Poultry Studies, 20(1), 1-12
http://doi.org/10.34233/jpr.1302416

PROOF
RESEARCH PAPER

POULTRY STUDIES

Microorganisms Associated with Poultry Feeds in South West, Nigeria

Ismail Babatunde ONAJOBI[1], Oluwatosin Lara ADEBANJO[1], Oyindamola John SAMSON[1,*], Titilayo Omolade ABIONA[1], Abdulrazaq Omotunde OGUNMOYE[2], Stephen Olaosebikan MAKANJUOLA[3], Lawrence Olubukunade ADEBAJO[1]

[1]Department of Microbiology, Olabisi Onabanjo University, Ago Iwoye, Nigeria
[2]Department of Chemical Sciences, Olabisi Onabanjo University, Ago Iwoye, Nigeria
[3]Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University, Sagamu, Nigeria

Article History
Received: 27 Feb 2023
Accepted: 14 May 2023
First Online:25 May 2023

***Corresponding Author**
Tel: +2347065311985
E-mail:
oyindamolasamson1997@gmail.com

Keywords
Poultry
Animal
Feeds
Pathogenic
Microorganisms

Abstract

The rate of mortality of poultry as a result of contaminated feeds is of the increase in the recent times. This study was carried out to determine the level of contamination, microbial loads and spoilage of selected poultry feeds in south west Nigeria. Rabiu Feeds, Caps Feeds, Ayo Best Feeds and Top feeds were selected and sampled. Standard pour plate methods were used for the analyses. Results obtained revealed average range of total viable count, coliform counts, *Staphylococus* counts and fungal counts of 2.35 - 7.10x 10^4 Cfu/g, 0.55-2.65x10^4, 0.50-2.90 Cfu/g and 1.30-3.30 x 10^4 Cfu/g respectively. Microorganisms obtained include fifteen bacteria, eight yeast and five mould isolates. The genera are *Bacillus, Pseudomonas, Klebsiella, Enterobacter, Escherichia, Staphylococus, Micrococcus, Alcaligenes, Acinetobacter, Salmonella, Serratia, Corynebacterium, Clostridium, Erwinia, Flavobacterium*, *Saccharomyces, Candida, Geotrichum, Rhodotorula, Kluyveromyces, Torulopsis, Hansenula, Pichia*, *Aspergillus*, *Rhizopus, Fusarium, Mucour* and *Talaromyces.* Fifty percent of the isolates were known pathogenic microorganisms. This study therefore, concludes and recommends that stringent hygienic measures during production and storage of poultry feeds should be followed and enforced to the later. Constants inspection by the Standard Regulatory Bodies to the production sites should be encouraged.

Introduction

Poultry are birds of economic value contributing significantly to human food as a primary supplier of meat, egg, raw materials to industries (feathers, waste products), source of income and employment to people compared to other domestic animals (Demeke, 2004; Onajobi et al., 2020). Food for farm fowl, such as chickens, ducks, geese, and other domestic birds, is known as poultry feed (Bonnie, 2013).

Prior to the 20th century, grain, kitchen scraps, calcium supplements like oyster shell, and garden waste were frequently added to chicken diet as supplements. To maintain healthy birds, the feeds are kept fresh as much as possible at all times. However, in Southwest of Nigeria, it is quite difficult at times to maintain the freshness of the feed, where high temperature and oxidation destroy certain vitamins.

Feed spoilage is caused by the growth of undesirable molds and bacteria. Poultry feed spoilage reduces the feed value and palatability. Poultry feed is known to contain Salmonella, and other microorganisms which are detrimental to the health of poultry animals (Eugene, 2012). As a result, this study aim is to evaluate the microbial quality of poultry feeds in south west region of Nigeria.

Materials and Methods

Study area

The study area is three reputable commercial poultry feed Companies within the southwest Nigeria. These includes; Rabiu Feeds – Ijebu-Ode, Ogun State, F.A. Feeds–Ijebu–Ode, Ogun States, Hybrid Feeds–Osogbo, Osun State and Top Feeds–Ibadan, Oyo State.

Sample collection

A total of 12 samples were used. Samples consisted of Chicks Mash, Grower Mash, and Layers Mash from each commercial poultry feed companies respectively. The samples were collected in a clean polythene bag and transported to Federal Institute of Industrial Research, Oshodi, Lagos State (FIIRO) for further analysis. Each selected commercial feed depot was visited two times for sample collections during the study period.

Preparation of media diluents

23 g of Nutrient agar, 67g of MarConkey agar and 38g of Potato dextrose agar is weighed using a digital chemical balance and suspended into 1 liter amount of distilled water homogenized on hot plate magnet stirrer to form a uniform solution. Diluents (dilution blanks 0.1%) made up of 90ml and 9ml amount of distilled water were made sterilized at 121℃. 15 pounds per pressure (PSI) for 15 minutes in the autoclave. At the end of the sterilization period, media were cooled to 45^{o}C in water bath preset at 45℃ order to inhibit bacterial growth, streptomyan (0.14w/v) was aseptically weighed and added to potato dextrose agar only.

Isolation of microorganisms from sample

Ten grams (10g) of sample were weighed with sterile spatula using chemical balance. The samples were put into a sterile pestle and mortar, crushed with 90 milliliters of sterile distilled water. The sample was aseptically poured into the bottle of 90ml of sterile distilled water above burner. This was properly mixed together-1ml portion from the above dilution was aseptically taken with a sterile pipette and introduced into 9ml-amount of sterile water 10^{-1} dilution and from this dilution the samples were serially diluted up to the required dilution 10^{-5} dilutions according to Onajobi et al. (2015).

Disposable petri dishes were set out and labeled accordingly while inoculation was carried out using pour plate method. From the 10^{-4} and 10^{-5} dilutions, avqurt (1.0ml) of inoculums was aseptically pipette and inoculated into sterile petri dishes, cool molten of Nutrient agar, MacConkey agar and potato dextrose agar poured onto the inoculums respectively and mixed clock wisely and anticlockwise for evenly distribution of the inoculums. The plates were allowed to set properly and incubated in an incubator at 35+2℃ for 24 hours for bacteria and 28 +2℃ for 3 – 5 days for fungi.

At the end of incubation period, the colony observed on the culture plates is counted using coulter colony counter. The colony or viable count per ml was calculated by multiplying the average number of colonies per countable plate by the reciprocal of the dilution. Report as Colony forming units/ml (Cfu) or (Cful/g) was according to Onajobi et al. (2017).

Casein hydrogen

Nutrient agar (250ml) was prepared only 1%w/v (2.5g % casein) casein powder was added to Nutrient agar homogenized on hot plate magnetic stirrer. The medium was sterilized in an autoclave at 115^{o}C for 10 mins allowed to cool to about 45–50^{o}C and poured aseptically in petri dishes. The plates were allowed to set and dry at 45^{o}C. Fresh culture or isolation of 18 -24 hours were inoculated into plates of casein agar. Incubated at 35+/-2^{o}C for 5 days. Plates were examined for clearing of the medium around the bacteria growth using 20% and mercuric chloride (HCl and $HgCl_2$) solution (Onajobi et al., 2020).

Identification of moulds

The observed moulds growth was subcultured on fresh potato dextrose agar (PDA) plates and incubated at 28+2^{o}C 5 days and therefore an accurate description of the fungus as grown on the medium was observed and examined at frequent intervals for colonial or cultural characteristics.

The colonial morphology of the mold isolates was performed based on the size, colour and aerial mycelia growth. Microscopy morphology is determined using blue stain. The fungal growth was stained using wet mount techniques. With a sterile inoculating needle, mycelia growth is picked from the culture plates and placed onto cleaned grease free glass slide on which a drop of saline water had been dropped. The fungal mycelia were teased out properly. One drops of lactophenol cotton blue stain was added and the preparation was covered with clean cover slips. The preparation was subsequently viewed under the X40 microscope objective (Cheesbrough, 2010).

Screening of poultry samples for toxins

Chromatographic method was used to screen for poultry samples for the presence of toxins. 50 ml of 80% methanol was added to 10g of inoculated poultry feed each and were grinded into fine particles using a high-speed blender for 3 minutes. They were transferred back into conical flask and was shaken for 30 minutes on a shaker. The mixture was then filtered.

through Whitman paper and the extract collected in a 250ml flask, 20ml distilled water was added to ease separation. 15ml dichloromethane was added and shaken for proper mixture.

After separation, dichloromethane layer was filtered out through 20g of anhydrous sodium Sulphate to remove residual H_20. The extraction was collected in polypropylene cup and evaporated to dryness in a fume cupboard. The residue was redissolved in 1ml of dichloromethane. Aflatoxin standards and extracts were separated on thin layer chromatography plate. Aflatoxin plate was observed under long wavelength U.V light pitted in a black cabinet (Cheesbrough, 2010).

Characterization and identification of bacterial isolates

Pure cultures of bacterial isolates from feeds are identified based on their colonial morphology, cellular morphology and biochemical characteristics whereby the following analysis were carried out gram and spore staining, catalase production (Ramachandran et al., 2014), gelatin hydrolysis, starch hydrolysis, carbohydrate utilization oxidase test, indole production, nitrate reduction, coagulase test, urease test and methyl-red voges proskauer test (Cheesbrough, 2010).

Results and Discussion

Average range of count of 2.35-7.1×10^4cfu/g for total viable bacteria 0.55-2.65 x 10^4 for coliforms counts, 0.5-2.9 Cfu/g for *Staphylococcus* and 1.3-3.3×10^4Cfu/g for fungi (yeast counts were recorded respectively in all the samples analyzed from all poultry feeds investigated in table 1, 2 and 3 below. Slight variations were observed amongst the group of microorganisms within each poultry feeds. The average rate of occurrence and distribution of ten (10) members of the fungi group (Yeasts) were significantly different from the bacteria group.

Table 4, 5, 6 and 7 revealed vast array of microorganisms were detected and isolated among the various groups of microorganisms isolated were *Bacillus* species, *Corynebacterium* species, *Clostridium* species, *Flavobacterium* species, *Pseudomonas* species, *Micrococcus* species, *Alcaligenes* species, *Acinetobacter* species, *Proteus* species, *Staphylococcus* species, *Erwinia* species, *Enterobacter* species, *Klebsiella* species, *Serratia* species, *Citrobacter* species, *Salmonella* species, *Escherichia* species, *Sporosarcina* species and *Xanthomonas* species were among the bacteria group while the fungi group included: *Saccharomyces cererisiae*, *Saccharomyces exigins*, *Saccharomyces rouxii*, *Candida* species, *Pichia* species, *Geotrichum* species, *Rhodotorula glutinis*, *Hansenula anomala*, *Torulopsis stellate* and *Kluyveromyces maxians*.

Table 1. Physicochemical characteristics of poultry feed samples

Sample Location	Types of Feed	Sample No	Dtae of Collection	Moisture Content	PH	Colour	Texture	Odour
Rabiu Feeds	i.Layers Mash	1	1 – 8 – 16	1. 16.5%	6.6	Light	Coarse	Faint
(Ijebu Ode)	ii.Grower Mash	2	"	2. 17.0%	6.5	Brown	Coarse	Faint
Ogun State	iii.Chick Mash	3	"	3. 15.5%	6.7	Light Brown Light Brown	Coarse	Very Faint
F. A Feeds	i.Layers Mash	4	"	1. 16.3%	6.6	Brown	Coarse	Faint
(Ijebu Ode)	ii.Grower Mash	5	"	2. 16.7%	6.5	Brown	Coarse	Faint
Ogun State	iii.Chick Mash	6	"	3. 16.0%	6.6	Brown	Coarse	Faint
Hybrid Feeds	i.Layers Mash	7	"	1. 16.6%	6.7	Light	Coarse	Faint
Osogbo Osun	ii.Grower Mash	8	"	2. 17.0%	6.4	Brown	Coarse	Faint
State	iii.Chick Mash	9	"	3. 15.8%	6.7	Light Brown Light Brown	Coarse	Faint
Top Feeds	i.Layers Mash	10	"	1. 16.8%	6.4	Brown	Coarse	Faint
(Ibadan)	ii.Grower Mash	11	"	2. 16.4%	6.5	Brown	Coarse	Faint
Oyo State	iii.Chick Mash	12	"	3. 16.0%	6.6	Brown	Coarse	Faint

Table 2. Total mesophile aerobic microbial population of poultry fee

Sample Location	Types of Feed	Total Viable Count	Coliform Count	*Stapylococcus* Count	Fungi Count
Rabiu Feeds (Ijebu Ode) Ogun State	i. Chick Mash	71×10^3	11×10^3	29×10^3	33×10^3
	ii. Layer Mash	31×10^3	09×10^3	07×10^3	17×10^3
	iii. Grower Mash	24×10^3	06×10^3	05×10^3	14×10^3
F. A Feeds (Ijebu Ode) Ogun State	i. Chick Mash	33×10^3	06×10^3	10×10^3	16×10^3
	ii. Layer Mash	47×10^3	17×10^3	13×10^3	22×10^3
	iii. Grower Mash	64×10^3	23×10^3	21×10^3	25×10^3
Hybrid Feeds Osogbo Osun State	i. Chick Mash	30×10^3	09×10^3	07×10^3	13×10^3
	ii. Layer Mash	31×10^3	13×10^3	19×10^3	21×10^3
	iii. Grower Mash	46×10^3	10×10^3	14×10^3	25×10^3
Top Feeds (Ibadan) Oyo State	i. Chick Mash	41×10^3	11×10^3	10×10^3	20×10^3
	ii. Layers Mash	47×10^3	20×10^3	12×10^3	20×10^3
	iii. Grower Mash	57×10^3	27×10^3	14×10^3	27×10^3

Table 3. Mean of total mesophile aerobic microbial population of poultry feeds

Sample Location	Types of Feed	Total Viable Count	Coliform Count	*Stapylococcus* Count	Fungi Count
Rabiu Feeds (Ijebu Ode) Ogun State	1. Chick Mash	39×10^3	10×10^3	09×10^3	19×10^3
		42×10^3	12×10^3	10×10^3	21×10^3
	2. Layer Mash	48×10^3	19×10^3	11×10^3	18×10^3
		45×10^3	20×10^3	12×10^3	22×10^3
	3. Grower Mash	55×10^3	25×10^3	13×10^3	25×10^3
		58×10^3	28×10^3	15×10^3	28×10^3
F. A Feeds (Ijebu Ode) Ogun State	1. Chick Mash	28×10^3	09×10^3	06×10^3	12×10^3
		32×10^3	08×10^3	07×10^3	14×10^3
	2. Layer Mash	53×10^3	14×10^3	20×10^3	20×10^3
		48×10^3	11×10^3	18×10^3	22×10^3
	3. Grower Mash	49×10^3	12×10^3	12×10^3	24×10^3
		42×10^3	08×10^3	14×10^3	26×10^3
Hybrid Feeds Osogbo Osun State	1. Chick Mash	68×10^3	13×10^3	26×10^3	30×10^3
		74×10^3	09×10^3	32×10^3	36×10^3
	2. Layer Mash	33×10^3	10×10^3	05×10^3	18×10^3
		28×10^3	08×10^3	08×10^3	16×10^3
	3. Grower Mash	25×10^3	07×10^3	04×10^3	13×10^3
		22×10^3	04×10^3	06×10^3	15×10^3
Top Feeds (Ibadan) Oyo State	1. Chick Mash	31×10^3	05×10^3	10×10^3	15×10^3
		34×10^3	07×10^3	09×10^3	17×10^3
	2. Layers Mash	46×10^3	15×10^3	12×10^3	20×10^3
		48×10^3	18×10^3	13×10^3	24×10^3
	3. Grower Mash	66×10^3	24×10^3	18×10^3	26×10^3
		62×10^3	22×10^3	24×10^3	23×10^3

Amongst the bacterial group *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus brevis*, *Bacillus cereus*, *Bacillus polymyxa*, *Staphylococcus aureus*, *Staphylococcus albus, Staphylococcus hominis, Micrococcus luteus*, *Micrococcus roseus*, *Enterobacter cloacea*, *Escherichia coli*, *Klebsiella oxytoca*, *Euterobacter intermedius*, *Alcaligenes faecalis*, *Acinetobacter mallei*, *Klebsiella aurogenes*, *Klebsiella liquefascieus*, *Pseudomonas aeruginosa* and *Flavobacterium rigense* were most prevalent while *Saccharomyces cerevisiae*, *Saccharomyces rouxii*, *Candida utilis*, *Hansenula anomala* and *Candida parapsilosis* were most prevalent among the fungi group.

Thirteen species of *Bacillus* were encountered and they were identified. They were all Gram-positive rods, catalase positive, motile, oxidase positive, most are citrate, starch, gelatin, casein and Proskaeur positive. Most species fermented glucose, sucrose, lactose, mannitol, fructose, Arabinose and Xylose (Table 4). They were mostly present in all poultry feeds samples.

Two species of *Clostridium* were isolated and identified as *Clostridium tertium* and *Clostridium septicum*. They were all gram-positive rods, catalase, oxidase, indole, methyl red, voges proskaeur, citrate, urease negative. They were both motile, casein, positive spore formers. *Clostridium tertuim* reduced nitrate to nitrite, fermented glucose, sucrose, lactose mannitol, maltose and fructose, while *Clostriduim septicum* liquefied gelatin, NO_3 reduction negative, fermented glucose, sucrose, lactose, xylose and fructose. The organism identified as *Flavobacterium* species, was yellow/orange, short rods, gram negative rods, catalase, oxidase positive *Flavobacterium rigense* is motile, urease positive and liquefied nutrient gelatin, fermented glucose, sucrose/mannitol.

Five species of *Pseudomonas* were encountered and they were identified as *Pseudomonas aeruginosa*, *P. mendocina*, *P. cepaciae*, *P. mallie* and *P. fluorescens*. They were all Gram-negative rods, catalase and oxidase positive, motile, urease negative. Most species liquefied nutrient gelatin. The species did not hydrolyse starch. Most species reduced nitrate to nitrite. *P. mendocina* fermented only glucose, other species fermented glucose, sucrose, mannitol, Arabinose / raffinose. *P. aeruginosa* did not ferment xylose, lactose and salicin whereas *P. fluorescens* fermented xylose, mannitol and salicin.

The next genus was identified as *Alcaligenes*, it was isolated from the five samples. The colonies were white, entire and raised. Cellular observation showed that they were coccibacilli in shape and Gram negative. The cells were motile, catalase, oxidase, citrate utilization and voges proskaeur positive, urease, indole, methyl red, starch and gelatin tests were all negative. The organisms did not ferment lactose, xylose, salicin, sorbitol, mannitol, maltose, arabinose, raffinose and fructose. It was subsequently identified as a strain of *Alcaligenes faecalis*.

The organism identified as *Acinetobacter iumiwoffi* was short rod, gram negative and non-motile. It was catalase, urease and citrate test positive and did not produce acid from most carbohydrate sugars tested, except glucose and mannitol sugars.

The next species were *Eriwinia*, they were identified as *Eriwinia carotovora* and *E. uredovor.* They were gram negative rods, motile, catalase positive, and voges proskaeur positive, liquefied nutrient gelatin, nitrate reduced. They both fermented carbohydrate sugars such as glucose, xylose, salicin, mannitol and arabinose, *E. uredovora*did not ferment sucrose, lactose sorbitol, maltose, raffinose and fructose. The organic identified as *Xanthomonas campestris* was yellow in colour, gram-negative rods in shape, motile, catalase positive, starch hydrolyzed and liquefied nutrient gelatin, they fermented glucose, sucrose, maltose, arabinose and fructose. They did not ferment lactose, xylose, sorbitol, Salicin, mannitol, and raffinose.

Two species of *Staphylococcus* were isolated and identified as *Staphylococcus albus*, and *S. ariettae*. Both were Gram-positive cocci, catalase positive, non-motile, oxidase negative except *S. albus*, indole, methyl red, citrate utilization tests negative, they did not hydrolysed and liquefied nutrient gelatin as shown in table 6. Both were voges proskaeur test positive and Urease positive. *Staphylococcus albus* did not ferment xylose, ribose, galactose, raffinose, arabinose, but *Staphylococcus ariettae* fermented almost all the sugars except galactose in which acid was not produced. *Micrococcus* species (*Intense, Candidus* and *Roseus*). They were all gram-positive cocci, catalase positive, oxidase positive except *Micrococcus roseurs* which was oxidase negative. *Micrococcus candidius* was urease, Voges proskaeur positive and liquefied nutrient gelatin. They all fermented glucose, sucrose, xylose and maltose sugars. Out of 12 poultry feed samples investigated, a total of 24 isolates were obtained. Gram negative isolates were about 12 isolates. Table 7 shows the identified fungi isolates (yeast isolates) *Saccharomyces* species (*cerevisiae, rouxii*, and *exigus*) *Candida* species (*parapsilosis, utilis, castelli, sphaenical,* and *glabaruta*) *Geotrichum* species (*Klebahnii* and *capitatum*) *Torulopsis stellate*, *Kluyveromyces maxicans*, *Hansenula anomola*, *Pichiaohmeri*, and *Rhodotorula glutinis*. They were all catalase positive, motile. Nitrate was not reduced except *Hansenula anomalla* in which nitrate was reduced to nitrite. *Saccharomyces* species, *Kluyveromyces* species, *Pichiaohmeri*, *Hansenula* species produced/formed ascospores from their asci. Carbohydrate sugars were fermented by the most yeast species. The fungi (molds) groups were significantly differently from the bacteria group and were in the order *Aspergillus niger*, *Rhizopus stolonifer*, *Mucorplumbeus*, *Fusarium oxysporium*, *Aspergillus chevalieri*, *Rhizopus arrhizus*, *Nigrospora oryzae*, *Absidiaspinosa*, *Aspergillus flavus*, *Aspergillus amstelodami*. Among the fungi (moulds) the most prevalent species were *A. niger*, *A. flavus*, *Fusarium oxysporum*, *Rhizopus arrhizus*, *Rhizopus stolonifer*, and *Absidias spinosa* as shown in Figure I.

