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Evaluation the Impact of Low Quality Rice as Corn Substitute in Broiler's Diet on Growth, Digestive Organ and Carcass Trait's

A Thesis

Submitted to the Council of the College of Veterinary Medicine at University of Kerbala as a Partial fulfillment of the Requirement for the Master Degree in the Sciences of Veterinary Medicine / Public Health

BY

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيم

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Dedication

To Allah the Almighty, Creator of the heavens and the earth.

I present my humble research as a gift to my masterand lord, the awaited Imam Mahdi (peace be upon him).

to those who sought to support me... my father, to my loved ones... my daughters, my brothers and sisters, to my friends, colleagues, and classmates and all my teachers

Acknowledgement

In the name of Allah, the most gracious, the most merciful Praise be to Allah, by whose grace good deeds are accomplished, and prayers and peace be upon the most honorable of prophets and messengers, our master Muhammad, and upon all his family and companions.

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Ameera Muhammed Said

Summary

This study was conducted to investigate the effect of different levels of low quality rice as alternative of corn on broiler chicken performance by measuring some productive performance, digestive organs measurement, carcass equality characterization, gene expression of insulin-like growth factor-1 and intestinal morphology. The experiment lasted five weeks (35days) that started from 22 November 2023 to 26 December 2023. One hundred and sixty broiler chicks (Ross308), the birds were fed on basal diet for two weeks, at day 15^{th} of the experiment the birds were divided randomly into 4 groups, each group contain 40 birds, each group subdivided into two replicates (20 birds for each) in a sector design. Control group (T0) was fed on the basal diet second group (T1) as treatment fed on basal diet with replace corn with 10% rice. Third group (T2) as treatment fed on basal diet with replace corn with 20% rice. Forth (T3) group as treatment (T3) fed on basal diet with replace corn with 30% rice.

The productive traits measured weekly, at day 5^{th} and 25^{th} of age, blood samples from jugular vein were collected from ten (10) birds from each group to obtain the serum to determine immunological test, and carcass characteristics measured after slaughtering. The results revealed that live body weight showed no significant difference between control group and T1group in all periods of the experiment that increased as compared with T2 and T3groups.There was no significant difference in feed intake in the 3^{rd} week in control group, while at 4^{th} and 5^{th} week, T3 showed a significant ($p<0.05$) increase in feed intake, while T2 showed a significant ($p< 0.05$) increase in feed in take at 4^{th} and 5^{th} week. Weight gain showed a significant ($p<0.05$) increase in

all period of the experiment in the control group and T1, comparing with T3 and T4.

Feed conversion ratio showed a significant ($p<0.05$) improve in the T0 and T1 groups as compared with other treated groups and did not difrent from control group. The antibody titers of Newcastle disease, Gomboro, infectious bronchitis and avian influenza showed a significant ($p<0.05$) increase in the control and T1 groups. There was a significant ($p<0.05$) increase in the relative weight of liver, gizzard, proventiculus and crop in the control group comparing with other groups, while spleen showed a significant ($p<0.05$) increase in T3.The sensory evaluation results showed a significant increase in T3 ,T4 comparing with T0,T1 as tenderness and colour, whereas juiciness showed no significant difference among groups. The flavor and palatability showed a significant increase in the control comparing with other groups. The contents of mineral in meat showed a significant increase in Calcium, Phosphorus, Iron, Zinc and Potassium in control and T1, comparing with T2 and T3. Gene expression data showed a significant ($p<0.01$) difference among experimental groups. Also villi length, crypt depth, villi width and muscular thickness were recorded a significance in duodenum and jejunum villi length, crypt depth, villi width showed a significant ($p<0.05$) increase in control group while T2 decreased significantly ($p<0.05$) as compared with other groups. Ilium villi showed a significant ($p<0.05$) increase in the control group. In conclusion, diet of chickens that fed on 10% rice yield the best growth performance compered to diet with 20% and 30 % rice. As well as rice content beyond 10% regularly effect chickens growth and health.

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List of abbreviations

Abbreviations	Full Form
AI	Avian influenza
BW	Body weight
Ca	Calcium
FCR	Feed convertion ratio
Fe	Iron
FI	Feed intake
GALT	Gut-associated lymphoid tissue
HOC	High oil corn
IB	Infectious bronchitis
IBD	Infectious bursal disease
IGF-1	Insulin like growth factors-1
JAK	Janus kinase
K	Potassium
Mg	Magnesium
Mn	Manganese
ND	Newcastle disease
P	Phosphorus
QTL	Quantitative trait loci
SCFAs	Short chain fatty acids
STAT	Signal tranducer and activator of transcription

WG	Weight gain
Zn	Zinc

Chapter One
Introduction

Introduction

Broiler production is important for several reasons; firstly, it plays a crucial role in meeting the increasing global demand for poultry meat, which is essential for feeding the growing population. Secondly, broiler production provides income and employment opportunities, particularly for small-scale farmers in rural areas. Additionally, the industry has seen advancements in genetic selection and management practices, leading to improved productivity and reduced disease incidences in chicken diets (Mottet & Tempio., 2017).

It seems that the developing world's use of poultry products such as meat and eggs is rising. The worldwide corn order for use in the production of agricultural feeds and fuel is increasing rapidly (Popp *et al.*, 2016). In Japan, almost 100% of the corn used for animal feeds is imported (Statistics Department, Ministry of Agriculture, Forestry and Fisheries, 2019), resulting in a very low level of feedstuff self-sufficiency nationwide. As a result, the cost of producing chicken would rise due to an increase in worldwide demand for the primary feedstuffs for poultry, rice stands out as a versatile and energy-dense option, offering potential benefits to broiler chickens when incorporated into their diets.

The use of rice in chicken diets has grown in popularity being an excellent source of protein, energy, vitamins, and minerals, has a lot of promise as a nutritional element (Attia *et al.*, 2023).

Rice has a significant amount of protein including four different fractions such as prolamin, glutelin, globulin, and albumin with different solubility characteristics (Jayaprakash *et al.*, 2022). These proteins exhibit a higher amino acid profile, so they are nutritionally important and possess several functional properties. Compared with

many other cereal grains, rice protein is hypoallergic due to the low percent of gluten (Al-Doury *et al.*, 2018).

Rice is considered a good source of energy for poultry, and it can contribute to the overall nutritional profile of the feed, rice is highly digestible for poultry, providing a good source of carbohydrates, the digestibility of rice can positively influence the growth and performance of broiler chickens (Jha & Mishra, 2021).

Rice is particularly high in fiber compared to some other grains, it does contain some dietary fiber. The presence of fiber can influence the gut environment and may have positive effects on the intestinal microflora by promoting the growth of beneficial bacteria (Abazari *et al*., 2016).

The impact on intestinal microflora can be indirect, as the overall diet composition influences the microbial balance in the gut. A well-balanced diet that includes rice along with other ingredients like proteins, vitamins, and minerals can contribute to a healthy gut microbiota (Singh & Kim., 2021).

Study objectives**:**

The purpose of the current study is to assess the effects of different levels of rice that will be supplemented instead of corn to broiler chickens diet by evaluating the following parameters:

1- Productive performance: body weight, body weight gain, feed intake, feed conversation ratio.
2- Immune system.
3- Digestive organs measurements: (crop, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, colon, and pancreas).
4- Carcass characterization and sensory evaluation of meat and skin color.

5- Gene expression of insulin-like growth factor-1.

6- Intestine morphology: villus height, crypts depth, villus width and muscular thickness.

Chapter Two

Review of Related Literature

2- Review of Related Literatures

2-1 Feeding of Broiler chickens

The primary source of protein in broiler feed is typically derived from plant-based ingredients, such as soybean meal, corn gluten meal, and canola meal. These ingredients are rich in essential amino acids, which are the building blocks of protein. Amino acids such as lysine, methionine, and threonine are particularly important in broiler diets as they are limiting amino acids, meaning their availability can affect overall protein synthesis (Giannenas *et al*., 2017).

To meet the nutritional requirements of broilers, feed formulations are carefully balanced to provide the necessary levels of protein and amino acids. The cost of feed for broiler chickens depends on several factors as the age of the broilers, desired growth rate, and market specifications. (Kidd and Hackenhaar, 2006).

Protein quality and digestibility can vary among different feed ingredients, which can affect the overall performance and efficiency of broiler production. Therefore, feed manufacturers and nutritionists carefully select and formulate feed ingredients to ensure that broilers receive a balanced and nutritionally complete and economic diet. In the early stages of broiler growth, starter feeds are formulated to contain a higher percentage of protein, typically ranging from 20% to 24%. This higher protein content helps support the rapid growth and development of broilers during this critical phase. As the broilers grow older, the protein content in the feed is gradually reduced, while the energy content increased to support weight gain and minimize excessive fat deposition (Shehu *et al*., 2021).

2-2 Corn

According to Kumaravel and Natarajan (2014), maize makes up around 50–60% of most chicken feeds, and it is more common in poultry diets (Panda *et al*., 2010). Its output, however, is insufficient to satisfy the poultry industrys always rising demand. Intense rivalry for its use by humans and other domesticated animal species (Agbede *et al*., 2002), as well as the starch and related businesses (Egbunike and Achiobong, 2002), are another reason why its price is always rising.

Maize is the primary feed grain used in chicken diets globally. This is mostly due to the fact that poultry can easily digest starch, which serves as its energy supply. It is also devoid of anti-nutritional elements, very tasty, and a high- density source of easily accessible energy. In general, the metabolizable energy value of maize is used as a benchmark for comparing different energy sources (Zaefarian *et al* ., 2015)

The feed business has benefited from surplus maize in North America and Brazil as a consequence of greater automation and the use of genetic and agronomic approaches to boost production. However, maize yield per hectare is poor in Asia and Africa, and output has never been enough in most of these places to fulfill the demands of a rising human society. The final effect is that these areas continue to have a lack of maize for feed (Shiferaw *et al*., 2011) .

One of the most popular energy-producing components in poultry diets is maize. 3,350 kcal ME/kg and 8.5% crude protein are found in dry dent corn grain. Producers of maize have adjusted the energy content by adding or removing oil. Increasing the grains germ percentage results in higher oil content, which also raises the grain's protein content (Watson and Freeman, 1975). High oil corn (HOC)

types already on the market have oil contents of 6 to 8% and yields that are on par with commercial. By making it easier to formulate higher energy diets that should enhance feed conversion and maybe growth in broilers, the use of HOC can benefit the poultry business. By using HOC, less additional fat would be needed, which would result in less feed(hood *et al*., 2012).

2.3 Rice

Rice is produced by grass plants (Poaceae family) and is an edible starchy cereal grain. Approximately 50% of the global populace, encompassing nearly all of East and Southeast Asia, is entirely reliant on rice as their primary food source; humans consume 95% of the world's rice harvest. Rice may be crushed into flour or cooked by boiling it. In Asian, Middle Eastern, and many other cuisines, it is consumed both on its own and in a wide range of soups, side dishes, and main courses in 2022, Hanghas *et al*.

Rice serves as a vital source of nutrition, primarily contributing carbohydrates protein and minimal fat the main energy source for the body(Mohidem *et al*., 2022).

Scientific classification of rice according to (Wang & Li., 2005):

Kingdom: ***Plantae***

Phylum: ***Magnoliophyta***

Class: ***Liliopsida***

Subclass: ***Commelinidae***

Order: ***Cyperales***

Family: ***Poaceae***

Genus: ***Oryza***

Subject: ***Oryza sativa L.***

Rice contributes to the amino acid profile of broiler feed. Though it is not a complete protein source, rice contains some essential amino acids, including small amounts of lysine and threonine. Supplementing rice with other protein sources can help create a balanced amino acid profile, ensuring that broilers receive the necessary building blocks for muscle development. (Lee *et al.,* 2023).

In addition to its nutritional benefits, rice can serve as an economical and sustainable component in broiler diets as a widely cultivated crop rice is often more readily available and cost-effective compared to some alternative grains. (Bodie *et al.,* 2019) Rice inclusion in feed formulations can contribute to the overall affordability of broiler production, an essential consideration for poultry farmers. (Babatunde *et al.,* 2021).

Rice is more readily available than corn and has comparable protein and metabolized energy levels; it may be a good substitute (Daghir, 2008). Because of rise has a high content of vitamins, minerals, and fiber, as well as their phenolic base compounds which can help decrease cholesterol and boost antiatherogenic activity, rice by-products have drawn more attention as functional foods in recent years (El-Sabrout *et al.,* 2023)as shown in tables (2-1) .

