7	2.2	0.9	12.1^{b}	9.8
6			75.9	29.2^{a}	13.1	15.5^{b}	3.1	2.3	0.7	12.6^{b}	9.3
12			75.6	27.7^{ab}	14.0	16.8^{a}	2.7	2.1	0.9	13.6^{a}	9.7
18			73.6	24.7^{bc}	14.4	17.1^{a}	3.1	2.4	1.0	13.5^{a}	9.8
24			73.1	23.5^{c}	14.5	16.7^{a}	3.1	2.5	1.0	13.5^{a}	9.9
SEM±			1.22	1.38	0.44	0.31	0.30	0.14	0.08	0.26	0.33
Enzyme											
0			74.8	28.3	13.9	16.2	2.6	2.4	1.0	12.9	9.5
R			75.2	25.1	14.1	16.3	3.1	2.3	0.8	13.0	10.0
T			75.0	27.1	13.5	16.5	3.1	2.3	0.9	13.3	9.6
SEM±			0.94	1.07	0.34	0.24	0.24	0.11	0.06	0.20	0.25
P-value											
DDGS			0.954	0.011	0.125	0.002	0.680	0.403	0.229	0.001	0.677
Enzyme			0.174	0.117	0.116	0.697	0.255	0.709	0.281	0.247	0.395
Enzyme x DDGS			0.952	0.73	0.900	0.395	0.164	0.891	0.525	0.131	0.939

$^{a-d}$Means within a column with different superscript letters differ ($P<0.05$)

TABLE 10: Effect of DDGS and enzyme supplementation on blood parameters and liver enzymes of broiler chickens given experimental diets during fifth week of age. Parameters

Treatment	DDGS %	Enzyme	Protein (g/dl)	Albumin (g/dl)	Globulin (-)	ALT (U/L)	AST (U/L)
1	0	0	3.0	1.5	1.6	7.4	229.0
2	6	0	4.7	1.9	2.8	6.3	258.5
3	12	0	2.6	1.4	1.2	7.1	336.6
4	18	0	2.7	1.2	1.5	6.3	234.7
5	24	0	2.7	1.5	1.3	6.8	182.6
6	0	R	2.9	1.9	1.0	9.0	259.8
7	6	R	2.7	1.6	1.1	6.3	306.0
8	12	R	2.9	1.6	1.2	6.4	265.4
9	18	R	3.3	1.5	1.7	7.2	203.7
10	24	R	2.8	1.3	1.5	5.6	190.8
11	0	T	2.7	1.7	1.0	7.3	245.4
12	6	T	2.8	1.7	1.1	4.8	295.6
13	12	T	2.5	1.3	1.2	5.3	212.8
14	18	T	2.6	1.5	1.1	4.9	217.0
15	24	T	2.8	1.7	1.1	8.6	280.3
SEM±			0.60	0.12	0.61	1.08	42.37
DDGS average							
0			2.9	1.7^{ab}	1.2	7.9	244.7
6			3.4	1.7^{a}	1.7	5.8	286.7
12			2.6	1.4^{c}	1.2	6.2	271.6
18			2.8	1.4^{c}	1.4	6.1	218.5
24			2.8	1.5^{bc}	1.3	7.0	217.9
SEM±			0.35	0.07	0.35	0.63	24.46
Enzyme average							
0			3.1	1.5	1.7	6.8	248.3
R			2.9	1.6	1.3	6.9	245.1
T			2.7	1.6	1.1	6.2	250.2
SEM±			0.27	0.05	0.27	0.48	18.95
Statistical probabilities							
DDGS			NS	**	NS	NS	NS
Enzyme			NS	NS	NS	NS	NS
Enzyme x DDGS			NS	*	NS	NS	NS

*p<0.05, **p<0.01, ***p<0.001, NS: Not significant, SEM: Standard error of the mean

Table 11: Effect of DDGS and enzyme supplementation on muscle and liver antioxidant and MDA at 35 d of age.