Table 4. Biochemical test on isolated bacteria

Isolate code	Colour/ pigment	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	Mr-methyl/ red	Vp-voges prosphase	Urease	Citrate utilization	Gelutin hydrohyes	Stenrch hydrolyis	No_3 reduction	Probable identification
Top Feed															
1	Cream	+ve	Rods	-	-	-	+	-	-	-	-	-	-	+	*Clostridium tertium*
1	Cream	+ve	Rods	-	-	-	+	-	-	-	-	+	-	-	*Clostridium septicum*
2	Yellow Orange	-ve	Rods	+	+	-	+	-	-	+	+	+	-	-	*Flavobacterium rigense*
2	Yellow Orange	-ve	Rods	+	+	-	-	-	-	-	-	-	-	+	*Pseudomonas mendocina*
3	Cream White	+ve	Cocci	+	+	-	-	-	+	-	-	-	-	-	*Staphylococcus albus*
3	Cream White	-ve	Rods	+	+	-	-	-	-	-	-	+	-	+	*Pseudomonas cepaciae*
Hybrid Feed															
1	Yellow	+ve	Cocci	+	+	-	-	-	-	+	-	+	-	-	*Micrococcus intense*
1	Cream	-ve	Rods	+	-	-	+	-	+	-	-	+	-	+	*Erivinia carotovora*
2	Pinkish	-ve	Rods	+	-	-	-	-	-	+	+	-	-	+	*Acinetobacter iwoffi*
2	Orange Yellow	+ve	Cocci	+	-	-	-	-	+	-	-	-	-	-	*Staphylococcus ariettae*
3	Red	+ve	Cocci	+	-	-	+	-	+	-	-	-	-	+	*Micrococcus roseus*
3	Yellow	-ve	Rods	+	-	-	+	-	-	-	-	+	+	-	*Xanthomonas campestris*
Rabiu Feed															
1	Green	-ve	Rods	+	+	-	+	-	-	+	-	+	-	-	*Pseudomonas mallei*
1	Green	-ve	Rods	+	+	-	+	-	-	-	+	+	-	+	*Pseudomonas aeruginosa*
2	Bluish Green	-ve	Rods	+	+	-	+	-	-	-	-	+	-	+	*Pseudomonas fluorescens*
2	Yellow	+ve	Cocci	+	+	-	-	-	+	+	-	+	-	-	*Micrococcus candidus*
3	Yellow	+ve	Cocci	+	+	-	-	-	+	+	-	+	-	-	*Micrococcus candidus*
3	Pink	+ve	Cocci	+	-	-	+	-	+	-	-	-	-	+	*Micrococcus roseus*
F.A. Feed															
1	Yellow	+ve	Cocci	+	+	-	-	-	+	+	-	+	-	-	*Micrococcus candidus*
1	Yellow	-ve	Rods	+	-	+	+	-	+	-	-	+	-	+	*Erivinia uredovora*
2	Cream	-ve	Rods	+	-	-	+	-	+	-	-	+	-	+	*Erivinia carotovora*
2	Pinkish	-ve	Rods	+	+	-	+	-	+	-	-	-	-	-	*Alcaligenes faecalis*
3	Cream Butter	+ve	Rods	+	+	-	+	-	+	-	+	+	+	+	*Bacillus cereus*
3	Cream Butter	+ve	Rods	+	+	-	+	-	+	-	+	+	+	+	*Bacillus cereus*

CODE: 1 = Chick Mash 2 = Layers Mash 3 = Growers Mash

Table 5. Biochemical test on isolated coliforms

Sample location	Isolate code	Colour/ pigment	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	Mr-methyl /red	Vp-voges prosphase	Urease	Citrate utilization	Starch	No_3 reduction	Probable identification
Top Feed															
	1	Pink	-ve	Rods	+	-	+	-	-	+	+	+	+	+	*Klebseiella oxytoga*
	1	Pink	-ve	Rods	+	-	-	+	-	+	+	-	+	-	*Enterobacter aerogeres*
	2	Pink	-ve	Rods	+	-	-	-	+	-	+	+	-	-	*Klebseiella liquefezium*
	2	Black	-ve	Rods	+	-	-	+	-	-	-	+	-	-	*Salmonella arizonic*
	3	Black	-ve	Rods	+	-	+	+	+	-	-	-	-	+	*Escherichia coli*
	3	Red	-ve	Rods	+	-	-	+	+	+	-	+	-	-	*Serratia liquefasciens*
Hybrid Feed															
	1	Pink	-ve	Rods	+	-	-	+	+	+	-	+	-	+	*Enterobacter intermedines*
	1	Cream	-ve	Cocci	+	-	+	+	+	-	-	+	-	+	*Citrobacter diversus*
	2	Cream	-ve	Rods	+	-	+	-	-	-	-	+	-	-	*Citrobacter koseri*
	2	Pinkish	-ve	Rods	+	-	-	+	-	+	+	+	+	-	*Enterobacter cloacae*
	3	Pinkish	-ve	Rods	+	+	-	+	-	-	-	+	-	-	*Alkaligenes faecalis*
	3	Pink	-ve	Rods	+	-	-	-	+	+	+	+	+	+	*Klebseiella planticola*
Rabiu Feed															
	1	Cream	-ve	Rods	+	-	-	+	+	-	+	+	-	-	*Citrobacter freundii*
	1	Pinkish Cream	-ve	Rods	+	-	-	-	-	-	-	+	-	-	*Acinetobacter mallei*
	2	Pink	-ve	Rods	+	-	-	+	-	+	-	-	-	+	*Enterobacter amigenus*
	2	Black	-ve	Rods	+	-	-	+	+	-	-	+	-	+	*Salmonella bougori*
	3	Pink	-ve	Rods	+	-	-	+	+	+	-	+	-	+	*Enterobacter agglomerans*
	3	Black	-ve	Rods	+	-	+	+	+	-	-	-	-	+	*Escherichia coli*
F.A. Feed															
	1	Cream	-ve	Rods	+	-	-	+	+	+	-	+	+	-	*Proteus mirabilis*
	1	Black	-ve	Rods	+	-	+	-	+	-	-	-	-	+	*Escherichia coli*
	2	Pink	-ve	Rods	+	-	+	+	-	+	+	+	+	+	*Klebseiella oxytoca*
	2	Pink	-ve	Rods	+	-	-	+	+	+	-	+	-	+	*Enterobacter intermedium*
	3	Cream	-ve	Rods	+	+	-	+	-	-	-	+	-	-	*Alcaligenes faecalis*
	3	Pink	-ve	Rods	+	-	-	+	-	+	+	+	+	-	*Enterobacter cloacae*

CODE: 1 = Chick Mash 2 = Layers Mash 3 = Growers Mash

Table 6. Biochemical characterization of isolated *Staphlococcus* species

Staphlococcus	Colour	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	Mr-methyl/ red	Vp-voges prosphase	Urease	Citrate utilization	No3 reduction	Probable identity
Isolate Code													
Top Feed													
1	White	ve	Cocci	+	+	-	-	-	+	-	-	-	*Staphlococcus albus*
1	Orange	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus aureus*
2	White	ve	Cocci	+	+	-	-	-	+	-	-	+	*Staphlococcus albus*
2	Orange	ve	Cocci	+	-	-	-	-	+	-	-	+	*Staphlococcus ariettae*
3	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus carnosus*
3	White	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus simulans*
Hybrid Feed													
1	Yellow	ve	Cocci	+	+	-	-	-	-	-	+	+	*Micrococcus varians*
1	Orange	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus aureus*
2	Yellowish	ve	Cocci	+	+	-	-	-	+	+	-	+	*Micrococcus kristinae*
2	White	ve	Cocci	+	+	-	-	-	+	-	-	+	*Staphlococcus albus*
3	Yellow	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus epidermid*
3	Orange	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus aureus*
Rabiu Feed													
1	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus hominis*
1	Yellow	ve	Cocci	+	+	-	-	-	+	+	-	+	*Micrococcus candidus*
2	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus hominis*
2	Red	ve	Cocci	+	-	-	+	-	+	-	-	+	*Micrococcus roseus*
3	Yellow	ve	Cocci	+	+	-	-	-	-	+	-	+	*Micrococcus luteus*
3	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus hominis*
F.A. Feed													
1	Red	ve	Cocci	+	-	-	+	-	+	-	-	+	*Micrococcus roseus*
1	Cream	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus hominis*
2	Orange	ve	Cocci	+	-	-	-	-	+	+	-	+	*Staphlococcus aureus*
2	Yellow	ve	Cocci	+	+	-	-	-	-	+	-	+	*Micrococcus luteus*

CODE: 1 = Chick Mash 2 = Layers Mash 3 = Growers Mash

Table 7. Biochemical characterization of isolated yeasts

Yeast	Colour	Cellular morphology	Catalase test	No3 reduction	Ascospaze formation	Motility test	Urease test	Psedomycillui m production	Glucose	Maltose	Meubrose	Probable Oranism
ISOLATE CODE												
Top Feed												
1	Cream white	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomyes cerevisiae*
1	Cream dull	Oval	+	-	-	+	-	-	+	+	-	*Candida parapsilopsis*
2	Cream rough	Cylindrical	+	-	-	+	-	-	+	-	-	*Geotrichum klebahnii*
2	Cream	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
3	Cream	Ellipsoidal	+	-	-	+	-	-	+	-	-	*Saccharomye s rouxi*
3	Cream white	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
Hybrid Feed												
1	Cream dull	Oval budding (small)	+	-	+	+	-	-	+	+	-	*Candida utilis*
1	Cream	Round budding	+	-	+	+	-	-	+	-	-	*Saccharomye s exigus*
2	Cream	Round	+	-	-	+	-	-	+	-	-	*Torulopsis stellata*
2	Cream	Round	+	-	+	+	-	-	+	-	-	*Kluyveromyc es maxians*
3	Cream	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
3	Cream	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
Rabiu Feed												
1	Cream	Round budding	+	+	+	+	-	-	+	-	-	*Hansenula anomola*
1	Cream	Round budding	+	-	+	+	-	-	+	-	-	*Pichiaohmeri*
2	Cream	Oval small	+	-	+	+	-	+	+	-	-	*Candida castelli*
2	Cream	Ellipsoidal	+	-	+	+	-	-	+	-	-	*Saccharomye s cerevis*
3	Cream white	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
3	Cream	Oval budding	+	-	+	+	-	-	+	+	-	*Saccharomye s cerevisiae*
F.A. Feed												
1	Red/Pink	Elongated	+	-	-	+	-	-	+	-	-	*Rhodoforula glutinis*
1	Cream	Cylindrical	+	-	-	+	-	-	+	+	-	*Geotrichum capitatum*
2	Cream	Oval small	+	-	-	+	-	-	+	+	-	*Candida sphaxrica*
2	Cream	Cylindrical	+	-	-	+	-	-	+	-	-	*Candida glabrata*

CODE: 1 = Chick Mash 2 = Layers Mash 3 = Growers Mash

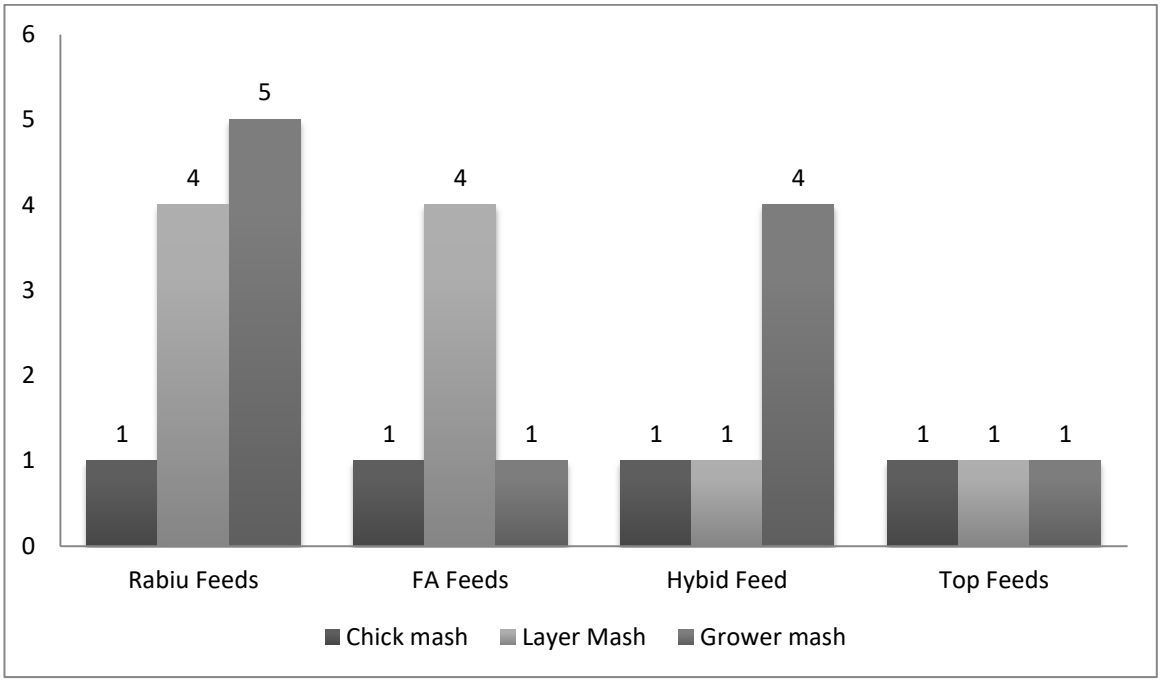

Figure 1. Histogram showing level of afflatoxins in (3) different poultry feed

Keys: 1 – No Aflatoxin detected, 4 – Aflatoxin (B1) detected, 5 – Aflatoxin (G1) detected
NOTE: X- Axis – Sample Names (Rabiu feeds, FA feeds, Hybrid feeds and lastly Top feeds)
Y- Axis- Toxins Status (1- Not detected, 4- AF(B1), 5- AF (G1)

This study revealed the microorganisms associated with poultry feeds that are commonly used within the south western region of Nigeria. The presence and isolation of these microorganisms depict that they are the causal agents responsible for the spoilage of poultry feeds. The slightly high viable staphylococcus counts bacteria, fungi (yeast/moulds), recorded may be associated with inadequate post-processing handling practices as spreading on the floor, mat and sometimes on high density polythene spread on the floor during and after pre-mixed bagging and packaging and during haulage and storage. These may also be responsible for the vast array of microorganisms detected and isolated. These finding corroborate with the report of Lund et al. (2000).

Low counts of coliforms and *Salmonella* were detected. However, there presence appeared transient since no growth was detected on agar plate following analysis after 24 hours. This may be due to their inability to with stand the micro environmental condition. The high rate of occurrence and distribution of moulds such as a *Aspergillus, Fusarium, Rhizopus, Taloromyces, Absidia* and others may be traced to the inadequate post-processing handling practices, storage in high environmental conditions, the ubiquitous nature of their moulds and their ability to withstand and tolerate harsh environmental conditions such as low pH and low moisture content of the poultry feeds (Beatriz and Eliana, 2000).

It is a mandate of the World Health Organization (WHO) that chicken products be safe for human consumption. Important potential route for infections to enter the supply of food for humans is through microbial contamination of chicken feed (Kashiwazwki, 1999). To ascertain the load and the microorganisms connected with chicken diets in southwest Nigeria, this study was planned and executed. These are the primary industries that deal with poultry feeding. Despite the apparent similarity in contamination, market and factory-sourced feeds were analyzed independently due to the effects of storage, time, and environmental factors. A total of 132 isolates were discovered and acquired from the twelve (12) feeding samples that were analyzed. There were somewhat fewer Gram-negative isolates than Gram-positive ones. Gram-negative bacteria, particularly Salmonella, are more dangerous than Gram-positive bacteria, hence only a very small fraction of them were discovered here (Olajuyigbe et al., 2006 and Onajobi et al., 2017).

Salmonella arizonae and *Salmonella bongori* were both isolated from samples from Top feeds layer mash and Rabin feeds layer mash. This finding is consistent with previous work (Kidd et al., 2002). Feeds have been noticed to be the source of human infection due to eating chicken fed salmonella-contaminated feeds. Other feeds sources or samples were found free of salmonella but can be contaminated if stored in environment with about 20–25% moisture content.

The result showed that grower mash and chick mash were most contaminated due to period of feed storage and storage conditions are suspected to be behind the higher level of contamination. It was found that chick mash feed samples are the most contaminated followed by grower mash feed samples, followed by layer mash which was the least contaminated feed samples. This is mainly attributed to the high nutritive value of the feed samples (Sakazaki, 2000 and Onajobi et al., 2017).

Amongst Gram-negative bacteria *Escherichia coli*, followed by *Klebsiella* species *Enterobacter* species, *Citrobacter* species, *Pseudomonas* species, *Alcaligenes* species, *Acinetobacter* species, *Serratia* species, and *Proteus mirabilis* isolated from poultry feed was reported by Wadu (2002), additionally discovered in a poultry shop (Quinn et al., 1999).

The majority of feed sample isolates contained *Bacillus* species. According to, *Bacillus* spp. may be pollutants in poultry feed (Bryan and Doyl, 1995). In this experiment, the results were consistent with those established by Wadu (2002), who discovered that *Bacillus* species are the most prevalent isolate in chicken feed. Nada (2005) successfully isolated *Bacillus* species from chicken feed. The samples also included isolated *Staphylococcus* species, including *Staphylococcus aureus, Staphylococcus albus, Staphylococcus simulans*, and *Micrococcus* species. The public's health may be impacted by these organisms. The microflora in poultry feeds may be different and come from a variety of environmental factors, such as soil, temperature, dust, and insects. Pathogens may infect poultry feed ingredients at any moment when they are being grown, harvested, processed, or stored (Watkins et al., 2003).

Seventeen fungal isolates were recovered from the twelve feed samples. The yeasts found to spoil the feed samples were identified as *Saccharomyces roxii*, *Saccharomyces exigus*, *Geotrichum klebahaii*, *Candida utilis*, *Torulopsis stellata*, *Kluyveromyces maxians, Hansenula anomala*, *Candida castelli*, *Candida glabrata*, *Candida sphaerica*. *Geotrichum capitatum* and *Rhodotorula glutinis, Saccharomyces*, *Candida*, and *Geotrichum* species are dominant organisms in cereal-based foundation species of the genus *Saccharomyces* and *Candida* are widespread in nature and can be found on plants or material of plant origin in fermenting or spoiling food (Belgin and Kathryn, 2006).

Fungal colonies (moulds) selected from each plate were based on colony appearance. Colonies having characteristic features such powdery appearance, fluffy, velvety texture, low mycelia with colour ranging from white, gray to pinkish, pink, greenish yellow, black yellow, green, gray green and others were selected; fifteen fungal (moulds) isolated were selected, examined microscopically and identified by their cellular morphology and culture characteristics.

The moulds isolates encountered and identified are *Aspergillus niger*, *Tolaromyces thermophilus*, *Fusaruim oxysporium*, *Absidia spinosa, Mucor plumbeus, Aspergillus amstelodami, Nigrosporaoryzae*, *Aspergillus chevalieri, Rhizopus arrhizus* and others. These groups of moulds have been variously linked with the production of various types of mycotoxins under various condition (Tournas, 1994). Exposure of mycotoxins through ingestion of contaminated foods of poultry feeds by birds or chicken and inhalation to toxins produced have been linked to acute and chronic toxicity in animals. Since poultry feeds require little or no further processing or treatment prior to consumption by the chicken, there is the possibility of ingesting large dosage over a period of time with possible health hazards. Hence the need to develop adequate processing, handling and storage techniques for this relish poultry feeds (Kayode and Oworunubi, 1988 and Onajobi et al., 2020).

Conclusion

The present investigation or work revealed slightly high bioload and vast array of microorganism associated with poultry feeds and high rate of occurrence and prevalence of fungal producing mycotoxins. This is alarming and suggests early warning signals indicating the level of safety of available poultry feeds. It also warrants renewed vigilance on the efficacies of food processing conditions, feed handling and handlers' technical knowhow, hygiene practices and safety storage conditions.

References

Beatriz, H.P. and Eliana, B.F. (2000). The occurrence of moulds, yeast and micotocins in pre-cooked pizza dough sold in Southern Rio grande. Desul. Brazillian Journal of Microbiology. 31(2): 1-8

Belgin, D. and Kathryn, J.B. (2006). Genetic Diversity and spoilage potentials among *Pseudomonas* spp. Polated from fluid milk products and dairy processing plants, Journal of Applied Microbiology. 101:72-79

Bonnie, J.M. (2013). Gardening with Free-Range Chickens for Dummies. Journal of Science. 2: 78-79

Bryan, F. L and Doyl, M.P. (1995). Health risk and consequences of *Salmonella* and *Campylobacter jejumi* in raw poultry. Journal of Feed Production. 58:326-344

Cheesbrough, M. (2010). Adaptive or Specific Immune Response. In:*District Laboratory in Tropical Countries*. 2nd Ed. Cape Town: Cambridge University Press. pp11.

Demeke, S. (2004). Egg Production and Performance of local white leghorn hens under intensive and rural household conditions in Ethiopia. LRRD 16: 2

Eugene, N. O. (2012). Food, Nutrition and Toxicology: Is your life in your Hands. Inaugural Lecture Series No. 99 University of Port Harcourt Press Limited (UPPL), 138 ppp.