Table (2-1): chemical compositions Value of Uncooked Rice (per 100 grams) according to (Lyu *etal*., (2018)

Calories	364 kcal
Carbohydrates	80 grams
Sugars	0.1 grams
Dietary Fiber	1.3 grams
Protein	7.1 grams
Fat	0.6 grams

Saturated Fat	0.2 grams
Iron	1.5 mg
Sodium	5 mg
Potassium	115 mg
Thiamine (Vitamin B1)	0.07 mg (6% of the Daily Value)
Niacin (Vitamin B3)	1.6 mg (8% of the Daily value)

2.4 Effect of Rice on Broiler productive performance

Rice is main food for a large part of the global population and has been utilized in broiler diets due to its nutrient composition, palatability, and availability (Babatunde *et al.,* 2021).

The effect of rice on broiler productive performance is an important consideration in poultry nutrition as dietary ingredients play a significant role in influencing growth, feed efficiency, and overall production outcomes in broiler chickens (Nanto-Hara *et al.,* 2021).

Rice serves as a valuable source of energy due to its carbohydrate content, carbohydrates are a primary energy source for broilers, supporting essential metabolic functions and providing the energy needed for growth, activity, and maintenance. The energy derived from rice contributes to overall feed efficiency and weight gain in broilers (Bodie *et al.,* 2019).

Rice can complement protein-rich components in the broiler diet. A balanced amino acid profile is crucial for supporting muscle development and overall growth in broilers. Properly formulated diets that include both rice and protein sources contribute to optimal performance (Sibanda *et al.,* 2023).

In examining the essential amino acids in rice, it becomes apparent that it is deficient in lysine, a crucial amino acid for protein synthesis and overall growth. Lysine plays a vital role in various physiological functions, including the formation of collagen, the absorption of calcium, and the production of enzymes and antibodies (Yang *et al.,* 2016).

Valine and isoleucine are two additional essential amino acids present in rice that play roles in muscle metabolism, energy production, and immune system function (Kumar *et al.,* 2016).

Rice inclusion in broiler diets can influence growth rate which is a critical parameter in broiler production. The energy content of rice, primarily derived from carbohydrates, provides a readily available source of energy for broiler chickens. Diets containing rice may support optimal growth performance by providing the necessary energy for metabolic processes and tissue growth (Selim *et al.*, 2021).

Rice inclusion in broiler diets can influence FCR through its nutrient composition and digestibility. Diets containing rice with high digestibility and energy content may result in improved feed efficiency and lower FCR values (Zare-Sheibani *et al.,* 2015).

The composition of broiler carcasses, including meat yield, fat deposition, and muscle development, can be influenced by rice inclusion in the diet. Rice-derived nutrients and bioactive compounds may affect metabolic pathways involved in protein synthesis, lipid metabolism, and muscle growth. Diets containing rice may result in desirable carcass characteristics, such as higher breast meat yield, lower abdominal fat deposition, and improved meat quality attributes (Selim *et al.,* 2021 and Al-Abdullatif *et al*., 2023).

Rice inclusion in broiler diets can influence feed intake, which is a critical factor in determining overall productivity and nutrient

utilization efficiency. Diets containing rice with high palatability and digestibility may stimulate feed intake and promote voluntary feed consumption in broiler chickens. However, excessive inclusion of rice or imbalances in nutrient composition may lead to reduced feed intake and impaired growth performance. Therefore, careful formulation of rice-based diets is necessary to ensure optimal feed intake and productivity in broiler production systems (González-Alvarado *et al.*, 2007 and Effiong *et al.*, 2019).

Rice inclusion in broiler diets can have significant effects on productive performance, including growth rate, feed conversion ratio, body composition, mortality rate, feed intake, and economic efficiency (Edea Muleta, 2020).

2.5 Effect of Rice on Broiler immune system

Rice provides essential nutrients like vitamins (particularly vitamin E), minerals, and antioxidants; these micronutrients play a crucial role in immune cell function and the production of immune mediators (Mir *et al.*, 2017).

Polyphenols that present in rice has been shown to exhibit antioxidant and anti-inflammatory properties, which can potentially benefit immune function. Flavonoids found in rice have also been reported to modulate immune responses by regulating cytokine production and immune cell activity (Liu *et al.*, 2023).

Phytic acid is another bioactive compound in rice that has been studied for its immunomodulatory effects, including its ability to enhance immune cell proliferation and antibody production. Moreover, rice-derived polysaccharides have been shown to stimulate immune cell activity and enhance host defense mechanisms (Alagawany *et al.*, 2021).

The results demonstrated that broilers fed diets supplemented with rice bran exhibited increased production of pro-inflammatory cytokines and higher antibody titers compared to control groups, indicating enhanced immune function (Komiyama *et al.*, 2011).

Rice especially its bran and hulls contains various polyphenolic compounds such as ferulic acid, caffeic acid, and quercetin which are Polyphenols possess antioxidant properties and can modulate immune responses by reducing oxidative stress and inflammation. These compounds have been reported to enhance immune function in various animal species by promoting the production of cytokines and increasing the activity of immune cells (Wanyo *et al.*, 2014).

Rice contains various polysaccharides including arabinoxylans, β-glucans, and pectins. These polysaccharides have been studied for their immunomodulatory properties, which include stimulating immune cell activity and enhancing host defense mechanisms. Polysaccharides derived from rice may contribute to improving immune function in broiler chickens by activating immune cells and promoting the production of cytokines and antibodies (Choct,. 2015 and Nguyen *et al.*, 2021).

Rice is a good source of vitamins and minerals, including vitamin E, selenium, and zinc, which are essential for immune function. Vitamin E acts as an antioxidant and plays a role in maintaining immune cell integrity, while selenium and zinc are involved in the regulation of immune responses. Adequate levels of these vitamins and minerals in the diet can support the development and function of the immune system in broiler chickens (Selim *et al.*, 2021).

2.6. Effect of rice on the sensory evaluation of broiler meat

Rice inclusion in broiler diets can affect meat texture through several mechanisms. The carbohydrate content of rice can influence meat tenderness and juiciness (Rahman *et al.,* 2022). Diets containing rice may result in meat with a softer texture due to differences in muscle development and fat deposition. Additionally, the presence of bioactive compounds in rice, such as polysaccharides and polyphenols, may affect meat texture by altering muscle fiber structure and water-holding capacity (Sinambela *et al.,* 2023).

Utilizing rice in broiler diets can impart subtle flavor profiles to the meat due to its own flavor characteristics. The type of rice used in the diet can contribute different flavors to the meat. Aromatic rice varieties may impart a distinctive aroma and flavor to broiler meat. Additionally, rice-derived compounds such as amino acids, carbohydrates, and lipids may undergo Millard reactions during cooking, resulting in the development of desirable flavor compounds in the meat (Hashem *et al.,* 2023).

Broiler diets containing rice may affect meat color due to differences in pigments and antioxidant compounds present in rice. For example, pigmented rice varieties (e.g., red rice, black rice) contain anthocyanins and other phytochemicals that can influence meat color (Rahman *et al.,* 2022). Incorporating rice into broiler chicken feed can influence meat juiciness through its effect on moisture retention and fat content. Diets containing rice may result in meat with higher moisture content and juiciness due to differences in nutrient composition and water-holding capacity. Additionally, rice-derived compounds such as polysaccharides and polyphenols may enhance meat juiciness by improving water-binding properties and reducing cooking loss (Parmar *et al.,* 2019).

2.7 Effect of Rice on gene expression

Rice contains carbohydrates, proteins, vitamins, and minerals that can influence nutrient signaling pathways involved in gene expression regulation (Zaghum *et al.*, 2022).

Carbohydrate metabolism-related genes such as those encoding enzymes involved in glycolysis, gluconeogenesis, and glycogen metabolism may be modulated by rice inclusion in broiler diets. Genes involved in lipid metabolism, amino acid metabolism, and energy metabolism may also be affected by rice-derived nutrients (Shi *et al.*, 2022).

Rice nutrients and bioactive compounds can affect the expression of genes involved in growth and development processes in broiler chickens. Genes encoding growth factors, growth hormone receptors, and insulin-like growth factor binding proteins may be influenced by rice inclusion, impacting growth performance, body composition, and skeletal development. Additionally, genes involved in muscle growth, adipogenesis, and bone formation may be regulated by rice-derived factors, influencing broiler phenotype and carcass quality (Fujimoto *et al*., 2020).

2.7.1 Insulin-like growth factors I (IGF-1) gene expression:

The polypeptide hormone family preproinsulin, which included proinsulin, IGF-I, IGF-II, and C peptide had several metabolic roles, included insulin-like growth factor-1 (IGF-1). Insulin-like growth factor-1 (IGF-I) is one of the most prominent hormones required to sustain adequate development in chickens. It is found on chromosome 1 inside a linkage area where various quantitative trait loci (QTLs) influencing growth have been discovered.

According to (Fujita *et al*., 2019), insulin-like growth factors have been thoroughly investigated and are known to be essential for the development of chicken muscles. IGF1 has autocrine or paracrine actions is mostly of local origin. It may be obtained from enhanced synthesis in the liver under the influence of growth hormone (Kanački *et al*., 2012). Furthermore, IGF1 is known to have a significant role in the metabolism of fat, protein, and carbohydrates in a variety of tissues, including the liver, muscle, and fat. IGF1 promotes protein synthesis and glucose absorption in skeletal muscle cells in(2022, Vaccaro *et al*).

Chicken insulin-like growth factor-1 has been identified as a biological candidate gene responsible for body composition, growth, fat deposition and metabolic activities in chickens (Hosnedlova *et al*., 2020) and it is a critical regulator of satellite cell proliferation and skeletal muscle hypertrophy (Yu *et al*., 2015) . Although the liver is the primary organ where they produce IGF- 1, some organs such as the pituitary, brain, ovary, spleen, and muscle, are also known to synthesize IGF. By binding to the growth hormone receptor (GHR), growth hormone (GH) induces the janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway activation and then triggers IGF-1 synthesis (Kita *et al*., 2005).

2.8.Effect of rice on broiler intestine

Rice is a rich source of carbohydrates providing a readily digestible energy source for chickens. The starch in rice is broken down into sugars during digestion and the resulting glucose is absorbed in the small intestine (Li, *et al*., 2019).

Fibers that found in rice play a crucial role in maintaining a healthy gut environment. It promotes the growth of beneficial gut bacteria, contributing to a balanced gut microbiota (Llanes &

Ramirez., 2022). A well-balanced microbiota is associated with improved nutrient absorption, enhanced immune function, and resistance to pathogenic bacteria (Wickramasuriya *et al.*, 2022).

The fermentation of dietary fibers including those present in rice by beneficial bacteria in the ceca and colon results in the production of short- chain fatty acids (SCFAs) (Ali, *et al.,* 2022).

short- chain fatty acids are essential for gut health as they provide an energy source for the epithelial cells lining the intestine and contribute to the maintenance of a low pH environment, inhibiting the growth of harmful pathogens (Rinttilä, T., & Apajalahti ., 2013).

The presence of a robust population of beneficial bacteria can help in reducing the colonization and proliferation of pathogenic bacteria in the gut. By competing for resources and producing antimicrobial compounds, these beneficial microbes contribute to a healthier gut environment and reduce the risk of infections in broilers (Wigley, 2015).

Rice is a good source of energy but it lacks certain nutrients essential for chickens. Therefore, it is important to balance the diet with other feed ingredients that provide the necessary vitamins, minerals, and proteins (Gunaratne *et al.*,1993).

Rice like other grains contain anti-nutritional factors such as phytic acid this can hinder the absorption of certain minerals, and strategies such as proper processing or combining with other feed components can mitigate their effect (Isah & Okosun., 2023).

The Gut-Associated Lymphoid Tissue (GALT)is a crucial component of the immune system and the nutrients derived from rice along with other dietary components play a role in supporting the GALT, A healthy GALT is essential for efficient immune responses, helping chickens resist infections and diseases (Rubio, 2019).

Research by (Liu *et al*.**,** 2014) focused on the digestibility and nutrient utilization of broilers fed with rice versus corn found that starch Rice starch was found to be more digestible than corn starch, leading to higher energy availability. Also, the protein digestibility of rice was higher contributing to better growth performance and nutrient utilization.

Also, study conducted by (Kheravii *et al*., 2017) investigated the effects of replacing corn with rice on intestinal morphology and broiler performance found that Villous Height: Broilers fed with rice-based diets had significant ly higher villous height in the small intestine, indicating improved nutrient absorption. Crypt Depth: Reduced crypt depth was observed, suggesting lower cellular turnover and better intestinal health. Growth Performance: Broilers on rice-based diets showed improved body weight gain and feed efficiency compared to those on corn-based diets.