			Muscle Liver			
Treatment	DDGS(%)	Enzyme	AOC (U/mg protein)	MDA(nmol/mg protein)	AOC(U/mg protein)	MDA(nmol/mg protein)
1	0	No	2.24	2.25	31.98	1.19
2	0	Rovabio	2.23	2.20	32.64	1.20
3	0	Tomoko	2.16	2.13	31.62	1.21
4	6	No	2.17	2.21	31.86	1.24
5	6	Rovabio	2.17	2.24	31.71	1.26
6	6	Tomoko	2.27	2.10	33.30	1.17
7	12	No	2.18	2.20	31.47	1.12
8	12	Rovabio	2.17	2.25	32.33	1.14
9	12	Tomoko	2.03	2.21	31.07	1.22
10	18	No	2.18	2.29	32.24	1.21
11	18	Rovabio	2.17	2.26	31.10	1.18
12	18	Tomoko	2.03	2.27	30.53	1.30
13	24	No	2.09	2.35	31.10	1.36
14	24	Rovabio	2.11	2.47	31.2	1.39
15	24	Tomoko	2.10	2.51	30.70	1.50
SEM±			0.07	0.05	0.77	0.05
DDGS average						
0			2.21	2.19^{bc}	32.10	1.20^{b}
6			2.20	2.18^{c}	32.29	1.22^{b}
12			2.12	2.22^{bc}	31.62	1.16^{b}
18			2.13	2.27^{b}	31.30	1.23^{b}
24			2.10	2.44^{a}	31.01	1.42^{a}
SEM±			0.04	0.03	0.44	0.03
Enzyme average						
0			2.16	2.26	31.73	1.23
R			2.17	2.28	31.80	1.23
T			2.13	2.24	31.44	1.28
SEM±			0.03	0.02	0.34	0.02
P-value						
DDGS			0.720	0.184	0.542	0.635
Enzyme			0.554	0.459	0.473	0.230
Enzyme x DDGS			0.248	<0.001	0.250	<0.001