Kashiwazwki, M. (1999). Contamination of aerobic bacteria in materials for feed and commercial feed for animals *J. Hyg*. 1:21-26

Kayode, J.G. and Oworunubi, J.J., (1988). Crop science and production in warm climates, 1st edition, London; Macmillian publishers, 185-188

Kidd, R.S., Rossignol, A.M. and Grmroth, M.J. (2002). *Salmonella* and other Enterobacteriaeceas in animal feed ingredients and antimicrobial resistance in Western Oregon, *Journal of Environs.Health* 64: 9-16

Lund, B.M., Parker, T.C. and Gould, G.W (2000). Toxigenic fungi and mycotoxins. Aspen Inc publishers; Themcrobiological and safety and quality of food pp. 1490-1517

Nada, E.A.F. (2005). Aerobic Bacteria in Rendered slaughter by products used as poultry feed. MSC. Dissertation, university of Khartoun

Olajuyigbe, O.O., Oluremi. B.B and Umaru, D.G (2006). Bacterial spoilage of fresh meat in some selected markets. *Ife Journal of science*, 8(2) 193-198.

Onajobi I.B., Adam A.I., Adeyemi S.A. and Agbaje A. B. (2017). Antibacterial Activity of *Anacardium occidentale* leaf Extracts on some Microorganisms Associated with dental Plaque. *SMU Medical Journal* 2(4), 2349 – 1604.

Onajobi, I.B., Agbaje, A.B., and Alaba, S.A. (2015). In vitro Antibacterial Assessment of *Elaeis guineenis* and *Bambusa vulgaris* Extracts against Some selected Pathogens. *Nigeria Journal of Microbiology* 27(1): 2701-2708.

Onajobi, I. B., Idowu, E. O., Adeyemi, J. O., Samson, O. J. Ogunyinka, P. I. and Fagade, O. E. (2020). In vitro antibacterial activities and molecular characterization of bacterial species isolated from farmlands against selected pathogens. Biotechnology Reports (Elsevier) 27 e00513. www.elsevier.com/locate/btre

Quinn, P.J., Carter, M.E., Marley, B.K. and Carter, G.R. (1999). Clin. Vet. Microbial.Mosby Inc, U.S.A.

Ramachandran, R., Chalasani, A.G., Lal, R. and Roy, U. 2014. A Broad-Spectrum Antimicrobial Activity of *Bacillus subtilis* RLID 12.1. *The Scientific World Journal.* 2014(968487):1-10.

Sakazaki, R.E. (2000). The number of contaminating bacteria in a finlin eatery *Exp. Anim*. 9: 85 86

Tournas, V. (1994). Heat-resistant fungi of importance to the food and beverage industry. *Grit. Rev Microbial* 20(4), 243-63

Wadu, A.A (2002). Bacterial load of Animal and poultry feed concentrate M.Sc. Dissertation, University of Khartoun

Watkins, J.R., Flower, A.Z. and Grumbler, L.C. (2003). *Salmonella* organism in animal products used in poultry feed. *Avtan. Dis. J.* 3: 210-306.

Poultry Studies, 20(1),13-18
http://doi.org/10.34233/jpr.1302448

PROOF

RESEARCH PAPER

Molecular Prevalence of Three Chicken Gastrointestinal Tract Pathogens and Phylogenetic Characterization of *Tetratrichomonas gallinarum* in Türkiye

Nuri ERCAN[1,*], Alparslan YILDIRIM[2], Onder DUZLU[2]

[1] Kirsehir Ahi Evran University, Faculty of Agriculture, Kirsehir, Türkiye
[2] Erciyes University, Faculty of Veterinary Medicine, Department of Parasitology, Kayseri, Türkiye

Article History
Received: 14 Apr 2023
Accepted: 11 May 2023
First Online: 23 May 2023

***Corresponding Author**
Tel: +90 386 280 48 00
E-mail: nuri.ercan@ahievran.edu.tr

Keywords
Histomonas meleagridis
Heterakis gallinarum
Tetratrichomonas gallinarum
Molecular prevalence
18S rRNA

Abstract

Several species of parasites infect chickens and can cause economic losses. *Histomonas meleagridis*, its intermediate host *Heterakis gallinarum,* and *Tetratrichomonas gallinarum* are the most common parasites of poultry. The current study aimed to determine the molecular prevalence and phylogenetic relationships of *H. meleagridis, He. gallinarum,* and *T. gallinarum* in chickens in Central Anatolia Region of Türkiye. For this, a total of 100 fecal specimens from chickens were analyzed by using a PCR assay targeting the 18S rRNA gene region of *H. meleagridis* and *T. gallinarum* and the mtDNA COI gene region of *He. gallinarum*. PCR analysis identified *T. gallinarum* in 11 (1.1%) specimens, whereas other species were not found in all the examined specimens. Positive specimens were detected only in backyard chickens. Sequence analyses identified two novel genotypes named TRTgal1 and TRTgal3, and one known genotype (TRTgal2) of *T. gallinarum*. The TRTgal2 genotype was found to be identical to the human genotype (AY247746) and the four avian genotypes. Phylogenetic analyses of the obtained genotypes clustered in separate monophyletic clades. Our study provides the first data on *T. gallinarum* in chickens in Türkiye and contributes to public health with the identified zoonotic genotype and knowledge of the epidemiology of this parasite.

Introduction

Poultry plays an important role in meeting animal protein needs, with short production cycles and low ecological requirements (Marangon and Busani, 2007). The poultry breeding is faced with diseases such as necrotic enteritis, coccidiosis and avian influenza that cause adverse economic effects. (Galarneau et al., 2020). Histomoniasis, mostly known as blackhead disease, caused by *Histomonas meleagridis*, represented by losses in turkeys with pathological lesions in the caeca and liver (Grabensteiner and Hess, 2006). In chickens, histomoniasis lesions are often confined to the caeca and associated with less severe production losses and some mortality (Esquenet et al., 2003). Hu et al. (2006) reported that the transmission dynamics of histomoniasis in chickens differ from turkeys. In general, infections are transmitted by intermediate hosts such as mainly *Heterakis gallinarum*, an enteric nematode parasite that primarily affects turkeys and chickens. However, some reports suggest that transmission also occurs without vectors (Hu and McDougald, 2003). *Heterakis* worms are small, white, and thin, and found mostly in the ceca of birds, and several species infect poultry (McDougald, 2020). *Heterakis gallinarum* infections may cause weight loss in heavy infections, like enteric nematodosis. Moreover, the damage of *He. gallinarum* infection may increase depending on co-infection with histomoniasis (Cupo and Beckstead, 2019). *Tetratrichomonas gallinarum* is a widespread enteric flagellate that can colonize the digestive tract of galliform and anseriform

birds (Cepicka et al. 2005). The pathogenicity of this species is controversial (Amin et al., 2011). *T. gallinarum* is considered nonpathogenic but diarrhea and inflammation of the caecum have been reported in chukar partridges (Wichmann and Bankowski, 1956), mockingbird (Patton and Patton S, 1996), white pelican (Burns et al., 2013), and chickens (Landman et al., 2016). The first infection with *T. gallinarum* was described in chickens in 1911 (Martin and Robertson, 1911). Therewithal, *T. gallinarum*-like organisms were isolated from human patients in 2005 (Cepicka et al., 2005).

Molecular methods such as polymerase chain reaction and DNA sequencing have been successfully used for species identification and phylogenetic relationships (Cepicka et al., 2010). Different gene regions such as ITS1/5.8S/ITS2, 18S rRNA, and protein-coding genes are commonly used to describe the phylogenetic relationships of the species (Grabensteiner et al., 2010). Analysis of 18S rRNA gene is a good marker for species identification and characterization of trichomonads (Delgado-Viscogliosi, et al., 2000; Dimasuay and Rivera, 2013).

Studies on the current epidemiological status and molecular characterization of these three species in Türkiye are limited. Therefore, we aimed to determine the molecular prevalence, phylogenetic characterization of *H. meleagridis*, *He. gallinarum*, and *T. gallinarum* in backyard chickens and laying hens in Kirsehir province, Türkiye.

Materials and Methods

Collection of fecal samples

A total of 100 fresh fecal droppings from chickens, including 80 backyard and 20 laying hens, were collected, and placed in plastic individual containers, and labeled. Ethics approval was not required. All samples were stored at -20 °C until DNA extraction.

DNA extraction and PCR amplification

Genomic DNA (gDNA) extraction was carried out from each fecal sample by using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The final nucleic acids were precipitated in a 50 µL elution buffer and stored at -20 ℃. The DNA quality of each isolate was assessed by Qubit Fluorometric Quantitation (Thermo Fisher Scientific, Waltham, USA). All gDNA samples were examined with PCR analyses to determine the *H. meleagridis* and *T. gallinarum* by amplifying the partial sequence of the 18S rRNA gene region and *He. gallinarum* mtDNA COI gene region. Primer pairs used in PCR analyses are shown in Table 1. A total of ~25 ng of gDNA of each sample was included in the ready to use PCR Master Mix (Dream Taq Hot Start Green PCR Master Mix, Thermo Scientific, USA) in a final reaction volume of 25 µL. The same cycling conditions for the PCR analyses were used as the references given in Table 1. The PCR amplifications were performed in a Sensoquest Labcycler (SensoQuest, Germany). Positive controls of each species and nuclease-free water as a negative control were used in all analyses. PCR products were electrophoresed on 1.5% agarose gel and visualized using the gel documentation system (Fig. 1).

Sequencing and phylogenetic analysis

All PCR positive amplicons were purified by using GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced in both directions via Sanger sequencing (BMLabosis, Ankara, Türkiye). The raw sequence reads (~500 bp) were checked, aligned and assembled using Geneious Prime 2020.0.3 (https://www.geneious.com) software to create consensus sequences. Final sequences (469-490 bp) were compared with reference sequences in the GenBank database by BLAST analyses (Fig. 2). The phylogenetic analyses of the identified genotypes were performed by the Maximum Likelihood (ML) method with the HKY genetic distances model in MEGA X (Kumar et al., 2018). The best DNA model was determined according to the Akaike Information Criterion (AIC) algorithm by using jModeltest v.0.1.1. (Posada, 2008). Branch support was assessed with 1000 bootstrap replicates. For phylogenetic analyzes, the twenty-four *T. gallinarum* isolates from China, Philippines, France, Austria, Czechia, and Germany that had been found from a variety of hosts, including chickens, humans, and lemur, and had been registered in the Genbank database were employed. *Tetratrichomonas gallinarum* 18S rRNA gene region partial sequences obtained in the study were deposited in the GenBank database under accession numbers OP379566-68.

Results

Molecular prevalence of *H. meleagridis, He. gallinarum* and *T. gallinarum* in chickens

In the present study, 100 specimens were examined. *Tetratrichomonas gallinarum*-positive samples were found in 11 (1.1%), whereas *H. meleagridis* and *He. gallinarum* were not detected. All positive specimens were collected from backyard chickens.

Nucleotide Sequence and phylogenetic analyses of *T. gallinarum*

Owing to sequence analysis of the partial 18S rRNA region of 11 *T. gallinarum* isolates, two novel genotypes (TRTgal1 and TRTgal3) and one (named as TRTgal2 by us) known genotypes were characterized.

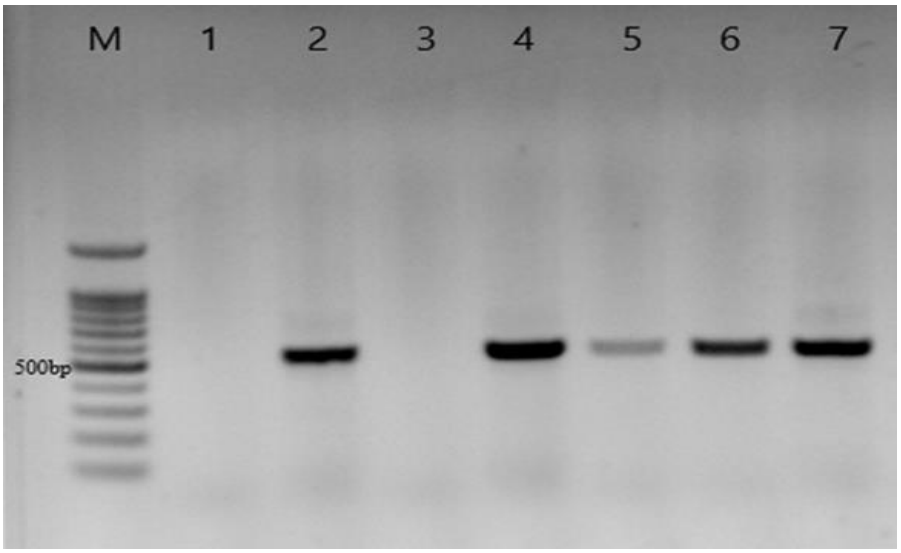

Figure 1. Some *T. gallinarum* positive samples on agarose gel. M: Marker (100bp), 4-7: Positive samples, 3: Negative samples, 2: Positive control, 1: Negative control

Table 1. Primer pairs were used in this study.

Species	Primers	Sequences	Amplicon size (bp)	References
H. meleagridis	Hmf Hmr	5'-GAAAGCATCTATCAAGTGGAA-3' 5'-GATCTTTTCAAATTAGCTTTAAA-3'	574	Grabensteiner and Hess, 2006
He. gallinarum	P1 P2	5'- ATATCCTCTAGGTGGTAAGCTCTTG-3' 5'-AGCCCCAAATAAAAACTTCAAAACACAC-3'	696	Gu et al. 2018
T. gallinarum	Tgf Tgr	5'-GCAATTGTTTCTCCAGAAGTG-3' 5'-GATGGCTCTCTTTGAGCTTG-3'	526	Grabensteiner and Hess, 2006

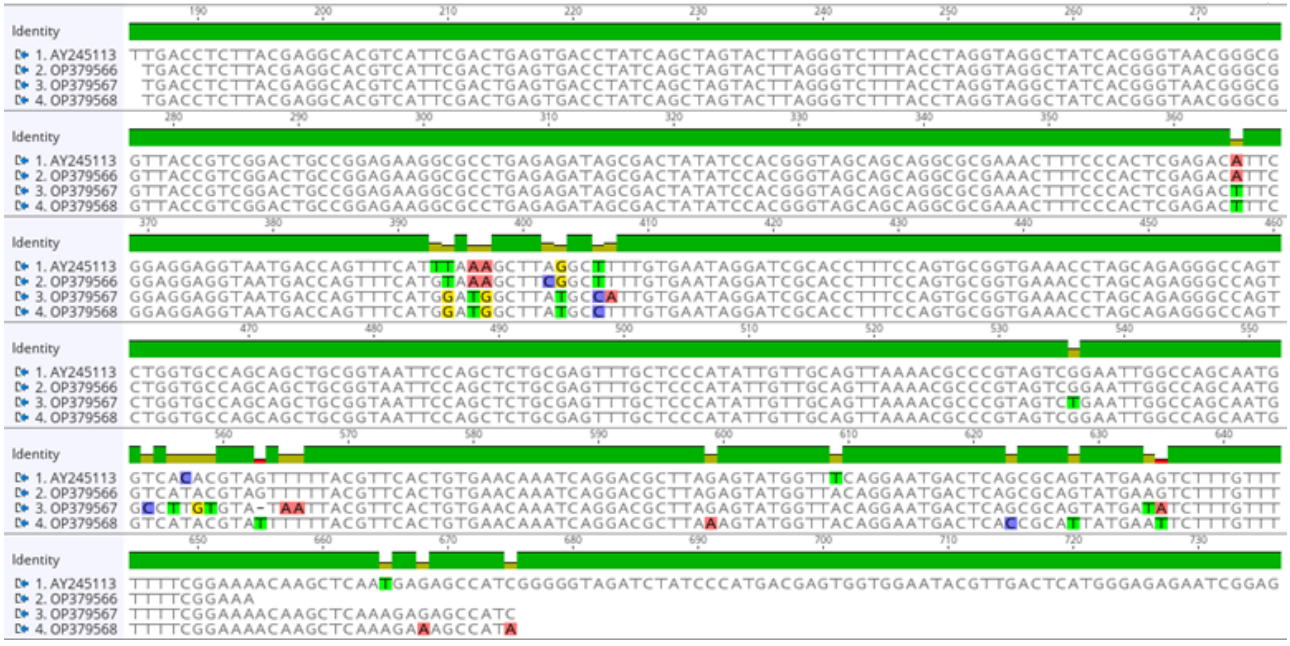

Figure 2. Sequence variation in the 18S rRNA region of *T. gallinarum* isolates. Polymorphic sites are represented by color

The identified genotypes contain 12-17 variable sites among them. The TRTgal2 (OP379567) isolate was found to be identical to isolates identified from duck (JX565083, JX565081), human (AY247746), turkey (AJ920324) and swan (AY245111). The molecular prevalence and identified genotypes with GenBank accession number are presented in Table 2.

Phylogenetic analyses of *T. gallinarum* 18S rRNA gene region clearly separated all the isolates in two monophyletic clades (53%). The first clade comprised of the genotypes from different hosts and countries while the second clade comprised of mainly chicken genotypes is shown in Fig. 3.

Table 2. Molecular prevalence, identified genotypes and GenBank accession number of *T. gallinarum*

Tetratrichomonas gallinarum					
Location	No. of samples	No. of positive	Prevalence (%)	Identified genotypes	GenBank Acc. No.
Kirsehir	100	11	11	TRTgal1 (n=4)	OP379566
				TRTgal2 (n=3)	OP379567
				TRTgal3 (n=4)	OP379568

Figure 3. Phylogenetic relationships between *T. gallinarum* genotypes obtained in this study (in red) and known genotypes previously reported from different countries and hosts. The tree was constructed using Maximum Likelihood analyses of 18S rRNA gene partial sequences. Numbers at the branches indicate bootstrap values (1000 replicates). The *Blastocystis* sp. genotype identified from chicken (MW093219) is used as an out group

In phylogenetic analysis, the TRTgal1 and TRTgal3 genotypes differed from the subgroup E (AY245113) in 4 and 17 nucleotides, respectively, and TRTgal2 genotype is identical to subgroup B1 (AY245111) of *T. gallinarum* reported by Cepicka et al. (2005).

Discussion

Backyard chickens are often found in outdoors where biosecurity is poor, and they have close contact with each other, wild birds, other animals (especially turkeys), and humans. This makes them susceptible to sources of infection. In this study, the molecular prevalence of *H. meleagridis, He. gallinarum*, and *T. gallinarum* was investigated in backyard chickens and laying hens. We were unable to detect *H. meleagridis* and its intermediate host *He. gallinarum* in any of the specimens. In contrast, some reports of histomoniasis in chickens have been published in different countries. (Patra et al., 2013; van der Heijden and Landman 2011; Grafl et al., 2011; Nguyenet al., 2015). Das et al. (2021) reported in an experimental study that an average of one hundred histomonads were excreted from 1 g of feces and three to four thousand histomonads were excreted within 24 hours from chickens infected with *H. meleagridis*. In the same study, the average daily egg excretion varied from five hundred to two thousand from a bird infected with *He. gallinarum*. Grabensteiner and Hess (2006) investigated PCR specificity for identification of *H. meleagridis* and *T. gallinarum* in tissue samples from infected birds and reported that PCR assays (using the same primers as in our study) demonstrated to be a useful and sensitive diagnostic tool for identification of *H. meleagridis* and *T. gallinarum*. In this context, it can be presumed that the studied chickens were not infected with *H. meleagridis* and *He. gallinarum*.

Cepicka et al. (2005) analyzed 29 isolates of *T. gallinarum* sequences and divided them into five groups (A-E) with eleven subgroups and suggested that groups A and B predominate in domestic fowls. In addition, human tetratrichomonads were assigned to groups A and B, hypothesizing that infections in humans are transmitted by avian genotypes or host-specific genotypes. In this study, the TRTgal2 genotype (OP379567) was found to be identical to the human genotype (AY247746) and the avian genotypes including the group B. In this perspective, it can be suggested that human tetratrichomoniasis was originate from avian genotypes. However, this assumption needs to be supported by the detection of more common genotypes. The TRTgal1 and TRTgal3 genotypes were exhibited highest identities of 96.9% to 99.1% to the genotypes reported in chicken in Czechia, China, Iran, and Philippines. This similarity is probably due to high gene flow with migratory hosts or neglected submission of sequences to genetic databases and low sampling. Novel genotypes are likely to be identified with studies that include new and large numbers of samples.

In conclusion, our results contribute to the molecular characterization and transmission dynamics of *T. gallinarum*. Moreover, to our knowledge, this is the first report on molecular characterization of *T. gallinarum* genotypes in chickens in Türkiye. Considering the zoonotic potential of *T. gallinarum*, chickens could pose a risk for public health. To better understand the molecular epidemiology, zoonotic potential, and transmission dynamics of *T. gallinarum*, further studies in a larger number of species with large-scale sampling are required.