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Chapter Three

Methodology

3.1. Experimental design:-

A total of one hundred sixty (160) at one-day-old unsexed chicks of the Rose 308 breed were purchased from local hatchery. The birds were fed on basal diet for two week (14 days). Blood was collected at 5 days of age from ten birds randomly to examine immunity. At day 25 of the experiment the birds were randomly divided into 4 groups each group contains forty (40) birds, and each group was subdivided into two groups, each group contains 20 birds, the group was divided as flowing:

The first group (control group) (T0) was fed on the basal diet

The second group (T1) as treatment fed on basal diet with replace10 % corn with 10% rice

The Third group (T2) as treatment fed on basal diet with replace20 % corn with 20% rice

The Forth (T3) groups as treatment (T3) fed on basal diet with replace30 % corn with 30% rice

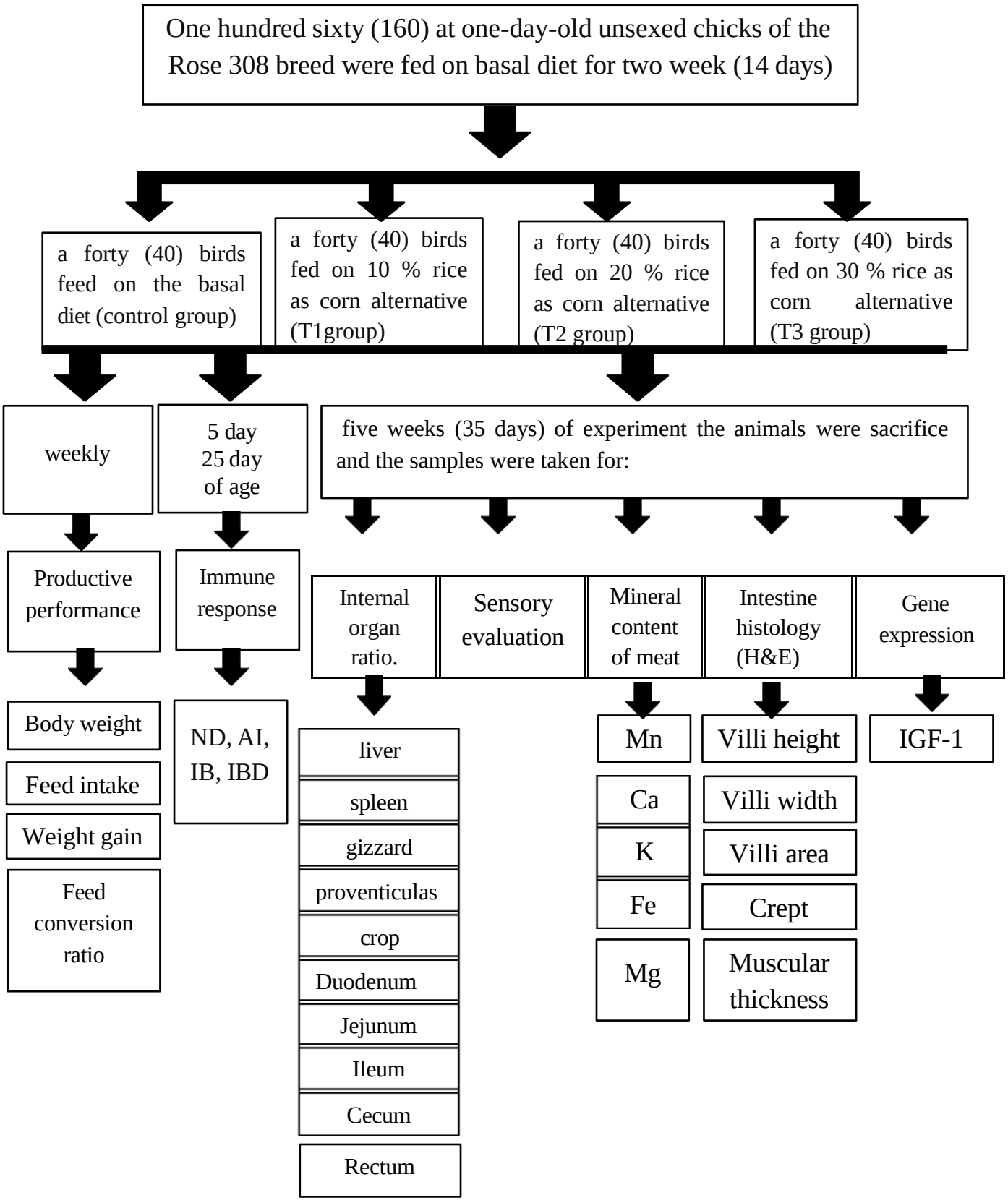


Figure (3-1): experiment design

3.2 Preparation of poultry house

All windows were opened and ventilation was turned on to ensure that all harmful gases were completely removed before the chicks were admitted. Drinker and feeders were also cleaned and disinfected before being distributed among the groups. The walls, ceiling, and floor cleaned using clean water and an approved disinfectant (sodium hypochlorite). The appropriate litter (wood sawdust) was given to each hall group, and the lighting and ventilation were adjusted in accordance with recommendations. Every chick was raised in accordance with the Aviagen manual. (Neeteson *et al*., 2016).

3.3. Vaccination Program

Distal water was used to administered all required live attenuated vaccinations (1000 doses), which were then administered by multiplying the number of birds by the age of each bird and then diluted with water. Count of birds × age of birds equals volume of water (ml) The LaSota strain of live attenuated Newcastle Disease (ND) vaccination. Boehringer, a German company, administered via drinking water (1000 doses) vial. The live attenuated strain of the infectious bursal disease (IBD) virus was sourced Ceva –Hungary was given throw drinking water. Table (3-1) shows that the age Route of administration of vaccines.

Table (3-1) vaccines, which used in this study

Age (days)	Strain	Origin	Route of administration
(10 & 20 days)	ND La sota	Boehringer Germany	Drinking water
(14 days)	IBD GumboL	Ceva -Hungary	Drinking water
(1 day)	AI	Boehringer Germany	Injection
(1 day)	IB	Boehringer Germany	Injection

3. 4 .Blood sampling

At 25 days all blood samples were taken from the heart in a test tube without the use of EDTA anticoagulant. In order to extract serum, the blood was allowed to coagulate and centrifuged for 10 minutes at 3000 rpm. The serum was refrigerated at -20°C until examination. Antibody titer against ND, IB, IBD, and AI illnesses is measured by ELISA.

3.5 .Sensory evaluation:

Slices of breast flesh, about 2 to 3 cm in size, were placed in an electric oven and roasted to 177 °C before being lowered to the sensory assessment. According to the scores shown in the table (3-2), the sensory evaluation of five adult women was conducted for flavor, tenderness, juiciness, color, and palatability (Abdul aali & alobadi., 2018).

Table (3-2) Sensory evaluation

appreciation	Tenderness	Juicy	Color	Flavor	Palatability
1	Very soft	very juice	light	Very good	Very palatable
2	Soft	juice	pale	good	palatable
3	Middle	Middle	Middle	Middle	Middle
4	Hard	Dry	dark	weak	unpalatable
5	very hard	very dry	very dark	Very weak	rejected

3.6 Gene expression samples

All Gene expression samples were collected at days 35 of age from all groups randomly. The sample were obtained from the liver and stored at liquid nitrogen until analysis.The samples were used for detection of

Gene expression for (IGF-1).

3.7 .Organs collection for Histological section

After the birds were sacrificed ,the birds were dissected to remove samples (duodenum ,jejunum & illume), and the organs were preserved in formalin at a concentration of 10% in clean plastic containers after numbering them and the formalin was changed after 24 h for the histological examination .

3.8. Instruments and Equipment

All the devices utilized as a part of this study are summarized in table(3.3)

Table 3.3. Apparatus and equipment with their manufactures.

No.	Apparatus & Equipment	Company	Manufactures
1.	Anatomicalset(Scissors, Forceps, Scalpel)	Chemo lab	China
2.	Balance	Denver	Germany
3.	Beakers (100, 250, 500, 1000)	Chemo lab	India
4.	Centrifuge	Hettich	Germany
5.	Colony flask	Chemo lab	India
6.	Cotton	Entrepreneur	India
7.	Digital balance	Denver	Germany
8.	Digital camera	Canon	China
9.	ELIZA printer	epson	japan
10.	ELIZA reader	biotek	USA
11.	Eppendorf's tubes	Chemo lab	India
12.	Filter paper	Chemo lab	India
13.	Gel tube	Chemo lab	India
14.	Incubator	Lab tech	Korea
15.	Light Microscope	Olympus	Japan
16.	Micropipettes(different volumes)	dragonmed	China
17.	Microscope with camera	Olympus	Japan
18.	Microtome	Leica RM	USA
19.	Pipette tips (10 – 1000) µl volume	Chemo lab	China
20.	Refrigerator	denka	japan
21.	Sensitive balance	Sartorius	Germany
22.	Slide & cover slip	Chemo lab	China
23.	Spectrophotometer	EMCLAB	Germany
24.	Surgical gloves	Chemo lab	China
25.	Syringe (1 ml, 5 ml)	Chemo lab	China
26.	Test tubes	Chemo lab	China
27.	Vortex	Sturat	United kingdom

28.	Water bath	labtech	Korea

3.9 Nutritional Information about the Rice used in the experiment

The Nutritional compositions about 100g of rice used in the experiment is shown in table (3.4) and in apandix 1.

Table (3.4): Nutritional information of the rice

No.	Name	Con.
1	Protein %	8.24
2	Lipid%	1.1
3	CHO %	77.44
4	Energy (Kcal)	3525
5	Ca (µg/gm)	98.5
6	Fe(µg/gm)	12.9
7	Mg(µg/gm)	198.5
8	K(µg/gm)	689
9	Mn(µg/gm)	9.14
10	cu(µg/gm)	1.25

3.10- Composition of the broiler chickens diet

The composition of the broiler chickens diet is shown in table (3-5).

Table (3-5): composition of the broiler chickens diet

Ingredients	Starter 1-14 day	Finisher diet 15_35 day			
	%	T_0	T_1	T_2	T_3
Corn	50.5	58.0	47.5	38.0	28.0
Soy-bean meal	36.0	27.5	27.5	27	27
Rice	0	0	10.0	20.0	30.0
wheat	8.0	7.0	7.5	7.5	7.5
premix	2.5	2.5	2.5	2.5	2.5
Plant oil	1.5	3.5	3.5	3.5	3.5
di-calcium-PH	0.1	0.1	0.1	0.1	0.1
Lime	1.1	1.1	1.1	1.1	1.1
Salt	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100
Chemical analysis					
metabolizable energy	3015 kcal/kg	3210	3201	3225	3249
crude protein	23.1%	19.5%	19.16	18.97	18.78
Total Ca	1.10%	1.08%	0.66	0.68	0.69
available PH	0.74%	0.71%	0.42	0.45	0.49
Lysine	1.12%	1.02%	0.93	0.95	0.96
methionine + cystine	0.73%	0.65%	0.62	0.63	0.63
energy/protein	130.4%	163.9%	167.1	170.0	173

Premix composition per kg: vitamin D,2,400 IU; vitamin E, 60.0 mg; vitamin K, 3.0 mg; vitamin B1, 3.0 mg; vitamin B2, 8.0 mg; vitamin B6, 4.0 mg; vitamin B12, 0.02 mg; niacin 50.0 mg; pantothenic acid 15.0 mg biotin 0.04 mg; folic acid 2.0 mg; cu 15

mg; Fe 40 mg; Mn 100 mg; Zn 100 mg; I 1.0 mg; Se 1 mg

3.11 Parameters of the study

3. 11.1 Productive Parameters:-

3.11.1.1 Average body weight (BW) each week (gm/bird) :

Each chick was weighed on the first day of life and at the conclusion of each week using a delicate balance to determine the weight. The total weight of all the chicks divided by the number of chicks yielded the mean body weight.

3.11.1.2 Weekly intake of feed (F.I.) in grams :

Feed intake was calculated weekly using the equation provided by Al-Zubaidi (1986). This was accomplished by weighing the leftover feed at the conclusion of each week and deducting it from the total amount provided at the start of the week.

3. 11.1.3 Mean weekly growth in weight (WG) in grams/birds

Using the following formula, the weight growth was recorded at the start and end of each week to determine the mean body weight gain for each group. Body weight at the end of the week minus body weight at the start of the week equals the mean weekly weight increase (Al-Fayadh and saad, 1989).

3. 11.1.4 Feed Conversion Ratio (F.C.R)

Until the completion of the trial, the feed conversion ratio for each group was determined on a weekly basis. The FCR measurement equation was described in (AL-Fayadh and Naji, 1989).

$$\textbf{FCR=}\ \frac{\textbf{mean}\ weekly\ feed\ intake\ (gm)}{\text{mean}\ \textbf{weekly body weight gain (gm)}}$$

3.12 Content of minerals in meat

3.12.1 Content of Ca, P, Fe, Zn and K in meat

Calcium, P, Fe, Zn and K were determined by use special Colorimetric method kit as shown in appendix II, III, IV, V, VI.