[a-d]Means within a column with different superscript letters differ (P <0.05

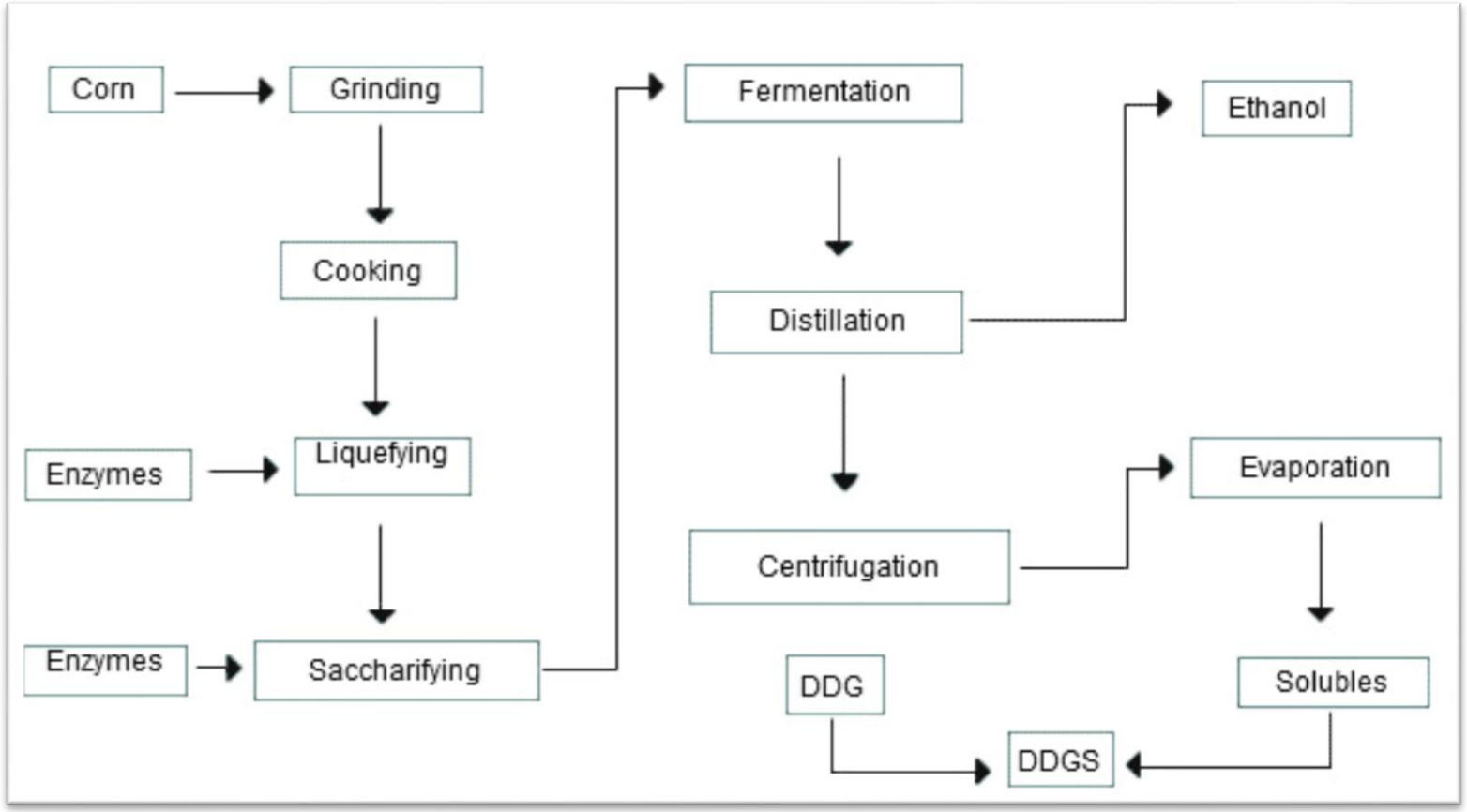

Figure 1. DDGS formation as byproducts of Corn Fermentation to Ethanol. (Adapted from MAIZAR (www.maizar.org.ar))

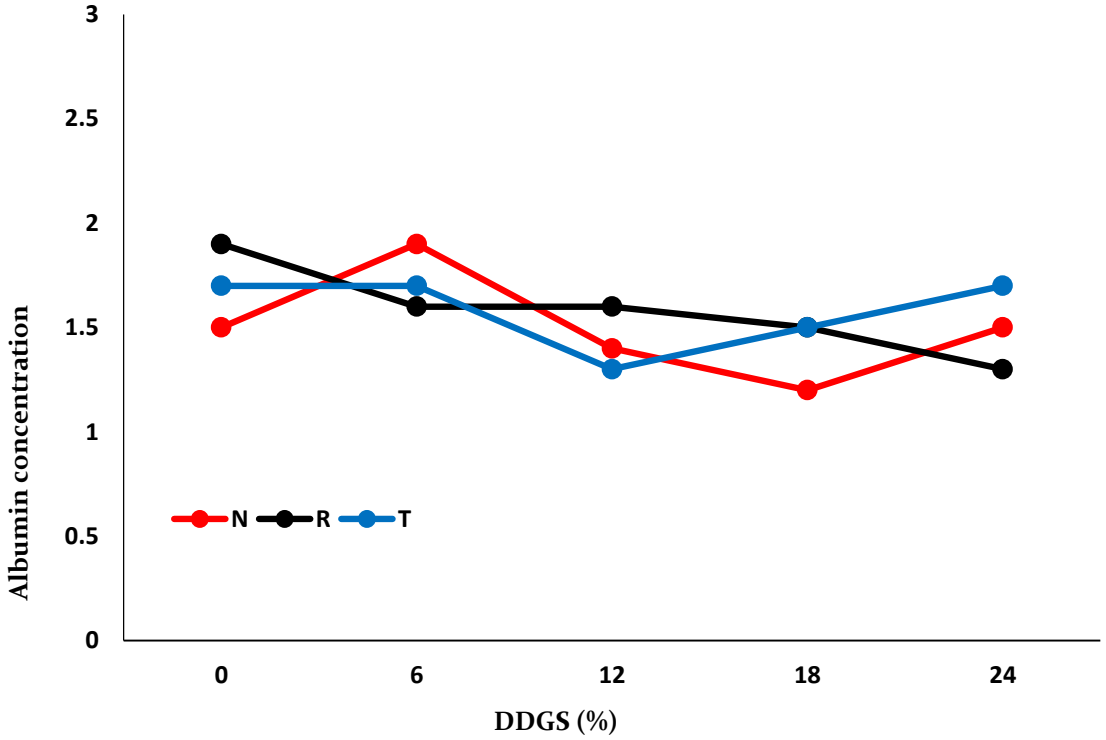

Figure 2: Interaction effect between enzyme and DDGS on albumin level of broiler at fifth week

CONCLUSION

There was no health hazard affect the birds during the study. Based on the performance result it could be concluded that 6% and 12% DDGS may be used without harm in starter and grower/finisher diets, respectively. An inclusion level of 12% and 18% DDGS may be excessive during the starter and grower/finisher periods, respectively. The results suggest that enzyme supplementation to DDGS diets can improve growth performance in broilers.