References

Amin, A., Liebhart, D., Weissenböck, H., & Hess, M., (2011). Experimental infection of turkeys and chickens with a clonal strain of *Tetratrichomonas gallinarum* induces a latent infection in the absence of clinical signs and lesions. *Journal of comparative pathology, 144(1)*, 55-62. https://doi.org/10.1016/j.jcpa.2010.06.002

Burns, R. E., Braun, J., Armién, A. G., & Rideout, B. A., (2013). Hepatitis and splenitis due to systemic tetratrichomoniasis in an American white pelican (*Pelecanus erythrorhynchos*). *Journal of Veterinary Diagnostic Investigation, 25(4)*, 511-514. DOI: 10.1177/1040638713488368

Cupo, K. L., & Beckstead, R. B., (2019). *Heterakis gallinarum*, the cecal nematode of gallinaceous birds: a critical review. *Avian Diseases, 63(3),* 381-388. https://doi.org/10.1637/0005-2086-63.3.381

Cepicka, I., Kutišová, K., Tachezy, J., Kulda, J., & Flegr, J., (2005). Cryptic species within the *Tetratrichomonas gallinarum* species complex revealed by molecular polymorphism. *Veterinary Parasitology, 128(1-2),* 11-21. https://doi.org/10.1016/j.vetpar.2004.11.003

Cepicka, I., Hampl, V., & Kulda, J. (2010). Critical taxonomic revision of parabasalids with description of one new genus and three new species. *Protist*, *161(3)*, 400-433. https://doi.org/10.1016/j.protis.2009.11.005

Daş, G., Wachter, L., Stehr, M., Bilic, I., Grafl, B., Wernsdorf, P., & Liebhart, D. (2021). Excretion of *Histomonas meleagridis* following experimental co-infection of distinct chicken lines with *Heterakis gallinarum* and *Ascaridia galli*. *Parasites & Vectors*, *14(1)*, 1-15.

Delgado-Viscogliosi, P., Viscogliosi, E., Gerbod, D., Kulda, J., Sogin, M. L., Edgcomb, V. P. (2000). Molecular phytogeny of parabasalids based on small subunit rRNA sequences, with emphasis on the Trichomonadinae subfamily. *Journal of Eukaryotic Microbiology, 47(1)*, 70-75. https://doi.org/10.1111/j.1550-7408.2000.tb00013.x

Dimasuay, K., G., B., Rivera, W., L., (2013). Molecular characterization of trichomonads isolated from animal hosts in the Philippines. *Veterinary Parasitology*, *196(3-4),* 289-295.

Esquenet, C., De Herdt, P., De Bosschere, H., Ronsmans, S., Ducatelle, R., Van Erum, J., (2003). An outbreak of histomoniasis in freerange layer hens. *Avian Pathology, 32*, 305–308. https://doi.org/10.1080/0307945031000097903

Galarneau, K. D., Singer, R. S., Wills, R. W., (2020). A system dynamics model for disease management in poultry production. *Poultry science, 99(11)*, 5547-5559. https://doi.org/10.1016/j.psj.2020.08.011

Grabensteiner, E., Hess, M., (2006). PCR for the identification and differentiation of *Histomonas meleagridis, Tetratrichomonas gallinarum* and *Blastocystis* spp. *Veterinary Parasitology, 142(3-4)*. https://doi.org/10.1016/j.vetpar.2006.07.011

Grabensteiner, E., Bilic, I., Kolbe, T., & Hess, M. (2010). Molecular analysis of clonal trichomonad isolates indicate the existence of heterogenic species present in different birds and within the same host. *Veterinary Parasitology, 172(1-2)*, 53-64. https://doi.org/10.1016/j.vetpar.2010.04.015

Grafl, B., Liebhart, D., Windisch, M., Ibesich, C., & Hess, M., (2011). Seroprevalence of *Histomonas meleagridis* in pullets and laying hens determined by ELISA. *Veterinary record*, *168(6),* 160-160. https://doi.org/10.1136/vr.c6479

Gu, X. B., Wang, B. J., Zhao, X. B., Li, Y. F., Yang, G. Y., Lai, W. M., ... & Peng, G. N., (2018). Genetic variation in mitochondrial cox2 of *Heterakis gallinarum* from poultry in Sichuan, China. *Mitochondrial DNA Part A, 29(4),* 629-634. https://doi.org/10.1080/24701394.2017.1334771

Hu, J., Fuller, L., Armstrong, P. L., McDougald, L. R., (2006). *Histomonas meleagridis* in chickens: attempted transmission in the absence of vectors. *Avian Diseases, 50(2)*, 277-279. https://doi.org/10.1637/7431-090605R.1

Hu, J., L. R., (2003) McDougald. Direct lateral transmission of *Histomonas meleagridis* in turkeys. *Avian Dis. 47*:489–492. https://doi.org/10.1637/0005-2086(2003)047[0489:DLTOHM]2.0.CO;2

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution, 35(6),* 1547.

Landman, W. J. M., Molenaar, R. J., Cian, A., Van der Heijden, H. M. J. F., Viscogliosi, E. (2016). Granuloma disease in flocks of productive layers caused by *Tetratrichomonas gallinarum*. *Avian Pathology, 45(4),* 465-477. https://doi.org/10.1080/03079457.2016.1163325

McDougald, L.R,. (2020). Internal Parasites. In Diseases of Poultry (eds D.E. Swayne, M. Boulianne, C.M. Logue, L.R. McDougald, V. Nair, D.L. Suarez, S. Wit, T. Grimes, D. Johnson, M. Kromm, T.Y. Prajitno, I. Rubinoff and G. Zavala).

Marangon, S., Busani, L., (2007). The use of vaccination in poultry production. *Revue Scientifique et Technique-Office International des Epizooties, 26(1),* 265.

Nguyen, D. T., Bilic, I., Jaskulska, B., Hess, M., Le, D. Q., Le Hua, L. N., ... & Vu-Khac, H. (2015). Prevalence and genetic characterization of *Histomonas meleagridis* in chickens in Vietnam. *Avian diseases, 59(2),* 309-314. https://doi.org/10.1637/10964-102414-Reg

Patra, G., Prasad, H., Lalsiamthara, J., Kataria, J., Malsawmkima, D., & Lalrinkima, H., (2013). Prevalence of *Histomonas meleagridis* in broiler chicken in different parts of Mizoram, India. *International Journal of Poultry Science, 12*, 98-101.

Patton, C. S., & Patton, S., (1996). *Tetratrichomonas gallinarum* encephalitis in a mockingbird (*Mimus polyglottos*). *Journal of Veterinary Diagnostic Investigation, 8(1),* 133-137.

Posada, D., (2008). jModelTest: phylogenetic model averaging. *Molecular biology and evolution, 25(7)*, 1253-1256. https://doi.org/10.1093/molbev/msn083

van der Heijden, H. M., & Landman, W. J., (2011). High seroprevalence of *Histomonas meleagridis* in Dutch layer chickens. *Avian Diseases, 55(2),* 324-327. https://doi.org/10.1637/9609-120610-ResNote.1

Wichmann, R. W., Bankowski, R. A., (1956). A report of *Trichomonas gallinarum* infection in chukar partridges (*Alectoris graeca*). The Cornell Veterinarian, 46(3), 367-369.

Poultry Studies, 20(1), 19-24
http://doi.org/10.34233/jpr.1302523

PROOF

RESEARCH PAPER

Effects of Different Viol Types on Egg Qualities in Table Eggs at Different Storage Temperatures

Zeynel Abidin PARMAK[1], Ali AYGÜN[1,*]

[1] Faculty of Agriculture, Department of Animal Science, Selcuk University, 42250, Konya, Turkey

Article History
Received: 14 Apr 2023
Accepted: 11 May 2023
First Online: 23 May 2023

***Corresponding Author**
Tel: +90 505 5470821
E-mail: aaygun@selcuk.edu.tr

Keywords
Cardboard viol
Plastic box viol
Cardboard box viol
Shell strength
Haugh unit

Abstract

The purpose of this research was to examine the impact of storing table hen eggs in egg cardboard viol, plastic box viol, and cardboard box viols at various temperatures on the egg qualities. A total of 300 table chicken eggs were used in the study. The eggs were randomly distributed into three groups: standard cardboard viol, plastic box viol, and cardboard box viol. The eggs were stored at room temperature and in the refrigerator for 28 days. On the 0, 7, 14, 21, and 28 days of the experiment, ten eggs from each group were analyzed for egg weight loss, egg shell strength, Haugh unit, yolk index, and albumen pH. Viol type x storage temperature interaction effect was significant ($P < 0.05$) only on egg weight loss, but its effect on other properties was insignificant. After 28 days of storage, egg weight loss was statistically insignificant between eggs stored in plastic box viols at 25℃ and eggs stored in cardboard viols at 4℃. The effect of viol types on egg shell strength, yolk index, Haugh unit, and albumen pH was insignificant. Egg weight loss was found to be better in plastic box viols than in other groups ($P < 0.05$). Eggs stored in refrigerator conditions are better than egg stored room conditions in terms of egg quality criteria except eggshell strength ($P < 0.05$). As a result, it would be more appropriate to store eggs in plastic box viols in order to preserve egg quality for a longer period of time.

Introduction

Although eggs are an animal protein source, their importance in human nutrition is high due to containing unsaturated fatty acids, vitamins, and minerals (Şenköylü 2001; Sarıca and Erensayın 2014; Puglisi and Fernandez 2022; Tian *et al.,* 2022). An egg obtained from a healthy animal has the highest quality value at the moment it is laid, but its quality may decrease depending on storage conditions, leading to marketing problems (Aygun 2017; Brasil *et al.,* 2019; Yenilmez and Bulancak 2020; Sariyel *et al.,* 2022).

Depending on the storage conditions, it is possible to observe a decrease in egg weight (Akyurek and Okur 2009; Sariyel *et al.,* 2022), a decrease in albumen height and Haugh unit (Aktan and Kampus 2011; Baylan *et al.,* 2011) , an increase in egg yolk index (Copur *et al.,* 2008), and an increase in albumen pH (Silversides and Budgell 2004). When eggs are collected from egg production companies, they need to be stored appropriately.

In the market, cardboard viols with a capacity of 30 eggs are used for storing or transporting eggs. However, in recent years, cardboard box viol and plastic box viol with a capacity of 6, 10, and 15 eggs have also started to be used. The use of cardboard box viol with a capacity of 6, 10, and 15 eggs during the sale of eggs makes it difficult for consumers to choose eggs

based on their outer appearance, as the dirt on the surface of the eggshell cannot be seen. On the other hand, plastic viols provide convenience for consumers in terms of choice. However, foreign materials on the surface of eggs in plastic box viol can be seen more easily, making it easier to evaluate the eggs based on their appearance. Additionally, during storage or sales, there is a possibility of condensation on the egg surface or inside the viols, which can lead to the eggs becoming wet and causing the preference for viols more important.

In our literature review, it was observed that there are a limited number of studies on the effect of storing table chicken eggs in cardboard box viols or plastic box viols (with a capacity of 6, 10, and 15 eggs) on egg quality. Therefore, the main question of the research is which tray type can preserve egg quality for a longer period of time during storage under different storage conditions. Therefore, the aim of this study is to investigate the changes in egg quality by storing table chicken eggs in carton, cardboard box viol, and plastic box viols under room and refrigerator conditions.

Materials and Methods

Table eggs that were purchased daily from the Konya commercial egg producer were used in the study. A total of 300 table eggs were used. The research was carried out in the Egg Quality Laboratory of the Department of Animal Science, Faculty of Agriculture, Selcuk University. Eggs were randomly distributed into 3 groups: cardboard viol, plastic box viol, and cardboard box viol. For 28 days, eggs were stored at ambient temperature (25 ℃) and in the refrigerator (4 ℃) in viols. On days 0, 7, 14, 21, and 28 of the experiment, egg weight loss, eggshell strength, yolk index, Haugh unit, and albumen pH analyses were conducted on 10 eggs from each group. All eggs were weighed before the experiment and written on the egg. Egg weights were weighed with a precision digital balance (0.01 g). In the specified analysis periods, eggs (10 eggs from each group) were weighed again before the analysis, and egg weight loss was determined with the formula below.

Egg weight loss (%) = [initial egg weight (g) – end of storage egg weight (g)] / initial egg weight (g) x 100

Shell breaking strength was measured with the Egg Force Reader device (Orka Food Tech.i China) and its unit was expressed as kg (Sariyel *et al.,* 2022). After the albumen height was determined with the Egg Analyzer (Orka Food Tech.i China) device, the Haugh unit was determined according to Haugh (1937).

The egg yolk was separated on a flat surface and the height of the egg yolk was determined with a digital height gauge. Egg yolk diameter was determined with a digital micrometer and calculated according to Funk (1948). After separating the egg albumen from the yolk, it was measured with a pH meter (Mettler Toledo, Switzerland).

The experiment was carried out in 2x3 randomized plots according to a factorial design in order to compare the qualities of eggs packed in plastic, cardboard, and standard viols to be stored at room temperature and in the refrigerator (Düzgüneş *et al.,* 1987). The MINITAB 16 statistic software was used in the analysis, and the Tukey multiple range test was used to determine the different groups. The significance level of 0.05 was used for statistical evaluation.

Results and Discussion

Table 1 summarizes the effects of storage temperature, viol types, and the storage temperature x viol type interaction on egg weight loss (%). During storage, storage temperature, viol types, and storage temperature x viol types interaction had a significant effect on egg weight loss. At the end of storage, the lowest weight loss was found in the C group (6.94%) stored at 25 ℃, and the lowest egg weight loss was found in the CB group (2.20%) at 4 ℃. After 28 days of storage, egg weight loss was statistically insignificant between eggs stored in plastic box viols at 25°C and eggs stored in cardboard viols at 4℃. When the effect of the viol type on the egg weight loss was examined, the lowest egg weight loss was detected in the PL group during the storage period.

When the effect of storage temperature on egg weight loss was examined, the weight loss of eggs stored at 4 ℃ during storage was found to be lower than that of those stored at 25 ℃. Egg weight loss during storage occurs as a result of the evaporation of water from the egg (Drabik *et al.,* 2021; Eroglu *et al.,* 2021). Egg weight loss is desired to be minimal during storage. The most important criterion around the classification of eggs is egg weight. In general, the market shelf life of eggs around the world is 28 days. Egg weight loss gains even more importance as eggs are sold in markets in room conditions in Turkey. According to the result obtained from our study, it would be more appropriate to store eggs in plastic viols when it is necessary to store eggs in room conditions in terms of egg weight loss. Table 2 summarizes the effects of storage temperature, viol types, and the storage temperature x viol type interaction on egg shell strength (kg).

Viol type, storage temperature, and the storage temperature x viol type interaction effect were not significant in any period of storage. It is consistent with the research indicating that the eggshell strength during storage is not significantly impacted by the storage temperature (Jo *et al.,* 2011; Sert *et al.,* 2011; Sariyel *et al.,* 2022). For egg producers, the strength of a table egg's shell is a crucial economic factor. The greater the breaking strength, the less breakage will occur during egg collection, transport, and storage, resulting in reduced economic losses.

Table 1. The effect of storage temperature, viol types and storage x viol type interaction on egg weight loss (%)

Treatment		Initial Egg Weight	Egg Weight Loss (%)			
			7 days	14 days	21 days	28 days
Storage Temperature (℃)	25	63.46	1.44	2.66	3.97	5.86
	4	61.75	0.64	1.28	1.84	2.62
	SEM	0.93	0.33	0.07	0.12	0.14
	P value	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Viol type[1]	C	61.98	1.31^{a}	2.39^{a}	3.66^{a}	4.99^{a}
	CB	62.84	1.16^{b}	2.08^{b}	3.01^{b}	4.51^{a}
	PL	63.01	0.65^{c}	1.45^{c}	2.04^{c}	3.23^{b}
	SEM	1.14	0.04	0.08	0.14	0.17
	P value	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Storage Temperature (℃) x Viol Type	25 x C	62.11	1.66^{a}	3.02^{a}	4.92^{a}	6.94^{a}
	25 x CB	63.05	1.74^{a}	3.15^{a}	4.28^{a}	6.83^{a}
	25 x PL	65.24	0.93^{b}	1.80^{b}	2.70^{b}	3.81^{b}
	4 x C	61.83	0.95^{b}	1.75^{b}	2.39^{bc}	3.03^{bc}
	4 x CB	62.61	0.58^{c}	1.00^{c}	1.74^{cd}	2.20^{c}
	4 x PL	60.77	0.37^{c}	1.09^{c}	1.37^{d}	2.65^{c}
	SEM	1.61	0.05	0.12	0.21	0.24
	P value	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 2. The effect of storage temperature, viol types and storage x viol type interaction on egg shell strength (kg)

		Initial Egg Shell Strength (kg)	Egg Shell Strength (kg)			
			7 days	14 days	21 days	28 days
Storage Temperature (℃)	25	4.45	4.39	4.27	4.48	4.61
	4	4.42	4.60	4.46	4.56	4.58
	SEM	0.106	0.09	0.09	0.11	0.08
	P value	0.866	> 0.05	> 0.05	> 0.05	> 0.05
Viol type[1]	C	4.45	4.41	4.48	4.32	4.69
	CB	4.37	4.47	4.37	4.67	4.62
	PL	4.48	4.61	4.25	4.56	4.48
	SEM	0.130	0.11	0.11	0.13	0.11
	P value	0.822	> 0.05	> 0.05	> 0.05	> 0.05
Storage Temperature (℃) x Viol Type	25 x C	4.56	4.45	4.48	4.26	4.67
	25 x CB	4.41	4.38	4.18	4.38	4.63
	25 x PL	4.37	4.34	4.16	4.79	4.53
	4 x C	4.35	4.38	4.47	4.38	4.72
	4 x CB	4.33	4.56	4.54	4.95	4.61
	4 x PL	4.59	4.88	4.34	4.32	4.42
	SEM	0.18	0.16	0.16	0.19	0.15
	P value	0.481	> 0.05	> 0.05	> 0.05	> 0.05

[1]C: Cardboard viol; CB: Cardboard box viol; PL: Plastic box viol; SEM: Mean of standard errors

Table 3 summarizes the effects of storage temperature, viol types, and the storage temperature x viol type interaction on the Haugh unit. The storage temperature x viol type interaction effect did not have a significant effect on the Haugh unit during storage. The effect of viol type on the Haugh unit was only significant on day 21 of storage (P<0.05). On the 21st day of storage, the Haugh unit of the eggs in the PL group (74.12) was higher than the Haugh unit of the eggs in the C group (67.24) (P<0.05), but the difference between the Haugh unit of the eggs in the PL group (74.12) and the Haugh unit of the eggs in the CB group (70.96) was statistically insignificant.

The effect of storage temperature on the Haugh unit in all periods was found to be statistically significant. The effect of storage temperature on the Haugh unit in all periods was found to be statistically significant.

It was determined that the Haugh unit value of eggs stored at 4 ℃ during storage was higher than that of those stored at 25 ℃. In the egg industry, the Haugh unit is the main criterion for quality, and the greater the Haugh unit, the higher the albumen quality of the egg. During storage, the Haugh unit decreases due to the increase in clusterin and ovoinhibitory concentrations in albumen and the disordering of ovalbumin structure (Sheng *et al.,* 2018).

Table 4 summarizes the effects of storage temperature, viol types, and the storage temperature x viol type interaction on the yolk index. The effect of storage temperature x viol type interaction on the yolk index of eggs was significant only on day 28 of the storage period (P<0.05). On the 28th day of storage, the lowest yolk index value was found in group C eggs stored at 25℃, and the highest yolk index value in group C eggs stored at 4°C (P<0.05).

Viol type did not have a significant effect on the yolk index value in all periods of storage. The effect of storage temperature on the yolk index in all periods was found to be statistically significant. It was determined that the yolk index value of eggs stored at 4 ℃ during storage was higher than those stored at 25 ℃. This finding appears to be consistent with research that demonstrates that as storage temperatures increase, the yolk index dramatically declines (Samli *et al.,* 2005; Akarca *et al.,* 2021; Güler *et al.,* 2022; Sariyel *et al.,* 2022). The egg yolk index, which assesses the resistance to stretching of the vitelline membrane in the yolk, can be used to determine how fresh an egg is. The quality of the egg yolks improves as the egg yolk index increases (Stadelman 1995). The egg yolk index drops as a result of the water in the egg white diffusing into the egg yolk as the vitelline membrane of the egg yolk deteriorates (Didar 2019). Table 5 summarizes the effects of storage temperature, viol types, and the storage temperature x viol type interaction on albumen pH. Storage temperature x viol type interaction and viol type did not have a significant effect on the albumen pH in all periods of storage. The effect of storage temperature on the albumen pH in all periods was found to be statistically significant. It was determined that the albumen pH of eggs stored at 4 ℃ during storage was lower than that of those stored at 25 ℃. This result is consistent with the studies stating that the pH value of egg whites also increased due to the increase in storage temperature (Sariyel *et al.,* 2022; Tan *et al.,* 2022). Albumen's pH increases as a result of a shift in the carbonic acid-bicarbonate buffer system caused by the release of carbon dioxide from the eggshell's pores during storage (Shin *et al.,* 2012; Mathew *et al.,* 2016).