3.13 Immune response

The Immune response for ND, IB, IBD& AI were determined by use a special ELISA Kit as shown in (Appendix VII, VIII, IX & X)

3.14 Carcass characterization

Carcass characterization was measured after sacrificed of the animal, liver spleen, gizzard, proventiculas, crop, duodenum, jejunum, illume, cecum & rectum were measured by use electric balance.

3.15 Insulin-like growth factors I (IGF-1) Gene expression

Insulin-like growth factors I (IGF-1) gene expression was measured as shown in (appendix XII).

3. 16 Histological examination

Intestine histology preparation and measuring as show in (appendix XI)

3.17 Statistical analysis

Graph Pad software Prism version (8.0) was used to conduct the statistical analysis one way anova with LSD The standard of significance for the analysis was $p < 0.05$, and the data points were reported as mean and Standard Error(SAS, 2018).

Chapter Four
Results and Discussion

4. Results and Discussion

4.1. Production Performance

4.1.1. Effect of Replacing Corn With Different Levels of Low Quality Rice on Productive Performance of Broiler Chickens

Live body weight showed no significant difference between control group and 10 % rice group in all periods of the experiment. On the other hand there was a significant ($p< 0.05$) increase as compared with 20% & 30% rice group, while there were no significant differences between 20 % rice group & 30 % rice group as compare to each other in week 3 rd & week 4th while 30 % rice group show a significant ($p< 0.05$) decrease in in live body weight in the 5th week as compared with the other groups , as shown in table (4-1)

Table (4-1): Effect of replaced diet with different levels of low quality rice on live body weight.

Time	Control group	10 % rice Group(T1)	20% Rice Group(T2)	30% Rice Group(T3)
3rd week	718.9 ± 13.65 A	713.4 ± 16.18 A	695.4 ± 12.83 B	697.1 ± 26.47 B
4th week	1226.4 ± 16.32 A	1227 ± 26.45 A	1184.1 ± 32.14 B	1176.8 ± 20.45 B
5th week	1930 ± 34.24 A	1916.5 ± 23.47 A	1829 ± 55.24 B	1783.1 ± 43.71 C

Different letters next to means horizontally indicate a significant differences ($p< 0.05$)

In the current study, there was no significant difference in feed intake in the week 3 in the control group as compare with the other groups. In the week 4 & 5, the 30 % rice showed a significant ($p< 0.05$) increase in feed intake as compare with the other group. Also 20% rice show a significant ($p < 0.05$) increase in feed intake in 4th & 5th week as compared with the control & the 10% rice ,while control

group demonstrate significant difference ($p< 0.05$) decrease in the 5th week as compared with the other groups, as shown in table (4-2).

Table (4-2) effect of basal diet, different levels of low quality rice on broiler feed intake.

Time	control	10%	20%	30%
3rd week	630 ± 5.4 A	640 ± 5.3 A	634 ± 4.3 A	640 ± 4.88A
4th week	875 ±12.7 C	880 ± 13.2 C	900 ± 11.5 B	925 ± 12.7 A
5th week	1120 ± 20.4 D	1165 ± 23.4 C	1180 ± 17.7 B	1200 ±28.9 A

Different letters next to means horizontally indicate a significant differences ($p< 0.05$)

Weight gain showed a significant ($p< 0.05$) increase in all the period of the experiment in the control group & 10% rice group as compared with the 20 % & 30 % rice group , while 30 % rice group showed a significant ($p<0.05$) decrease as compeer with the other group in the 5th week of the study as shown in the table (4-3)

Table (4-3) effect of basal diet different levels of low quality rice on broiler weight gain.

Time	Control group	10 rice group	20% Rice group	30% Rice Group
3rd week	361.1917 ± 21.14A	355.6917 ± 15.64A	337.6917 ± 22.81 B	339.3917 ± 25.31B
4th week	507.5 ±15.84 A	513.6 ± 22.14 A	488.7 ± 16.82 B	479.7 ± 28.21 B
5th week	703.6 ± 30.43 A	689.5 ± 30.78 A	644.9 ± 40.16 B	606.3 ± 36.12 C

Different letters next to means horizontally indicate a significant differences ($p< 0.05$)

Feed conversion ratio showed a significant ($p< 0.05$) increase in the 20 % & 30% rice groups as compeer with the control group and the 10 % rice group in all the period of the experiment ,while the control group demonstrate significant difference ($p< 0.05$) decrease in the control group as compared to other group in the 5th week, as shown in table (4-4).

Table (4-4) effect of basal diet different levels of low quality rice on Feed conversion ratio.

Time	Control group	10 rice group	20% Rice group	30% Rice Group
3rd week	1.744226 ± 0.13 B	1.771197 ± 0.11 B	1.865607 ± 0.14 A	1.856262 ± 0.14 A
4th week	1.724138 ± 0.12 C	1.70366 ± 0.12 C	1.790464 ± 0.15 B	1.824057 ± 0.16 A
5th week	1.591814 ±0.11 D	1.624365 ± 0.12C	1.736703 ± 0.13 B	1.84727 ± 0.14 A

Different letters next to means horizontally indicate a significant differences ($p < 0.05$)

In general, the current study found that there was a significant increase in the average live weight of broiler chickens in the control group and the group that was fed with 10% rice, in contrast to the groups that were fed 20% and 30% rice, as shown in table (4-2) in the third, fourth, and fifth weeks of the experiment periods. This result may occur due to the presence of substances in rice such as anti-nutrients factor and these substances play an important role in the process of absorbing and breaking down substances and enzymes within the intestine, when the amount of rice in the feed increases above 10%, the amount of these factors will be increasing this negatively affects on growth performance and live body weight (Erdaw & Beyene ., 2018).

Lectin that found in rice serve various functions in plants; some can interfere with nutrient absorption and digestion. Lectins bind to carbohydrates in the gut lining or to specific receptors on the surface of cells, potentially disrupting normal nutrient absorption processes (Kiarie *et al.*, 2022).

Tannins are polyphenolic compounds found in some varieties of rice. While they have antioxidant properties, high levels of tannins may also interfere with the absorption of certain minerals and

nutrients. Tannins can form complexes with proteins and minerals, limiting their bioavailability and absorption (Hassan *et al.,* 2003 ; Choi *et al*., 2022.

Rice is known to contain enzyme inhibitors that may affect the activity of digestive enzymes in the gastrointestinal tract. while part of the plant's natural defense mechanism These inhibitors, can influence the efficiency of nutrient digestion and absorption. Enzymes play a pivotal role in breaking down complex nutrients into forms that the body can absorb. When enzyme inhibitors are present in rice, they can hinder the normal digestive process, potentially affecting the absorption of essential nutrients like carbohydrates and proteins (Samtiya *et al*., 2020& Dhewa *et al.,* 2020).

It is noted from the current study that there is an increase in the amount of food intake by the broiler that were fed with 20% & 30% of rice compared to the control group and the 10 % rice group. This result may be due to the presence of a large amount of high carbohydrates in both the 20 % and 30 % rice groups that leads to an increase in the amount of insulin in the body leads to an increase in the amount of the ghrelin hormone which lead to hunger and leads to the consumption of larger quantities of food conception For this reason we notice an increase in the food eaten when compared with the control groups and the 10 % rice group during the fourth and fifth weeks of the experiment (Te Pas *et al*., 2020 ; Chuang *et al*., 2020).

High-carbohydrate meals, especially those with a high glycemic index, can lead to a rapid increase in blood sugar levels, triggering a subsequent insulin response. The insulin surge may contribute to a quick drop in blood sugar, potentially leading to feelings of hunger (Woods *et al*., 2006). In the short term, insulin can contribute to a decrease in hunger by promoting the storage of glucose in tissues.

However, an exaggerated insulin response, often associated with rapidly digestible carbohydrates, may lead to a subsequent drop in blood sugar levels, potentially triggering hunger.

The study also show that there is a significant increase in the weight gained in the control group and the 10% rice group when compared with the 20% and 30% rice group during the fourth and fifth weeks of the experiment. Perhaps the reason is due to the presence of anti-nutrients, which are present in rice and play an important role in the process of absorbing all of the minerals, proteins and vitamins, which affects the weight of the bird and thus a decrease in the weight of the bird when compared to the control group (Abbas, 2020).

The study also showed a decrease in the feed conversion ratio for the control group and the 10% rice group compared to the 20% & 30% rice group. These results appeared due to the increase in the amount of feed intake eaten by the 20% and 30% with a decrease in weight gained by these birds. The control and 10% group, because of its increase in the amount of feed eaten and the decrease in the amount of weight gained by the bird, leads to a higher feed conversion ratio, as the higher this percentage is, the more it is a negative factor, which leads to economic losses.

4.1.2. Effect of Replacing Corn With Different Levels of Low Quality Rice on immune response.

In the current study the ND, IB, IBD and AI antibody titer showed a significant ($p < 0.05$) increase in the control group as compared with the other group, also 10 % rice group show a significant ($p<0.05$) increase as compared with 20% and 30 % rice groups, on the other hand 20% rice group showed a significant ($p < 0.05$) increase as

compared with 30 % rice group, as shown in table (4-5)

Table (4-5) Effect of replacing corn with different levels of low quality rice on antibody titer

5 day old broiler				
Group	ND	IB	IBD	AI
Control	4297.4±120	7140.2 ± 161	13304 ±220	3877.8
25 day old broiler				
Group	ND	IB	IBD	AI
Control	3177.4±142 A	7150.2 ± 168 A	11264 ± 240 A	3755 ± 146 A
10% rice	2239.4±132 B	5220 ± 133 B	8963.6 ± 170 B	3524 ± 192 B
20%rice	1920.4 ± 88 C	4720 ± 147 C	6835± 155 C	3219 ± 185 C
30% rice	1338.6 ± 96 D	2207.4 ± 129 D	6287.6 ± 128 D	2955 ± 165 D

Different letters next to means horizontally indicate a significant differences ($p < 0.05$)

Through the current study, there is a decrease in immunity in birds that were fed with 20% and 30% rice when compared with both the control group and the 10% group, and thus reflects a decrease in the birds' immunity.

Corn contains a higher amount of vitamin C compared to rice and this can contribute to an increase in birds' immunity. Vitamin C enhances the function of various immune cells, including neutrophils, lymphocytes, and phagocytes. It aids in the production and function of white blood cells, which are crucial for fighting infections. Studies showed that vitamin C supplementation can enhance the immune response in animals, leading to improved resistance to infections and quicker recovery from illnesses (Lohakare *et al.,* 2005).Rice contains higher levels of anti-nutrients like phytic acid and lectins, which can interfere with mineral absorption and potentially impact immune function indirectly. Phytic acid reduces the bioavailability of essential minerals, such as zinc and iron, which are crucial for immune system function. Zinc, for example, plays a critical role in the development and activation of immune cells and the production of antibodies.

4.2Effect of Replacing Corn With Different Levels of Low Quality Rice on carcass characterization in Broiler chickens.

In the current study there was a significant ($p< 0.05$) increase in the relative weight of liver, gizzard, proventiculas & crop in the control group as compared with the other group as shown in the table (4-6)

Table (4-6) effect of basal diet different levels of low quality rice on relative weight of internal organ.

group	Liver	Spleen	Gizzard	Prove-nticulas	Crop	Duod-enum	Jejunum	Ileum	Rectum	Cecum
control	2.02 ± 0.1 A	0.155 ± 0.01 B	1.66± 0.1 A	0.62± 0.01 A	0.36± 0.01 A	0.36 ± 0.01 D	0.72 ± 0.01B	0.77 ± 0.01A	0.207± 0.01 A	0.31 ± 0.01B
10%	1.67 ± 0.1 C	0.104 ±0.01 C	1.3 ± 0.1 C	0.41± 0.01 B	0.104± 0.01B	0.73 ± 0.01A	0.73± 0.01 B	0.57 ± 0.01B	0.104± 0.01 C	0.46 ± 0.01A
20%	1.53± 0.1 D	0.109 ±0.01C	1.64 ± 0.1 A	0.43± 0.01B	0.109± 0.01B	0.49 ± 0.01 B	0.87 ± 0.01A	0.71 ± 0.01A	0.164 ± 0.01 B	0.21 ± 0.01C
30%	1.73± 0.01 B	0.168± 0.01 A	1.45 ± 0.1 B	0.33 ± 0.01C	0.112 ± 0.01 B	0.39 ± 0.01 C	0.84 ± 0.01A	0.78± 0.01A	0.168 ± 0.01B	0.39 ± 0.01 B

Different letters next to means horizontally indicate a significant differences ($p< 0.05$)

The study showed that there was a significant ($p< 0.05$) increase in the ratio of liver, gizzard, proventiculas & crop in the control group as compared with the other groups as shown in the table (4-6) This result may occur due to the large weight of the broiler that obtained at the end of the experiment. As the percentage of weight of the internal organs is proportional to the weight of the total body, as the body size increases, the weight of the internal organs increases with it (Martínez *et al.*, 2021) . On the other hand liver in the 30 % rice group demonstrate significant difference ($p< 0.05$) increase as compared with the 10% and 20 % rice. This result may occur due to carbohydrate consumption, excess glucose is converted into glycogen and stored in the liver, muscles and other tissues for future energy needs. Increased carbohydrate intake can lead to greater glycogen storage in the liver, potentially contributing to an increase in liver weight. (Karacay *et al.*,

2008). On the contrary, the weight of the liver decrease in the group were fed rice due to the rice contains ctina as anti-nutrient factor which inter fear with metabolism of carbohydrate (abbas 2020).

