BOOKLET

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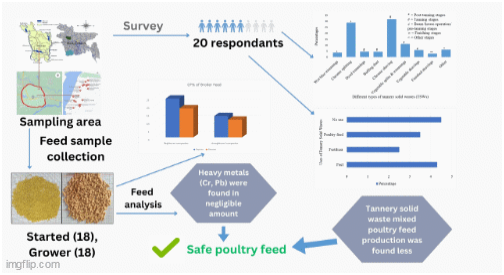
Research Paper

Using tannery wastes in poultry feed: a matter of concern for safe poultry production in Bangladesh

Islam Md.S, Protik AS, Zannat Mst.A, Naim Z, Kabir Md.E, Asaduzzaman Md., and Akter M.

Online J. Anim. Feed Res., 14(1): 1-12, 2024; pii: S222877012400001-14

DOI: https://dx.doi.org/10.51227/ojafr.2024.1



Abstract
Nowadays tannery waste is a matter of concern because if it used as livestock feed, it could cause health hazards to humans. Therefore, this study was conducted to know the generation rates, utilization, disposal method of tannery solid wastes (TSWs), and inclusion level of it into the poultry feed. Moreover, this study determined the physical and chemical parameters of interest including moisture content, crude protein (CP) levels, and the presence of heavy metals such as chromium (Cr) and lead (Pb) in the poultry feeds that were sold in the studied area. For these purposes, a field survey was conducted with twenty tannery industries. Broiler feed samples were procured from multiple farmers situated in the Savar area of Dhaka. The feed source was classified into two categories, namely registered and unregistered feed mills. According to the study, wet blue trimmings was generated in 3.84% that was used as an ingredient of poultry feed. In addition, 55% of tanneries landfilled their waste, while 30% of them sold it for poultry feed. The utilization of TSWs in poultry feed production in this country was limited to a maximum of 1.314% of the total annual production. Besides, the CP% was determined in the range of 24.24 - 13.32 % and 18.15 - 11.01 % for broiler starters and growers, respectively, where lower CP content was found only in unregistered feed mills. Trace amounts of Cr and Pb were identified in each of the feed samples at very low concentrations. In conclusion, it can be stated that the percentage of tannery solid wastes mixed poultry feed was generated in negligible amounts and the registered companies' feed was found normal in all aspects of the quality tested in the study.
Keywords: Broiler feed, Feed mill, Heavy metal, Physical quality, Tannery solid waste

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Research Paper

Effects of dietary substitution of Soybean meal by dried Azolla on blood and serum parameters, productive and reproductive traits, and economic efficiency of rabbit does as well as semen quality of bucks

El-Deeb MM, Alazab AM, Shazly SA, Fahim HN, and Ragab MA.

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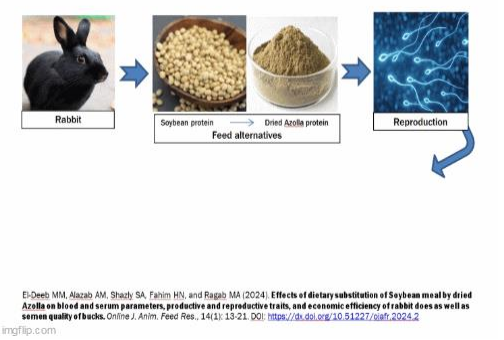

Abstract
An experiment was carried out to investigate the effects of dietary substitution of dried Azolla (*Azolla pinnata*) in different ratios by soybean meal on productive and reproductive performances, hematological and serum traits, and economic efficiency of rabbit does as well as semen quality of males. Forty mature does and eight males of Black Balady rabbits aged seven and eight months were assigned to 1 of 4 dietary groups: 0 (control), 20, 30, and 40% of soybean protein substituted with dried Azolla protein. Data were analyzed using repeated measures of statistical software computer program package. There were no significant differences among groups in number of services per conception as well as parturition intervals (days) with a superiority of 30% group over the control and other two groups. Average litter weight was significantly ($P<0.05$) superior in the 30% replacement group. There were no significant differences in live body weight either at birth or at weaning among the four tested groups and the 30% replacement groups recorded the highest significant daily weight gain of bunnies during the whole experimental period. All Azolla groups recorded better results in the studied traits of bucks' semen compared to the control. Azolla diets did not show any adverse effects on the studied blood parameters. The 30% replacement of the soybean protein group showed the best economic return compared to the other two

replacement groups and the control. In conclusion, Azolla can safely and economically replace soybean protein at the rate of 30% in adult female rabbits' diets.
Keywords: Azolla, Body weight, Rabbit, Reproduction, Semen.

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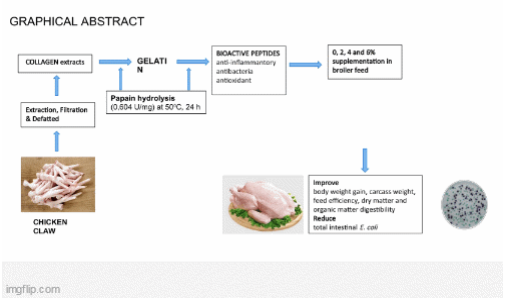
Research Paper

Performance, carcass weight, total intestinal bacteria, and feed digestibility of broilers fed chicken foot-derived bioactive peptides

Rahayu S, Suhartati FM, Hartoyo B, Bata M, Widiyastuti T, Rimbawanto EA, Hidayat N, and Prihambodo TR.

Online J. Anim. Feed Res., 14(1): 22-28, 2024; pii: S222877012400003-14

DOI: https://dx.doi.org/10.51227/ojafr.2024.3



Abstract
The aim of this study was to evaluate the supplementation of chicken claw-derived bioactive peptides on performance, carcass weight, total intestinal bacteria, and feed dry matter (DMD) and organic matter (OMD) digestibility of broilers. A completely randomized design and five repetitions were applied in this experiment. The research material consisted of 200 DOC strain CP-707 grown up to 35 days of age, and the biopeptide was produced by hydrolyzing chicken claws protein with a commercial papain enzyme. Bioactive peptides were added to feed treatments in amounts of 0, 2, 4, and 6%. The differences between treatments were tested using the honestly significant difference test. The addition of chicken claws biopeptides had a significant influence ($P<0.01$) on OMD and carcass weight, as well as a significant effect ($P<0.05$) on body weight gain, feed efficiency, DMD and total bacteria. Addition of chicken claws-derived peptides in rations up to 6% enhanced body weight gain, feed efficiency, carcass weight, DMD, OMD, and total intestinal bacteria in broiler chickens.

Keywords: Biopeptides, Chicken, Claw, Digestibility, Carcass quality.

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Research Paper

Genotypic characterization of *Escherichia coli* isolated from infected chicken in Basrah, Iraq

Lateif BM, Ahmed JA, Najem HA.

Online J. Anim. Feed Res., 14(1): 29-39, 2024; pii: S222877012400004-14

DOI: https://dx.doi.org/10.51227/ojafr.2024.4

Abstract
This study aimed to detect the presence of *Escherichia coli* in broiler and layer hens in the Basrah province, Iraq using macroscopic and microscopic diagnosis and bacterial isolation that causes infection insome internal organs (liver and heart), and by polymerase chain reaction. Randomly chosen samples were taken from different places within Basrah province for further investigation (poultry fields in Al-Qurnah and Al-Hartha). The bacteriological analysis revealed that the presence of *Escherichia coli* is responsible for causing fibrinous pericarditis and perihepatitis in birds. The macroscopic examination revealed hemorrhagic lesions and a significant buildup consisting of a white fibrous accumulation in the pericardial sac of the infected birds' hearts. The livers of infected birds exhibited significant deposition of white fibrous exudate on the liver surface, along with hepatomegaly. The afflicted heart displays a microscopic appearance marked by a notable aggregation of inflammatory cells in the pericardial sac and the release of fibrinous exudate. Additionally, there is an accumulation of edematous exudate in the cardiac muscle fibers, accompanied with congestion of blood vessels in the myocardium. The microscopic examination of the infected liver revealed the existence of a significant infiltration of inflammatory cells in the liver capsule, as well as the presence of a thick fibrinous exudate encapsulated on the liver surface and congestion of the central vein. The histological analysis of the affected heart and liver revealed a significant buildup of collagen and fibrin fibers, which exhibit a prominent dark bluish staining. This buildup is widely distinguished in the pericardial and hepatic capsules. The study indicated that fibrinous pericarditis and perihepatitis affected birds, as indicated by the examination of bacterial results. *Escherichia coli* emits endotoxins that induce vascular damage in the heart and liver, resulting in an elevated presence of fibrin exudate around the affected

tissue. The histological analysis supported this conclusion.
Keywords: Fibrinous, Pericarditis, Perihepatitis, Pathology, Biological.

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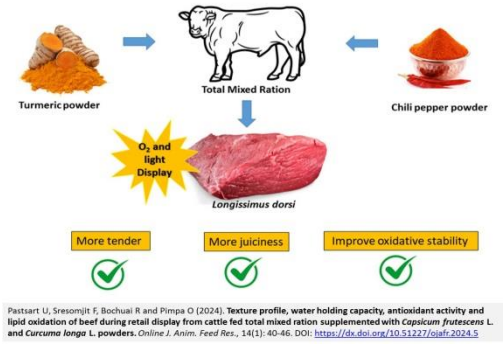
Research Paper

Texture profile, water holding capacity, antioxidant activity and lipid oxidation of beef during retail display from cattle fed total mixed ration supplemented with *Capsicum frutescens* L. and *Curcuma longa* L. powders

Pastsart U, Sresomjit F, Bochuai R and Pimpa O.

Online J. Anim. Feed Res., 14(1): 40-46, 2024; pii: S222877012400005-14

DOI: https://dx.doi.org/10.51227/ojafr.2024.5



Pastsart U, Sresomjit F, Bochuai R and Pimpa O (2024). **Texture profile, water holding capacity, antioxidant activity and lipid oxidation of beef during retail display from cattle fed total mixed ration supplemented with *Capsicum frutescens* L. and *Curcuma longa* L. powders.** *Online J. Anim. Feed Res.*, 14(1): 40-46. DOI: https://dx.doi.org/10.51227/ojafr.2024.5

Abstract
This study aimed to elucidate the effects of supplementation of *Capsicum frutescens* L. or chili pepper (ChP) and *Curcuma longa* L. or turmeric (T) powders combination in total mixed ration (TMR) on texture profile, water holding capacity (WHC) and oxidative stability of beef during days 0, 5 and 10 of retail display. The experiment was carried out on 16 crossbred bulls (Brahman and Charolais) of about 2 years in age. The bulls were randomly assigned to 4 dietary treatment groups as follows: 1) TMR as control, 2) TMR + 1%ChP powder, 3) TMR + 1%T powder, and 4) TMR supplemented with a mixed powder of 1%ChP + 1%T, over a 6 months feeding period. The results revealed that the hardness and gumminess of control beef were higher than other groups, and the cohesiveness of beef from cattle fed a mixed powder of 1%ChP + 1%T was lower than other groups ($P<0.05$). Regarding WHC, the results showed that, on days 0 and 5 of storage, the control group meat had higher cooking losses than either the 1%T or a mixture of 1%ChP + 1%T groups ($P<0.05$). Also, on 0 and 5 days of retail display, the 1%ChP + 1%T group showed the highest antioxidant activity when compared to other groups ($P<0.05$). As for the lipid oxidation in beef, on day 5 of storage MDA level in control beef was higher than the 1%T or a mixture of 1%ChP + 1%T groups ($P<0.05$). It can be concluded that the combination of chili pepper and turmeric powder in TMR can improve texture, water holding capacity, and oxidative stability of beef during refrigerated storage.
Keywords: Beef, Chili pepper, Cooking loss, Oxidative stability, Texture profile, Turmeric powder.

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Research Paper

Solid state fermentation characteristic of rice straw using herbivore's cecum microbes

Wahyudi A, Hendraningsih L, Mahmud A, Mulatmi SNW, and Prima A.