Table 3. The effect of storage temperature, viol types and the storage temperature x viol type interaction on Haugh unit

Treatment		Initial Haugh Unit	Haugh Unit			
			7 days	14 days	21 days	28 days
Storage Temperature (℃)	25	83.06	71.29	63.84	62.02	55.94
	4	83.35	81.87	84.24	79.53	77.00
	SEM	1.07	1.005	0.991	1.263	1.067
	P value	0.849	< 0.05	< 0.05	< 0.05	< 0.05
Viol type[1]	C	81.80	76.07	71.9	67.24[b]	65.27
	CB	83.20	76.55	75.37	70.96[ab]	67.28
	PL	84.60	77.10	74.78	74.11[a]	66.84
	SEM	1.31	1.22	1.21	1.54	1.31
	P value	0.334	> 0.05	> 0.05	< 0.05	> 0.05
Storage Temperature (℃) x Viol Type	25 x C	82.49	72.39	61.03	58.30	53.91
	25 x CB	81.91	69.82	64.77	63.84	57.92
	25 x PL	84.79	71.66	65.72	63.91	56.00
	4 x C	81.13	79.77	82.91	76.19	76.63
	4 x CB	84.51	83.31	85.96	78.06	76.68
	4 x PL	84.43	82.54	83.84	84.31	77.72
	SEM	1.85	1.74	1.72	2.19	1.85
	P value	0.543	> 0.05	> 0.05	> 0.05	> 0.05

Table 4. The effect of storage temperature, viol types and storage x viol type interaction on yolk index

Treatment		Initial Yolk Index	Yolk Index			
			7 days	14 days	21 days	28 days
Storage Temperature (℃)	25	0.44	0.38	0.33	0.27	0.20
	4	0.42	0.45	0.45	0.43	0.41
	SEM	0.01	0.010	0.010	0.010	0.010
	P value	0.068	< 0.05	< 0.05	< 0.05	< 0.05
Viol type[1]	C	0.42	0.41	0.40	0.34	0.32
	CB	0.44	0.41	0.39	0.35	0.30
	PL	0.43	0.42	0.39	0.36	0.29
	SEM	0.01	0.01	0.01	0.01	0.01
	P value	0.365	> 0.05	> 0.05	> 0.05	> 0.05
Storage Temperature (℃) x Viol Type	25 x C	0.43	0.37	0.34	0.26	0.19[c]
	25 x CB	0.44	0.37	0.32	0.26	0.20[c]
	25 x PL	0.45	0.39	0.33	0.27	0.21[c]
	4 x C	0.41	0.46	0.46	0.41	0.46[a]
	4 x CB	0.44	0.46	0.46	0.43	0.40[ab]
	4 x PL	0.41	0.45	0.44	0.44	0.37[b]
	SEM	0.01	0.02	0.01	0.01	0.02
	P value	0.442	> 0.05	> 0.05	> 0.05	< 0.05

[a-c] Significant differences exist between the means of a column using different superscripts (P<0.05); [1]C: Cardboard viol; CB: Cardboard box viol; PL: Plastic box viol; SEM: Mean of standard errors

Table 5. The effect of storage temperature, viol types and storage x viol type interaction on albumen pH

Treatment		Initial Albumen pH	Albumen pH			
			7 days	14 days	21 days	28 days
Storage Temperature (℃)	25	9.08	9.59	9.75	9.63	9.77
	4	9.10	9.16	9.21	9.12	9.35
	SEM	0.04	0.01	0.01	0.02	0.01
	P value	0.731	< 0.05	< 0.05	< 0.05	< 0.05
Viol type[1]	C	9.07	9.37	9.47	9.40	9.57
	CB	9.14	9.41	9.47	9.36	9.57
	PL	9.06	9.34	9.48	9.36	9.55
	SEM	0.05	0.02	0.01	0.02	0.01
	P value	0.538	> 0.05	> 0.05	> 0.05	> 0.05
Storage Temperature (℃) x Viol Type	25 x C	9.09	9.56 [a]	9.74	9.64	9.77
	25 x CB	9.07	9.63 [a]	9.75	9.64	9.78
	25 x PL	9.08	9.59 [a]	9.76	9.60	9.74
	4 x C	9.04	9.19 [b]	9.21	9.16	9.36
	4 x CB	9.20	9.18 [b]	9.19	9.09	9.35
	4 x PL	9.06	9.10 [b]	9.23	9.10	9.33
	SEM	0.07	0.03	0.02	0.03	0.02
	P value	0.399	< 0.05	> 0.05	> 0.05	> 0.05

[1]C: Cardboard viol; CB: Cardboard box viol; PL: Plastic box viol; SEM: Mean of standard errors

Conclusions

According to the results obtained from our study, it was determined that the type of viol affects only egg weight loss. In terms of egg weight, it was observed that storage in plastic box viol preserves egg weight better than other viol types. It is seen that storing eggs in plastic box viols will preserve their shelf life longer.

Acknowledgments

This research is a summary of Zeynel Abdin Parmak's master's thesis, which was funded by Selcuk University (project number: 18201140).

References

Akarca, G., Istek, Ö., Tomar, O. 2021. The Effect of Resin Coating on the Quality Characteristics of Chicken Eggs During Storage. Journal of Food Science, *86*(4): 1243-1257.

Aktan, S., Kampus, D. 2011. Effects of Age and Storage Duration on Relationships among Albumen Quality Traits and Egg Weight in Japanese Quails. Journal of Animal and Veterinary Advances, *10 (Supplement)*: 3340 - 3344.

Akyurek, H., Okur, A. A. 2009. Effect of Storage Time, Temperature and Hen Age on Egg Quality in Free-Range Layer Hens. Journal of Animal and Veterinary Advances, *8*(10): 1953-1958.

Aygun, A. (2017). The Eggshell Microbial Activity. In P. Hester (Ed.), *Egg Innovation and Strategies for Improvement* (pp. 135-144). Academic Press, Elsevier.

Baylan, M., Canogullari, S., Ayasan, T., Copur, G. 2011. Effects of Dietary Selenium Source, Storage Time, and Temperature on the Quality of Quail Eggs. Biological Trace Element Research, *143*(2): 957-964.

Brasil, R., Cruz, F., Rufino, J., Oliveira, P., Freitas, B., Viana, G. 2019. Physical-Chemical and Sensorial Quality of Eggs Coated with Copaiba Oil Biofilm and Stored at Room Temperature for Different Periods. Brazilian Journal of Poultry Science, *21*.

Copur, G., Camcil, O., Sahinler, N., Gul, A. 2008. The Effect of Propolis Egg Shell Coatings on Interior Egg Quality. Archiv Fur Geflugelkunde, *72*(1): 35-40.

Didar, Z. 2019. Effects of Coatings with Pectin and Cinnamomum Verum hydrosol included Pectin on Physical Characteristics and Shelf Life of Chicken Eggs Stored at 30°C. Nutrition and Food Sciences Research, *6*(4): 39-45.

Drabik, K., Próchniak, T., Kasperek, K., Batkowska, J. 2021. The Use of the Dynamics of Changes in Table Eggs During Storage to Predict the Age of Eggs Based on Selected Quality Traits. Animals, *11*(11): 3192.

Düzgüneş, O., Kesici, T., Kavuncu, O. 1987. Ve Gürbüz, F. 1987. Araştırma ve Deneme Metodları.

Eroglu, M., Baykalir, Y., Erisir, Z. 2021. The Effect of Different Storage Period on Some Egg Quality Characteristics and Ovalbumin Levels in Goose Eggs. Emirates Journal of Food and Agriculture, *33*(8): 699-703.

Funk, E. 1948. The Relation of the Yolk Index Determined in Natural Position to the Yolk Index as Determined after Separating the Yolk from the Albumen. Poultry science, *27*(3): 367-367.

Güler, G., Ayşe, Ş., Turgud, F. K., Tahtabiçen, E., Aylin, A., Samli, H. E. 2022. Effects of Propolis Extract Coating on Shelf Life and Quality Parameters of Eggs Obtained from Free-Range Hens. Journal of Tekirdag Agricultural Faculty, *19*(1): 89-100.

Haugh, R. R. 1937. The Haugh Unit for Measuring Egg Quality. The US Egg Poultry Magazine, *43*: 522-573.

Jo, C., Ahn, D., Liu, X., Kim, K., Nam, K.-C. 2011. Effects of Chitosan Coating and Storage with Dry Ice on the Freshness and Quality of Eggs. Poultry science, *90*(2): 467-472.

Mathew, A. O., Olufemi, A. M., Foluke, A., Abel, O. 2016. Relationship of Temperature and Length of Storage on Ph of Internal Contents of Chicken Table Egg in Humid Tropics. Biotechnology in Animal Husbandry, *32*(3): 285-296.

Puglisi, M. J., Fernandez, M. L. 2022. The Health Benefits of Egg Protein. Nutrients, *14*(14): 2904.

Samli, H., Agma, A., Senkoylu, N. 2005. Effects of Storage Time and Temperature on Egg Quality in Old Laying Hens. Journal of Applied Poultry Research, *14*(3): 548-553.

Sarıca, M., Erensayın, C. (2014). Tavukçuluk Ürünleri. In M. Türkoğlu M. Sarıca (Eds.), Tavukçuluk Bilimi Yetiştirme, Besleme ve Hastalıklar (Vol. 4, pp. 110-139.). Bey Ofset Matbaacılık.

Sariyel, V., Aygun, A., Coklar, H., Narinc, D., Akbulut, M. 2022. Effects of Prestorage Application of Gum Arabic Coating on the Quality of Table Eggs During Storage. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, *28*(3): 363-370.

Sert, D., Aygun, A., Demir, M. 2011. Effects of Ultrasonic Treatment and Storage Temperature on Egg Quality. Poultry science, *90*(4): 869-875.

Sheng, L., Huang, M., Wang, J., Xu, Q., Hammad, H., Ma, M. 2018. A Study of Storage Impact on Ovalbumin Structure of Chicken Egg. Journal of Food Engineering, *219*: 1-7.

Shin, D., Narciso-Gaytán, C., Regenstein, J. M., Sánchez-Plata, M. X. 2012. Effect of Various Refrigeration Temperatures on Quality of Shell Eggs. Journal of the Science of Food and Agriculture, *92*(7): 1341-1345.

Silversides, F., Budgell, K. 2004. The Relationships among Measures of Egg Albumen Height, Ph, and Whipping Volume. Poultry science, *83*(10): 1619-1623.

Stadelman, W. (1995). The Preservation of Quality in Shell Eggs. In W. Stadelman O. J. Cotterill (Eds.), *Egg Science and Technology* (Vol. 4, pp. 67-79). Food Products Press.

Şenköylü, N. (2001). *Modern Tavuk Üretimi* (Vol. 3. Baskı). Anadolu Matbaacılık.

Tan, F., Rungruengpet, W., Simsiri, U., Kaewkot, C., Sun, Y., Chumngoen, W. 2022. Influences of Egg Washing and Storage Temperature on Quality and Shelf Life of Duck Eggs During Storage. Brazilian Journal of Poultry Science, *24*(4): 1-8.

Tian, Y., Zhu, H., Zhang, L., Chen, H. 2022. Consumer Preference for Nutritionally Fortified Eggs and Impact of Health Benefit Information. Foods, *11*(8): 1145.

Yenilmez, F., Bulancak, A. 2020. Microbiological Quality of Table Eggs Sold at Different Sales Location. Çukurova Tarım ve Gıda Bilimleri Dergisi, *35*(2): 115-124.

Poultry Studies, 20(1), 25-31
http://doi.org/10.34233/jpr.1314476

PROOF
RESEARCH PAPER

Relationships Between Morphological Characteristics of Roosters in Broiler Breeders at Different Age Periods

Saliha SABAH[1], Bilgehan YILMAZ DİKMEN[1, *]

[1]Bursa Uludağ University, Faculty of Agriculture, Animal Science Department, 16059 Bursa, Türkiye.

Article History
Received: 09 Jun 2023
Accepted: 12 Jun 2023
First Online: 14 Jun 2023

***Corresponding Author**
Tel: +902242941569
E-mail: bilgehan@uludag.edu.tr

Keywords
Morphological characteristics
Fertility
Age
Broiler breeder

Abstract

The aim of study was to determine the relationships between morphological characteristics of roosters and fertility in broiler breeders at different age periods. A total of 48 roosters and 480 hens Ross 308 broiler breeder were used. The body weight, comb width and length, wattle width and length, and tarsus length of roosters were measured for morphological characteristics at 42, and 53 weeks of age. A total of 1500 eggs were incubated at 37.7 °C and 50-55% relative humidity for 18 d, then eggs were incubated at 36.6 °C and 60% relative humidity for 3 d. The effect of age on comb length, comb index (P=0.002), wattle width (P=0.008), wattle area (P=0.015) and tarsus length were found significant (P=0.000). There were correlations between body weight and fertility at 53 weeks of age (r = 0.663; P<0.05). There was no correlation between fertility and comb length, comb width, comb area, comb index, wattle width, wattle area, wattle index, and tarsus length at 42 and 53 weeks of age (P>0.05). In conclusion; some characteristics based on external appearance are used for male selection in broiler breeders, but no relationship was found between investigated morphological characteristics and fertility, except for body weight.

Introduction

The fertility outcomes of broiler breeders are influenced by a variety of factors. Although both male and female breeders play important roles in fertility, genetic and non-genetic factors that occur in both males and females and are thought to be the most important factors influencing egg fertilization and embryo development. Especially, sperm motility, concentration, amount, and rate of abnormal and dead sperm are the main sperm characteristics that affect male fertility performance (Bansal and Cheema, 2014). In broiler breeders, roosters must be physically and behaviorally mature in order to be able to mate and successfully evoke the sexual response in females.

Fertility issues, particularly in lines descended from Cornish breeds can be brought on by an inability to mate successfully due to poor physical health and inappropriate sexual behavior (Wilson et al., 1979). Furthermore, the behavioral characteristics that allow roosters to successfully mate with chickens are dependent on the rooster's weight and leg health (Brillard, 2003). When it comes to mating behavior and optimum fertility, breeding males must be at the target body weight, should have the right leg lengths, and also should have a smooth leg structure without curled toes or abrasion problems on the sole (Anonymous, 2018). The length of the tarsus has a major influence on

fertility (Abad, 2020). Thus, tall males with a higher tarsus length may be more likely to win male-male competition, so they may have extra mating opportunities (Lei et al., 2019).

Secondary sexual characteristics in roosters are thought to play a role in increasing the frequency of mating with females. As a matter of fact, it was observed that females preferred males with large combs in case of Red Jungle Chicken (Zuk et al., 1995). If the secondary sexual characteristics of the roosters are fully developed, it indicates that they are healthier (Hamilton and Zuk, 1982), have better reproductive characteristics (Møller, 1994), and the relationship between fertility and testis weight is positive (McGary et al., 2002). Secondary sexual traits in poultry are regarded to be crucial in the selection of female mates. Comb size has been claimed to be an indicator of the amount of androgens in male's body (Verhulst et al., 1999). The exhibition of roosters has a phenotypic relationship with fertility (McGary et al., 2002), particularly during the early mating season females are attracted towards the roosters having the largest comb thus large combs may be associated to reproductive behavior in birds (Bilcik et al., 2005). It has been observed, for example, that roosters with larger combs exhibit more aggressive behavior and are more dominant against roosters with smaller combs (Bilcik et al., 2005; Mukhtar and Khan, 2012). However, in some studies; no significant link between the dimension of secondary sexual features, mating frequency, and sperm quality in predicting broiler breeder reproductive performance was reported (Wolanski et al., 2004; Bilcik et al., 2005). Roosters are often selected in breeding flocks based on characteristics such as posture, body structure, maturity, body size, leg and foot condition, head appearance, overall health and vigour. Although these traits aid in male selection, they do not guarantee a high fertility rate (Wilson et al., 1979).

In general, low-level fertility is an important problem in old aged broiler breeder flocks. Thus, this study was carried to investigate the relationships between morphological characteristics of roosters and fertility in broiler breeders at 42 and 53 weeks of age. Also, the correlations among the morphological characteristics were investigated.

Materials and Methods

This study was conducted in a private broiler breeder company. A total of 48 roosters and 480 hens Ross 308 broiler breeders were placed in a separate compartment in breeder house. The flock age was followed to obtain morphological characteristics and incubation parameters. In the house a chain type channel feeder, nipple drinker system and pine sawdust litter were used. The broiler breeders were fed with a standard broiler breeder feed containing (14.5% CP and 2750 ME) and water provided was ad libitum. The 13L: 11D light duration and 60 lux light intensity were applied according to broiler breeder standards.

All roosters© morphological characteristics such as; body weight, comb width and length, wattle width and length, and tarsus length were measured at 42 WOA (weeks of age), and then again measured at 53 WOA. The body weight of the roosters recorded by weighing 0.1 g with a precision balance then comb, wattle and tarsus measurements were taken from the same roosters. The comb area and wattle area were calculated according to Chung (2010); for this, the length and width of the comb, the length and width of the wattle were measured with a digital caliper from the left side of the roosters. The comb and wattle index were calculated according to Francesh et al. (2011). The length of the left tarsus bone was determined by measuring the distance between the knee joint and the sole of the foot with a digital caliper (Souza et al., 2017). The formulas used in the calculation of morphological characteristics are given below.

$$\text{Comb area} = \text{comb width} \times \text{comb height}$$
$$\text{Wattle area} = \text{wattle width} \times \text{wattle height}$$
$$\text{Comb index} = (\text{comb length}) / (\text{comb width})$$
$$\text{Wattle index} = (\text{wattle length}) / (\text{wattle width})$$

Eggs were obtained from separated experimental compartment at 42 WOA, then again obtained at 53 WOA. A total of 1500 eggs were incubated at each age period for fertility results. Eggs were stored at 18 ℃ temperature and 70-75% relative humidity (RH) for 5-7 d. Each tray in the incubator held 150 eggs, with 10 trays being used for each age. Eggs pre-warmed before incubation at 24 ℃ and 70-75% RH during 8 hours. Eggs were incubated in the setter at 37.7 ℃ and 50-55% RH for 18 d. Eggs were incubated in the hatcher at 36.6 ℃ and 60% RH for 3 d. At hatch chicks were counted and all unhatched eggs were examined for fertility and embryonic mortality. Then fertility was calculated.

The data were analyzed by using two sample T-test using the Minitab software (Minitab, 2013). After arcsine transformation, the percentage data analyses were carried out. The correlations between morphological characteristics and fertility were analyzed with Pearson correlation analysis (Minitab, 2013). Differences were considered significant at $P \leq 0.05$ and the statistical difference at $P < 0.10$ was described as a tendency.

Results and Discussion

The morphological characteristic of roosters and fertility in broiler breeders at 42 and 53 WOA are given in Table 1. In the study, the effect of age on

Table 1. The morphological characteristics of roosters and fertility in broiler breeders at different age (mean ± StDev)

Characteristics	42 WOA	53 WOA	P
Body weight, g	4693.9 ± 314	4737.5 ± 236	0.454
Comb length, mm	128.8 ± 9.74[b]	135.5 ± 7.35[a]	0.000
Comb width, mm	73.6 ± 7.42	73.1 ± 7.17	0.737
Comb area, mm^2	9535 ± 1618	9913 ± 1226	0.212
Comb index	1.76 ± 0.12[b]	1.87 ± 0.19[a]	0.002
Wattle length, mm	54.5 ± 5.91	52.3 ± 5.03	0.056
Wattle width, mm	54.9 ± 5.73[a]	52.1 ± 4.29[b]	0.008
Wattle area, mm^2	3030.9 ± 639[a]	2742.3 ± 462[b]	0.015
Wattle index	0.99 ± 0.03	1.00 ± 0.06	0.239
Tarsus length, mm	122.4 ± 6.57[a]	116.9 ± 3.89[b]	0.000
Fertility, %	96.07 ± 2.30	94.27 ± 2.27	0.096

[a,b]; Differences between means with different letters in the same column are significant (P<0.05). WOA: Weeks of age

comb length (P=0.000), comb index (P=0.002), wattle width (P=0.008), wattle area (P=0.015) and tarsus length were significant (P=0.000). But, effect of age on wattle length and fertility tend to be significant (P=0.056 and P=0.096; respectively). The effect of age on body weight, comb width, comb area, wattle index was not found significant (P>0.05).

The body weight is highly heritable and simple to determine (Groeneveld et al., 2010). Bird growth rates vary depending on age and species. In the study, body weight was not affected by age, but body weight at 53 WOA was numerically higher than 42 WOA. It was to be expected thus body weight is a trait affected by age (Hocking and Bernard, 1997). The results are in accordance with the Leão et al. (2017) who showed that body weight increased with age (25 to 45 weeks of age) in male broiler breeders. Meanwhile, Özdemir (2019) reported that an increase in bodyweight with age in both İspenç genotype roosters and chickens.

Along with the increase in age, comb length and comb index were increased. But the other comb characteristics such as, comb width and comb area were found similar in investigated age periods. However, Leão et al. (2017) revealed that there was no difference for histological comb score between the 25 and 45 WOA groups.

Along with the increase in age wattle width and wattle area decreased and, also wattle length tended to be lower at 53 WOA than in 42 WOA. But wattle index was found similar in investigated age periods. However, Nääs et al. (2008) reported that there was an increase in wattle width with increasing age in male broilers of Cobb 500. They also reported that there was no significant morphological difference found at 28 day of age, but at 35 and 48 day of age a difference in wattle width was found when compared to the other morphological characteristics. Also, Özdemir (2019) reported that a significant increase in wattle length, width and comb length, width, with the increased age in İspenç genotype roosters.

The tarsus length was found lower at 53 WOA than 42 WOA, and tarsus length was decreased with the increased age. A slight decrease in tarsus length was found at 53 WOA, it might be result of body weight getting heavier and deformation in the bones with age. But, Özdemir (2019) reported that there was no difference between tarsus lengths of İspenç roosters (small genotype) at different age periods.

In the study, fertility tend to be decreased with increased age. This finding in accordance with Yılmaz Dikmen and Şahan (2009) who reported that fertility rate decreased with increased age in broiler breeder. The decrease in fertility with increased age was also reported by some authors (Gumułka and Kapkowska, 2005; Abudabos, 2010; Mahammad et al., 2018).