In the current study, spleen showed a significant ($p< 0.05$) increase in the 30% rice group as compared with the other group, this result may occur due to high level of anti-nutrient such as phytic acid and tannins have been associated with inflammatory responses in the gastrointestinal tract (Chuang *et al.*, 2019) . Chronic inflammation may lead to alterations in organ size and function, including the spleen. Increased spleen weight could be a reflection of systemic inflammation. Changes in spleen weight may serve as an indicator of immune function or inflammatory responses in experimental studies (Kiarie *et al*., 2022).

There is a decrease in the weight of the intestine weight in the control group when compared to the rest of the other groups. This is due to the large weight of the control group, and this led to a reduction in the ratio of the weight of the intestine organs to the body weight it (Martínez *et al*., 2021).

As broiler chickens consume a high-energy diet to promote rapid growth, the gizzard adapts to the increased workload by growing in size and developing stronger muscular walls. This adaptation allows for better grinding and digestion of the feed aiding in nutrient absorption (David *et al*., 2023).

4.3 Effect of Replacing Corn with Different Levels of Low Quality Rice on sensory evaluation

The tenderness showed a significant decrease in the control group and 10 % rice group as compared with 20% and 30 % rice group, the juiciness show no significant difference among groups all the experiment. On the other hand the color of the control group showed a

significant decrease as compared with the 10 %, 20 % and 30 % rice groups. The flavor and the palatability showed significant increase in the control group as compared with the other group.as shown in table (4-7).

Table (4-7): Sensory score test of poultry meet fed different levels of local rice

Group	Tenderness	Juiciness	Color	Flavor	Palatability
Group 1 (CON)	3.2± 0.8 B	2.4 ±0.2 A	3.2±0.6 C	3.3 ± 0.4 B	3.0± 0.5 B
Group 2 (10 % rice)	3.2 ± 0.7 B	2.4 ± 0.5 A	4.0 ±0.5 B	3.8 ±0.7 A	3.2 ± 0.8 B
Group 3 (20 % rice)	3.5 ±0.6 A	2.6 ± 0.6 A	4..6 ±0.3 A	3.2± 0.5 B	3.8 ± 0.9 A
Group 4 (30 % rice)	3.6 ± 0.4 A	2.2 ±0.4 A	4.8±0.7 A	3.2 ± 0.4 B	3.4 ± 0.7 B

Different letters next to means horizontally indicate a significant differences ($p < 0.05$)

High-carbohydrate diets may influence glycogen reserves, which in turn can impact the rate of lactic acid production post-slaughter. Proper post- slaughter pH decline is essential for meat tenderness (Smulders *et al*., 2014). If pH decline is too slow or incomplete, it can result in tougher meat. Also Carbohydrates can influence the water-holding capacity of meat. Adequate glycogen reserves in muscles can help retain water, leading to juicier and tenderer meat. High-carbohydrate diets can lead to increased glycogen storage in muscle tissue. (Sultana *et al.*, 2022).

Maillard reaction is a chemical reaction between amino acids and reducing sugars that gives browned foods that change flavor and color. Carbohydrates, particularly reducing sugars like glucose and fructose, are essential for this reaction to occur. Therefore, a high-carbohydrate diet may result in increased levels of reducing sugars in the muscle tissue, which could contribute to more pronounced browning during cooking (Chansataporn *et al*., 2019). During cooking, glycogen can

break down into glucose, which then participates in the chicken meat, leading to browning of the meat surface. Caramelization of sugars can also contribute to browning. High-carbohydratediets may increase the availability of sugars in meat, which can undergo caramelization when exposed to high heat during cooking (Gibis, 2016, zozo 2020 and Nath 2022).

4.4 Effect of Replacing Corn With Different Levels of Low Quality Rice on some minerals content of meat (Ca,p,Fe,Zn & K)

The current study showed no significant deference in the meat Ca level in the control group and 10% rice group. There was a significant ($p< 0.05$) decrease in the 20 % rice and 30 % rice group as compared with the control and 10% rice group .

The level of phosphorus in the meat of the current study showed a significant ($p< 0.05$) increase in the control group as compared with other group, also 10 % rice group showed a significant ($p< 0.05$) increase as compared with 20% and 30 % rice group and 2% showed a significant ($p< 0.05$) increase as compared with 30 % rice group.

The iron showed no significant difference between control group and 10% rice group, while it showed a significant ($p< 0.05$) increase as compared with 20% and 30% rice group, 20% rice showed a significant ($p< 0.05$) increase as compared with 30% rice.

The zinc and potassium showed no significant difference between control group and 10% rice group , while it showed a significant ($p< 0.05$) increase as compared with 20% and 30% rice group , 20% rice show a significant ($p< 0.05$) increase as compared with 30% rice

Table (4-8): Effect of replacing corn with different levels of low quality rice on mineral content of meat mg/kg .

Group	Ca	P	Fe	Zn	K
Control	65.27± 2.4A	2241 ± 66 A	4.8 ± 0.4 A	6.88 ±0.3 A	3290 ± 141A
10% rice	66.35± 3.5 A	2158 ± 56 B	4.78 ± 0.2 A	6.24 ± 0.4 A	3150 ± 124 A
20%rice	56.33± 5.6 B	2033 ± 35 C	4.28 ± 0.2 B	4.24 ± 0.3 B	2975 ± 85 B
30% rice	54.27± 7.6 B	1970 ± 42 D	2.93 ± 0.3 C	3.88 ± 0.3 C	2750 ± 94 C

Different letters next to means horizontally indicate a significant differences ($p < 0.05$)

Through the current study, there is a significant increase in minerals in the control and 10% rice groups when compared to the other groups. This reason may be due to the fact that rice contains smaller amounts of minerals than corn. Thus this leads to a decrease in the amount of minerals that the bird consumes which leads to a decrease in the amount of minerals present within the muscles.

Corn contains substances that help in better digestion and absorption of minerals through the intestines. Corn contains small amounts of vitamin C which can enhance the absorption of iron, particularly non-heme iron which is found in plant-based foods. Soluble fibers in corn can help create a favorable gut environment for the absorption of minerals by promoting healthy gut bacteria. Additionally Inulin and Fructooligosaccharides (FOS) prebiotics found in corn can enhance calcium absorption by stimulating beneficial gut microbiota.

On the other hand, rice contains certain substances that can inhibit the absorption of minerals. These substances include phytic acid, oxalates, and dietary fiber, which can bind to minerals and reduce their bioavailability. phytic acid, also known as phytate, is a compound found in rice that can bind to minerals such as calcium, iron, and zinc, forming insoluble complexes that are not easily absorbed by the body.

Oxalates are naturally occurring substances in rice that can bind to calcium and form insoluble salts, reducing calcium absorption. While beneficial for digestion, the fiber in rice can also bind to minerals and reduce their absorption in the digestive tract.

4.5 Effect of Replacing Corn With Different Levels of Low Quality Rice on IGF-1 gene expression

At the gene expression, the control group showed a significant ($p < 0.05$) decrease in the IGF-1 gene folding change as compared with the other groups. Also 10 % rice group showed a significant ($p < 0.01$) decrease in the folding change as compared with 20 % & 30 % rice group, while there were no significant difference between 20 % and 30 % rice groups as compared with each other as shown in figure (4-1) and figure (4-2).

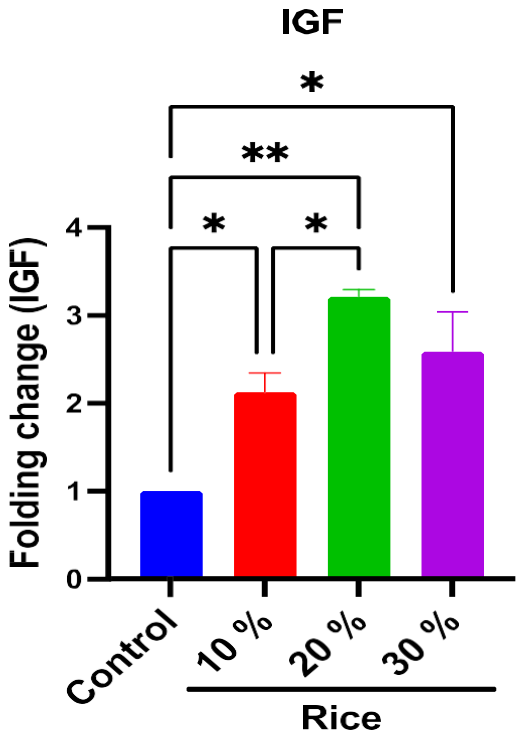
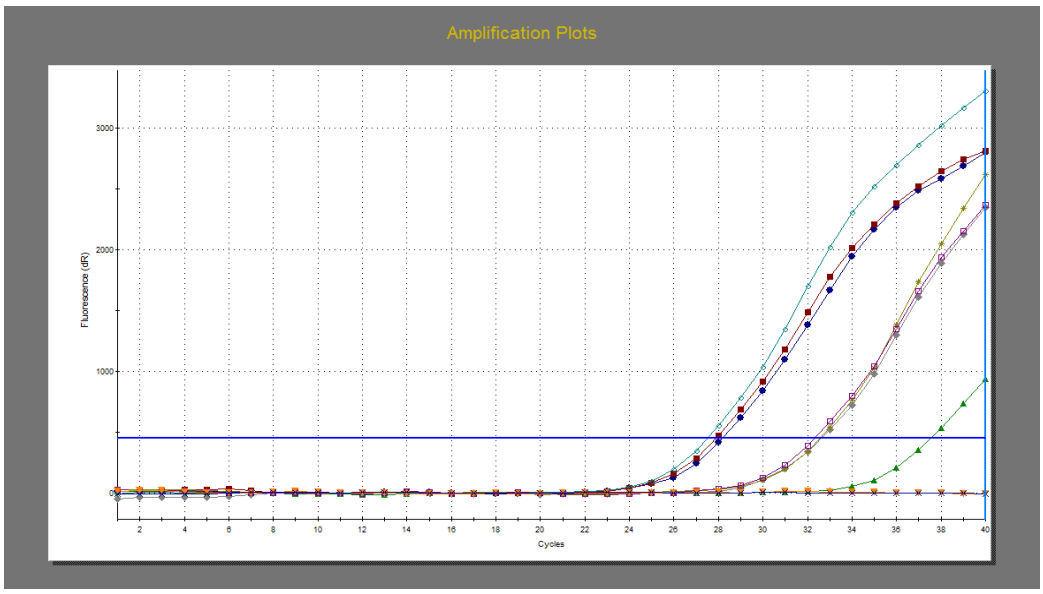


Figure (4-1): Fold change comparison between the groups expressed igf-1 gene. This shows significant upregulation of the rice group compared with control groups. (*): Indicates a p-value < 0.05, (**): Indicates a p-value < 0.01.



Figure(4-2): Amplification plot of IGF gene by the Mx3005P Stratagene system

There was an increase in the IGF-1 in the experiment groups as compared with the control group. High-carbohydrate diets can indeed stimulate IGF-1 production in broiler chickens (Melnik, 2012). This effect is partly due to the fact that carbohydrates, especially simple carbohydrates like glucose, can stimulate insulin secretion which can stimulate the production of IGF-1 in various tissues, including the liver. Increased IGF-1 levels can then promote growth in broiler chickens (Chen *et al*., 2022).

Carbohydrates can influence IGF-1 production, other factors such as protein intake, specific carbohydrate sources, and overall diet composition also play significant roles in regulating growth. On the other hand if there's an excess of carbohydrates in the diet, leading to chronically elevated insulin levels. It can potentially lead to insulin resistance, where cells become less responsive to insulin's effects. This can affect glucose metabolism and may indirectly influence IGF-1 signaling, in a high-carbohydrate diet, both insulin and IGF-1 levels increase due to the carbohydrate-induced release of insulin and subsequent stimulation of IGF-1 production, while insulin helps regulate glucose metabolism

(Begum *et al.*, 2014).