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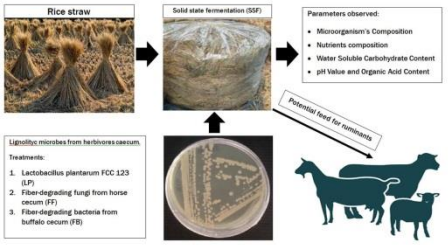
DOI: https://dx.doi.org/10.51227/ojafr.2024.6



Wahyudi A, Hendraningsih L, Mahmud A, Mulatmi SNW, and Prima A (2024). **Solid state fermentation characteristic of rice straw using herbivore's cecum microbes.** *Online J. Anim. Feed Res.*, 14(1): 47-52. DOI: https://dx.doi.org/10.51227/ojafr.2024.6

Abstract
This study was aimed at identifying highly capable lignolytic microbes from nature for use in Solid State Fermentation (SSF) of rice straw. The SSF silage was prepared in laboratory scale, as the following treatments: uninoculated (control), *Lactobacillus plantarum* FCC 123 (LP), fiber-degrading fungi (*Aspergillus* sp.) from horse cecum (FF), and fiber-degrading bacteria (*Enterococcus casseliflavus*) from buffalo cecum (FB). Incubation was carried out for a month at room temperature. The observed parameters were: organic acids, water-soluble carbohydrate (WSC), microorganism and nutrient composition. Rice straw SSF that was inoculated with LP showed the highest quality of fermentation, indicated by significant highest lactic acid bacteria (LAB) population, and has the lowest of poor bacteria indicators (coliform, aerobic bacteria, and bacilli). The LP treatment also has the highest LAB content and lowest WSC. Among treatments, FB treatment seems to have given a similar result with LP followed by FF. While the chemical composition seems unaffected by treatments. Compared with the fresh material, all fermentation with and without inoculants has reduced neutral detergent fiber (NDF) and increased acid detergent fiber (ADF), but there were no differences among all treatments. Inoculation of both LP and FB could improve rice straw SSF silage quality, but this system could not improve fiber degradation as well as in liquid state fermentation (LSF).
Keywords: Microorganism, Organic acid, Silage, Straw, Water soluble carbohydrate

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Research Paper

Effects of probiotic *Enterococcus faecium* and raw, fermented and sprouted pearl millet based diets on performances, carcass traits, hematological and biochemical indices of broiler chickens

Olasehinde O and Aderemi F.

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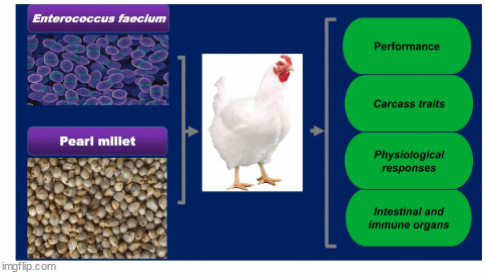
DOI: https://dx.doi.org/10.51227/ojafr.2024.7



Abstract
Probiotics, recognized as a safe substitute for antibiotics in the animal industry, have been acknowledged for their growth-enhancing properties. This study assessed the impact of *Enterococcus faecium* strain NCIMB 11181 and diets incorporating Raw, Sprouted, and Fermented pearl millet on the performance, carcass traits, organ weights, and blood parameters of broiler chickens. In a randomized design, 120 one-day-old Arbor Acre broiler chickens were assigned to five groups: 1) No supplement, negative control (N-con); 2) Control + antibiotics, positive control (P-con); 3) Raw pearl millet + probiotics in drinking water (RPM + PRO); 4) Sprouted pearl millet + probiotics in drinking water (SPM+PRO); 5) Fermented pearl millet + probiotics in drinking water (FPM + PRO). Probiotic supplementation did not significantly impact body weight gain (BWG) but influenced feed intake (FI) ($P<0.05$). FPM+PRO increased feed conversion ratio (FCR), thigh yield, and drumstick yield. Thymus weight is reduced in the RPM+PRO and SPM+PRO groups compared to the control groups. Serum high-density lipoprotein (HDL) levels decreased ($P<0.01$) in the P-con and FPM+PRO groups. No treatment effect ($P>0.05$) was observed on hematological indices. Overall, pearl millet diets supplemented with probiotics demonstrated no adverse effects on the health status of broiler chickens, suggesting their potential as viable alternatives to antibiotics.
Keywords: Pearl millet, Broiler chickens, Blood, Carcass traits, Growth performance, Probiotics.

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Research Paper

Processing of slaughterhouse blood for antianemic food products

Omarov R and Shlykov S.

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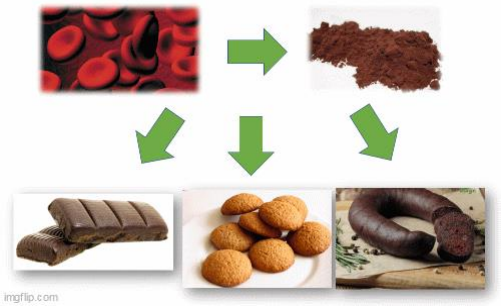
DOI: https://dx.doi.org/10.51227/ojafr.2024.8



Abstract
Currently, rational processing of blood from slaughterhouses remains as a waste fluid in many regions. Traditional approaches to use the blood for food are significantly limited because of specific and non-favorable organoleptic characteristics. Present study provides a comparison of various methods for modifying the red blood cell (RBC) mass of animals and a more in-depth study of acid hemolysis. The solution of ascorbic acid has been proposed as a hemolyzing agent. There has been established experimentally that the addition of equal volumes of RBC and a solution of an ascorbic acid with a concentration of 0.75 mol/dm3 can effectively destroy the stroma more up 90% of red blood cells within 15 minutes. By this, the hemoglobin oxidation degree to methemoglobin is about 50%, which forms the desired color of the resulting hydrolysate. The dry semi-finished product has a neutral odor and brown color with high functional and technological properties. It also contains 0.9% organic iron with good biological value. Thus, the study shows that blood products can effectively use in various foods such as meat products, and also as a dietary supplement for various proposes. Consumption of these products has potent positive effect on hemoglobin levels and it is recommending for people with iron deficiency anemia.
Keywords: Antianemia products, By-products, Heme iron, Farm animals, Slaughterhouse.

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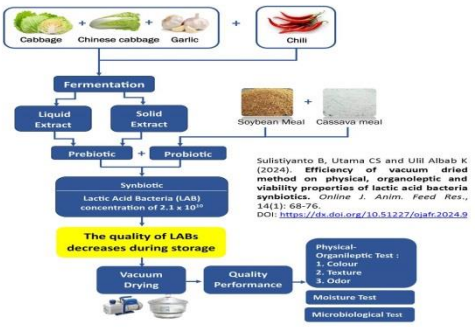
Research Paper

Efficiency of vacuum dried method on physical, organoleptic and viability properties of lactic acid bacteria synbiotics

Sulistiyanto B, Utama CS and Ulil Albab K.

Online J. Anim. Feed Res., 14(1): 68-76, 2024; pii: S222877012400009-14

DOI: https://dx.doi.org/10.51227/ojafr.2024.9



Abstract
Vacuum drying storage is a more efficient storage method for synbiotic feeds, compared to fresh storage. The current study aimed to examine the effect of vacuum drying on the physical, organoleptic, and microbiological qualities of synbiotics made from cabbage and Chinese cabbage greens. The study was conducted using a completely randomized design with a 5x3 factorial pattern with two replications consisting of two factors, namely five levels of drying time (24, 48, 72, 96, and 120 hours) and three levels of storage time (4, 8 and 16 weeks). The variables observed were physical-organoleptic quality in water content, color, odor, and texture, and microbiological quality in the form of total lactic acid bacteria. The results showed no interaction between the two treatments in terms of vacuum drying method, drying time, and storage time. The recommended treatment is drying for 48 hours, as evidenced by the moisture content factor supporting the viability of the lactic acid bacteria and maintaining the sensory properties. This study suggests a more efficient storage method of synbiotics for food applications.
Keywords: Lactic Acid Bacteria, Orgoleptic, Storage, Synbiotic, Vacuum Drying.

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Research Paper

Effects of fermented palm kernel cake in acidic and basic solutions on the performance of broiler chickens

Teufack S, Noumbissi MNB, Ngouana TR, Tchouan DG, Edie NLW, Taboumda E, Tindo TR, and Kana JR.

Online J. Anim. Feed Res., 14(1): 77-85, 2024; pii: S222877012400010-14

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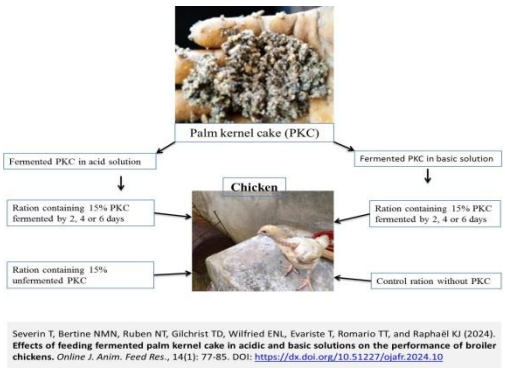

Abstract
Palm kernel meal is a by-product used very sparingly in poultry feed, due to its low nutritional value and gravelly appearance which could be improved by physical or chemical treatments. The aim of this study was to assess the effect of palm-kernel meal fermentation period on its nutritional value and growth performances of broiler chickens. The treatment consisted of fermenting palm kernel meal in a solution of humic acid (HA) or limestone, for 0, 2, 4 and 6 days. A control ration without palm kernel meal (R0) was compared rations containing 15% unfermented palm kernel cake (R0+) and 15% fermented palm kernel cake in humic acid and limestone solutions. Each experimental ration was randomly assigned to 8 chicks in 4 experimental units of 02 chicks each, repeated 4 times per a 2×3 factorial design (2 fermentation modes and 3 fermentation period). The main results showed that fat content (13.04%) and metabolizable energy (5314 Kcal/kg DM) of palm kernel meal were higher when fermented in humic acid for 6 days. Fermentation in the basic solution for the same period (6 days) increased protein (13.52%) and cellulose (24.21%) contents. Whatever the fermentation mode, the digestive utilization coefficient of dry matter, organic matter, crude protein and crude cellulose increased with the fermentation period. Fermentation mode and period had no significant effect on growth performance. However, growth characteristics tended to improve with fermentation period. In conclusion, fermentation of palm kernel in humic acid and limestone solutions improved significantly ($P<0.05$) the digestibility of all feed components, enabling chickens to take advantage of the nutrients for better growth performances.
Keywords: Chemical composition, Digestibility, Fermentation, Humic acid, Metabolizable energy, Palm kernel meal.

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USING TANNERY WASTES IN POULTRY FEED: A MATTER OF CONCERN FOR SAFE POULTRY PRODUCTION IN BANGLADESH

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Nowadays tannery waste is a matter of concern because if it used as livestock feed, it could cause health hazards to humans. Therefore, this study was conducted to know the generation rates, utilization, disposal method of tannery solid wastes (TSWs), and inclusion level of it into the poultry feed. Moreover, this study determined the physical and chemical parameters of interest including moisture content, crude protein (CP) levels, and the presence of heavy metals such as chromium (Cr) and lead (Pb) in the poultry feeds that were sold in the studied area. For these purposes, a field survey was conducted with twenty tannery industries. Broiler feed samples were procured from multiple farmers situated in the Savar area of Dhaka. The feed source was classified into two categories, namely registered and unregistered feed mills. According to the study, wet blue trimmings was generated in 3.84% that was used as an ingredient of poultry feed. In addition, 55% of tanneries landfilled their waste, while 30% of them sold it for poultry feed. The utilization of TSWs in poultry feed production in this country was limited to a maximum of 1.314% of the total annual production. Besides, the CP% was determined in the range of 24.24 - 13.32 % and 18.15 - 11.01 % for broiler starters and growers, respectively, where lower CP content was found only in unregistered feed mills. Trace amounts of Cr and Pb were identified in each of the feed samples at very low concentrations. In conclusion, it can be stated that the percentage of tannery solid wastes mixed poultry feed was generated in negligible amounts and the registered companies' feed was found normal in all aspects of the quality tested in the study.