The correlations between morphological characteristics of roosters and fertility in broiler breeders at different age are shown in Table 2. In the study, correlation between body weight and comb width (r = 0.265; P= 0.068) was found tend to be significant at 42 WOA, but there was no correlation between body weight and investigated comb characteristics at 53 WOA (P>0.05). According to these findings, there might be a slight relationship between body weight and comb characteristics at 42 WOA. Also negative correlations between body weight and comb index (r = -0.281; P=0.053) was found tend to be significant at 42 WOA. Kumar et al. (2022) in a study carried on Aseel and Kadaknath chickens at 24 week of age reported a correlation between body weight and comb length. Almost similar study was carried by Leão et al. (2017) that showed positive significant correlation between histological comb score and live weight. Also, Rizzi and Verdiglione (2015) reported that there was a correlation between comb and wattles weight and body weight in Italian purebreds of Robusta lionata. There were no significant correlations found between body weight and all investigated wattle characteristics, also tarsus length in investigated age periods (P>0.05). In a study, Dahloum et al. (2016) showed males having stronger

Table 2. Correlations between morphological traits of roosters and fertility in broiler breeders at different age

	WOA	CL	CW	CA	CI	WL	WW	WA	WI	TL	F
BW	42	0.131^{NS}	0.265$^{0.068}$	0.212^{NS}	-0.281$^{0.053}$	-0.049^{NS}	-0.099^{NS}	-0.062^{NS}	0.195^{NS}	-0.009^{NS}	0.097^{NS}
	53	0.242^{NS}	0.038^{NS}	0.136^{NS}	0.111^{NS}	0.125^{NS}	0.087^{NS}	0.107^{NS}	0.091^{NS}	0.018^{NS}	0.663*
CL	42		0.769****	0.926****	-0.040^{NS}	0.091^{NS}	0.077^{NS}	0.092^{NS}	0.076^{NS}	-0.211^{NS}	0.226^{NS}
	53		0.176^{NS}	0.603****	0.365*	0.542****	0.513***	0.571****	0.156^{NS}	0.102^{NS}	0.234^{NS}
CW	42			0.951****	-0.667****	0.091^{NS}	0.063^{NS}	0.081^{NS}	0.123^{NS}	-0.145^{NS}	0.038^{NS}
	53			0.890****	-0.848****	0.362*	0.213^{NS}	0.323*	0.288$^{0.065}$	-0.003^{NS}	-0.015^{NS}
CA	42				-0.409**	0.103^{NS}	0.078^{NS}	0.097^{NS}	0.116^{NS}	-0.174^{NS}	0.149^{NS}
	53				-0.518****	0.556****	0.405**	0.531****	0.329*	0.043^{NS}	0.103^{NS}
CI	42					-0.031^{NS}	-0.007^{NS}	-0.015^{NS}	-0.094^{NS}	-0.008^{NS}	0.194^{NS}
	53					-0.070^{NS}	0.042^{NS}	-0.027^{NS}	-0.173^{NS}	0.047^{NS}	0.288^{NS}
WL	42						0.972****	0.992****	0.268$^{0.065}$	0.339**	0.131^{NS}
	53						0.760****	0.943****	0.542 ****	0.049^{NS}	0.178^{NS}
WW	42							0.989****	0.034^{NS}	0.308*	0.160^{NS}
	53							0.928****	-0.133^{NS}	-0.008^{NS}	0.254^{NS}
WA	42								0.162^{NS}	0.310*	0.158^{NS}
	53								0.238^{NS}	0.043^{NS}	0.217^{NS}
WI	42									0.179^{NS}	-0.215^{NS}
	53									0.080^{NS}	-0.406^{NS}
TL	42										0.192^{NS}
	53										0.103^{NS}

Correlation coefficient values (r). *: P<0.05; **: P<0.01; ***: P<0.001; ****: P< 0.0001, NS: Not significant
WOA: Weeks of age; BW: Body weight, CL: Comb length, CW: Comb width, CA: Comb area, CI: Comb index,
WL: Wattle length, WW: Wattle width, WA: Wattle area, WI: Wattle index, TL: Tarsus length, F: Fertility

correlations between body length and either body weight, tarsus length, or comb height than females' indigenous chicken genotype. In both the Hubbard and Arboracre strains, a study by Yahaya et al. (2012) revealed a strong linear relationship between body weight and tarsus length. Another study conducted by Abdel-Latiff (2019) found that in White leghorn chickens, there was a highly significant correlation between body weight and shank length and also correlation with shank diameter. Also positive correlation between body weight and tarsus length was reported by Adeogun and Adeoye (2004). In the study, there was moderate positive correlation between body weight and fertility at 53 WOA ($r = 0.663$; $P<0.05$).

In the study correlations between comb length and comb width, comb area at 42 WOA ($r = 0.769$, $P<0.0001$; $r = 0.926$, $P<0.0001$); and comb area, comb index, wattle length, wattle width, wattle area at 53 WOA ($r = 0.603$, $P<0.0001$; $r = 0.365$, $P<0.05$; $r = 0.542$, $P<0.0001$; $r = 0.513$, $P<0.001$; $r = 0.571$, $P<0.0001$) found significant. Females preferred larger-combed roosters, especially during the initial mating season. Thus, males who attempted mating more frequently had larger combs (Bilcik et al., 2005). There were positive correlations between comb length and comb width, comb area at 42 WOA ($P<0.0001$). Thus, Joseph et al. (2003) reported that a significant correlation between comb height, length, area; and also significant correlation between investigated comb traits and age in female broiler breeders. There were positive correlations between comb length and comb area; comb index at 53 WOA ($P<0.0001$ and $P<0.05$). However, there were negative correlations between comb width and comb index ($P<0.0001$), comb area and comb index ($P<0.01$ and $P<0.0001$) at all investigated age periods.

The comb and wattle size are related to gonadal development and sex hormone secretion (Nesheim et al., 1979). According to Tabbaa and Hassanin (2018) a numerical difference in comb size frequencies among different breeds was observed however no significant difference was noticed. In the study, there were positive correlations between comb length and wattle length, width, area ($P<0.0001$; $P<0.001$; $P<0.0001$); also, there were positive correlations between comb width and wattle length, wattle area at 53 WOA ($P<0.05$). According to these findings, it could be said that comb characteristics and wattle characteristics were positively related to each other in broiler breeder roosters. However, there were no correlations between investigated comb characteristics and tarsus length in all investigated age periods ($P>0.05$). Rizzi and Verdiglione (2015) measured comb and wattle weights of male chickens from three different Italian purebreds (*Robusta maculate*, *Ermellinata di Rovigo* and *Robusta lionata*), the comb and wattle weight were significantly correlated in all purebreeds.

It has been observed, that roosters with larger combs exhibit more aggressive behavior and more dominant against roosters with smaller combs (Bilcik et al., 2005; Mukhtar and Khan, 2012). In the study, there were no significant correlation between investigated comb characteristics and fertility in investigated all age periods ($P>0.05$). Thus, Pizzari et al. (2004) reported that there was no relation between comb size and sperm quality in cross breeds of domestic fowl and red jungle fowl.

In the study there were positive correlations found between wattle length and wattle width, area; also, there were positive correlations between wattle width and wattle area in investigated all age periods ($P<0.0001$). It might be a result of investigated wattle characteristics were positively related to each other. There was no correlation between wattle width and wattle index; no correlation between wattle area and wattle index in investigated all age periods ($P>0.05$). The correlation between wattle length and wattle index was tend to be significant at 42 WOA ($P= 0.065$). The significant positive correlations were found between wattle width and wattle area at all investigated age periods ($P<0.0001$).

There were positive correlations between wattle length, width, area and tarsus length at 42 WOA ($P<0.01$, $P<0.05$, $P<0.05$; respectively). According to these findings, it could be said that some wattle characteristics such as; length, width, and tarsus length were positively related to each other in broiler breeder roosters. In the study, there were no correlation between investigated wattle characteristics and fertility in all investigated age periods ($P>0.05$). Similar to our findings Bilcik and Estevez (2005) reported that there was no correlation between wattle width and fertility in broiler breeders. However, McGary et al. (2003) reported that comb width, wattle length was positively related with sperm penetration and also fertility in male broiler breeder. Also, in the study, there were no correlation between tarsus length and fertility in all investigated age periods ($P>0.05$). These findings accordance with McGary et al. (2003) who measured that the leg and pelvic fluctuating asymmetry of male broiler breeder, and reported that there were no correlations between these traits and fertility or sperm penetration. Also, Yılmaz Dikmen and İpek (2006) reported that shank length had no discernible impact on fertility or hatchability in Japanese quails.

Conclusion

In conclusion morphological characteristics such as; comb length, index, wattle length, width, area and tarsus length were affected by breeder age. Although

some characteristics based on external appearance are used for male selection in broiler breeders, but no relationship was found between investigated morphological characteristics and fertility, except for body weight.

Highlights

The body weight, comb width and length, wattle width and length, and tarsus length of roosters were measured for morphological characteristics at 42, and then 53 weeks of age.

A total of 1500 eggs were incubated for fertility results at 42, and then 53 weeks of age.

Although some characteristics based on external appearance are used for male selection in broiler breeders, but no relationship was found between investigated morphological characteristics and fertility, except for body weight.

Ethical Statement

This study was approved by the Bursa Uludağ University Animal Experiments Local Ethics Committee (Approval no: 2019-12/06).

Acknowledgements

The authors would like to express their gratitude to the directors of the commercial breeder farm Hastavuk (Bursa, Türkiye), who provided the facilities for this study.

References

Abad, J.C. (2020). Management of males in broiler breeder operations. AviNews.com https://avinews.com/en/management-of-males-in-broiler-breeder-operations/

Abdel-Latiff, F.H. (2019). The linear association between live body weight and some body measurements in some chicken strains. Plant Archives, 19(1), 595-599.

Abudabos, A. (2010). The effect of broiler strain and parent flock age on hatchability and fertile Hatchability. International Journal of Poultry Science, 9(3), 231-235. https://doi.org/10.3923/ijps.2010.231.235

Adeogun, I.O., Adeoye, A.A. (2004). Heritabilities and phenotypic correlations of growth performance traits in Japanese quails. Livestock Research for Rural Development, 16(12), 103.

Anonymous (2018). Ross Parent stock management handbook. Aviagen, 0619-AVNR-087. https://en.aviagen.com/assets/Tech_Center/Ross_PS/RossPSHandBook2018.pdf

Bansal, A.K., Cheema, R.A. (2014). Analysis of sperm and relationship between conventional sperm parameters and hypo-osmotic swelling test/acrylamide penetration assay-crossbred cattle bulls. Advance in Applied Research, 6(1), 39-44. https://doi.org/10.5958/j.2349-2104.6.1.007

Bilcik, B., Estevez, I. (2004). Impact of male-male competition and morphological traits on mating strategies and reproductive success in broiler breeders. Applied Animal Behaviour Science, 92(4), 307-323. https://doi.org/10.1016/j.applanim.2004.11.007

Bilcik, B., Estevez, I., Russek-Cohen, E. (2005). Reproductive success of broiler breeders in natural mating systems: The effect of male-male competition, sperm quality, and morphological characteristics. Poultry Science, 84(9), 1453-1462. https://doi.org/10.1093/ps/84.9.1453

Brillard, J.P. (2003). Practical aspects of fertility in poultry. World's Poultry Science Journal, 59(4), 441-446. https://doi.org/10.1079/WPS20030027

Chung, K.M. (2010). Effect of double interspiking on Fertility, Behavior, and Blood, and Blood Parameters in Broiler Breeder Males Reared under Heat Stress Conditions [Unpublished MSc dissertation]. University of Tennessee, Knoxville, USA. 98 pp.

Dahloum, L., Moula, N., Halbouche, M., Mignon-Grasteau, S. (2016). Phenotypic characterization of the indigenous chickens (*Gallus gallus*) in the northwest of Algeria. Archives Animal Breeding, 59(1), 79-90. https://doi.org/10.5194/aab-59-79-2016

Francesch, A., Villalba, I., Cartañà, M. (2011). Methodology for morphological characterization of chicken and its application to compare penedesenca and empordanesa breeds. Animal Genetic Resource, 48(48), 79-84. https://doi.org/10.1017/S2078633610000950

Groeneveld, L.F., Lenstra, J.A., Eding, H., Toro, M.A., Scherf, B., Pilling, D., Negrini, R., Finlay, E.K., Jianlin, H., Groeneveld E., Weigend, S. (2010). Genetic diversity in farm animals-a review. Animal Genetics, 41(1), 6-31. https://doi.org/10.1111/j.1365-2052.2010.02038.x

Gumułka, M., Kapkowska, E. (2005). Age effect of broiler breeders on fertility and sperm penetration of the perivitelline layer of the ovum. Animal Reproduction Science, 90(1-2), 135-148. https://doi.org/10.1016/j.anireprosci.2005.01.018

Hamilton, W.D., Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? Science, 218(4570), 384-387. https://doi.org/10.1126/science.7123238

Hocking, P.M., Bernard, R. (1997). Effects of dietary crude protein content and food intake on the production of semen in 2 lines of broiler breeder males. British Poultry Science, 38(2), 199-202. https://doi.org/10.1080/00071669708417969

Joseph, N.S., Robinson, F.E., Renema, R.A., Thorsteinson, K.A. (2003). Comb growth during sexual maturation in female broiler breeders. Journal of Applied Poultry Research, 12(1), 7-13. https: //doi.org/10.1093/japr/12.1.7

Kumar, M., Dahiya, S., Ratwan, P., Sheoran, N. (2022). Evaluation of morphological traits in Aseel and Kadaknath breeds under backyard poultry farming using principal component analysis. Turkish Journal of Veterinary and Animal Science, 46(2), 356-365. https://doi.org/10.55730/1300-0128.4183

Leão, R.A.C., Castro, F.L.S., Xavier, P.R., Vaz, D.P., Grázia, J.G.V., Baião, N.C., Avelar, G.F., Marques Júnior, A.P. (2017). Comb, cloaca and feet scores and testis morphometry in male broiler breeders at two different ages. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 69(6), 1529-1538. https://doi.org/10.1590/1678-4162-9233

Lei, L., Zhang, Z., Groenewoud, F., Kingma, S.A., Li, J., Velde, M.V.V., Komdeur, J. (2019). Extra-pair mating opportunities mediate parenting and mating effort trade - offs in a songbird. Behavioural Ecology, 31(2), 421-431. https://doi.org/10.1093/beheco/arz204

Mahammad, A.M., Murali, L., Naga Raja Kumari, K., Narendra Nath, D., Saichaitanya, V. (2018). Effect of age on fertility and hatchability of RAJASRI birds. International Journal of Science, Environment and Technology, 7(6), 1970-1975.

McGary, S., Estevez, I., Bakst, M.R. (2003). Potential relationships between physical traits and male broiler breeder fertility. Poultry Science, 82(2), 328-37. https://doi.org/10.1093/ps/82.2.328.

McGary, S., Estevez, I., Bakst, M.R., Pollock, D.L. (2002). Phenotypic traits as reliable indicators of fertility in male broiler breeders. Poultry Science, 81(1), 102-111. https://doi.org/10.1093/ps/81.1.102

Minitab (2013). MINITAB release 17.1.0, Minitab Inc, USA.

Møller, A.P. (1994). Sexual selection and the barn swallow. Oxford Series in Ecology and Evolution, Oxford University Press, Oxford, UK.

Mukhtar, N., Khan, S.H. (2012). Comb: An important reliable visual ornamental trait for selection in chickens. World's Poultry Science Journal, 68(3), 425-434. https://doi.org/10.1017/S0043933912000542

Nääs, I.A., Sonoda, L.T., Romanini, C.E.B., Morello, G.M., Neves, H.A.F., Baracho, M.S., Souza, S.R.L.S., Menezes, A.G., Mollo Neto, M., Moura, D.J. & Almeida Paz, I.C.L. (2008). Morphological asymmetry and broiler welfare. Brazilian Journal of Poultry Science, 10(4), 209-213. https://doi.org/10.1590/S1516-635X2008000400003

Nesheim, M.C, Austic, R.E., Card, L.E. (1979). Poultry production (12th ed.). Lea and Febiger, Philadelphia.

Özdemir, D. (2019). Some morphological characteristics of İspenç roosters and chickens. Süleyman Demirel University Journal of Natural and Applied Sciences, 23(Special Issue), 155-162. https://doi.org/10.19113/sdufenbed.449617

Pizzari, T., Jensen, P., Cornwallis, C.K. (2004). A novel test of the phenotype linked fertility hypothesis reveals independent components of fertility. Proceedings of the Royal Society: B Biological Sciences, 271(1534), 51-59. https://doi.org/10.1098/rspb.2003.2577

Rizzi, C., Verdiglione, R. (2015). Testicular growth and comb and wattles development in three Italian chicken genotypes reared under free-range conditions. Italian Journal of Animal Science, 14(2), 3653. https://doi.org/10.4081/ijas.2015.3653

Souza, C., Jaimes, J.J.B., Gewehr, C.E. (2017). Equations of prediction for abdominal fat in brown egg-laying hens fed different diets. Poultry Science, 96(6), 1688-1695. https://doi.org/10.3382/ps/pew421

Tabbaa, M.J., Hassanin, H.H. (2018). Factors influencing the morphological characteristics of village chickens' genetic resources in the Abu Dhabi Emirate, UAE. Open Journal of Animal Sciences, 8(1), 87-103. https://doi.org/10.4236/ojas.2018.81007

Verhulst, S., Dieleman, S.J., Parmentier, H.K. (1999). A trade-off between immune competence and sexual ornamentation in domestic fowl. Proceeding of National Academy of Science USA, 96(8), 4478-4481. https://doi.org/10.1073/pnas.96.8.4478

Wilson, H.R., Piesco, N.P., Miller, E.R., Nesbeth, W.G. (1979). Prediction of the fertility potential of broiler breeder males. World's Poultry Science Journal, 35(2), 95-118. https://doi.org/10.1079/WPS19790008

Wolanski, N., Renema, R., Robinson, F., Wilson, J. (2004). End-of-season carcass and reproductive traits in original and replacement male broiler breeders. Journal of Applied Poultry Research, 13(3), 451-460. https://doi.org/10.1093/japr/13.3.451

Yahaya, H.K., Ibrahim, H., Abdulsalam, S. (2012). Correlation between body weight and body conformation of two broiler strains under the same dietary treatment. International Journal of Animal and Veterinary Advances, 4(3), 181-183.

Yılmaz Dikmen, B., İpek, A. (2006, September 10-14). The Effects of shank length on incubation results of Japanese Quails (*Coturnix coturnix japonica*) eggs and hatched chick shank length [Poster presentation]. XII European Poultry Conference, Verona, Italy. World's Poultry Science Journal, 62(supplement), 448 pp.

Yılmaz Dikmen, B., Şahan, Ü. (2009). The relationship among age, yolk fatty acids content, and incubation results of broiler breeders. Poultry Science, 88(1), 185-190. https://doi.org/10.3382/ps.2008-00068

Zuk, M., Pompa, S.L., Johnsen, T.S. (1995). Courtship displays, ornaments, and female mate choice in captive red jungle fowl. Behavior, 132(11-12), 821-836. https://doi.org/10.1163/156853995X00027

Poultry Studies, 20(1), 32-41
http://doi.org/10.34233/jpr.1317771

PROOF
RESEARCH PAPER

POULTRY STUDIES

Ileal Villi Morphological Characteristics of Cobb 500 Broilers Fed Phytase and Tannase Treated Sorghum Based Diets

Rutendo Paidamoyo MAGAYA[1,2,*], Tonderai MUTIBVU[1], Sharai NCUBE[1], Emmanuel NYAHANGARE[1], Paul MAPFUMO[2] Florence MTAMBANENGWE[2], Elijah NYAKUDYA[2], Ancilla NHAMO[2]

[1]Department of Livestock Sciences, University of Zimbabwe, P.O. Box MP167 Mount Pleasant, Harare, Zimbabwe
[2]The Innovation Hub, University of Zimbabwe, P. O. Box 167 Mount Pleasant, Harare, Zimbabwe

Article History
Received: 06 Jun 2023
Accepted: 20 Jun 2023
First Online: 22 Jun 2023

***Corresponding Author**
Tel: +263773955866
E-mail: pydaruemagaya@gmail.com

Keywords
Phytase
Sorghum diet
Tannase
Villus

Abstract

The effects of phytase and tannase enzyme treatment of sorghum-based broiler diets on ileal villi characteristics were evaluated in Cobb 500 broilers. A total of three sorghum levels, 0, 50, and 100%, with 4 enzyme levels; 0, 5% phytase, 5% tannase and 5% phytase+tannase combination were used to develop 12 different dietary treatments. Three hundred and sixty broilers were randomly allocated to the 12 dietary treatments in a completely randomized design experiment. On day 42, two birds from each replicate were randomly selected and slaughtered for ileal villi morphometry analyses. A 2cm tissue sample of the ileum was cut and prepared for histological analyses. Villus height and width, muscularis externa thickness, and crypt depth were measured on a light microscope using a calibrated eyepiece graticule. The total villi surface area was calculated, which indicates the digestive and absorptive capacity of the ileum. The General Linear Models (GLM) procedure of the Statistical Analysis System ver 9.4 (SAS Institute Inc., 2011) was used to analyse the data. All tests were performed at $p < 0.05$ significance. Villus height, width, and muscularis externa thickness significantly increased with increasing levels of sorghum in the diet ($p<0.001$). Birds fed complete sorghum diets supplemented with phytase enzyme had the longest villi ($p<0.001$). The 0% and 100% sorghum levels exhibited comparable crypt depth. Treatment significantly affected the apparent villi surface area ($p<0.0001$). The apparent villi surface area increased with increasing sorghum inclusion. Birds fed a complete sorghum diet supplemented with phytase had the highest villi surface area (15.48±0.241 mm). It can be concluded that phytase and tannase can be added to complete sorghum broiler diets without compromising ileal villi integrity. Hence, we recommend the addition of phytase and tannase in sorghum-based broiler diets to counteract the effects of sorghum antinutrients.