Recommendations

1. We recommend that every DDGS shipment should be nutritionally analysed even the shipments from the same plant and source.
2. Inclusion of DDGS in young birds feed should be calculated carefully.
3. With the high % of DDGS inclusion rate we recommend to use feed enzymes to enhance digestibility and correct the nutritional values of the utility.
4. We recommend encouraging researchers to study the effect of the different inclusion rate of DDGS in the layer chickens feed.
5. We recommend to make further studies on the Moulds pollution in different types of DDGS included in the poultry feed formulas.

REFERENCES

Aalhus, J.L., and Dugan, M.E.R. (2004). Spoilage, factors affecting (b) oxidative and enzymatic. In W. K. Jensen, C. Devine, & M. Dikeman (Eds.), *Encyclopedia of Meat Sciences* (pp. 1330–1336). Oxford: Elsevier.

Abudabos, A. (2012) Effect of enzyme supplementation to normal and low density broiler diets based on corn-soybean meal. *Asian Journal Animal and Veterinary Science Advances*,**7**:139-148.

Adeola, O., Jendza, J.A. Southern,L.L. Powell,S., and Owusu-Asiedu A. (2010). Contribution of exogenous dietary carbohydrases to the metabolizable energy value of corn distillers grains for broiler chickens. *Poultry Science*,**89**(9): 1947-54.

Aldai, N.,Aalhus J.L, Dugan M.E,Robertson W.M, McAllister T.A, Walter L.J, and McKinnon, J.J.(2010a). Comparison of wheat- versus corn-based dried distillers' grains with soluble on meat quality of feedlot cattle. *Meat Science*,**84**: 569-577

Aldai, N., Dugana, M.E.R. Aalhusa,J.L. McAllisterb,T.A. Walterc,L.J. and McKinnon, J.J. (2010b). Differences in the trans-18:1 profile of the backfat of feedlot steers fed wheat or corn based dried distillers' grains. *Animal Feed Science and Technology*,**157**: 168–172.

AOAC International. 1984. Official Methods of Analysis of AOAC International. 14th ed. AOAC int., Gaithersburg, MD.

Arce, J., Avila,E. Rosales, E. Charraga,S., and Fernández, S.R. (2010). Effect of adding protease and xylanase feed enzymes activities on broilers fed corn-SBM-DDGS diets. *Proceedings of International Poultry Scientific Forum 2010*, Atlanta, USA. T108.

Asplin, F. D., and Carnaghan, R.B.A. (1961). The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. *Veterinary Record*,**73**: 1215.

Bach Knudsen, K.E. (2001). The nutritional significance of dietary fiber analysis. *Animal of Feed Science and Technology*,**90**:3–20.

Batal, A.B., and Parsons, C.M. (2002). Effects of age on development of digestive organs and performance of chicks fed a corn–soybean meal versus a crystalline amino acid diet. *Poultry Science*,**81:** 1338–1341.

Beckman, J., Keeney, R., and Tyner, W. 2011. Feed demands and co product substitution in the biofuel era. Agribusiness 27(1), 1-18.

Belyea, R.L., Rausch,K.D., and Tumbleson, M.E. (2004). Composition of corn and distillers' dried grains with solubles from dry grind ethanol processing. *Bioresource Technol*. **94**: 293–298.

Binder, E.M., Tan,L.M. Chin,L.J. Handle, J., and Richard, J. (2007). Worldwide occurrence of mycotoxins in commodities feed and feed ingredients. *Animal Feed Science Technology*,**137**: 265-282.

Bingham, A.K., Phillips, T.D., and Bauer, J.E. (2003). Potential for dietary protection against the effects of aflatoxins in animals. *Journal of American Veterinary Medical Association*,**222**: 591-596.