Keywords: Broiler feed, Feed mill, Heavy metal, Physical quality, Tannery solid waste

INTRODUCTION

The Tannery industry holds major economic significance in Bangladesh, despite being recognized as one of the most environmentally hazardous industries globally (WorstPolluted.org, 2016; Abebaw and Abate, 2018; Moktadir et al., 2018). The generation of solid waste in leather industries presents significant hazards to both the atmosphere and public health. The tannery business in Bangladesh produces approximately 175-232 Metric Ton (MT) of solid trash per day while the beam house or pre-tanning process produces most of the total solid wastes (Kanagaraj et al., 2006; Moktadir and Rahman, 2022). Over the past decade, livestock farming and aquaculture industries have been significantly impacted by industrial waste, particularly tannery waste (Shaibur et al., 2019). Conversely, a number of studies have demonstrated that a particular group of people with deceitful tendencies are producing animal feed, specifically for poultry, through the amalgamation of TSWs though this activity is banned by the Supreme Court of Bangladesh (Hossain et al., 2007; Hossain and Hasan, 2014; Mazumder, 2013; Parvin et al., 2017). Moreover, the same scenario is seen in our neighboring countries - India and Pakistan (Sudha, 2010; Mahmud et al., 2011). Various types of TSWs exist, including raw skin cuttings, wet blue shaving dust, low chrome wet-blue scraps, tanned skin-cut wastes, and protein-rich leather shaving dust (Hossain et al., 2007; Ahmed et al., 2017). A significant issue associated with these practices pertains to the utilization of heavy metals, specifically chromium, in the tanning procedures (Hossain et al., 2007). Moreover, it is noteworthy that the concentration of heavy metals in poultry feed, meat, and eggs exceeds the permissible threshold, thereby warranting further consideration (Bari et al., 2015; Ahmed et al., 2017; Hossain et al., 2017; Haque et al., 2021; Ullah et al., 2021; Samad et. al., 2023; Hossain et al., 2023). People in Bangladesh get a significant portion of their required protein from poultry (Haque et al., 2021). The ingestion of chromium and other potentially harmful metallic elements occurs inadvertently through the consumption of contaminated poultry meat and eggs (Ullah et al., 2021; Samad et al., 2023; Hossain et al., 2023). Besides, the Government of Bangladesh aimed to alleviate pollution and promote economic growth

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through the relocation of the tannery industry upstream to a newly established tannery town situated in the Harindhara area of Savar (Whitehead et al., 2019; Moktadir and Rahman, 2022). After the replacement of the tanneries in a new area, the scenario of solid waste generation, utilization, and disposal has not been studied yet. Even the possibility of mixing TSWs to the poultry feed in aspect of total production of poultry feed in Bangladesh has not been determined till now, based on published and available studies. This study can be useful for solid waste management strategies, as it is crucial to obtain information regarding the process steps from which these solid wastes are generated, the desired end product of these processes, and the waste characteristics.

Therefore, the present study was conducted to investigate the generation and utilization scenarios of TSWs, as well as the possible inclusion rate detection in poultry feed, and the nutritional constituents, including physical, chemical, and heavy metal determination in feeds that are currently available in Bangladesh. The hypothesis posited that the utilization of poultry feed containing heavy metal-infused TSWs is infrequent. If it uses for poultry feed, the heavy metal concentration would be low and tolerable for humans.

MATERIALS AND METHODS

Study area and sample size

The study area was Hemayetpur, Savar, Dhaka – the tannery area of Bangladesh – with 23.7986° N, and 90.2680° E coordinates. A total of 120 tanneries operate under the Savar Tannery Estate (Sarkar, 2022). Among them, twenty respondents from twenty tanneries were taken as a sample of the survey by adopting the PPRS (Proportional Probability Random Sampling) techniques (Ridgman, 1990). A total of thirty-six samples of broiler feeds, consisting of eighteen starters and eighteen growers, were collected from farmers located in the vicinity of the tannery area in Savar for laboratory analysis.

Sample categorization

The twenty tanneries under study were categorized into three distinct types based on their respective capacities for processing raw hides and skins. These types include small, medium, and large tanneries. Small tanneries have a capacity of less than 800 kg/day, medium tanneries have a capacity ranging from 800 to 1050 kg/day, while large tanneries have a capacity exceeding 1050 kg/day. A registered feed company was termed as a company that has a license for feed production by the Department of Livestock Services (DLS), with an approximate production of 125000MT/ year. Whereas, a company that has no license from DLS and produces feed locally was categorized as an unregistered (local) feed company.

Questionnaire development

A carefully designed interview schedule was devised, comprising of a combination of open-ended and close-ended structured queries. The study examined several variables, including the types of raw materials used, the condition of the raw materials upon receipt, the sources and channels of raw material acquisition, the availability of modern facilities for solid waste storage and recycling, the mode of solid waste disposal, the maintenance protocol of the tannery, and the challenges faced by the tannery authority in improving the industry.

Evaluation of physical properties of feed sample

Organoleptic observations were conducted to assess the physical characteristics of the samples, including color, smell, particle size, and the presence of extraneous substances.

Determination of chemical properties and heavy metals of feed sample

For the purpose of determining moisture content and crude protein, the feed samples underwent proximate analysis using the standardized method (Helrich, 1990). The quantification of protein was conducted through the Kjeldahl method, which involves a three-step process consisting of digestion, distillation, and titration following the previous procedure (Rahman et al., 2014). The Analytik Jena novAA 400P Atomic Absorption Spectrophotometer (Analytik Jena, Germany) was used to measure the total levels of chromium and lead (Islam et al., 2022).

Statistical analysis

The descriptive statistics, frequencies, and percentages were used to examine the qualitative data. Chi-square test (Tables 7 and 8) and independent t-test (Tables 9 and 10) were applied to compare the data between registered and unregistered feed companies using SPSS 25 (Assefa and Melesse, 2018). Statistical significance was determined for group differences when the p-value was less than 0.05. The values were presented in the form of mean ± SD (Standard Deviation).

RESULT AND DISCUSSION

TSWs are generated from tanneries pose a significant risk to both environment and human health while the waste products are used for livestock feeds (Parvin et al., 2017). Since the tannery industry has a great economic impact on Bangladesh's finance, its challenges are needed to be studied. In addition, the tannery waste must be dumped, recycled, and reused properly according to the law of the country. In this study, a vast investigation has been carried out to explore the characteristics, waste generation and utilization, challenges, and opinions to overcome the problems of the tannery industry, the possible portion of TSW that can be used in poultry feed, and the quality of poultry feed in Bangladesh.

Tannery characteristics

The present study has unveiled significant characteristics pertaining to the examined tanneries, as depicted in Figure 1. According to Figure 1, the majority of the studied tanneries, specifically 90%, were privately owned while the remaining 10% were categorized as mergers. Approximately 90% of tanneries were relocated from Hazaribagh to the Hemayetpur tannery industrial area which agree with the fact of relocation history of Bangladesh's tannery industry in 2016 (Moktadir and Rahman, 2022). The findings indicated that a significant majority (95%) of the tanneries had established protocols for managing tannery waste. Besides, not only tannery industries but also footwear-making units work with leather in Bangladesh (Rahman, 2017). So, TSWs can be generated from those areas too. However, here we only considered the tanneries for our investigation.

In Figure 2, this experiment gave an overall view of the research tanneries' raw material receiving procedures, characteristics of raw materials, and way of the receiving channel. It was demonstrated that only the Dhaka division provided raw materials to 70% of the sample tanneries, while 85% came from middlemen. However, none of these tanneries obtained their raw materials exclusively from the owner (Figure 2). Because of being a Muslim country, a large portion of raw materials are collected in the season of Eid-ul-Adha – the religious festival of Muslims – by the middleman from all over the country (Khan, 2016). Most of cases, they cured it with salt because salt curing is the popular way of hide preservation. For these reasons, though the raw materials could be received in cured or not cured conditions, this study revealed that 85% of the study tanneries received raw (hides & skin) materials in cured conditions. There was no such tannery that had simply dealt with unprocessed raw materials (Figure 2).

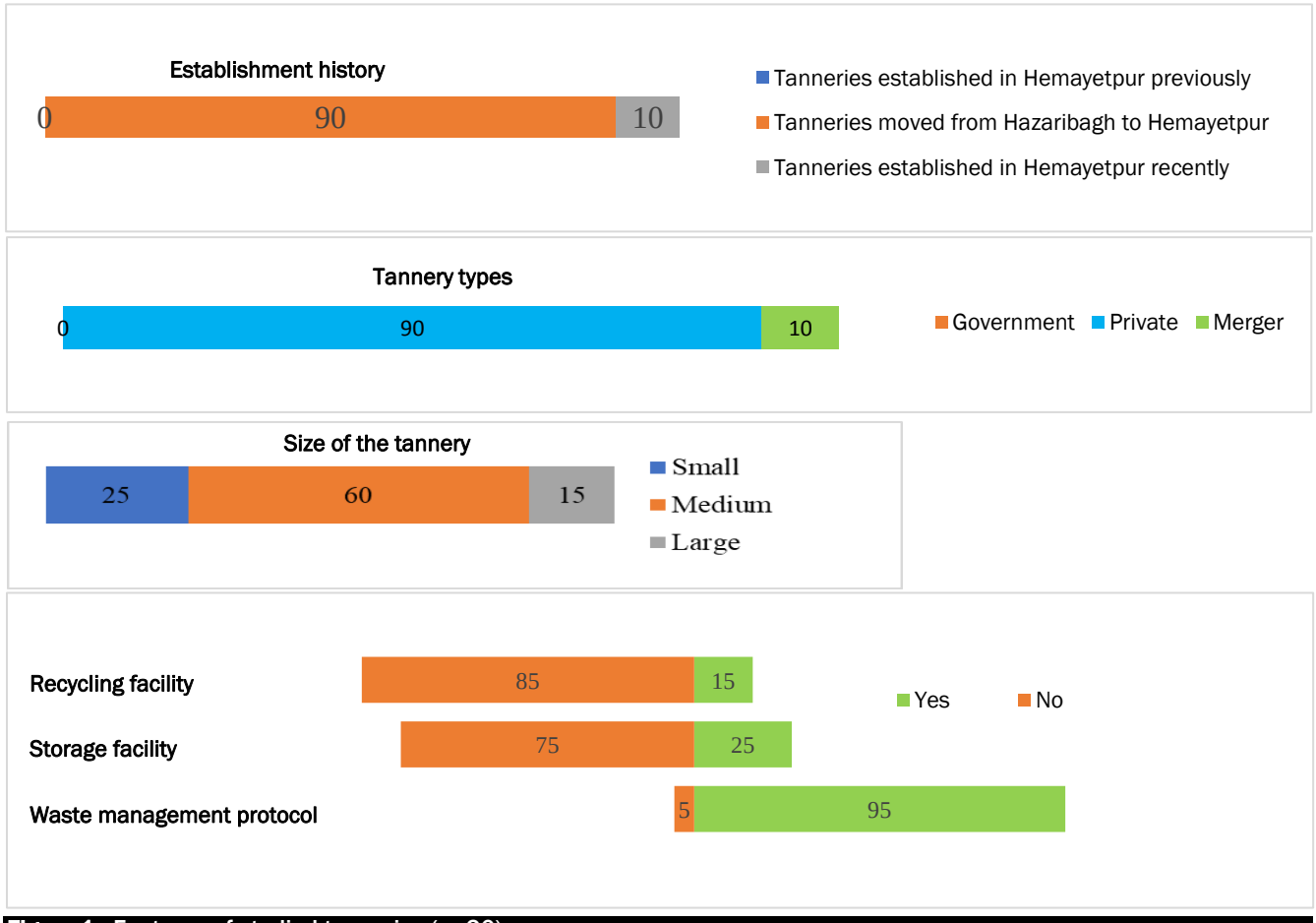

Figure 1 - Features of studied tanneries (n=20)