4.6 Effect of Replacing Corn With Different Levels of Low Quality Rice on Intestine Histology

In the current study, duodenum Villi length showed a significant (P< 0.05) increase in the control group as compared with the other groups. 10 % rice & 20 % rice show no significant deference between them, while 30 % showed a significant (p< 0.05) increase as compared with the 20 % group. The Villi width shows a significant decrease in 10 % rice group as compared with the other group. Villi area showed no significant deference between the control group and 20 % rice group, while there were a significant (p< 0.05) increase as compared with the 10 % rice group and 30 % rice group, 10 % showed a significant (p< 0.05) decrease as compared with the other groups, crypt depth showed a significant increase in control group as compared with the other groups. Also the 10 % & 30 % show no significant as compared with each other , while 20% group showed a significant (p< 0.05) decrease as compared with other groups . Muscular thickness showed a significant (p< 0.05) increase in the control group as compared with the other groups, as shown in the table (4-9).

Table (4-9) effect of basal diet different levels of low quality rice on Duodenum Villi

Groups	Duodenum Villi length(mm)	Villi width(mm) Mean ±S.D	Villi area$(mm)^2$ Mean ±Se	Crypt depth	Muscular thickness
Control	1,085.78 ± 102.13 A	95.9175 ± 7.9 A	104145.3 ± 140.13 A	152.1285 ± 12.6 A	131.0805 ± 14.2 A
10% rice	963.703 ± 80.12 B	69.2355 ± 9.6 B	66722.46 ± 122.6 C	143.7285 ±13.7 B	95.2165 ± 7.4 B
20% rice	948.16 ± 60.4 BC	113.73 ± 14.2 A	107834.2 ± 144.13 A	116.20 ± 16.5 C	94.53 ± 9.2 B
30% rice	986.237 ± 8.74 B	91.69 ± 11.2 A	90428.07 ± 130.9 B	149 ± 19.8 AB	98.89 ± 8.4 B

Different letters next to means horizontally indicate a significant differences (p< 0.05)

In the current study jejunum Villi length, width and villi area show a

Jejunum villi length, width and Villi area showed a significant ($p<0.05$) increase in the control group as compared with the other group. Also crypt depth showed a significant increase in the control group as compared with the other group, on the other hand muscular thickness showed a significant increase in the 30 % rice group as compared with the control group as shown in the table (4-10).

Table (4-10) effect of basal diet different levels of low quality rice on jejunum Villi

Groups	Villi length(mm)	Villi width(mm) Mean ±S.D	Villi area(mm)^2 Mean ±S.D	CRYPT DEPTH	MUSCUAR THICKNESS
Control	815.81 ±18.5 A	107.82 ± 17.6 A	87968.05 ± 190.24 A	142.50 ± 12.6 A	79.85 ± 12.3 C
10% rice	610.33 ± 16.8 B	59.43 ± 15.7 C	36277.63 ±163.45 C	119.86 ± 19.3 B	99.44 ± 14.2 B
20% rice	503.31 ±15.3 C	73.75 ± 17.2 B	37122.39 ±127.9 B	112.96 ±19.6 B	91.76 ± 14.6 B
30% rice	483.72 ± 18.4 D	43.68 ± 14.8 D	21130.26 ± 116.77 D	86.66 ± 12.4 C	114.12 ± 11.7 A

Different letters next to means horizontally indicate a significant differences ($p<0.05$)

Illume Villi length showed a significant ($p< 0.05$) increase in the control group as compared with the other groups, while villi width demonstrate significant difference ($p< 0.05$) increase in the 30 % rice group as compared with the other groups, Villi area showed a significant ($p< 0.05$) increase in the control group as compared with the other group. Crypt depth showed a significant ($p< 0.05$) increase in the 10 % group as compared with the other group as shown in table (4-11).

Table (4-11) effect of basal diet different levels of low quality rice on Ilium Villi

Groups	Villi length(mm)	Villi width(mm)	Villi area(mm)^2	crypt depth	muscular thickens
Control	428.42 ±26.4 A	90.81 ± 12.1C	38905.72 ± 120.6 A	99.22 ± 8.2 C	74.385± 12.2 B
10%	344.11 ± 31.7B	106.91 ± 16.4 B	36792.3 ± 145.7 B	124.82 ± 12.6A	108.352± 18.6 A
20%	347.64 ± 24.6 B	102.43 ± 9.4 B	35610.56 ± 160.9 B	75.55 ± 6.9 D	72.8685 ± 11.8 B
30%	207.85 ± 19.4 C	122.8 ± 16.9 A	25525.59 ± 198.4 C	105.32 ± 10.4B	106.4415 ± 14.8 A

Different letters next to means horizontally indicate a significant differences ($p< 0.05$)

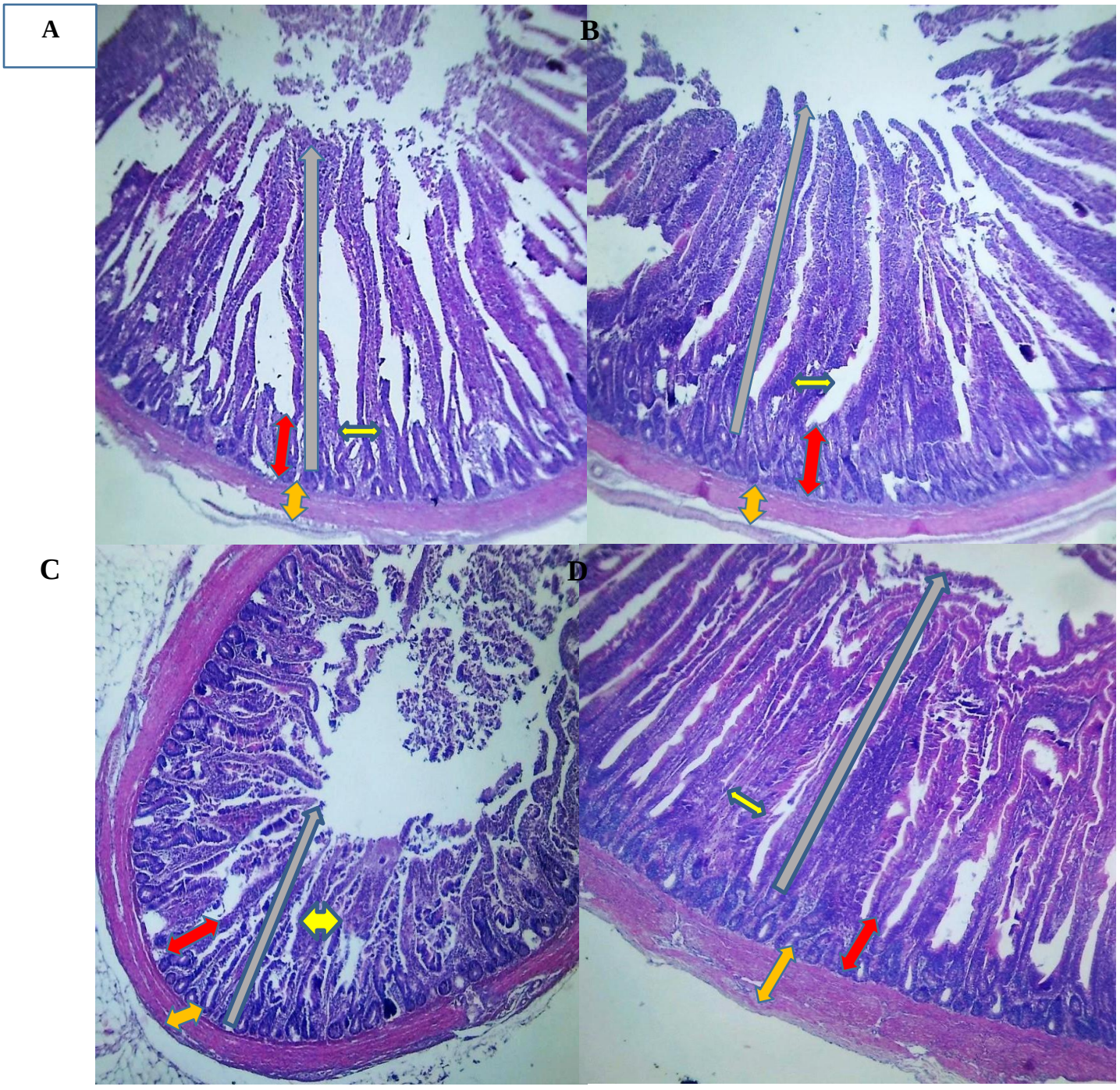


Figure (4-2): effect of basal diet(A) , 10% rice (B), 20% rice (C) and 30 % rice (D) on duodenum villi length (brown arrow) , villi width (yellow arrow), crept depth (red arrow) and muscular thickness(orange arrow)(H&E 40X) .

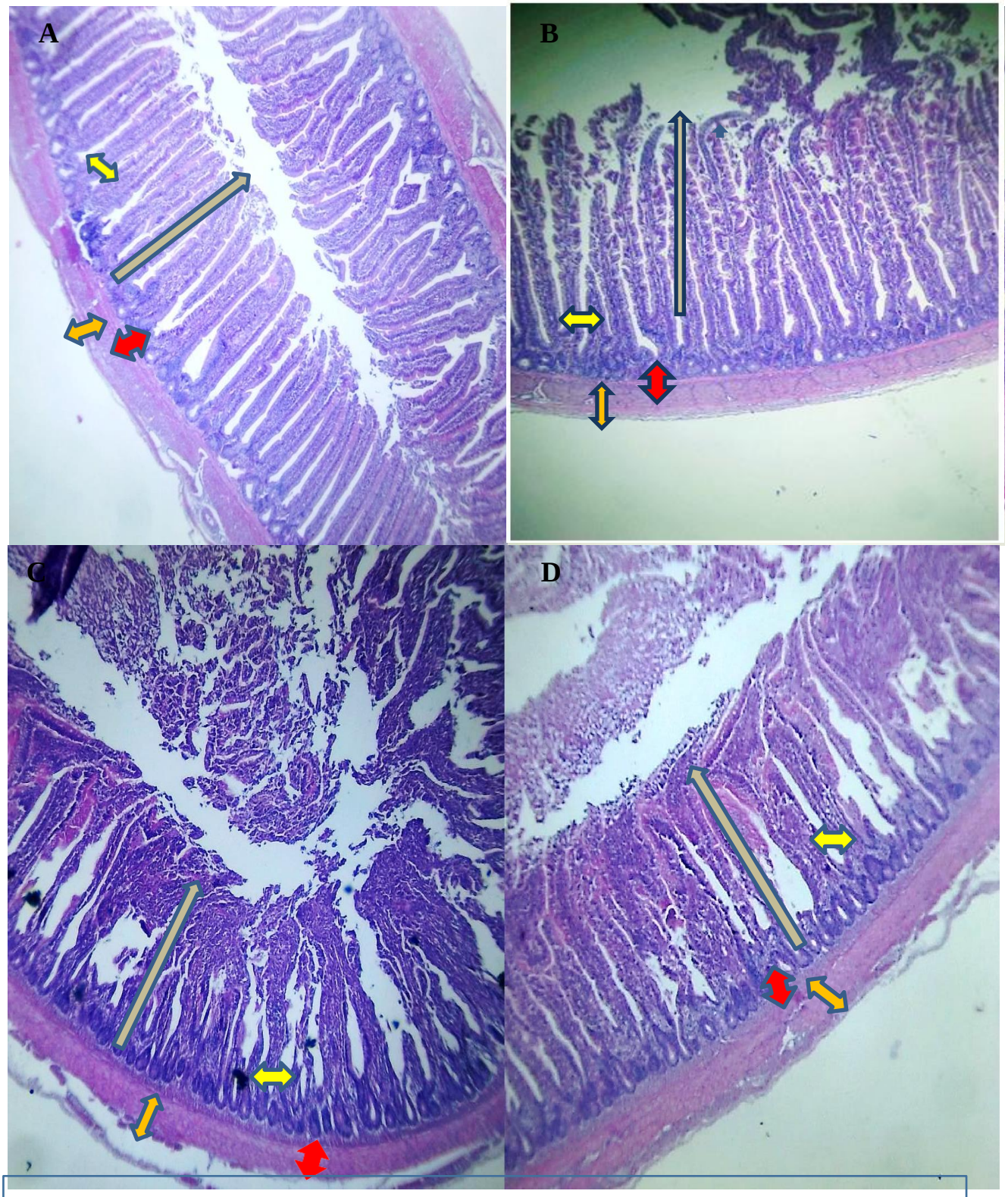


Figure (4-3): effect of basal diet(A) , 10% rice (B), 20% rice (C) and 30 % rice (D) on jejunum villi length (brown arrow) , villi width (yellow arrow), crept depth (red arrow) and muscular thickness(orange arrow) (H&E 40X) .