Botsoglou, N.A., Grigoropoulou, S.H, Botsoglou, E, Govaris, A., and Papageorgiou, G. (2003). The effects of dietary oregano essential oil and [alpha]-tocopheryl acetate on lipid oxidation in raw and cooked turkey during refrigerated storage. *Meat Science*, **65**: 1193-200.

Busby, W.F.Jr., and Wogan.G.N. (1981). Aflatoxins. Pages 3–27.In:*Mycotoxins and N-Nitrosocompounds, Environmental Risks*. Vol. 2. R. C. Shank, ed. CRC Press Inc., Boca Raton, FL.

Calkins, C. R., and Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*,**77**: 63–80.

Commission Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Off. J. Eur. Union L 285/33.

Cromwell, G.L., Herkelman,K.L., and Stahly, T.S. (1993). Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. *Journal Animal Science*,**71**: 679-686.

Diaz, D. E., Hagler,W.M. Jr., Blackwelder, J.T. Eve, J.A. Hopkins,B.A. Anderson,K. L. Jones,F.T., and Whitlow, L.W. (2004). Aflatoxin adsorbents II: Reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia*,**157**: 233–241.

Estrada-Angulo, A., Lopez, E.J., Contreras, G., Castro, B.I., Obregon, J.F., Perez, A.B. (2008). Two levels of dried distillers grains with soluble on growth performance and carcass characteristics of Pelibuey sheep. *Journal of Animal Science***86** (Suppl. 2): 498 (Abstr.).

Gibreel, A., Sandercock, J.R. Lan J. Goonewardene, L.A. Scott, A.C. Zijlstra, R.T. Curtis, J.M. Bressler,D.C. (2011). Evaluation of value-added components of dried distiller's grain with solubles from triticale and wheat. *Bioresource Technology*,**102**(13): 6920-6927

Heincinger, M., Balogh, K., Febel, H., Erdelyi, M., and Mezes, M. (2011). Effects of diets of diets with different inclusion levels of distillers dried grain with soluble combined with lysine and methionine supplementation on the lipid peroxidation and glutathione status of chickens. *Acta Veterinaria Hungaria*,**59**(2): 195-204

Huff, W.E., Kubena, L.F. Harvey, R.B. Hagler Jr., W.M. Swanson, S.P. Phillips, T.D. and Creger., C. R. 1986. Individual and combined effects of aflatoxin and deoxynivalenol (DON, Vomitoxin) in broiler chickens. Poultry Science 65, 1291–1298.

Ingledew, M.W., (2006). Improvements in Alcohol Technology Through Advancements in Fermentation Technology**.** In: *The Alcohol Textbook,* edited by: Lyons, T.P., Kelsall, D.R., and Murtagh, J.E. Nottingham University Press**.**

Inglett, G.E., Chen, D., and Berhow, M. (2010). High-shear, Jet-cooking, and Alkali Treatment of Corn Distillers' Dried Grains to Obtain Products with Enhanced Protein, Oil and Phenolic Antioxidants. *Food and Science Technology International*, **16**(4): 297-304

Jenschke, B.E., James, J.M. Vander Pol, K.J. Klopfenstein, T.J., and Calkins, C.R. (2007). Wet distillers' grains plus solubles do not increase liver-like off-flavors in cooked beef from yearling steers. *Journal of Muscle Foods*,**18**: 341–348.

Jung, B., Hoerler, M. Batal A.B. and Mitchell, R. (2011). Evaluation of feeding distillers dried grains with solubles and the effects of dietary enzymes on broiler performance and carcass characteristics. Poultry Science Association Annual Meeting, July 16–19, St. Louis, Missouri. *Poultry Science*,**90**: 18.

Kim, Y., Mosier, N. S. Hendrickson, R. Ezeji, T. Blaschek, H. Dien, B. Cotta, M. Dale, B., and Ladisch., M. R. (2008). Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. *Bioresource Technology*,**99**: 5165–5176

Liu, K., (2011). Chemical composition of distillers grains, a review. *Journal of Agricultural and Food Chemistry*, **59**(5): 1508-1526

Liu, N., Ru, Y.J. Tang, D.F. Xu, T.S. Partridge, G.G. (2011). Effects of corn distillers dried grains with solubles and xylanase on growth performance and digestibility of diet components in broilers. *Animal Feed Science and Technology*, **163**: 260–266.