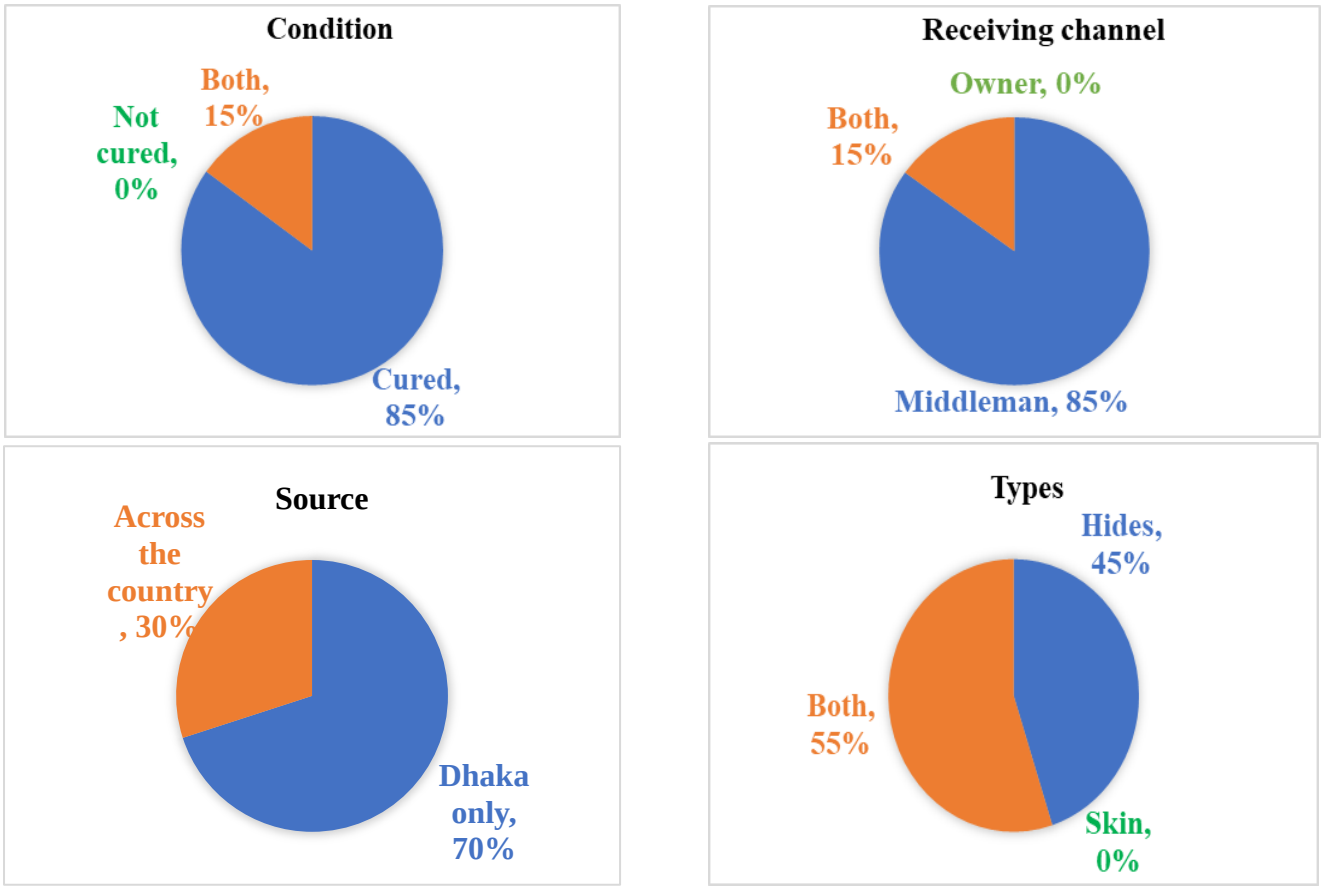

Figure 2 - Characteristics of raw materials and their acceptance rate in studied tanneries (n=20)

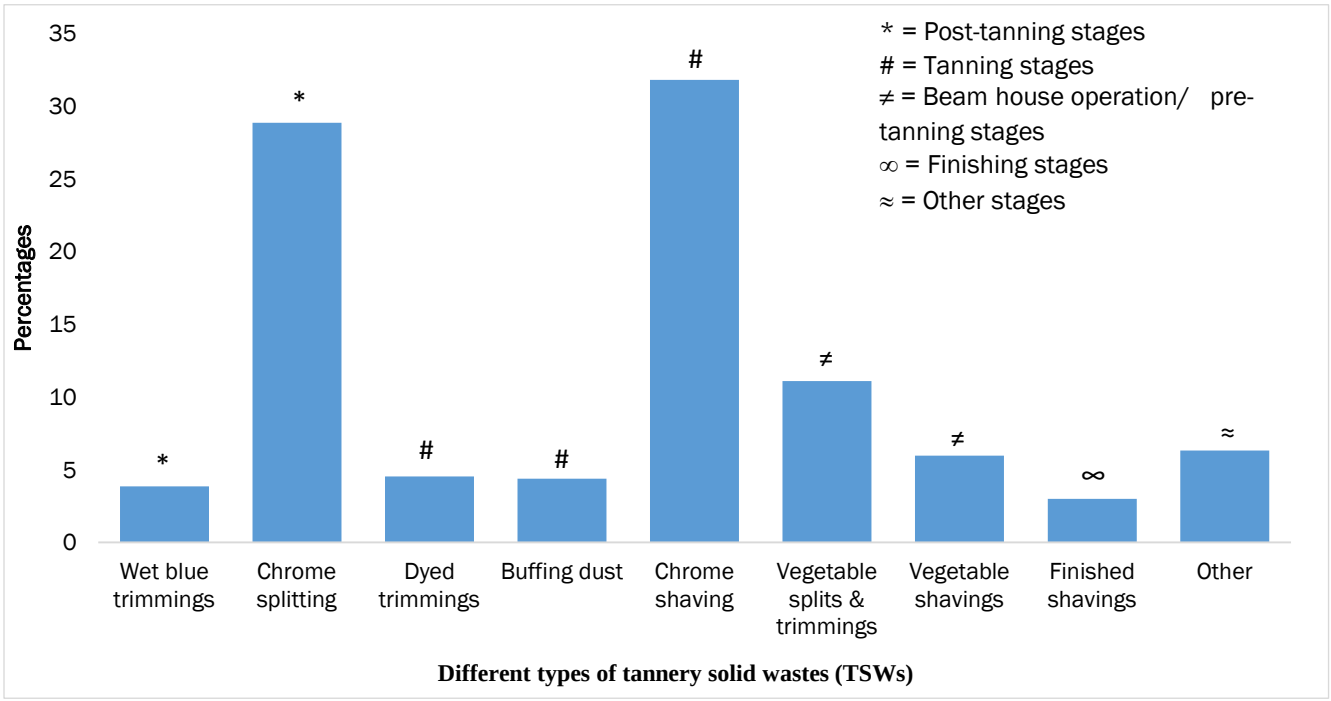

Figure 3 - Different types of TSWs generated in the studied tanneries per day (n=20)

Generation of solid waste and finished leather

The present study provided information regarding the percentages of various forms of TSW generation depending on its overall volume (Figure 3). The most prevalent type of waste generated in the TSWs was chrome shaving, accounting for 31.8% of the total waste. The study demonstrated that various types of solid wastes are produced at different stages of

leather production. For instance, vegetable shavings and splits were identified during the Beam House operation or pre-tanning phase, while wet blue trimmings and chrome splitting were detected during the post-tanning and tanning stages, respectively. Prior research indicates that pre-tanning procedures involve the preparation of animal skin or hide for collagen tanning, which is known to produce a significant amount of TSWs (Kanagaraj et al., 2015; Muralidharan et al., 2022). However, the TSWs' generation rate was found highest in the tanning process which was contradictory to the past study (Humayra, 2020; Moktadir and Rahman, 2022). In Table 1, the average percentage of solid waste generation was 28.88% while the medium-sized tanneries produced the highest amount of TSWs. The finished leather was produced on an average of 22.27 % (Table 1).

Table 1 - Generation rate of the total amount of TSW and final products in study tanneries

Variables	Type of the tannery	Percentage (%)	Average percentage
Waste generation	Small	27.94	28.88
	Medium	30.01	
	Large	28.69	
Final product generation	Small	21.69	22.72
	Medium	22.62	
	Large	23.85	

Table 2 - Utilization mode of TSW

Variables	Frequency	Percentage (%)
Fuel	12	60
Fertilizer	4	20
Poultry feed	6	30
No use	11	55

Table 3 - Disposal mode of TSW

Variables	Frequency	Percentage (%)
Landfilling	11	55
Open dumping	7	35
Incineration	9	45
No specific /documented method	13	65

The utilization and disposal of solid tannery waste

This research provided the overall concept of the utilization and disposal mode of TSW (Table 2 and 3). According to Table 2, a proportion of 30% of participants revealed that TSW was utilized as poultry feed, while there was no reported usage of TSW as cattle feed. The research findings indicated that solely wet blue trimmings were utilized and marketed for poultry feed among the various categories of solid waste examined, while most of the people utilized it as a means of fuel production (Table 2). The experiment demonstrated that the tanneries disposed of the solid waste by landfilling mostly, followed by incinerating and open dumping (Table 3) which agreed with the previous study (Moktadir and Rahman, 2022).

Contribution of TSW (wet blue trimmings) in poultry feed

The present investigation has revealed that wet blue trimmings were utilized solely as a source of poultry feed or as a constituent of the poultry feed among all of the TSWs, as indicated in Table 4. This experiment only took into account the annual quantities of wet blue trimmings and poultry feed production to assess the percentage of TSW mixed poultry feed. According to previous studies, the daily production of TSWs is determined to be 210 Metric tons (MT) (Saha et al., 2021) and 200-250 tons per day (Hossain et al., 2007; Ahmed et al., 2017). By assuming that producers incorporated wet blue trimmings at a ratio of 10% of the total volume of poultry feed, the estimated annual production capacity of TSWs mixed poultry feed would be 29433.6 MT. On the other hand, according to the report of IDLC (2020), total poultry feed production per year is 2240000 MT. According to Table 4, the annual proportion of TSW mixed poultry feed production was 1.314%. This percentage was deemed insignificant in terms of potential human health hazards. According to the

previous reports, the percentage was found to be significantly high for both animal and public health (Rahman et al., 2014; Ahmed et al., 2017; Hossain et al., 2017). The observed disparities between the outcomes of the present investigation and prior reports may be attributed to variations in sample size and composition, as well as differences in the methodology employed for data analysis. The high percentages of heavy metals could be readily discerned if the samples were exclusively obtained from the tannery waste mixed feed and if the computation was not predicated on the overall yearly production of livestock feed.

Table 4 - Contribution of TSW (wet blue trimmings) in livestock feed

Parameter	Quantity
Total TSWs production per day	210 MT (Saha et al., 2021)
Total wet blue trimmings waste production per day (3.84% of TSWs) (Figure 3)	8.064 MT
Total wet blue trimmings waste production per year (8.064 × 365 days)	2943.36 MT
Probable amount of TSW (wet blue trimmings) mixed poultry feed production per year [maximum 10% inclusion level]	29433.6 MT
Total poultry feed production per year	2240000 (IDLC, 2020)
Percentage of TSW mixed feed per year	$\frac{29433.6}{2240000} \times 100 = 1.314\ \%$

Table 5 - Challenges of the tannery industry in Bangladesh

Challenges	Frequency	Percentage (%)
Lack of skilled manpower	7	35
Difficulty in assessing buffer zone/dumping site	13	65
Lack of sufficient land for tannery expansion	9	45
High transportation cost	14	70
High labor cost	17	85
Unrest political situation in Tannery Worker Union	18	90
Unstable leather market	6	30
Fluctuation of raw hides & skin supple	4	20
High availability of synthetic goods in the market	9	45
Lack of Govt. support	8	40

Common challenges

The study focused on the difficulties and obstacles encountered by tannery industries in their efforts to improve the industry (Table 5). The present study identified political unrest within the tannery workers union as the primary obstacle, with a significance level of 90%. The tannery labor union are formed 58 years ago to ensure workers' rights but this organization's activities are sometimes hampered by political unrest and labor disputes (Sohel, 2019; Tuhin et al., 2022). After that, the high labor cost was placed in the second position may be due to the increasing livelihood cost of the people. The high transport cost both inside and outside of the country was identified as a major problem too (Table 5). The small number of effluent treatment plants (ETPs) in our country, rocketing up fuel prices, dependency on a single mode of transportation, also the raising labor cost are fueled the soaring transportation cost (Sakamoto et al., 2019; Shahriar et al., 2021; Bhowmick and Ghosh, 2022).