Introduction

Chicken meat has become the world's most consumed meat type (OECD, 2021). This is attributed to the fact that there are no negative cultural or religious perceptions associated with chicken consumption (Barbut and Leishman, 2022) and it has remarkable nutritional benefits (Farrell, 2022). However, the high cost of feed because of the unavailability of traditional feed ingredients limits poultry production (Ntuli and Oladele, 2013; Amponsah et al., 2015). Of major concern is the unavailability of maize, a key energy source used in poultry diets. Overall, 61% of global maize is used as livestock feed; however, its production is reduced by increasing trends of heat stress, severe droughts, and changing agroecological conditions (Challinor et al., 2014). Maize deficit because of climate change elicited the need to evaluate other potential energy sources.

Sorghum is drought tolerant and has a low risk of failure under drought conditions compared with maize (Amelework et al., 2017; Widiyono et al., 2021; Ali et al., 2023) and can thus potentially substitute maize in poultry diets to rump up broiler feed production. The use of sorghum in broiler feeds remains low due to the presence of tannins and phytates, which reduce nutrient digestibility, absorption, and bioavailability (Rahman and Osman, 2011).

The secondary negative effects associated with phytic acid involve altering the digestion process and this may negatively affect the intestinal health and intestinal microbiota of broilers (Ptak et al., 2015; Moita et al., 2021), resulting in poor performance.

The benefits of phytase supplementation in reducing the effects of phytate are well documented (Amerah et al., 2014; Moita et al., 2021; Selle et al., 2023). Exogenous phytases liberate phytate-bound phosphorus and reduce the direct antinutritive properties of phytate or myo-inositol hexaphosphate (IP6) (Selle et al., 2023). The improved bioavailability of phosphorus and calcium may express different effects on intestinal health, morphology, and microbiota diversity of broiler chickens (Moita et al., 2021). The use of tannase to curb the effects of tannins in sorghum-based broiler feeds remains unexplored.Additionally, the effects of phytase and tannase enzyme combination on gastrointestinal tract organs and ileal villi morphology are poorly understood.

Morphology of the small intestine mucosa is an important indicator of intestinal health and integrity (Paiva et al., 2014) and hence has a significant effect on feed digestion, nutrients absorption, immune status (animal health), and animal performance at large (Kogut and Arsenault, 2016). The aim of the current study was to evaluate the effects of phytase and tannase supplementation alone or in combination on the morphology of the ileal villi of Cobb 500 broilers fed sorghum-based diets.

Materials and Methods

Animal ethics

All procedures followed in this study comply with national guidelines for the good care and management of research animals. The Zimbabwe's National Animal Research Ethics Committee approved the procedures outlined in this study and granted the animal ethics (Reference Number: 013/22).

Study site

The study was conducted at Henderson Research Station, Poultry Section in Mazowe district, Zimbabwe. It is located in the agroecological region IIb characterized by an annual rainfall range of 750-1000 mm (Mavhura et al., 2021). The latitude and longitude for the area are 17.35^{o}E and 300.58^{o}S, respectively, while the altitude is 1300m. The temperature range for this area is between 15 and 29^{o}C. The area is suitable for all farming systems, including dairy, piggery, horticulture, poultry, beef, and crop production (Mavhura et al., 2021).

Experimental design and diets

All the ingredients were milled and analysed for dry matter, gross energy, crude protein, ash, fat, calcium, and phosphorus composition according to the Association of Official Analytical Chemists standards (AOAC, 1995). Condensed tannins in sorghum were quantified using the method of Folin-Denis (Pratik et al., 2016). Formulation was done using the IDT Feed Formulation software® through substitution by weight of maize with sorghum at three levels (0, 50 and 100%). There were four enzyme inclusion levels (none, 5% Phytase, 5% Tannase as well as a combination of 5% phytase and 5% tannase) at each sorghum inclusion level. A total of 12 diets were developed comprising a starter, grower, and finishing diets.

Diets were developed to meet the recommended nutrient levels for broilers (NRC, 1994). The diets were analysed for dry matter, crude protein, gross energy, fat, calcium, and phosphorus using the AOAC procedures (AOAC, 1995) and the analysed nutrients composition are shown in Tables 1-3. Diets 1 to 4 are complete maize-based diets with different enzyme treatments (1 has no enzyme, 2 has 5% phytase, 3 has 5% tannase and 4 has 5% of both phase and tannase). Additionally, diets 5 to 8 are described by 50% substitution of maize with sorghum on weight bases and have different enzyme levels (5 has no enzyme, 6 has 5% phytase, 7 has 5% tannase and 8 has 5% of both phase and tannase). Diets 9 to 12 are complete sorghum-based diets with variations in enzyme levels; thus, 9 has no enzyme, 10 has 5% phytase, 11 has 5% tannase, and 12 has 5% of both phase and tannase.

A total of 360 unsexed Cobb 500-day-old chicks were randomly allocated to thirty-six 1 m × 2 m pens. The experiment followed a completely randomized design experiment with 12 dietary treatments replicated three times. A total of ten birds were placed in each pen. A 10 cm thick layer of dry grass was placed on the floor as bedding in the pens. Heat and lighting were provided using 75 W infra-red lamps. The starter, grower, and finisher diets were fed from day 1 to 14, day 15 to 28, and day 29 to 42, respectively. Feed and water were offered *ad libitum* throughout the feeding trial. Chicks were offered vitamins C and E as well as biotin (Stress pac®, Irvine's Zimbabwe) in drinking water on arrival to combat the stress experienced during transportation. A foot bath drenched with disinfectant (Virukill®, Veterinary distributors, Pvt Ltd, Zimbabwe) was placed at the entrance to the brooding house. Mortality was recorded during the entire experimental period.

Data collection

On day 42, seventy-two birds from each dietary treatment comprise 2 birds/replicate were randomly selected for ileal villi morphology analyses. Birds were fasted overnight to limit intestinal throughput. The birds were humanely slaughtered using the neck dislocation method, carcasses were scalded in hot water at about 60°C for approximately 63 s, and the feathers were plucked manually.

Table 1. Ingredients and chemical composition of starter diets

Ingredient (gkg^{-1})	The percentage inclusion level of sorghum											
	0%				50%				100%			
Diet	1	2	3	4	5	6	7	8	9	10	11	12
Maize meal	560	560	560	555	289	286	286	282	0	0	0	0
Sorghum meal	0	0	0	0	289	286	286	282	585	585	585	580
Soya meal	400	395	395	395	382	383	383	386	375	370	370	370
Limestone flour	12	12	12	12	12	12	12	12	12	12	12	12
Broiler maxipack	28	28	28	28	28	28	28	28	28	28	28	28
Phytase	0	5	0	5	0	5	0	5	0	5	0	5
Tannase	0	0	5	5	0	0	5	5	0	0	5	5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
					Proximate Composition (%)							
DM	92.9	92.9	92.9	92.9	93.0	93.0	93.0	93.0	91.8	91.8	91.8	91.8
CP	22.0	21.9	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
Fat (EE)	2.20	2.20	2.20	2.20	1.99	1.99	1.99	1.99	2.14	2.14	2.14	2.14
CF	2.73	2.73	2.73	2.73	3.88	3.88	3.88	3.88	4.21	4.21	4.21	4.21
Ca	0.88	0.88	0.88	0.88	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
P	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
CT	0.02	0.02	0.02	0.02	0.65	0.65	0.65	0.65	1.02	1.02	1.02	1.02
GE(MJ/Kg)	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7

Table 2. Total mesophile aerobic microbial population of poultry feeds

Ingredient (gkg^{-1})	The percentage inclusion level of sorghum											
	0%				50%				100%			
Diet	1	2	3	4	5	6	7	8	9	10	11	12
Maize meal	644	644	644	640	324	322	322	320	0	0	0	0
Sorghum meal	0	0	0	0	324	322	322	320	644	644	644	640
Soya meal	316	311	311	310	312	311	311	310	316	311	311	310
Limestone flour	16	16	16	16	16	16	16	16	16	16	16	16
Broiler maxipack	24	24	24	24	24	24	24	24	24	24	24	24
Phytase	0	5	0	5	0	5	0	5	0	5	0	5
Tannase	0	0	5	5	0	0	5	5	0	0	5	5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
					Proximate Composition (%)							
DM	93.0	93.0	93.0	93.0	92.5	92.5	92.5	92.5	92.9	92.9	92.9	92.9
CP	19.0	19.0	19.0	19.0	19.5	19.5	19.5	19.5	20.0	20.0	20.0	20.0
Fat (EE)	2.81	2.81	2.81	2.81	2.39	2.39	2.39	2.39	2.12	2.12	2.12	2.12
CF	3.61	3.61	3.61	3.61	4.04	4.04	4.04	4.04	4.39	4.39	4.39	4.39
Ca	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.84	0.84	0.84	0.84
P	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.41	0.41	0.41	0.41
CT	0.02	0.02	0.02	0.02	0.69	0.69	0.69	0.69	1.11	1.11	1.11	1.11
GE(MJ/Kg)	17.4	17.4	17.4	17.4	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1

Table 3. Ingredients and chemical composition of finisher diets

	The percentage inclusion level of sorghum											
	0%				50%				100%			
Diet	1	2	3	4	5	6	7	8	9	10	11	12
Maize meal	700	700	700	697	361	358	358	356	0	0	0	0
Sorghum meal	0	0	0	0	360	358	358	357	721	716	716	711
Soya meal	264	259	259	257	243	243	243	241	243	243	243	243
Limestone flour	12	12	12	12	12	12	12	12	12	12	12	12
Broiler maxipack	24	24	24	24	24	24	24	24	24	24	24	24
Phytase	0	5	0	5	0	5	0	5	0	5	0	5
Tannase	0	0	5	5	0	0	5	5	0	0	5	5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
	Proximate composition %											
DM	93.0	93.0	93.0	93.0	95.4	95.4	95.4	95.4	94.3	94.3	94.3	94.3
CP	17.17	17.17	17.17	17.17	17.0	17.0	17.0	17.0	17.8	17.8	17.8	17.8
Fat (EE)	2.32	2.32	2.32	2.32	2.42	2.42	2.42	2.42	2.13	2.13	2.13	2.13
CF	3.56	3.56	3.56	3.56	3.50	3.50	3.50	3.50	4.05	4.05	4.05	4.05
Ca	0.77	0.77	0.77	0.77	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
P	0.37	0.37	0.37	0.37	0.38	0.38	0.38	0.38	0.37	0.37	0.37	0.37
CT	0.02	0.02	0.02	0.02	0.73	0.73	0.73	0.73	1.38	1.38	1.38	1.38
GE(MJ/Kg)	17.77	17.77	17.77	17.77	17.8	17.8	17.8	17.8	17.6	17.6	17.6	17.6

	The percentage inclusion level of sorghum											
	0%				50%				100%			
Diet	1	2	3	4	5	6	7	8	9	10	11	12
maize meal	700	700	700	697	361	358	358	356	0	0	0	0
Sorghum meal	0	0	0	0	360	358	358	357	721	716	716	711
Soya meal	264	259	259	257	243	243	243	241	243	243	243	243
Limestone flour	12	12	12	12	12	12	12	12	12	12	12	12
Broiler maxipack	24	24	24	24	24	24	24	24	24	24	24	24
Phytase	0	5	0	5	0	5	0	5	0	5	0	5
Tannase	0	0	5	5	0	0	5	5	0	0	5	5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
	Proximate composition %											
DM	93.0	93.0	93.0	93.0	95.4	95.4	95.4	95.4	94.3	94.3	94.3	94.3
CP	17.17	17.17	17.17	17.17	17.0	17.0	17.0	17.0	17.8	17.8	17.8	17.8
Fat (EE)	2.32	2.32	2.32	2.32	2.42	2.42	2.42	2.42	2.13	2.13	2.13	2.13
CF	3.56	3.56	3.56	3.56	3.50	3.50	3.50	3.50	4.05	4.05	4.05	4.05
Ca	0.77	0.77	0.77	0.77	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
P	0.37	0.37	0.37	0.37	0.38	0.38	0.38	0.38	0.37	0.37	0.37	0.37
CT	0.02	0.02	0.02	0.02	0.73	0.73	0.73	0.73	1.38	1.38	1.38	1.38
GE(MJ/Kg)	17.77	17.77	17.77	17.77	17.8	17.8	17.8	17.8	17.6	17.6	17.6	17.6

Note: DM=Dry Matter, CP= Crude Protein, EE= Ether Extract, CF= Crude Fibre, Ca= Calcium, P= Phosphorus, CT= Condensed Tannins, GE= Gross Energy

The ileum was separated from the rest of the segments at the Meckel's diverticulum to ileocecal-colonic junction (Incharoen et al., 2010). A 2-cm - long ileal (1m proximal to the ileocecal junction) segment sample was collected, placed in a sterile plastic container, and was cleaned twice with saline solution (1% NaCL) to remove intestinal digesta. The samples were fixed in 10% formalin, dehydrated using ethyl alcohol, embedded in paraffin wax, and cut into 4-m thick sections using a microtome (Brudnicki et al., 2017). The tissue samples were stained in eosin and hematoxylin on a glass slide and examined under a Trinocular Research Microscope Model B-5127 of 2015, India at × 40 magnifications. Digital images were captured using IS capture model S300 that was on computer fitted to the microscope. Villi height and width, crypt depth, and muscularis externa thickness was measured according to the specifications of Nain et al. (2012). Villi absorptive surface area was calculated as follows: (Nain et al., 2012).

Total villi surface area= Average villus width × villus height

Statistical analyses

The data were tested for normality using the Shapiro-Wilk test and log10-transformed wherever necessary. The General Linear Models (GLM) procedure of the Statistical Analysis System ver 9.4 (SAS Institute Inc., 2011) was used to analyse the data. The following model was used:

$$Y_{ijkl} = \mu + T_i + + \varepsilon_{ijk}$$

Where: Y_{ijkl} = response variable (villi height, villi width, crypt depth, muscularis externa thickness and apparent villi surface area)

μ = general mean common to all observations

T_i = effect of the ith dietary treatment (0, 50 and 100% sorghum level with no enzyme or phytase only or tannase only or phytase plus tannase enzyme combination)

ε_{ijk} = random error term

Comparison of means was done using Tukey's test. All tests were performed at $p< 0.05$ significance.

Results

The significance of treatment on the villi parameters studied are shown in Table 4. Treatment had a significant effect ($p<0.0001$) on villi height. Villus height significantly increased ($p<0.001$) with increasing levels of sorghum in the diet (Table 5). Birds fed 100% sorghum supplemented with phytase had the tallest villi, which was not significantly different from the 100% diet supplemented with phytase and tannase enzyme combination (Table 5). The 0% sorghum diet supplemented with phytase only recorded the shortest villi, and this was not significantly different ($P > 0.05$) from the 0% sorghum diet supplemented with tannase and the control diet (Figure 1). Birds fed a 0% sorghum diet supplemented with a phytase and tannase enzyme combination resulted in significantly taller villi compared with those fed 0% sorghum with (without) enzyme supplementation (Table 5).

The 50% sorghum inclusion diets exhibited moderately taller villi, shorter than those from the 100% sorghum diets, and taller than the 0% sorghum diets. There were no significant differences in villi height for the 50% sorghum diets with or without enzyme supplementation (Table 5).

Apical and basal villi widths significantly increased with increasing sorghum inclusion in the diet ($p<0.0001$). The 100% sorghum without enzyme diet resulted in the widest apical villi (Table 6). There were no significant differences in apical villi width for 100% sorghum supplemented with either phytase or tannase as single enzymes or used in combination (Table 6). The narrowest villi were recorded in birds fed 0% sorghum diet supplemented with a phytase and tannase enzyme combination. Basal villi width was also highest in birds fed the 100% sorghum diet, and no significant differences emanated from enzyme supplementation (Table 6). Again, the 0% sorghum diet supplemented with a phytase and tannase enzyme combination resulted in the narrowest villi width.

Treatment significantly affected the apparent villi surface area ($p<0.0001$). The inclusion of sorghum increased the apparent villi surface area. Broilers fed complete sorghum-based diets showed higher villi surface area. Birds fed a complete sorghum diet supplemented with phytase had the highest villi surface area (15.48 ± 0.241 mm). The birds fed 0% sorghum diets had lower villi surface area compared to the 50 and 100% sorghum diets (Figure 2). The least clear villi surface area was recorded in broilers fed 0% sorghum supplemented with a phytase and tannase enzyme combinations (5.63 ±0.241mm). Data were tested for normality using the Shapiro - Wilk test and all the collected data were normal ($S>0.90$ for villus height, apical villi width, basal villi width, crypt depth, muscularis externa, and clear villi surface area). The crypt depth was highest in birds fed the 50% sorghum level with no significant differences resulting from enzyme supplementation (Table 5). The 0% and 100% sorghum levels exhibited comparable crypt depth. However, the 100% sorghum diet supplemented with a phytase and tannase enzyme combination had the least crypt depth measurement.

Dietary treatment significantly influenced live body weight at slaughter and carcass weights ($p< 0.05$). Generally, the highest live body and carcass weights were observed in birds fed diet at 50% sorghum inclusion level with no enzyme supplementation and this was not significantly different from the weights recorded in broilers fed 50% sorghum diets with

Table 4. Effects of phytase and tannase inclusions in sorghum-based broiler diets on villi parameters

Parameter	Effect
	Treatment
Villus height	**
Villi surface area	*
Apical villi widths	**
Basal villi widths	**
Muscularis externas thickness	**

*P < 0.001; **P < 0.0001. All tests were performed at p < 0. 05 level of significance.

Table 5. Effect of sorghum level and phytase and tannase supplementation on ileal villi parameters

The ileal villus	Dietary treatment											
Parameter (mm)	1*	2*	3*	4*	5*	6*	7*	8*	9*	10*	11*	12*
VH	5.35^{ghi}	5.30^{ghi}	5.40^{ghi}	4.41^{f}	5.73^{e}	5.54^{eg}	5.74^{e}	5.76^{e}	6.43^{bcd}	6.71^{abd}	6.40^{bcd}	6.60^{abcd}
aVW	1.49^{h}	1.57^{h}	1.40^{h}	1.11^{k}	2.09^{bde}	2.00^{bdefg}	1.89^{efg}	1.86^{efg}	2.47^{a}	2.15^{bde}	2.15^{bde}	2.09^{bde}
bVW	1.47^{ef}	1.64^{ef}	1.64^{ef}	1.45^{f}	1.63^{ef}	2.15^{bcd}	2.07^{cd}	2.02^{cd}	2.30^{abc}	2.47^{ab}	2.34^{ab}	2.28^{bcd}
CD	1.80^{defg}	1.85^{defg}	1.70^{efg}	1.78^{defg}	2.12^{abcfg}	2.15^{abcf}	2.13^{abc}	2.12^{abc}	2.06^{bcd}	1.89^{cdef}	1.70^{efgh}	1.52^{h}
ME	1.17^{ghi}	1.22^{ghi}	1.26^{gh}	1.11^{hi}	1.13^{ghi}	1.80^{f}	1.97^{e}	2.13^{cd}	2.16^{cd}	2.47^{a}	2.30^{bc}	2.24^{bcd}
VSA**	7.92^{f}	8.48^{f}	8.20^{f}	5.63^{g}	10.67^{de}	11.48^{cd}	11.36^{cd}	11.15^{cde}	15.3^{a}	15.48^{a}	14.37^{b}	14.44^{b}

Note: Means in the same row with different superscripts differ significantly (P < 0.05). VH=villi height, Avw =apical villi width, b VW=basal villi width, CD=crypt depth, ME= muscularis externa thickness, VSA**= villi surface area in mm2

Description of treatments: 1 =100% maize with no enzyme, 2* =100% maize with 5% phytase, 3*= 100% maize with 5% tannase, 4*= 100% with 5% phytase and tannase combination, 5*=50% maize and 50% sorghum with no enzyme, 6*= 50% maize and 50% sorghum with 5% phytase, 7*= 50% maize and 50% sorghum with 5% tannase, 8*= 50% maize and 50% sorghum with 5% phytase and tannase combination, 9* =100% sorghum with no enzyme, 10* =100% sorghum with 5% phytase, 11*= 100% sorghum with 5% tannase, 12*= 100 sorghum with 5% phytase and tannase combination

Table 6. Effect of dietary treatment on live body and carcass weights on day 42

Dietary treatment	Live body weight	SEM	Carcass weight	SEM
1	2100.5^{abc}	63.50	761.5^{abcde}	6.43
2	1926.5^{cdef}	63.50	747.1^{cde}	6.43
3	1952.3^{cdef}	63.50	754.5^{abcde}	6.43
4	2096.8^{abcde}	63.50	757.0^{abcde}	6.43
5	2159.7^{abc}	63.50	767.9^{abcd}	6.43
6	2004.2^{abcdef}	63.50	758.4^{abcd}	6.43
7	1967.5^{bcdef}	63.50	756.4^{abcde}	6.43
8	2102.3^{abcd}	63.50	764.1^{abcde}	6.43
9	1950.5^{cdef}	63.50	747.1^{abcd}	6.43
10	1912.8^{cdef}	63.50	752.9^{bcde}	6.43
11	1931.2^{cdef}	63.50	743.6^{de}	6.43
12	1920.2^{abcdef}	63.50	742.6^{de}	6.43