Loar, R. E. II., Moritz, J.S. Donaldson J.R., and Corzo, A. (2010). Effects of feeding distillers dried grains with solubles to broilers from 0 to 28

days posthatch on broiler performance, feed manufacturing efficiency, and selected intestinal characteristics.*Poultry Science*,**89**: 2242–2250.

Lumpkins, B.S., Batal, A.B., and Dale, N.M. (2004). Evaluation of Distillers Dried Grains with Solubles as a Feed Ingredient for Broilers. *Poultry Science*,**83**: 1891–1896.

Lumpkins, B.S., Mathis, G. F., Rao, S. K., and McIntyre, D.R. (2011). Influence of NSP enzyme (Rovabio) on energy sparing and growth performance of broilers fed corn-based diets containing corn distillers dried grain with solubles. Poultry Science Association Annual Meeting, July 16–19, St. Louis, Missouri.*Poultry Science*,**90**: 18.

Magnoli, A., Monge, P.M.P. Miazzo, R. D. Cavaglieri, L.R. Magnoli, C.E. Merkis, C. I. Cristofolini, A. L. Dalcero, A. M., and Chiacchiera. S.M. (2011). Effect of low levels of aflatoxin B1 on performance, biochemical parameters, and aflatoxin B1 in broiler liver tissues in the presence of monensin and sodium bentonite.*Poultry Science*,**90**: 48-58.

Malkki, A., (2001). Physical properties of dietary fiber as keys to physiological functions. *Cereal Foods World*,**46**: 196-199.

Miazzo, R., Peralta, M.F. Magnoli, C. Salvano, M. Ferrero, S. Chiacchiera, S.M. Carvalho, E.C.Q. Rosa, C.A.R. and Dalcero. A. (2005). Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. *Poultry Science*,**84**: 1-8.

Min, Y.N., Yan, F., Liu, F.Z.,Coto,C., and Waldroup. P.W. (2009). Effect of various dietary enzymes on energy digestibility of diets high in distillers dried grains with solubles for broilers. *Journal of Applied Poultry Research*,**18**: 734-740

Moran, E.T., and Lehman, R. (2008). Response to combined amylase-phytase-proteasexylanasesupplementation when 8 week broiler males

had received corn-soybean meal feeds devoid of antimicrobials with/without alfalfa and/or DDGS. *International Poultry Science Form Proceedings*, 26.

Murthy, G.S., Townsend, D.E. Meerdink, G.L. Bargren, G.L. Tumbleson M.E. and Singh, V. (2005). Effect of Aflatoxin B1 on dry-grind ethanol process. *Cereal Chemistry*,**82**(3): 302–304.

Pack, M., and Bedford, M. (1997). Feed enzymes for corn and soybean

Ramos, A.J., Fink-Gremmels, J., and Hernandez. E.(1996). Prevention of toxic effects of mycotoxins by means of nonnutritive adsorbent compounds. *Journal of Food Protection*,**59**: 631-641.

Renewable Fuels Association, (2007). Ethanol Industry Outlook 2007: Building New Horizons.

Rodrigues, I., (2008). Crucial to monitor mycotoxins in DDGS. *Feed Business Asia*, Jan/Feb: 36-39.

Roeber, D.L., Gill, R.K., and DiCostanzo, A. (2005). Meat quality responses to feeding distillers' grains to finishing Holstein steers. *Journal of Animal Science*,**83**: 2455–2460.

Saleh, F., Yamamoto, M., Tahir, M., Ohtsuka, A., and Hayashi, K. (2006). A new natural feed additive for broiler chickens. *Poultry Science Association Annual Meeting*. Edmonton, Canada. 36-54.

Schilling, M.W., Battula, V. Loar R. E. II. Jackson , V. Kin , S., and Corzo. A. (2010). Dietary inclusion level effects of distillers dried grains with soluble on broiler meat quality. *Poultry Science*,**89**: 752–760

Schwartz, M.H., Ward, N.E., Ferket, P.R.and Brake, J. (2010). Enzyme supplementation of corn-based diets with or without distillers dried grains with solubles (DDGS) for broilers. *Proceedings of International Poultry Scientific Forum 2010*, Atlanta, USA. M23.