Respondent's opinion

In this survey, participants were asked for their thoughts on how the industry as a whole may be improved (Table 6). The present investigation revealed that a majority of 85% of participants expressed a desire for the government to furnish tannery proprietors with low-interest loans. The majority vote was in favor of granting demesne for industry expansion and reducing the VAT percentage on imported instruments, tools, and machinery. Some believe that improving transportation infrastructure, regulating the leather market, preventing the import of synthetic leather products from abroad, and establishing institutions to educate tannery workers are the keys to the sector's growth (Table 6).

Physical characteristics of broiler starter and grower feeds

The color, smell, particle size, and presence of foreign particles in the broiler starter and grower feeds were observed organoleptically. Only the feed coloration was different in this study; the other attributes were discovered under normal conditions. The particle size of the feed samples depicted in Figures 4 and 5 were nearly identical. The samples were

devoid of any foreign particles or molds, although certain unregistered feed samples contained dusty particles. Figures 4 and 5 demonstrate that three types of color (yellowish, yellow, dark yellow) were found in broiler starter feed and two varieties of color were found in broiler grower feed (brown and light brown). No significant color differences were found between registered and unregistered feed mills for both starter and grower feed (Table 7 and 8). The ingredients used to make natural broiler feed may cause it to change color. Broiler feed is often made up of grains as well as supplements such as vitamins and minerals. These ingredients may come in a variety of colors, and they may also go through processing like as grinding or pelleting, which can alter the appearance of the final feed. Besides, the feed color, odor, form, and particle size have the ultimate effect on a bird's feed intake and performance (Chewning et al., 2012; Farghly, 2017; Kreis, 2019; Gulizia and Downs, 2021).

Table 6 - Opinions of the respondents for the betterment of the industry

Opinion	Frequency	Percentage (%)
Provision of a soft loan from the Govt.	17	85
Allowance of demesne/ land for the expansion of the tannery industry	11	55
Lessen the VAT% for the imported instrument, tools, and machinery	13	65
Establishment of institutions for the training of the laborer	9	45
Control the country's leather market	10	50
Resist the import of foreign synthetic leather goods	18	90
Development of the transportation system	8	40

Table 7 - Difference between registered and unregistered companies' broiler starter feed color

Feed color types / Company type	Yellowish	Yellow	Dark yellow	P-value
Registered	0	3	6	
Unregistered	3	4	2	0.076 ^{NS}
Total	3	7	8	

NS= non-significant (p>0.05)

Table 8 - Difference between registered and unregistered companies' broiler grower feed color

Feed color types / Company type	Brown	Light brown	P-value
Registered	4	5	
Unregistered	4	5	1.00 ^{NS}
Total	8	10	

NS= non-significant (p>0.05)

Table 9 - Comparison of the moisture values between the registered and unregistered companies' commercial broiler feeds

Feed categories	Registered companies' feeds (%)	Unregistered companies' feeds (%)	Level of significance
Starter feeds	12.89 ± 1.28	11.59 ± 0.46	NS
Grower feeds	10.83 ± 0.62	9.96 ± 0.48	NS

NS= non-significant (p>0.05)

Table 10 - Comparison of the crude protein values between the registered and unregistered companies' commercial broiler feeds

Feed categories	Registered companies' feeds (%)	Unregistered companies' feeds (%)	Level of significance
Starter feeds	24.24^{a} ± 1.07	13.32^{b} ± 8.39	*
Grower feeds	18.15^{a} ± 1.03	11.01^{b} ± 0.69	*

The different superscripts indicated a significant difference among the values in the same row; * = p <0.05 (significant)

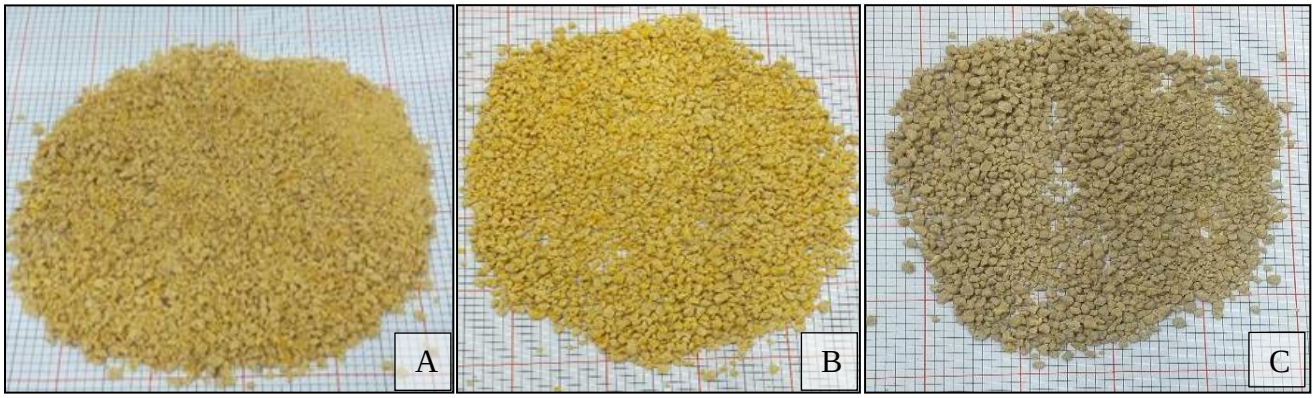

Figure 4 - Color variations of broiler starter feed. Yellowish (A), yellow (B), dark yellow (C)

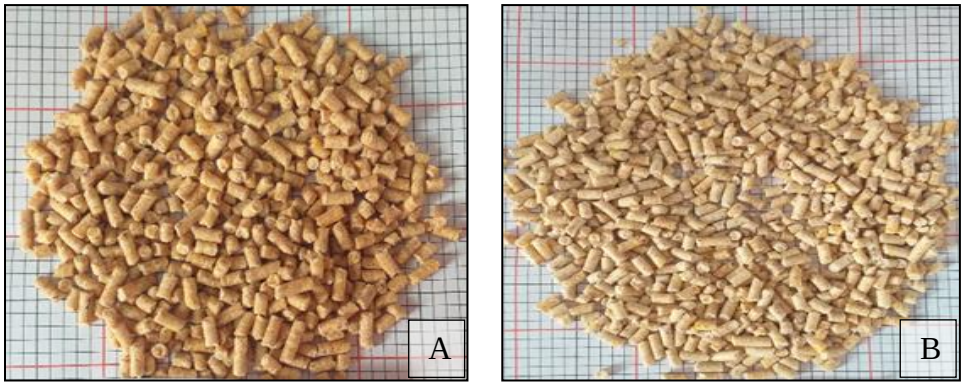

Figure 5 - Color variations of broiler grower feed. Brown (A), light brown (B)

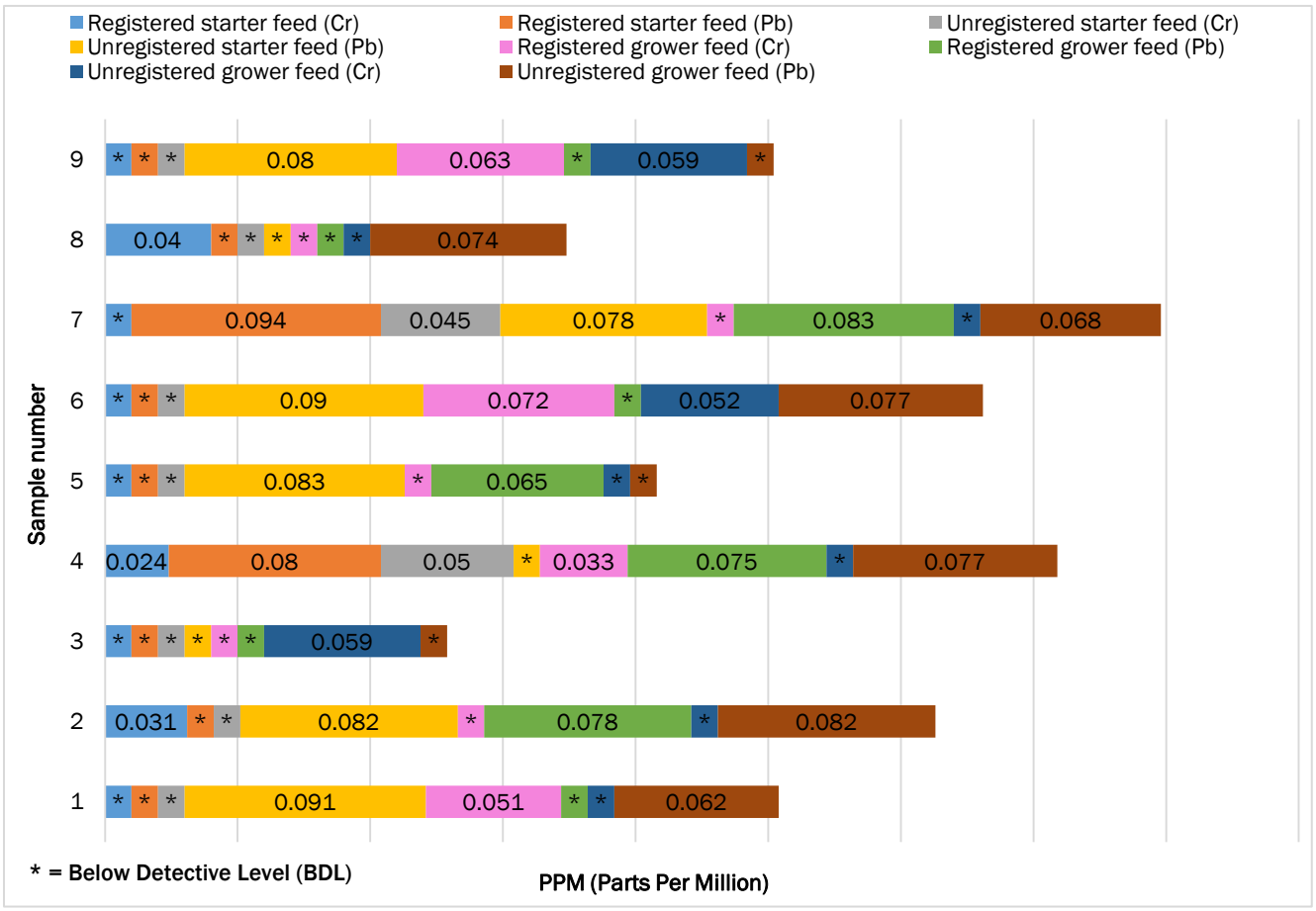

Figure 6 - Heavy metal (Cr & Pb) concentrations in starter and grower broiler feed at registered and unregistered feed companies.

Moisture percentages in broiler feed

The difference between moisture percentages of registered and unregistered poultry feed was non-significant (Table 9). Moisture percentages were found between the range of 10.83% to 12.89% and 9.96% to 11.59% in starter and grower feed, respectively which agreed with the previous studies (Rahman et al., 2014; Hossain et al., 2023). The moisture range of starter feed was observed slightly above the normal value of poultry feed compared to the previous report (Vakili et al., 2015). High moisture content is favorable for fungal development. Fungi contamination destroys the quality of feeds.

Protein percentages in broiler feed

Table 10 presents a comparison of CP percentages between the feeds of the registered and unregistered companies which differ significantly. The range of CP percentages was observed 13.32% to 24.24% and 11.01% to 18.15% in starter and grower feed, respectively which disagreed with the past studies (Hossain et al., 2023; Rahman et al., 2014). The unregistered company's feed had a low amount of protein percentages which indicated that the quality of the feed was poor (Table 10). The aforementioned differentiation can be attributed to the disparity in the caliber of broiler feed sourced from diverse producers, as well as the dissimilarity in the constituents employed for feed formulation. Additional research is advised to uncover the crude protein content of indigenous feed sources. The quantification of crude protein is a crucial task in assessing potential feed options due to its high cost and significant impact on growth and production in the event of a deficiency. Typically, starter rations are characterized by a high protein content, while grower and finisher regimens tend to feature a lower protein content, as mature fowl necessitate a reduced amount of protein (Vakili et al., 2015). According to Rahman et al. (2014), broiler diets typically exhibit a higher protein content in comparison to layer diets.