Through the current study, a decrease in the length, width area & crypt depth of the villi in the groups that contain a high percentage of rice when compared to the control group that does not contain rice. This is observed from tables (2, 3 &4) and the reason may be that anti-nutrients that found in the rice can bind to nutrients or enzymes in the gastrointestinal tract in the broilers forming complexes that are difficult to digest or absorb (Abbas, 2020). Prolonged exposure to these anti-nutrients may lead to impaired nutrient absorption, including essential vitamins, minerals, and amino acids, which are necessary for maintaining intestinal health and supporting growth in broiler chickens. (Zentek and Boroojeni, 2020).

Some anti-nutrients have pro-inflammatory properties and can induce damage to the intestinal mucosa. Chronic inflammation and tissue damage in the intestinal lining can disrupt the normal structure and function of intestinal villi, leading to a reduction in villi length and surface area available for nutrient absorption (Bedford & Apajalahti., 2022). Also high carbohydrate diets, especially those containing fermentable fibers, can promote microbial fermentation in the ceca and colon of broiler chickens, fermentation primarily occurs in the hindgut, it can influence the pH and microbial populations throughout the gastrointestinal tract. Changes in gut microbiota composition and metabolite production may indirectly affect villi length and morphology in the small intestine , imbalance in gut microbiota, has been associated with intestinal inflammation and impaired intestinal barrier function, which can contribute to villi atrophy and compromised nutrient absorption (Kouzounis *et al*., 2021and wu *et al*., 2018).

Some anti-nutrients possess antioxidant properties, while others may promote oxidative stress in the gastrointestinal tract. Excessive production of reactive oxygen species (ROS) can damage intestinal cells and

impair their ability to regenerate, leading to villi shortening and compromised nutrient absorption (Amir, 2021).

On the other hand high carbohydrate diets may contribute to nutrient imbalances, such as excessive energy intake relative to protein, vitamins, and minerals. Imbalances in dietary nutrients can impact gut health and villi morphology, as optimal nutrient availability is essential for maintaining the structural integrity of the intestinal epithelium and supporting villi growth and renewal (Adedokun & Olojede ., 2019).

Chapter Five
Conclusions and Recommendations

5.1. Conclusions

Depending on the results of the current study finding, the conclusions are as the following:

1. Incorporating more than 10% low-quality rice into broiler chicken diets results in reduced body weight and weight gain, while simultaneously increasing feed intake.

2. Using high quantities more than 10% of low-quality rice in the chicken feed leads to a decrease in birds' humeral immune response.

3. Using a diet containing more than 10% low-quality rice negatively impacts the growth rate of chickens, which in turn affects the development of their internal organs.

4. There was an increase in tenderness and color, with a decrease in flavor and Palatability of birds meat that were fed on 10%, 20% and 30% of low-quality rice.

5. It was found that there is a decrease in the amount of minerals found in chicken meat that fed low-quality rice at 10%, 20% and 30%.

6. Decrease in the length of the villi of the intestine in birds that fed containing low quality rice.

5.2 Recommendations

From the results of the present study, it can be recommended the following:

1- Avoid the use of low quality rice in poultry at level above 10%.

2- Utilize low quality rice in broiler ratio at constriction below 10%.

3- Studying the replacement of trait in layer.

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Appendix

بسم الله الرحمن الرحيم

وزارة العلوم والتكنولوجيا
دائرة البيئة والمياه والطاقات المتجددة
مركز بحوث تلوث الغذاء

العدد:
التأريخ: ٢٠٢٣/١٠/٢٤

م/ نتائج فحص

ادناه نتائج فحص وتحليل نموذج رز والخاص بطالبة الماجستير (اميرة محمد سعيد) جامعة كربلاء – كلية الطب البيطري

No	Name	Con.
1	Protein %	8.24
2	Lipid %	1.10
3	CHO %	77.44
4	Energy (kcal)	3525
5	Ca (µg\ gm)	98.5
6	Fe (µg\ gm)	12.9
7	Mg (µg\ gm)	198.5
8	K (µg\ gm)	689.0
9	Mn (µg\ gm)	9.14
10	Cu (µg\ gm)	1.25

المهندس
فرقد عبدالله رشيد
مدير مركز بحوث تلوث الغذاء
٢٠٢٣/١٠/٢٤

ر. مهندسين اقدم
فرقد عبدالله رشيد
مدير مركز بحوث تلوث الغذاء
٢٠٢٣/١٠/٢٤

Content of Ca in meat

Sample Preparation:

The chicken meat sample is prepared by homogenizing it and then subjecting it to an acid digestion process. Acid digestion, often with nitric acid or a mixture of acids, breaks down the organic matrix of the meat and releases calcium ions into solution.

Complexation Reaction:

Complexing agent, usually ethylenediaminetetraacetic acid (EDTA), is added to the solution. EDTA is a chelating agent that forms stable complexes with metal ions, including calcium ions (Ca^{2+}). The complexation reaction can be represented as:

$$Ca^{2}++EDTA^{4-}\rightarrow[Ca\text{-}EDTA]^{2-}$$

Indicator:

Calconcarboxylic acid, which form a colored complex with free calcium ions.

Titration Process:

The titration involves the gradual addition of a standardized EDTA solution to the sample solution containing the calcium ions and indicator. The EDTA reacts with all free calcium ions until they are completely complexed.

The endpoint of the titration is reached when all the calcium ions have reacted with EDTA, and the indicator changes color, signifying that no free calcium ions are left to form a comp
Eriochrome Black T, the color change typically goes from red (indicator-calcium complex) to blue (free indicator).

Calculation:

The amount of EDTA used to reach the endpoint is directly proportional to the amount of calcium in the sample. By knowing the concentration of the EDTA solution and the volume used, the calcium content in the original chicken meat sample can be calculated using the stoichiometry of the reaction.

Calcium (mg/L)=Volume of EDTA (L)×Concentration of EDTA (mol/L)×M olar mass of Ca (g/mol)/ Volume of sample (L)

Appendix III

Content of p in meat

1. Sample Preparation

a. Homogenization and Digestion:

The chicken meat sample is first homogenized to ensure uniformity. A 1 gram of the homogenized sample is then weighed.The sample undergoes acid digestion, typically using a combination of nitric acid (HNO_3) and perchloric acid (HClO4) or sulfuric acid (H,SO4). This process breaks down organic material and converts all forms of phosphorus into inorganic phosphate ($PO4^{3}$).

b. Filtration and Dilution:

The digested sample is filtered to remove any undissolved solids. The filtrate, containing the phosphate ions, is then diluted with distilled water to a known volume.

Formation of Phosphomolybdate Complex

c. Reagent Addition:

To the solution containing phosphate ions, an acidic solution of ammonium molybdate (NH4),MoO4 is added. The acidic environment is often maintained using sulfuric acid (H,SO4).Under these acidic conditions, phosphate reacts with ammonium molybdate to form a yellow complex known as phosphomolybdic acid or ammonium phosphomolybdate.

d. Reduction to Molybdenum Blue:

The yellow phosphomolybdic acid complex is then reduced to form a blue-colored complex called molybdenum blue. The reduction can be achieved using various reducing agents such as ascorbic acid, stannous chloride (SnCl,), or a mixture of sodium bisulfite and sodium sulfite.

$$PO43^{-}+12(NH4)2MoO4+21H^{+}\rightarrow(NH4)3[PMo12O40]+12NH3+12H2O$$

Spectrophotometric Analysis:

The solution's absorbance is measured using a spectrophotometer at a wavelength typically around 700-880 nm, depending on the exact composition of the molybdenum blue complex. The absorbance reading correlates with the amount of phosphorus present in the sample.

Content of Fe in meat

PRINCIPLE

The method is based on the properties of Chromazurol S (CAS), a chromogenic iron-binding dye, that under acidic conditions in presence of cetrimide (CTAB) forms an intense purple complex proportional to the concentration of iron present in the sample

$$Fe^{+3} + 3\ CAS + CTAB \xrightarrow{pH\ 4.7} Fe\ (CAS)_3\ complex$$

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labelled test tubes:

TUBES	Blank	Sample	CAL. Standard
R1.Reagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	50 µL	–
CAL. Standard	–	–	50 µL

3- Mix and let the tubes stand 10 minutes at 37ºC.

4- Read the absorbance (A) of the samples and the standard at 635 nm against the reagent blank.

Content of Zn in meat

PRINCIPLE OF TEST

Zinc reacts with the chromogen present in the reagent forming a coloured compound which colour intensity is proportional to the zinc concentration present in the sample.

SAMPLE PREPARATION

Tissue samples

Add 5% TCA solution, vortex 1 min. and incubate at 4 - 8°C for 30 min. Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use for the assay.

REAGENTS PREPARATION

Prepare the Work Reagent mixing in equal quantity the Reagent A with Reagent B. Reagents are stored at 2-8°C and are stable until expiration date on label. Work Reagent is stable 20 days at room temperature.

REAGENTS	BLANK	STANDARD	SAMPLE
Work Reagent Distilled Water Standard Sample	1 ml 50 µl -- --	1 ml -- 50 µl --	1 ml -- -- 50 µl
Mix and read the absorbance against blank at 578 nm. Colour is stable for 30 minutes.			

CALCULATION

Zn mg/dl = [A(sample) / A(standard)] x 200

Content of k in meat

The chicken meat sample is first homogenized to ensure uniformity. A 1 gram of the homogenized sample is then weighed. The sample undergoes acid digestion, typically using a combination of nitric acid (HNO_3) and perchloric acid This process breaks down organic material and converts all forms of phosphorus into inorganic phosphate ($PO4^3$).

PROCEDURE

Bring reagents and samples to room temperature. Pipette into labelled cuvettes:

Cuvettes	Blank	Sample	Calibrator
R1. Reagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	25 µL	-
Calibrator	–	–	25 µL

Mix, incubate for 5 minutes at 37ºC. And add

R2. Reagent	250 µL	250 µL	250 µL

Mix, incubate for 1 minutes at 37ºC and read (A1) at 405 nm. Mix, incubate for 3 minutes at 37ºC and read (A2) at 405 nm.

CALCULATIONS

$$\frac{(A2 - A1)_{\text{Sample}}}{(A2 - A1)_{\text{Calibrator}}} \times C_{\text{Calibrator}} = \text{mmol/L Potassium}$$

Immune response on ND

Newcastle Disease Virus Antibody titer

Preparation

1) BringELISA reagents to the room temperature (20-25℃) for 30 min to get best results.

2) Sample dilution: use the sample diluent to dilute the sample at 40 times(for example: 5ul serum sample+195ulSample diluent solution), mix the diluted sample evenly can get better result.

3) Washing solution preparation: Dilute the10×concentrated washing buffer with deionized water at10 times.(eg.10ml 10×concentrated washing buffer + 90mldeionized water), if there is crystallization in the 10×concentrated washing buffer, it is normal, dissolve it at 37℃.

Test procedure

1. Adding sample: Take out the required coated plates according to sample quantity (Can be detached)and record the sample position on a worksheet. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100μL/well. Others are sample wells, addthediluted sample,100 μl/well.

2. Incubation: cover withAdhesive Foil after adding sample,incubate at37℃for30 min.

3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution into each well fully, be static for about 10s, pour out directly. Repeat 3 times, at last time pat to dry on absorbent paper.

4. Add 100μL enzyme conjugate into each well.

5. Cover with adhesive foil andincubate at37℃for30 min.

6. Repeat step 3.

7. Add100μL substrate into each well, mix properly,Color for 10 min at37℃in the dark.

8. Add 50μL stop solution into each well, shake evenly for 10s, and

determine the result.

9. Read OD value of each well with ELISA Reader at double-wave length: 450/630nm.

Immune response on IB

Preparation

1) BringELISA reagents to the room temperature (20-25℃) for 30 min to get best results.

2) Sample dilution: use the sample diluent to dilute the sample at 40 times(for example: 5ul serum sample+195ulSample diluent solution), mix the diluted sample evenly can get better result.

3) Washing solution preparation: Dilute the10×concentrated washing buffer with deionized water at10 times.(eg.10ml 10×concentrated washing buffer + 90mldeionized water), if there is crystallization in the 10×concentrated washing buffer, it is normal, dissolve it at 37℃.

Test procedure

1. Adding sample: Take out the required coated plates according to sample quantity (Can be detached)and record the sample position on a worksheet. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100μL/well. Others are sample wells, addthediluted sample,100 μl/well.

2. Incubation: cover withAdhesive Foil after adding sample,incubate at37℃for30 min.

3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution into each well fully, be static for about 10s, pour out directly. Repeat 3 times, at last time pat to dry on absorbent paper.