Spiehs, M.J., Whitney, M. H., and Shurson. G. C. (2002). Nutrient database for distillers' dried grains with solubles produced from new ethanol

plants in Minnesota and South Dakota. *Journal of Animal Science*,**80**: 2639–2645.

Swiatkiewicz, S., and Koreleski, J. (2007). Effect of dietary level of maize- and rye distiller dried grains with solubles on nutrient utilization and digesta viscosity in laying hens. *Journal of Animal Feed Science*,**16**: 668–677.

U.S Grain council , A GUIDE TO Distiller's Dried Grains with Solubles (DDGS) ,3rd Edition.

Van Emon, M.L., Gunna, P.J. Nearya, M.K. Lemenagera, R.P. Schultzb, A.F., and Lake, S.L. (2011). Effects of added protein and dietary fat on lamb performance and carcass characteristics when fed differing levels of dried distiller's grains with soluble. *Small Ruminant Research*, doi:10.1016/j.smallrumres.2011.09.002

Verrips E., Klein, S. Lamptey, A., and Yersin. A.G. (2010). An overview of the prevalence of mycotoxins in feed and feed ingredients. *Proceedings of International Poultry Scientific Forum 2010*, Atlanta, USA. P233.

Wang, Z., Cerrate, S. Coto, C. Yan, F., and Waldroup, P.W. (2007a). Use of constant or increasing levels of distillers dried grains with solubles (cDDGS) in broiler diets. *International Journal Poultry Science*,**6**: 501–507.

Wang, Z., Cerrate, S. Coto, C. Yan, F., and Waldroup, P.W. (2007b). Effect of Rapid and Multiple Changes in Level of Distillers Dried Grain with Solubles (DDGS) in Broiler Diets on Performance and Carcass Characteristics. *International Journal Poultry Science*,**6**: 725–731.

Wang, Z., Cerrate, S. Coto, C. Yan, F., and Waldroup, P.W. (2007c). Utilization of Distillers Dried Grains with Solubles (DDGS) in Broiler Diets Using a Standardized Nutrient Matrix. *International Journal Poultry Science*,**6**: 470–477.

Ward, A.T., and Marquardt,R. R. (1983). The effect of saturation, chain length of pure triglycerides and age of bird on the utilization of rye diets. *Poultry Science*,**62**: 1054-1062.

Ward, N.T., R.T. Zijlstra, C. Parsons and C. Starkey, (2008). Non-Starch Polysaccharide (NSP) content of US commercial corn Distiller's Dried Grains with Solubles (DDGS). *Poultry Science*, **87**(Suppl1): 39.

Wen, X.X., Li, F.D. Peron, A., and Ru, Y.J. (2010). Fiber composition and crude protein content in 20 corn-DDGS samples from China. *Proceedings of International Poultry Scientific Forum 2010,* Atlanta, US. P213.

West, M.L., Corzo, A. Dozier, W. A. III. Blair, M. E., and Kidd, M. T. (2007). Assessment of dietary Rovabio Excel in practical United States broiler diets. *Journal Applied Poultry Research*,**16**: 313–321.

Wood, K.M., Salim, H. McEwen, P.L. Mandell, I.B. Miller, S.P., and Swanson. K.C. (2011). The effect of corn or sorghum dried distiller's grains plus solubles on growth performance and carcass characteristics of cross-bred beef steers. *Animal Feed Science and Technology*, **165**: 23–30.

Zhang, Y., Caupert, J. Imerman, P.M. Richard J.L., and Shurson,G.C. (2009). The occurrence and concentration of mycotoxins in U.S. distillers dried grains with solubles. *Journal of Agriculture Food Chemistry,* **57** (20): 9828–9837

Zhao, J., Shirley, R. B. Dibner, J. D. Uraizee, F. Officer, M. Kitchell, M. Vazquez-Anon, M., and Knight, C. D. (2010). Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. *Poultry Science*, **89**: 2147-2156.

Yaling, et al 2008. Similtaneous detection of airborne aflatoxin, ochratoxin and zearlaenone in poultry house by immunoaffinity column and high performance liquid chromatography. Environ. Res., 107: 139-144.