Heavy metals content of commercial broiler feeds

The study examined the Cr and Pb levels in the feeds of both registered and unregistered companies (Figure 6). The majority of the feed samples analyzed exhibited minimal quantities of Cr and Pb. Values that were below 0.05 mg/Kg, were identified as Below Detective Level (BDL) (Figure 6) (Rahman et al., 2014). That means it was not considered to be a cause for concern in terms of potential health implications. This study agreed with the past study where the Pb percentages are found in BDL (Rahman et al., 2014). In modest amounts, these metals are necessary for the maintenance of certain physiological and biochemical activities, but when they surpass particular levels, they can induce cell dysfunction and, ultimately, poisoning. Previously, the greatest concentration of chromium in broiler meat was discovered, which was greater than the permitted amount – 0 ppm for Cr and 5.00 ppm for Pb - proclaimed by WHO and FAO (Rahman et al., 2014; Islam et al., 2016). The aforementioned differentiation can be attributed to variances in the sampling methodology utilized during analysis, as well as increased awareness among feed producers. Heavy metal contamination sources vary from one element to the next (Sevik et al., 2020). These can vary depending on the kind of soil, environmental concerns, species of animals and product feeds, and geographic location (Khan et al., 2016). Several environmental components have the potential to contaminate poultry feed (Ukpe, 2018). The livestock production system can be exposed to heavy metals through multiple pathways such as inorganic fertilizer land application, air deposition, agrochemicals, and animal waste. This highlights the need for enhanced governmental quality monitoring of feedstuffs supply (Sarker et al., 2017). The utilization of animal waste as a fertilizer and soil amendment may result in the accumulation of hazardous substances in the soil and water due to the presence of heavy metals in it (Oyewale et al., 2019). It can spread to animals via the food chain. In contemporary times, individuals are increasingly aware of matters pertaining to food safety and the potential health risks associated with hazardous substances. A strong regulatory framework for food safety is necessary to guarantee the safety of food products for consumers. The lack of it in Bangladesh has persisted for a considerable duration, leading to significant implications for public health. In order to effectively tackle health challenges across every sector, laws must be applied in a suitable manner.

CONCLUSION AND RECOMMENDATIONS

A wide range of research shows that poultry products contain a significant portion of heavy metals due to the use of tannery solid wastes as an ingredient in poultry feed. This study aimed to clarify the fact that most of the poultry feed-producing companies may not generate tannery solid wastes or heavy metal-containing feed, and using their feed in poultry may not pose a health risk to human. The findings of this investigation demonstrated that the tannery sector produces solid waste of varying attributes depending upon the stages of processing. In a broad sense, the possibility of tannery solid wastes mixed feed production in Bangladesh was very rare. The unregistered companies' feeds contained low protein proving that the feeds' quality was low. Also, the negligible amount of heavy metal in poultry feed proved the fact that a small percentage of tannery solid wastes was present in poultry feed. These findings may be useful for appropriate solid waste management strategies and removing the myth of consuming poultry products.

A detailed study with a large amount of sampling is needed to investigate the true scenario of feed quality in Bangladesh. The TSWs can be a good protein source for feed if they can be collected before the tanning process.

Therefore, further research is needed to recycle the heavy metal-free TSWs as poultry feed. The heavy metals in poultry products may come from different sources other than poultry feed, such as water, soil, and air. So, concentration should also be given to those sources also. Since there is a potential health risk for those exposed to contaminated feed with different heavy metals, additional research is required to determine the quality and sources of heavy metals in feed by analyzing different types of raw ingredients that are used in livestock feed in different regions of Bangladesh.

DECLARATIONS

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Authors' contributions

The first four authors should be considered as first author. M. S. Islam planned, designed, reviewed the writing, and managed the fund of this research. A. S. Protik and M. A. Zannat collected data and samples, conducted laboratory analysis, and collaborated in data analysis. Z. Naim analyzed and visualized data, prepared the original draft, revised and edited the manuscript. M. E. Kabir curated the data and reviewed the manuscript. M. Asaduzzaman and M. Akter reviewed and approved the final version of the manuscript. All authors read and approved the submitted version of the manuscript.

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethics committee approval

This study had been carried out as a part of partial fulfillment of degree of Master of Science (MS) in Animal Science under the Department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. Consents of the sellers/shopkeepers had been taken before moving to the collection of the samples.

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EFFECTS OF DIETARY SUBSTITUTION OF SOYBEAN MEAL BY DRIED AZOLLA ON BLOOD AND SERUM PARAMETERS, PRODUCTIVE AND REPRODUCTIVE TRAITS, AND ECONOMIC EFFICIENCY OF RABBIT DOES AS WELL AS SEMEN QUALITY OF BUCKS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: An experiment was carried out to investigate the effects of dietary substitution of soybean meal in different ratios by dried Azolla (*Azolla pinnata*) on productive and reproductive performances, hematological and serum traits, and economic efficiency of rabbit does as well as semen quality of males. Forty mature does and eight males of Black Balady rabbits aged seven and eight months were assigned to 1 of 4 dietary groups: 0 (control), 20, 30, and 40% of soybean protein substituted with dried Azolla protein. Data were analyzed using repeated measures of statistical software computer program package. There were no significant differences among groups in number of services per conception as well as parturition intervals (days) with a superiority of 30% group over the control and other two groups. Average litter weight was significantly ($P<0.05$) superior in the 30% replacement group. There were no significant differences in live body weight either at birth or at weaning among the four tested groups and the 30% replacement groups recorded the highest significant daily weight gain of bunnies during the whole experimental period. All Azolla groups recorded better results in the studied traits of bucks' semen compared to the control. Azolla diets did not show any adverse effects on the studied blood parameters. The 30% replacement of the soybean protein group showed the best economic return compared to the other two replacement groups and the control. In conclusion, Azolla can safely and economically replace soybean protein at the rate of 30% in adult female rabbits' diets.

Keywords: Azolla, Body weight, Rabbit, Reproduction, Semen.

INTRODUCTION

Nowadays, the animals' performance and lower feeding costs are major concerns by researchers all over the world due to the global economic crises. In the diets, nearly 75% of livestock operation costs are associated with feed costs (Issa and Abo Omar, 2012; Tubb and Seba, 2021). The shortage of fodder is therefore substituted with concentrate feed, increases animal production costs. Fasuyi and Aletor (2005) stated that green plants early considered the most probable potential source and cheapest protein due to their power in synthesizing amino acids from various available materials. The technology of green fodder production is especially important in some countries where forage production is limited (Abo Omar et al., 2012). So far reasonable cost potential feed quality is a key to successful livestock production projects.

The need for using alternatives to concentrate feeds led scientists to one valued plant namely Azolla. Azolla is a floating fern grown in shallow water belongs to the family *Azollaceae* (DeFrank, 1995). Azolla has a unique symbiotic relationship that makes it a perfect plant with high protein content (Pillai et al., 2001; Mohamed et al., 2018). This plant is considered a promise of providing a cheap source of protein in livestock feeds (Samad et al., 2020; Nasir et al., 2022). Chemical analysis has shown that Azolla is not only very rich in proteins, but also it has valuable essential amino acids content, vitamins (such as A, B12, Beta-carotene) and growth promoter substances and minerals, e.g. calcium, phosphorus, potassium, ferrous, copper, magnesium, etc., as reviewed and discussed by Roy et al. (2016). Azolla is also characterized by phytochemical properties that make it with diverse pharmacological influences, e.g. antioxidant, anti-carcinogenic, anti-allergic, and anti-inflammatory due to its content from flavonoids, phenolic, tannins, and saponins (Mithraja et al., 2011; Thangaraj et al., 2022). From another side, Azolla carbohydrate and oil contents are very low and can be easily digested by the livestock, because of its high protein with low lignin content. Azolla found to

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improves the meat quality as well as health status and longevity of livestock. Feeding Azolla to poultry birds improved the weight of broiler chicken and increased egg production of layers. Several authors used Azolla in feeding different animals (Sheep, goats, pigs, and rabbits) as a feed substitute (Pillai et al., 2001). Moreover, El-Deeb et al. (2021a) concluded that feeding Azolla for growing rabbits positively affected its growth performance without adverse influence on blood biochemical and carcass traits when substituting 40% of soybean protein with dried Azolla protein in their diets. It is known that rabbits are considered pseudo-ruminants have the capability to utilize forage protein more efficiently than other kinds of livestock. Animals' capability of utilizing nutrients via feed conversion to meat, eggs, milk, etc. depends on several factors, e.g. immunity, its health status, and environmental conditions (stress factors) inside the animals' house, feed quality, feed intake, digestion and absorption rates of nutrients as well as its elements sufficiency (Halas and Babinszky, 2014). Scientists in the field of animal nutrition are asked to achieve this by reducing production costs and obtaining the optimum productive performance with minimizing or vanishing adverse effects (Zeng et al., 2015).

The purpose of this study was to investigate the effects of substituting various levels of soybean protein with dried Azolla protein on the productivity, reproductive performance, and economic efficiency of Black Balady rabbits.

MATERIALS AND METHODS

Ethical regulation of study

All the procedures in caring for animals and methods in this study followed the Animal Welfare regulations of the Animal and Poultry Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The current study was declared by the Local Experimental Animals Care Committee's Ethics Committee and done according to the rules of Animal and Poultry Production Research Institute, Egypt which complies with ARRIVE guidelines.

Animals' management

A number of forty female and eight males of Black Balady rabbits (Egyptian local breed) aged seven and eight months (live body weight; LBW, 2860±100 g and 3330±70 g, respectively, on average) were allotted for this work. The experimental animals were divided into 4 dietary groups (10 does and 2 males each) at the rabbit research branch, El-Serw Animal Production Research Station, Animal, and Poultry Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. The four dietary treatment groups were assigned as follows: The first group fed the control diet (without Azolla), while the second, third, and fourth groups were fed diets that included 20, 30, and 40% of soybean protein substituted with Azolla protein. The rabbits housed to have three parturitions in a wired cage of the dimensions 50 x 50 x 35 Cm where fed their assigned experimental diets. Drinking water via stainless-steel nipple was available all the time in one environment condition. Hot air (80^{o}C) dried Azolla plant samples as well as other feed ingredients were analyzed for its chemical composition using AOAC (2005) methods and the formulated diets as well as calculated chemical analysis of these tested diets are presented in Table 1.

The tested diets were formulated from pellets of 4 x 12 mm in size which prepared from feed constituents bought from the local market after grounded and mixing and fed *ad libitum* according to the NRC (1994) requirements. The light was allowed for 12-14 hours/day in the animals' houses and daily morning urine and faces dropped on the floor from the cages and cleaned.

For mating purposes, each 5 does were allotted one buck who received the same specified tested diet and each doe of the treatment was transferred to the cage of its assigned buck and returned to her cage after being mated, and after 10 days from mating does ware palpated to determine pregnancy. Litter size (LS) was detected and other rearing process protocol implemented including date of birth, number of kits, stillbirth removed, and weight of kits were recorded within the first 12 hours after kindling. Litter sizes were examined also each morning for recording during the suckling period. Young rabbits were weighed for litter weight (LW) at birth, three weeks of age (21 days) and at weaning (five weeks of age; 35 days).

Reproductive and productive parameters of does

The reproductive performance traits including number of services per conception (NSC), parturition interval (PI, days), LS (No.), LW (g), and litter weaning weight (g) were examined for each doe's rabbit. The productive performance of each doe was studied in terms of litter size and weight, and mean bunny weight (MBW) was measured at birth, 21 and 35 days of age. Thereafter, daily weight gain (DWG) was calculated for the whole period from birth till weaning (35 days) during both gestation and suckling periods. Live body weight (LBW) and total feed intake (FI; Kg/doe) were recorded.

Reproductive parameters of bucks

Semen samples of the experimental bucks were collected (3 times) using an artificial vagina at the beginning, middle, and end of the experimental period. The collected samples were tested for the traits of ejaculate volume (ml), motility (%), abnormal sperm (%), live sperm (%), dead sperm (%), total mass (5/5), and sperms' concentration (x 106/ml) according to Smith and Mayer (1955).