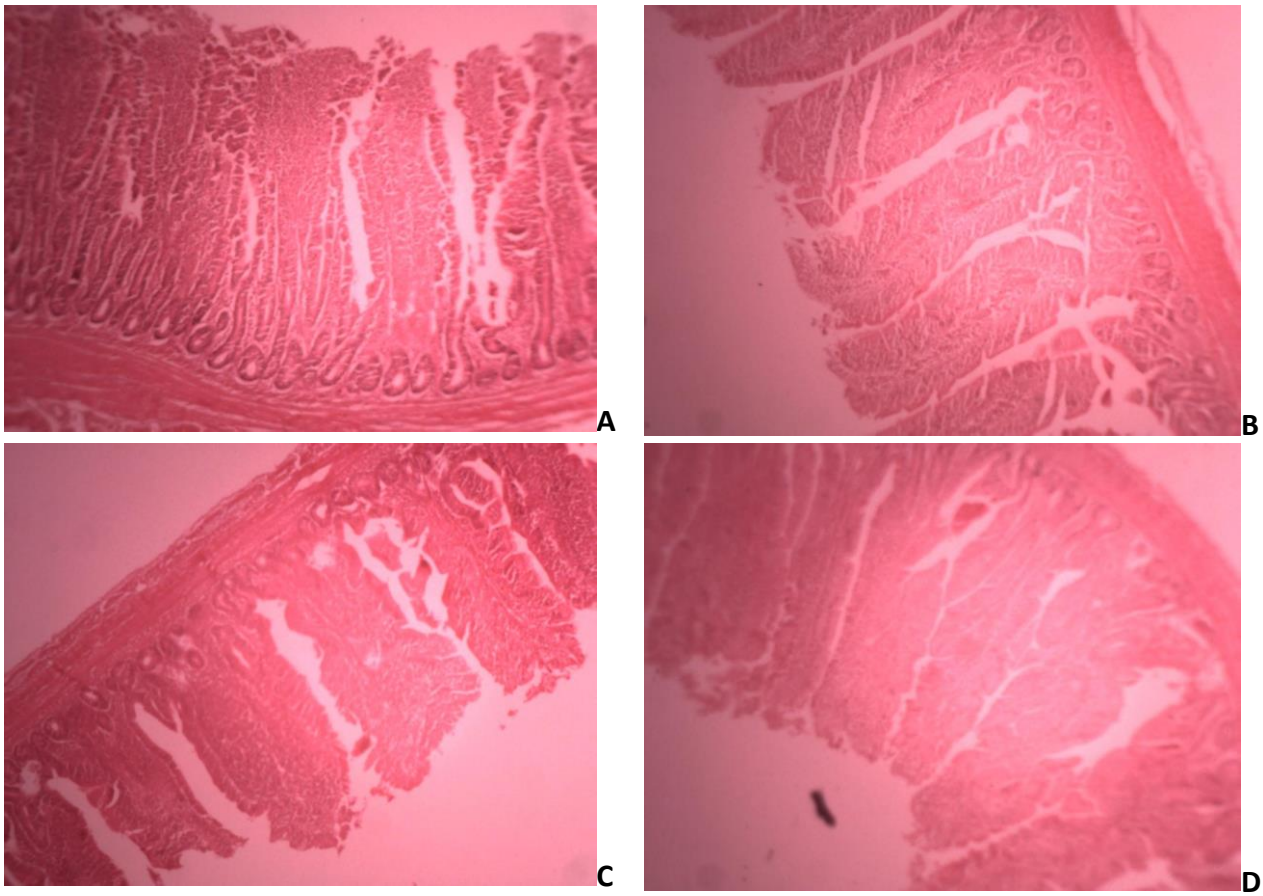

Figure 1. Ileal villi characteristics of Cobb 500 broilers fed: (A) 100% sorghum with phytase (B) 50% sorghum with phytase (C) 0% sorghum with phytase (D) 100% sorghum with phytase plus tannase enzyme combination at ×40 magnification

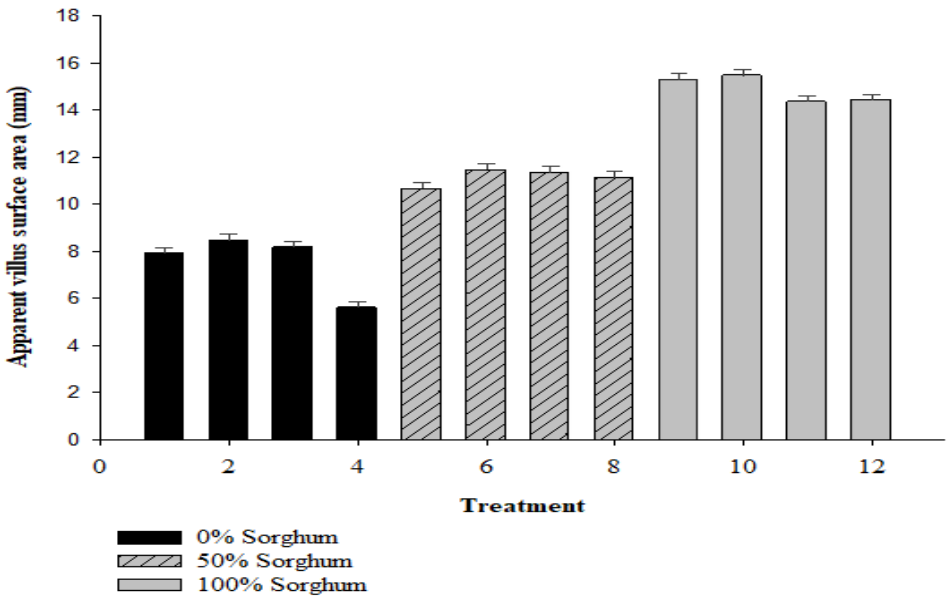

Figure 2. Effect of sorghum inclusion level and enzyme supplementation on apparent villus surface area

phytase only and with phytase and tannase enzyme combination as well as birds fed complete maize diets without enzyme, with phytase only and with phytase and tannase enzyme combination as shown in Table 6. The least live body and carcass weights were observed in broilers fed complete sorghum diets with a phytase and tannase enzyme combination.

Discussion

Generally, an increase in villi height is related to enhanced nutrient absorption, while deeper crypts are associated with higher tissue turnover rates (Choct, 2009; de Verdal et al., 2011). The results of the current study on villi height contradict previous results by Nyamambi et al. (2007). In their study, they observed that duodenal villus height and crypt depth were lowered with increasing tannin levels. Nyamambi et al. (2007) used Chirimaugute, Brown Tsweta, DC, and SV2 sorghum varieties with tannin contents of 1.68, 0.20, 2.48, and/g DM, respectively. They stipulated that day 7 and day 14 villi height of chicks fed 100% DC, Chirimaugute, and Brown Tsweta sorghum diets were lower than the villi height of chicks raised on total maize and 50% DC diets. Additionally, sorghum inclusion levels had no significant effect on villus height, width, and crypt depth of broilers at day 42 (Silva et al., 2015; Many*elo* et al., 2019). This is because the digesta arriving at the ileum had already been subjected to maximum enzyme activity and maximum absorption in the previous intestinal segments irrespective of the composition of feed consumed (Silva et al., 2015). Elongation of the villi with increasing sorghum level could be explained by the increase in tannins and their metabolites, which increase mitosis in the villi, implying increased proliferation (Jamroz et al., 2009; Brus et al., 2018). Tannins act as gut microbiota modulators (Tosi et al., 2013; Choi and Kim, 2020;) and their anti-oxidative traits enhance villus length (Buyse et al., 2022). The villi height obtained in this study was lower than that reported by Mutibvu (2016) in indigenous chickens. This can be attributed to the vitamin A supplemented in the diets, which enhances gut morphology (Kunisawa and Kiyono, 2013) as well as the indigenous chicken breed they used. The observation that broilers fed either complete maize or complete sorghum diets showed shallowest crypts is surprising given the fact that complete sorghum diets had higher levels of tannins and phytate which had negative effects on the intestinal epithelium. A shallower crypt depth indicates a decrease in the metabolic cost of intestinal epithelial renewal in the villi (Xu et al., 2022). On the other hand, deeper crypts promote rapid cell turnover, faster metabolism of tissue, and allow renewal of intestinal villi (Hamedi et al., 2011), resulting in rapid replacement of sloughed or inflamed villi (Jayaraman et al., 2013). The observation that completes substitution of maize by sorghum results in higher ileal villi surface area is contrary to previous findings (Silva et al., 2015).

They highlighted that sorghum level had no significant effect on the absorptive surface area in the jejunum; thus, the feed offered did not interfere with the absorption mechanisms. In their study, however, Silva et al. (2015) used a sorghum variety that contained 42.7 mg/kg equivalent to 0.00427% tannin and this is classified as a tannin free variety. It is possible that the impact of tannins in this variety on villi surface area could be negligible.

In a separate study, sorghum level had no effect on villus height and crypt depth in the small intestinal mucosa segments except for duodenal villus height, which was smaller in 7-d-old broilers fed the low-sorghum diet than in broilers fed the high-sorghum or control diets (Torres et al., 2013). Such a response is explained by the fact that during the initial stages of life, intestinal growth and differentiation occurs in the absence of exogenous feed nutrients and luminal and hormonal factors, thus the process is influenced by intrinsic factors (Drozdowski et al., 2010). However, Incharoen et al. (2010) highlighted that dietary nutrient composition influences intestinal development and mucosal architecture which subsequently influences digestive absorption and assimilation of digested nutrients. Shorter intestinal villi are related to a smaller number of absorptive cells, presence of toxic substances, and a larger number of secretory cells (Iwashita et al., 2003), whereas longer villi promote healthy digestion and high nutrients absorption efficiency (Itza-Ortiz et al., 2019). The current results showed villi widths higher than those reported by Ncube et al. (2017). This disparity could be a consequence of the protein source *Acacia angustissima*, which could have limited villi development in their study.

The observation that phytase and tannase enzyme supplementation had a significant effect on villi morphology is consistent with previous results. The addition of phytase maize-soyabean diet resulted in higher jejunal villi height (Pekel et al., 2017; Karami et al., 2020). The effects of phytase supplementation on villi width and crypt depth are inconsistent. The inclusion of phytase in broiler diets increased villi width (Karami et al., 2020; Moita et al., 2021). The effects of phytase or tannase enzyme combination in sorghum-based diets on intestinal villi morphology are still limited for comparison.

Although the total replacement of maize by sorghum-increased villi height and hence the absorptive capacity of the villi, complete sorghum diets negatively affected live body and carcass weights. The observation is consistent with previous results by Avila et al. (2009) reported that there was no positive association between villi height and nutrient absorption. However, this is surprising because longer villi provide higher absorptive area, better digestive enzyme activity and fast transport of nutrients (Wijtten et al., 2012) which translate to high live body weight.

Conclusion

It can be concluded that the broiler diet containing 50% sorghum with 5% phytase plus tannase enzyme combination can be fed to broilers without compromising ileal absorptive area, live body weight and carcass weight.

Acknowledgments

The work was funded by the Ministry of Higher and Tertiary Education Innovation Science and Technology Development through the University of Zimbabwe Vice Chancellor Research Fund. The authors would like to express their sincere gratitude to Dr. A. Nhamo, Dr. E. Nyakudya, and Prof F. Mutambanengwe and Prof. P. Mapfumo for their unwavering support toward the success of this work.

References

Ali, A. E. E., Husselmann, L. H., Tabb, D. L., Ludidi, N. (2023). Comparative Proteomics Analysis between Maize and Sorghum Uncovers Important Proteins and Metabolic Pathways Mediating Drought Tolerance. Life, 13, 170.

Amelework, B., Shimelis, H., Tongoona, P., Laing, M. (2017). Physiological mechanisms of drought tolerance in sorghum, genetic basis and breeding methods: A review. African Journal of Agricultural Research, 10, 3029-3040.

Amerah, A. M., Plumstead, P. W., Barnard, L.P., Kumar, A. (2014). Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed maize-based diets. Poultry Science, 93, 906–915.

Amponsah, S. K., Otoo, D., Kwaku, P. E., Ampofo, S. D. (2015). Modelling the feed mix for poultry production, the case of Adama Musa farms, in Ghana. International Journal of Applied Mathematical Research, 4(2), 404.

AOAC (Association of Analytical Chemists). (1995). Official methods of analysis. 16th Ed. Association of official analytical chemists. Washington DC, USA.

Avila, D., Nino, A., Parra, A, Tarazona, J., Rodriguez, P., Torres, G. (2009). Description morfometrica de Las vellosidades intestinales en bovine, Cultural cientifica, 7, 57-60

Barbut, S., Leishman, E. M. (2022). Quality and Processability of Modern Poultry Meat. Animals, 12, 2766.

Brudnicki, A., Brudnicki, W., Szymeczko, R., Bednarczyk, M., Pietruszynska, D., Kirkillo-Stacewicz K. (2017). Histo-morphometric adaptation in the small intestine of broiler chicken, after embryonic exposure to A Galactosides. Journal of Animal and Plant Science, 27, 1075–1082.

Brus, M., Gradišnik, L., Trapecar, M., Škorjanc, D., Frange, Ž. R. (2018). Beneficial effects of water-soluble chestnut (Castanea sativa Mill) tannin extract on chicken small intestinal epithelial cell culture. Poultry Science, 97, 1271–82. 5

Buyse, K., Noten, N. V., Delezie, E., Goethals, L., Janssens, G. P. J., Lourenço, M. (2022). Chestnut tannins in broiler diets: Affecting intestinal development in different feeding phases. Frontiers in Veterinary Science, 9, 996524.

Challinor, A. J., Watson, J., Lobell, D. B., Howden, S. M., Smith, D. R., Chhetri N. (2014). A meta-analysis of crop yield under climate change and adaptation. National Climate Change, 4, 287–291.

Choi, J., Kim, W. K. (2020). Dietary application of tannins as a potential mitigation strategy for current challenges in poultry production: A review. Animals, 10, 2389.

Choct, M. (2009). Managing gut health through nutrition. British Poultry Science, 50, 9-15. Doi:10.1080/00071660802538632.

De Verdal, H., Nancy, A., Bastianelli, D., Charles, H., Meme, N., Urvoix, S., Le Bihan-Duval, E., Migonon-Grasteau, S. (2011). Improving the efficiency of feed utilization in poultry by selection.Genetic parameters of anatomy of the gastro-intestinal tract and digestive efficiency. BMC Genetics, 6, 59. doi:10. 1186/1471-2156-12-59

Drozdowski, L. A., Clandon, T., Thompson, A. B. R. (2010). Oncogene, growth and development of the small intestine: Understanding pediatric gastroenterology. World Journal of Gastroenterology, 16, 787-799.

Farrell, D. (2022). The role of poultry in human nutrition: The nutritional benefits of chicken meat compared with other meats. Food and Agriculture Organization. https://www.fao.org/3/al714e/al714e.pdf. Accessed on March 21. 2023.

Hamed, S., Rezaian, M., Shomali, T. (2011). Histology changes of small intestinal mucosa of cocks due to sunflower meal single feeding. American Journal of Animal and Veterinary Science, 6, 171-175.

Incharoen, T., Yamauchi, K., Erikawa, T., Gotoh, H. (2010). Histology of intestinal villi and epithelial cells in chickens fed low-crude protein or low crude fat diets. Italian Journal of Animal Science, 9, 82.

Itza-Ortiz, M., Seguru-Correa, J., Parra-Suescun, J, Aguilar-Urquizo, E., Escobar-Gordillo, N. (2019). Correlation between body weight and intestinal villi morphology in finishing pigs. Acta Universitaria, 29.

Iwashita, J., Yukita, S., Hiroko, S., Nagatomo, T., Hiroshi, S., Tatsuya, A. (2003). mRNA of MUC2 is stimulated by IL-4, IL-13 or TNF-alpha through a mitogen- activated protein kinase pathway in human cancer cells. Immunology and Cell Biology, 81, 275-282.

Jamroz, D., Wiliczkiewicz, A., Skorupińska, J., Orda, J., Kuryszko, J., Tschirch, H. (2009). Effect of sweet chestnut tannin (SCT) on the performance, microbial status of intestine and histological characteristics of intestine wall in chickens. British Poultry Science Journal, 50, 687–99.

Jayaraman, S., Thangavel, G., Kurian, H., Mani, R., Mukkalil, R., Chiraḳkal, H. (2013). Bacillus subtilis PB6 improves intestinal health of broiler chickens challenged with Clostridium perfiringens-induced necrotic enteritis. Poultry Science, 92, 370-374.

Karami, M., Karimi, A., Zentel, J., Boroojeni, F. G. (2020). Evaluation of interactive effects of phytase and benzoic acid supplementation on performance, digestibility, tibia mineralisation, gut morphology and serum traits in male broiler chickens. Italian Journal of Animal Science, 19, 1428-1438.

Kogut, M. H., Arsenault, R. J. (2016). Gut health: The new paradigm in food animal production. Frontiers in Veterinary Science, 31, 71. Doi: 10.3389/fvets.2016.00071

Kunisawa, J., Kiyono, H. (2013). Vitamin-mediated regulation of intestinal immunity. Frontiers in Immunology, 4, 189

Manyelo, T. G., Ng'ambi, J. W., Norris, D., Mabelebele, M. (2019). Substitution of *Zea mays* by *Sorghum bicolor* on Performance and Gut histo-morphology of Ross 308 broiler chickens aged 1-42d. Journal of Applied Poultry Research, 28, 647-657.

Mavhura, E., Manyangadze, T., Aryl, K. R. (2021). Perceived impacts of climate variability and change: an exploration of farmers' adaptation strategies in Zimbabwe's intensive farming regions. Geo Journal, 87, 3669-3684.

Moita, V. H. C., Duarte, M. E., Kim, S. W. (2011). Supplemental effects of phytase on modulation of mucosa-associated microbiota in the jejunum and the impacts on nutrient digestibility, intestinal morphology, and bone parameters in broiler chickens. Animals,11, 3351.

Mutibvu, T. (2016). Behaviour, physiological responses, meat yield and gut morphology of free-range chickens raised in hot environment. PhD Thesis, University of KwaZulu-Natal, South Africa.

Nain, S., Renema, R. A., Zuidhof, M. J., Korver, D. R. (2012). Effect of metabolic efficiency and intestinal morphology on variability in n-3 polyunsaturated fatty acid enrichment of eggs. Poultry Science, 91, 888–898.

Ncube, S., Halimani, T. E., Mwale, M., Saidi, P. T. (2017). Effect of Acacia angustissima Leaf Meal on the Physiology of Broiler Intestines. Journal of Agricultural Science, 9, 53.

NRC (National Research Council). (1994). Nutrient requirements of broilers. 9th Rev. Ed. National Academy Press. Washington D. C., USA

Ntuli, V., Oladele, O. I. (2013). Analysis of constraints faced by small scale broiler farmers in Capricon District in Limpopo province. Life Sciences Journal, 10, 3990-3996.

Nyamambi, B, Ndlovu, L. R., Naik, Y. S., Kock, N. D. (2007). Intestinal growth and function of broiler chicks fed sorghum-based diets differing in condensed tannins levels. South African Journal of Animal Science, 37, 202-213.

OECD. (2021) https://data.oecd.org/. Accessed March 20. 2023.

Paiva, D., Walk, C., Melrose, A. (2014). Dietary calcium, phosphorus and phytase effects on bird performance, intestinal morphology, mineral digestibility and bone ash during a natural necrotic enteritis episode. Poultry Science, 93, 2752- 2762

Pekel, A. Y., Horn, N. L., Adeola, O. (2017). The efficacy of dietary xylanase and phytase in broiler chickens fed expeller-extracted camelina meal. Poultry Science, 96, 98–107.0

Pratik, D. T., Prakash, D. T., Chandrashekhar, K. C. (2016). Quantitative estimation of tannins from Pueraria the berosa by UV spectrophotometry. International Journal of Pharmaceutical Sciences Review and Research, 37, 210-212.

Ptak, A., Bedford, M. R., Swiatkiewicz, S., Zyła, K., Józefiak, D. (2015). Phytase modulates ileal microbiota and enhances growth performance of the broiler chickens. PLoS ONE, 10, 770.

Rahman, I. E. A., Osman, M. A. W. (2011). Effect of sorghum type (Sorghum bicolor) and traditional fermentation on tannins and phytic acid contents and trypsin inhibitor activity. Journal of Food Agriculture and Environment, 9, 163-166.

Selle, P. H., Macelline, S. P., Chrystal, P. V., Liu, S. Y. (2023). The Contribution of Phytate-Degrading Enzymes to Chicken-Meat Production. Animals, 13, v 603.

Silva, M. C. A., Caroline, A. C. X. G., Litz, F. H., Fagundes, N. S., Fernandes E de, A., Mendonca, G. A. (2015). Effects of sorghum on broilers gastrointestinal tract. Brazilian Journal of Poultry Science, 17, 95-102. http: dx.doi.org/10.1590/1516-635x170195-102.

Statistical Analysis System (SAS). (2011). Statistical Analysis System User's guide version 9.2. SAS Institute Inc, North Carolina, USA.

Torres, K. A. A., Pizauro, J. M., Soares, C. P., Silva, T. G. A, Nogueira, W. C. L., Campos, D. M. B., Furlan, R. L., Macari, M. (2013). Effects of maize replacement by sorghum in broiler diets on performance and intestinal mucosa integrity. Poultry Science, 92, 1564-1571.

Tosi, G., Massi, P., Antongiovanni, M., Buccioni, A., Minieri, S., Marenchino, L. (2013). Efficacy test of a hydrolysable tannin extract against necrotic enteritis in challenged broiler chickens. Italian Journal of Animal Science, 12, 62.

Widiyono, W., Nugrohob, S., Sudianaa, M., Sulistyowatia, D. D. (2021). Water stress and water requirement of sorghum: case study of dry areas in east nusa tenggara province. The 5th SATREPS Conference. Pages, 72-78

Wijtten, P. G. A., Langhout, D. J. Verstegen, W. A. (2012). Small intestine development in chicks after hatch and in pigs around time of weaning in relation with nutrition: Aa review. Acta Agricultural Scandavian Sect. A Animal Science, 62, 1-12.

Xu, H., Fu, J., Luo, Y., Li, P., Song, B., Lv, Z., Guo, Y. (2022). Effects of tannic acid on the immunity and intestinal health of broiler chickens with Clostridium perfringens-induced necrotic enteritis. Research Square.