4. Add 100μL enzyme conjugate into each well.

5. Cover with adhesive foil andincubate at37℃for30 min.

6. Repeat step 3.

7. Add100μL substrate into each well, mix properly,Color for 10 min at37℃in the dark.

8. Add 50μL stop solution into each well, shake evenly for 10s, and determine the result.

9. Read OD value of each well with ELISA Reader at double-wave

length: 450/630nm.

Immune response on IBD

Preparation

1) BringELISA reagents to the room temperature (20-25℃) for 30 min to get best results.

2) Sample dilution: use the sample diluent to dilute the sample at 40 times(for example: 5ul serum sample+195ulSample diluent solution), mix the diluted sample evenly can get better result.

3) Washing solution preparation: Dilute the10×concentrated washing buffer with deionized water at10 times.(eg.10ml 10×concentrated washing
buffer + 90mldeionized water), if there is crystallization in the 10×concentrated washing buffer, it is normal, dissolve it at 37℃.

Test procedure

1. Adding sample: Take out the required coated plates according to sample quantity (Can be detached)and record the sample position on a worksheet. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100μL/well. Others are sample wells, addthediluted sample,100 μl/well.

2. Incubation: cover withAdhesive Foil after adding sample,incubate at37℃for30 min.

3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution into each well fully, be static for about 10s, pour out directly. Repeat 3 times, at last time pat to dry on absorbent paper.

4. Add 100μL enzyme conjugate into each well.

5. Cover with adhesive foil andincubate at37℃for30 min.

6. Repeat step 3.
7. Add100μL substrate into each well, mix properly,Color for 10 min at37℃in e dark.
8. Add 50μL stop solution into each well, shake evenly for 10s, and determine the result.
9. Read OD value of each well with ELISA Reader at double-wave length: 450/630nm.

Appendix X

Immune response AI

Avian influenza antibody test

Preparation

Test procedure

1) Take pre-coated microplate (Can unseal for several time use as per sample quantity), add 100μL diluted serum to a well, meanwhile set 1 well for Negative control, 2 wells for Positive control separately. Add 100 μL Negative/Positive control to its wells. Shake softly (do not spill),incubate at 37℃ for 30 min.

2) Pour the liquid out of the wells, add 250μL diluted washing solution to each well, pour out. Repeat 4-6 times, then pat to dry on absorbent paper.

3) Add 100 μL Enzyme Conjugate to each well, andincubate at 37℃ for 30 min.

4) Repeat the step 2(washing). Remember pat to dry on absorbent paper at last.

5) Add 100μL substrate solution to each well, mix properly,react for 10 min at 37℃ in dark.

6) Add 50 μL stop solution in each well, and measure the result within 10 min.

Results

Read the OD value with microplate-reader at 450nm.

Appendix XII

Insulin-like growth factors I (IGF-1) Gene expression

Total RNA extraction using Easy-spin™ (DNA free) total RNA extraction Kit

1. Preparation of 50-100 mg of fresh tissue.
2. Add 1ml of Lysis Buffer (easy-BLUETM reagent) and homogenize tissue sample using a homogenizer or equivalent.
3. Vigorously vortex in room temperature for 10sec.
4. Add 200µl of Chloroform and apply vortex.
5. After centrifuging the solution at 13,000 rpm (4℃) for 10 min, transfer 400µl of the upper fluid to an empty 1.5ml tube.
6. Add 400µl of Binding Buffer and mix it well by pipetting or gently inverting the 2-3 times. Do not centrifuge and leave it for 1min at room temperature.
7. Load the upper solution to the column, but do not load the whole upper solution because the maximum volume of the column reservoirs is 800µl. After loading the optimum of the upper solution to the column, and centrifuge at 13,000rpm for 30sec. Discard the flow- through after centrifuging and place the spin column back in the same 2ml collection tube. And then repeat this step.
8. Add 700µl of Washing Buffer A to the column. Close the tubes gently, and centrifuge for 30 sec. at 13,000rpm to wash the column. Discard the flow-through and place the spin column back in the same 2ml collection tube.
9. Wash by adding 700µl of Washing Buffer B to the column and centrifuge for 30 sec. at 13,000rpm. Discard the filtrates and place the spin column back in the same 2ml collection tube.
10. Centrifuge for 1-2 min at 13,000rpm to dry the column membrane

11. Place the column in a clean 1.5ml microcentrifuge tube (not provided), and add 50µl of Elution Buffer directly onto the membrane. Incubate at RT for 1min, and centrifuge for 1min at 13,000rpm to elute.

Preparation of primers

According to instruction of the primer synthesiser company, the primers (originally lyophilized), were dissolved in the free ddH_2O to obtain a final concentration of 100 pM/µl which served as a stock solution that stored at -20 °C. A concentration of 10 pM/µl was prepared from the stock primers to be used as a work primer.

Primers used in this study

Gene	Accession	Sequence	Size (pb)
IGF1	M74176.1	F: 5'-CAGAGCAGATAGAGCCTGCG-3' R: 5'-TCTGCAGATGGCACATTCAT-3	655

Protocol of GoTaq® 1-Step RT-qPCR System for Real-Time qPCR (Gene expression assay):

1. Program a real-time instrument for standard or fast mode one-step RT-qPCR (Table 2).
2. Thaw the components of the GoTaq® 1-Step RT-qPCR System, the RNA templates and the primer pair on ice, at room temperature or at 37°C. Immediately mix each thawed component thoroughly. If using a vortex mixer, mix at low speed to minimize aeration. Keep thawed reagents on ice.

3. Prepare the RNA samples (mRNA [500fg–100ng]) in water or another qPCR compatible diluent.
4. Combine reaction components (Table 1) in a non-stick, sterile tube on ice. Mix gently after each addition. Carefully pipet reaction volumes to plate on ice.
5. Transfer plate from ice into the pre-programmed instrument. Start the run immediately.
6. When the run is complete, collect the data and analyse the results.

Table 1: Preparation of Real-Time PCR solutions

Components	**Concentration**	**Volume (20µl)**
GoTaq™ qPCR master mix, 2X	1X	10 µl
Forward primer	10 µM/µl	2µl
Reverse primer	10 µM/µl	2 µl
GoScript™ RT mix for 1-step RT-qPCR	1X	0.4 µl
ddH_2O	-	3.6 µl
RNA template	250 ng	2µl

Table 2: Real-Time PCR conditions (According to the instruction of GoTaq® 1-Step RT-qPCR System)

Stage	**Ta** (°C)	**Time**	**Cycles**
Reverse transcription	42	**15 min**	**1**
RT inactivation/Hot-start activation	95	**10 min**	**1X**
Denaturation	95	**10 sec.**	**40X**
Annealing/data collection	60	**30 sec.**	
Extension	72	**30 sec.**	
Dissociation	72	**2 min**	**1X**

Appendix XI

Histological examination

Histological sampling was collected from each replicate at 35 days. Section from the middle of duodenum, jejunum and ileum (about 0.6 cm in length) were excised longitudinally at the ant mesenteric attachment and gently flushed with NaCl (9 g.L1). These samples were fixed in a solution of formalin buffer (90 mL.L-1) for 12 to 24 24 h at 4 oC, then rinsed and stored in 70% ethanol at 4 oC until analysis. Villi and crypts were carefully individualized under a dissecting microscope. The preparation was then mounted between slides and coverslips, with addition of an aqueous agent for microscopy (Aqua mount improved gun, VWR, West Chester, PA). Ten villi and 10 crypts of Lieberkühn from each segment of each bird were measured using an optical microscope. The sample of duodenum, jejunum and ileum of 2 birds from each line, representative of the population on the basis of BW, were rehydrated with PBS and stored at 4 o C until analysis. Each sample was then embedded in medium in liquid nitrogen, cut at – 20 o C into – um-thick cross-section using a cryostat, and placed on gelatine –treated glass slides. Three cross-sections were obtained from each sample for further and observation. A routine procedure was carried out using Meyer hemalun and eosin (Sigma Chemical Company). The preparation were then mounted between slides and coverslips with the addition of an aqueous agent for microscopy. The slides were examined using an optical microscope. The thickness of the muscularis layer was measured on all section.

After the staining was completed the slide was examined by using a light microscope and a graduated lens was used to measure the villi height and crypt depth under magnified to X 10.

الخلاصه

اجريت هذه الدراسة لمعرفة تأثير اضافة مستويات مختلفة من الرز واطئ الجودة كبديل عن الذرة الصفراء على أداء دجاج اللحم من خلال قياس الصفات الإنتاجية، مؤشرات القناة الهضمية، صفات الذبيحة والتعبير الجيني لعامل النمو الشبيه بالأنسولين-1 اضافة الى مؤشرات الامعاء. استمرت التجربة لخمسة أسابيع (35 يومًا) بدأت من 2023/11/22 إلى 2023/12/26. تم تغذية مائة وستين فرخ دجاج لحم (Ross308) على العليقة الأساسية لمدة أسبوعين، وفي اليوم الخامس عشر من التجربة تم تقسيم الطيور عشوائيًا إلى 4 مجموعات، كل مجموعة تحتوي على 40 طائرًا، كل مجموعة مقسمة إلى مكررين (20 طائرًا لكل منهما)، تم تغذية مجموعة السيطرة (T0) على العليقة الأساسية، بينما غذيت الطيور في المجموعة T1 و T2و T3بنسب احلال 10٪ و20٪ و30٪ على التوالي من الرز بدل الذرة في العليقة. قيست الصفات الإنتاجية أسبوعياً، كما جمعت عينات الدم في اليوم الخامس والخامس والعشرين من العمر من عشرة طيور من كل مجموعة للحصول على المصل لتقدير المعيار الحجمي للاجسام المضادة، كما تم قياس خصائص الذبيحة بعد الذبح بعمر التسويق.

أظهرت النتائج أن وزن الجسم الحي لم يظهر فرقًا معنويًا بين مجموعة السيطرة و T1في جميع فترات التجربة والتي زادت مقارنة بـ T2 وT3، كما لم يكن هناك فرق معنوي في استهلاك العلف في الأسبوع الثالث، بينما في الأسبوع الرابع والخامس أظهرت T3 و T2زيادة معنوية ($p<0.05$) في استهلاك العلف .بينت الزيادة الوزنية زيادة معنوية ($p<0.05$) في جميع فترات التجربة في مجموعة السيطرة وT1 مقارنة مع T3وT4.

تحسنت نسبة تحويل العلف تحسناً معنوياً ($p<0.05$) في مجموعتي T0 و T1. أظهرت قيم الأجسام المضادة لمرض نيوكاسل والكمبورو والتهاب الشعب الهوائية المعدي وأنفلونزا الطيور زيادة معنوية ($p<0.05$) في مجموعة السيطرة و T1. كانت هناك زيادة معنوية ($p,<0.05$) في الوزن النسبي للكبد والقانصة والمعدة الحقيقية والحوصلة في مجموعة السيطرة مقارنة بالمجموعات الأخرى، بينما أظهر الطحال زيادة معنوية ($p<0.05$) فيT3 . كانت نتائج التقييم الحسي مرتفعة معنوياً في T3 و T4مقارنة بـ T0 و T1من حيث الطراوة واللون، في حين لم تظهر العصارة أي فرق معنوي بين المجموعات، كما أظهرت النكهة والمذاق زيادة معنوية في مجموعة السيطرة مقارنة بالمجموعات الأخرى

كان محتوى المعادن في اللحوم مرتفعاً بصورة معنوية بكل من الكالسيوم والفوسفور والحديد والزنك والبوتاسيوم في مجموعة السيطرة و T1مقارنة بـ T2 وT3. أظهرت بيانات التعبير الجيني وجود فروق معنوية ($p<0.01$) بين المجموعات التجريبية. كما تم تسجيل دلالة إحصائية لطول الزغابات وعمق الخبايا وعرض الزغابات وسمك العضلات في الاثني عشر والصائم. سجل طول الزغابات وعمق الخبايا وعرض الزغابات زيادة معنوية ($p<0.05$) في مجموعة السيطرة بينما

انخفضت T2 بشكل ملحوظ ($p<0.05$) مقارنة بالمجموعات الأخرى. خلاصة القول أن استبدال الذرة بكمية عالية (أعلى من 10٪) من الرز ردئ النوعية في علائق دجاج اللحم أدى إلى عدم تحسين المعايير المدروسة، بينما كانت T1 (10% الرز) مماثلة لمجموعة السيطرة الخالية من الاضافة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب البيطري

تقييم تأثير استخدام الرز منخفض الجودة كبديل للذرة في علائق فروج الحم على اداء النمو و الوظيفة المناعية و اعضاء الجهاز الهضمي وصفات الذبيحة

رسالة

مقدمة الى مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري في الصحة العامة

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