Table 1 - Experimental ingredients chemical composition, formulated diets and its chemical analysis

Ingredients	Chemical composition (%)				Digestible energy (Kcal/Kg)
	CP	CF	EE	Ash	
Barley	11	6.5	1.9	2.5	3300
Wheat bran	15	11	4.2	7	2410
Soybean meal (44% protein)	44	7.7	1.5	6.5	3270
Dried Azolla	28	18	2	25.8	2438
Alfalfa hay	18	27	2.7	9.7	2450
Mint straw	6	35	0.78	12	1850
Formulated diets	**Azolla protein substitution percentage**				
	Control	**20%**	**30%**	**40%**	
Barley	30	27.5	25	23.5	
Wheat bran	12	12	12	13	
Soybean meal (44 %)	18	12.34	9.51	6.68	
Dried Azolla	0	5.66	8.49	11.32	
Alfalfa hay	24.0	30	35.5	40	
Mint straw	12.5	9	6	2	
Di-calcium phosphate	1.5	1.5	1.5	1.5	
Lime stone	1	1	1	1	
Salt	0.4	0.4	0.4	0.4	
Premix*	0.3	0.3	0.3	0.3	
Yeast	0.1	0.1	0.1	0.1	
Antitoxin	0.1	0.1	0.1	0.1	
Methionine	0.1	0.1	0.1	0.1	
Total	100	100	100	100	
Chemical analysis of diets on dray matter basis (%)					
Crude protein (%)	18.44	18.07	18.09	18.11	
DE (digestible energy, Kcal/Kg)	2705	2662	2637	2630	
Crude fiber (%)	13.87	14.07	14.20	13.93	
EE (Ether extract, %)	2.22	2.49	2.68	2.90	
Ca (%)	1.12	1.24	1.34	1.38	
Av. Phosphorus (%)	0.47	0.49	0.49	0.50	
Lysine (%)	0.95	0.83	0.78	0.79	
Methionine (%)	0.32	0.30	0.28	0.29	
Methionine+Cystine. (%)	0.62	0.57	0.54	0.52	

* Permix= Vitamin & Mineral mixture contained: Vitamin A, 160,000 IU; Vitamin E, 125 mg; Vitamin K3, 17 mg; Vitamin B1, 13 mg; Vitamin B2, 43 mg; Vitamin B6, 18 mg; Pantothenic acid, 85 mg; Vitamin B12, 0.17 mg; Niacin, 230 mg; Folic acid, 12 mg; Biotin, 0.6 mg; Choline chloride, 4300 mg; Fe, 0.37 mg; Mn, 670 mg; Cu, 56 mg; Co, 3 mg; Se, 2.2 mg and Zn, 480 mg.

Blood hematology and serum biochemical parameters

At the end of the study, three blood samples were collected from the marginal ear vein of each treatment' does in two separate tubes, one of each provided with EDTA as an anticoagulant. The tubes without EDTA were kept at room temperature, and then centrifuged at 3500 rpm for 20 minutes to separate clear serum. Afterward, blood samples examined for some hematological traits included white blood cell (WBC) concentration ($WBCx10^3$), red blood cell count ($RBCx10^6$) according to Hawkeye and Dennett (1989), Hemoglobin (g/dl) according to Tietz (1982) and HCT (%). Serum samples were used to determine serum total protein (g/dl), albumin (g/dl), globulin (g/dl) by difference (total protein – albumin), total cholesterol (mg/dl), triglycerides (mg/dl), creatinine (mg/dl), high-density lipoprotein (HDL; mg/dl) and low-density lipoprotein (LDL, mg/dl) by using commercial kits (Diamond Diagnostics, Halliston, MA, USA).

Economic efficiency

For economic efficiency estimation, the model of input–output analysis outlined by Zeweil (1996) was used for calculating the return depending on the local market prices during the experimental time. The following equations were applied: Total feed cost (EGP) = Total feed intake (Kg) x price/kg feed (EGP); Total return (EGP) = Total weight rabbits (kg) x price/kg live body weight (EGP); Net return (EGP) = Total return (EGP) - Total feed cost (EGP); Economic Efficiency (EE) = Net return (EGP) / Total feed cost (EGP).

Statistical analysis

Data were analyzed by using repeated measures of statistical software computer program CoStat (2017) upon the following model: $Y_{ij} = \mu + T_i + P_j + (TP)_{ij} + e_{ijk}$, where: μ = General mean, T_i = Treatment effect, P_j = Period effect, $(TP)_{ij}$ = Interaction of treatment and period effect, and e_{ijk} = Error. Significance among treatment means was tested at ($P<0.05$) using the Least Significant Difference Test (Snedecor and Cochran 1990).

RESULTS AND DISCUSSION

The chemical composition of Azolla used in this study was higher in CP, CF, and ash (Table 1), but lower in EE content than that reported by Anitha et al. (2016b). The high content of Azolla CF in the present study is within the range values (from 15.17 to 19.85%) reported by Kavya et al. (2014). Also, the high ash content was within the values between 26.28 and 29.17% reported by Kathirvelan et al. (2015). This variation and changes in Azolla chemical constituents among different research works might be due to changes in its dry matter content. The low EE level detected in this study falls within the range of 1.6-5.05% reported by Mohamed et al. (2018). It is worth noting that the variation in the chemical composition of different Azolla species leads to differences in feed intake which is associated with energy consumption. In this concern, Alalade et al. (2007) attributed the poorer growth rate of birds at 15% Azolla to lower feed intake and consequently the reduction in metabolizable energy.

Rabbit doe reproductive performance

Table 2 represents the studied reproductive traits of the Black Baladi rabbit' does as well as the average feed intake (FI; Kg/doe) during the three parities studied periods. There were no significant differences among tested groups regarding No. of services/conception as well as parturition intervals (days) with a superiority of 30% group over the control and other two tested groups. It is obvious that FI (Kg/doe) was the lowest in the 30% group, while the highest one was in the 20% group followed by 40% and the control groups, respectively. The substitution of 30% soybean protein with dried Azolla protein reduced FI significantly (P<0.05) by about 2.1% while replacing 20 and 40% of soybean protein with dried Azolla protein raised significantly (P<0.05) FI by about 10.5 and 4.6% compared to the control group, respectively. This reduction in FI may be due to the bigger LS (6.63) recorded for the 30% substitution group compared to 4.88 and 5.00 for the control, 20 and 40% groups. Average LW was significantly (P<0.05) superior in the 30% replacement group over all the other three tested groups from birth till weaning.

This trend was confirmed by Jiao et al. (2014) who stated that physiological status as well as other factors, e.g. energy content affects FI. In this concern, Xiccato and Trocino (2010) stated that rabbits try to adjust the intake of digestible energy (DE) throughout regulating their feed intake if fed *ad libitum*. This adjustment can be achieved when the dietary energy concentration ranges between 9.00 and 11.50 MJ DE/kg which is in line with the energy content of the present tested diets (Ranging between 11.00 to 11.32 MJ DE/Kg diet). Moreover, the does during lactation are in a negative energy balance (lower body fatness), and according to Lebas (2004), a reduction in feed utilization with very high energy diets (>2650 kcal DE/Kg) is still to be noticed. Recently, Read et al. (2015) reported that a diet with levels of different fiber types maximizes digestive health and results in a favorable better feed utilization.

Table 2 - Some productive and reproductive traits of Black Baladi rabbit does, fed different ratios of dried Azolla

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
No. of services/conception	1.25	1.25	1.38	1.38	NS	-
Parturition interval (days)	50.25	52.50	49.25	54.00	NS	-
Feed intake (Kg/doe)	23.20^{ab}	25.63^{a}	22.72^{c}	24.26^{bc}	*	1.45
Average litter size at birth	4.88^{b}	5.00^{b}	6.63^{a}	5.00^{b}	*	1.21
at 21days	4.63^{b}	4.50^{b}	6.25^{a}	4.63^{b}	**	0.75
at weaning	4.25^{ab}	4.38^{ab}	4.69^{a}	4.50^{b}	**	0.92
Average litter weight at birth	283.75^{b}	297.75^{b}	384.75^{a}	303.13^{b}	*	72.90
at 21days	1289.25^{b}	1084.38^{b}	1735.13^{a}	1174.25^{b}	**	248.51
at weaning	3083.38^{b}	2556.25^{b}	3821.75^{a}	2598.75^{b}	**	649.56

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD=Least significant difference at P<0.05; NS = not significant; *= P≤0.05; **= P≤0.01.

Rabbit does productive performance

Information in Table 3 introduces the studied productive traits of bunnies resulting from Black Baladi does in the four tested groups in terms of average live body weight and weight gain (DWG) from birth till weaning. The figures reflected that, although there were no significant differences in live body weight either at birth or at weaning among the four tested groups, the 30% replacement groups recorded the highest significant DWG during the whole experimental period. During the suckling period, it is difficult to consider the feed conversion ratio for the resultant bunnies, the improvements in weight gain may be attributed to the benefit of does from feeds and providing its growers with enough milk. In this concern, the increase in EE% (Table 1) of the Azolla groups' tested diets may be helped in increasing milk production for their bunnies. This opinion is supported by Maertens and Gidenne (2016) who reviewed that moderate dietary fat addition for reproductive females in intensive rabbit breeding systems has a favorable impact on milk production. However, such effects on LW at weaning are not very noticeable. Moreover, growth rate and feed efficiency are higher if the feed is adjusted for essential nutrients such as amino acids (Read et al. 2015) which may be available in

Azolla-tested diets than the control one. On the other hand, Bovera et al. (2012) concluded that feed additives can have little or no effects in improving rabbit performance.

In general, it is of interest to note that young bunnies started to consume diets with their dams after 3 weeks of age, thus it is important to estimate LW at this time. In this concern, Fortun-Lamothe et al. (2006) demonstrated that with high production efficiency of the female rabbit, the kits consumed 4.17 Kg of feed from 18 to 35 days (Weaning), or 34% of the exclusive doe consumption (12.2 Kg from parturition to weaning). Alalade et al. (2007) attributed the differences in weight gain to the variation in Azolla strains and nutrient composition as well as the type and physiological status of the used experimental animal.

Table 3 - Growth performance of bunnies from does Black Baladi rabbit fed different ratios of dried Azolla

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
Average bunny weight at birth	59.68	59.53	57.91	61.15	NS	-
Average bunny weight at weaning	686.5	732.09	729.19	695.94	NS	-
Daily weight gain/bunny (0-21 days)	47.89^{b}	37.46^{b}	64.30^{a}	41.46^{b}	**	10.88
Daily weight gain/bunny (21-35 days)	128.14^{ab}	105.13^{b}	149.04^{a}	101.76^{b}	*	31.94
Daily weight gain/bunny (0-35 days)	79.99^{b}	64.54^{b}	98.20^{a}	65.60^{b}	**	17.99

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD= Least significant difference at P<0.05; NS = not significant; *= P≤0.05; **= P≤0.01.

Reproductive parameters of bucks

Table 4 introduces the effect of substituting soybean protein in the diet with 20, 30, and 40% of dried Azolla protein. Generally, all Azolla tested groups recorded better significant (P<0.05) results in terms of ejaculate volume (ml), motility, abnormal sperm, live sperm, and dead sperm percentages as well as sperm concentration, while total mass was not significantly differed among the four tested groups. A similar trend was recorded by El-Deeb et al. (2021b) who attributed this enhancement in the reproductive traits of rabbit bucks to the active components of feed additives, e.g. antioxidant factors and/or sufficient supplied nutrient elements which are available in Azolla. Moreover, several studies on rabbits, in this area (Gabbar et al. 2019, Kandeil et al. 2019 and Abdel-Wareth and Metwally 2020) concluded that thyme aqueous extracts can play a major role in enhancing the semen quality of bucks due to its diverse pharmacological properties.

Table 4 - Semen quality traits of Black Baladi rabbits' bucks fed different levels of dried Azolla

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
Ejaculate volume (ml)	0.77^{c}	0.83^{b}	0.83^{b}	0.90^{a}	**	0.05
Motility (%)	63.53^{b}	75.97^{a}	81.17^{a}	77.67^{a}	**	6.90
Abnormal sperm (%)	15.04^{a}	11.67^{b}	11.07^{b}	9.15^{c}	**	1.81
Live sperm (%)	76.20^{c}	83.63^{b}	78.60^{c}	87.33^{a}	**	3.02
Dead sperm (%)	23.80^{a}	16.70^{b}	22.73^{a}	12.67^{c}	**	2.95
Total mass	3.33	3.67	4.33	4.33	NS	-
Concentration	173.53^{b}	185.17^{ab}	196.60^{a}	195.83^{a}	*	14.27

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD= Least significant difference at P<0.05; NS = not significant; *= P≤0.05; **= P≤0.01.

Blood and serum parameters

Exploring figures of blood and serum studied parameters in Table 5 showed no significant differences among tested diets regarding both white and red blood cell count (WBC & RBC) as well as hematocrit % in which the 40% Azolla replacement group recorded the highest values of all parameters. While hemoglobin % significantly (P<0.05) varied among the four tested groups and followed a similar trend to the other three hematology-studied traits.The obtained values of white blood cell (WBC) and red blood cell (RBC) counts are close to the published ranges of the same used experimental breed by Beshara et al. (2018) and El-Deeb et al. (2021b). In this domain, Moore et al. (2015) demonstrated that New Zealand white rabbit adult females' blood contents of WBC, RBC, and hemoglobin ranged from 5.2-10.6, 5.11-6.51 (x103 and 106/µL) and 9.8-15.8%. Moreover, Hct. (%) was lower than that obtained by El-Deeb et al. (2021b), except for the 40% replacement group which was within their range (24.6-29.03), but almost similar to the results of Beshara et al. (2018). Moreover, Özkan et al. (2012) stated that values of both red blood cell count and hematocrit (HCT) level in rabbits are influenced by stress, age, season, and genus. Melillo (2007) and Jenkins (2008) reported that HCT values

under 30% accompanied by a reduction in hemoglobin are evaluated as anemia. Furthermore, they also denoted that WBC counts increased in rabbits rarely indicate an infection.

Generally, serum studied traits (Total protein, albumin, globulin, total cholesterol, and creatinine) contents did not show any significant differences among the four tested groups. But, triglyceride concentration significantly (P<0.05) increased in the 20 and 40% replacement groups, while significantly (P<0.050) decreased compared to the control. The HDL content significantly (P<0.05) increased in the three replacement groups compared to the control, while LDL was not significantly differed among the four tested groups. Serum chemistry traits are in agreement and close to ranges of 5-7.5 g/dl, 2.5-4 g/dl and 2.5-4 g/dl mentioned by Anitha et al. (2016a). They also added that the mean values of serum chemicals are generally affected by the quality and quantities of protein intake and these values can be used as an indicator for non-stress factors as well as for the nutritional adequacy of dietary protein.

Table 5 - Some blood and serum parameters of Black Baladi rabbit does fed different ratios of dried Azolla.

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
Hematology traits						
White Blood Cells (WBC×10^3/µL)	6.37	4.80	6.00	8.00	NS	-
Red Blood Cells (RBC×10^6/µL)	4.95	5.75	4.61	6.56	NS	-
Hemoglobin (Hb; g/dL)	11.73^{a}	11.98^{a}	10.88^{b}	12.50^{a}	*	0.83
Hematocrit (Hct. %)	27.00	28.43	24.06	32.10	NS	-
Serum traits						
Total protein (g/dL)	6.57	6.50	7.77	7.20	NS	-
Albumin (g/dL)	3.53	3.93	3.60	3.73	NS	-
Globulin (g/dL)	3.04	2.57	4.17	3.47	NS	-
Total cholesterol (mg/dL)	156.67	128.67	135.67	137.00	NS	-
Triglycerides (mg/dL)	119.33^{ab}	135.67^{a}	111.00^{b}	124.33^{ab}	*	24.66
Creatinine (mg/dL)	0.95	0.97	0.88	0.87	NS	-
HDL (mg/dL)	16.00^{b}	22.00^{ab}	24.67^{ab}	32.33^{a}	*	11.78
LDL (mg/dL)	15.33	12.67	13.00	14.33	NS	-

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD= Least significant difference at P<0.05; NS = not significant; *= P≤0.05.

Economic efficiency

Calculations of the economic efficiency for substituting the proportion of soybean protein with a cheaper source of good nutrient composition source like Azolla protein at the rate of 20, 30, and 40% are presented in Table 6. The data generally indicated that the 30% replacement of the soybean protein group showed the best economic return and utilization of feed compared to the other two replacement groups and the control. The good return from this group may be attributed to the reduction in feed intake accompanied by the highest average weight rabbits (Kg/doe). This low-cost production technology is believed to be taken up more widely by dairy farmers particularly with fodder production shortage (Pillai et al. 2001). They reported that Azolla increased the feed conversion ratio and thus milk yield in cattle.

Katole et al. (2017) reported that the use of Azolla as a feed substitute saved about 20-25% of concentrate mixture feeding cost. Moreover, Jentzer (2008) stated that the feed conversion ratio was responsible for 30%, on average, of the feed cost margin variation, while this was only about 9 and 7% for the feed price and the sale price of rabbits, respectively.

Table 6 - Economic efficiency of does Black Baladi rabbit fed different diets of dried Azolla

Items	Azolla protein substitution % groups				Significance	LSD
	Control	20	30	40		
Average feed intake (Kg/doe)	23.20^{ab}	25.63^{a}	22.72^{c}	24.26^{ab}	*	1.45
Average weight rabbits (Kg/doe)	6.167^{ab}	5.113^{c}	7.644^{a}	5.198^{bc}	*	1.53
Price / Kg feed (EGP)	5.24	5.25	5.27	5.28	NS	NS
Total feed cost / doe (EGP)	121.57^{bc}	134.56^{a}	119.73^{c}	128.09^{ab}	*	8.76
Price / Kg live body weight (EGP)	40	40	40	40	-	-
Total return / doe (EGP)	246.68^{b}	204.52^{c}	305.76^{a}	207.92^{c}	**	61.17
Net return / doe (EGP)$^{(1)}$	125.11^{b}	69.96^{c}	186.03^{a}	79.83^{c}	*	68.79
Economic Efficiency (EE)$^{(2)}$	1.03^{ab}	0.52^{c}	1.55^{a}	0.62^{bc}	*	0.61
Relative Economic Efficiency (REE)$^{(3)}$	100^{ab}	50.52^{c}	150.96^{a}	60.55^{bc}	*	59.25

$^{a, b, c}$ Means in the same row having different superscripts are significantly different. LSD= Least significant difference at P<0.05. EGP = Egyptian pound. $^{(1)}$ Net return (EGP) = Total return (EGP) - total feed cost (EGP); $^{(2)}$ Economic Efficiency (E.E) = Net return (EGP) / total feed cost (EGP); $^{(3)}$ Relative Economic Efficiency (REE) = (E.E / E.E of control) x 100.

CONCLUSION

It can be concluded from the obtained results that Azolla can safely and economically replace soybean protein at the rate of 30% for adult female rabbits without any adverse effects on their health status or their suckling bunnies. The study recommending the replacement of 30% of soybean protein with dried Azolla protein in rabbit diets to reduce the production costs of feeds and increase profitability.

DECLARATIONS

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Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors' contribution

A.M. Alazab; S.A. Shazly; H.N. Fahim and M.A. Ragab performed research, taking and preparing samples. M.M. El-Deeb performed literature overview, analyzing results, editing and article writing. All authors reviewed and approved the manuscript.

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Consent to publish

All authors selected the "Online Journal of Animal and Feed Research" for publishing this research article because it is in the scope (animal nutrition) of the journal.

Competing interest

The authors have not declared any conflict of interests.

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PERFORMANCE, CARCASS WEIGHT, TOTAL INTESTINAL BACTERIA, AND FEED DIGESTIBILITY OF BROILERS FED CHICKEN FOOT-DERIVED BIOACTIVE PEPTIDES

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The aim of this study was to evaluate the supplementation of chicken claw-derived bioactive peptides on performance, carcass weight, total intestinal bacteria, and feed dry matter (DMD) and organic matter (OMD) digestibility of broilers. A completely randomized design and five repetitions were applied in this experiment. The research material consisted of 200 DOC strain CP-707 grown up to 35 days of age, and the biopeptide was produced by hydrolyzing chicken claws protein with a commercial papain enzyme. Bioactive peptides were added to feed treatments in amounts of 0, 2, 4, and 6%. The differences between treatments were tested using the honestly significant difference test. The addition of chicken claws biopeptides had a significant influence ($P<0.01$) on OMD and carcass weight, as well as a significant effect ($P<0.05$) on body weight gain, feed efficiency, DMD and total bacteria. Addition of chicken claws-derived peptides in rations up to 6% enhanced body weight gain, feed efficiency, carcass weight, DMD, OMD, and total intestinal bacteria in broiler chickens.

Keywords: Biopeptides, Chicken, Claw, Digestibility, Carcass quality.

INTRODUCTION

One of the feed additives which is currently prohibited in chicken ration is antibiotic growth promoters (AGPs). It causes resistance effect of pathogenic bacteria that lives inside the digestive tract and their abandoned residues in animal products (Bahar and Ren, 2013) as laws in many nations forbid using antibiotics (Prihambodo et al., 2021). Therefore, efforts are required to substitute the role of AGP in feed through the use of various natural ingredients that are secure for consumers and environmentally friendly. Biopeptide are peptides that have a measurement of 2-20 kDa and have various biological functions, they could act as antioxidants, antimicrobials, antihypertensives drugs and immunomodulators (Hartmann and Meisel, 2007).

Biopeptides are produced by hydrolysis of proteins using enzymes or microorganisms. Livestock product such as chicken claw has been studied to generate biopeptides. Chicken claw contains 60% water, 12.87% fat, 17.17% protein, 9.94% ash (Santana et al., 2020), 9.07%-12.8% collagen (Santosa et al, 2018), 1.70 mg phosphorus, 2.87 mg calcium, 5.5 g unsaturated fat, 2.571 mg omega-6 and 187 mg omega-3 (Muyonga et al., 2004), and cholesterol 108 mg/100 g ceker (USDA, 2018). Glycine (33%), proline and hydroxyproline (22%) are the main amino acids that form collagen. The primary structure of collagen is a triple alpha-helix, each helical chain has a molecular weight of around 100 kDa and is made up of 1014 amino acids, so the overall collagen molecular weight is 300 kDa respectively (Leon-Lopez et al., 2019). Chicken water-soluble collagen has the role of increasing immunity (Tong et al., 2010), omega-3 as anti-inflammatory and beneficial for brain function (Soeparno, 2011). Enzymatic hydrolysis of collagen produces smaller gelatin proteins, and if gelatin is hydrolyzed further, it will produce smaller-sized peptides which have the ability as bioactive peptides.

Papain (EC.3.4.22.2) is a kind of cysteine protease enzyme that consists of a polypeptide chain with three disulphide bridges and one sulfhydryl group that is essential for catalysis (Bakar, 2010). Papain's molecular weight is 23 kDa (Ming et al., 2002). Pure papain has a specific activity of 0.119-0.347 mg/ml in distilled water. Papain was found in papaya sap, leaves, seeds and fruit skin (*Carica papaya*) it has maximum activity at 60^{0}C and pH 8.0 and is stable at 60^{0}C for 30 minutes (Khatun et al., 2023). Papain was reported to have a selective advantage for hydrolyzing fishbone collagen (Hidayat et al., 2016). The use of the enzyme papain for the hydrolysis of chicken claws potentially produces bioactive peptides as antioxidants and antimicrobials. The antibacterial activity of papain was reported to inhibit the growth of acne-causing bacteria (Ardina, 2012). The degree of papain hydrolysis in the amount of 20% was reported to be able to produce bioactive peptides of 2-5 kDa respectively (Hema et al., 2017).

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An immune system that was maintained by biopeptide intake was indicated by increased growth and carcass weight of broiler chickens. Good immunity causes the use of feed protein more efficient so it improves meat protein deposition (Jamilah and Mahfudz, 2013). On the other hand, the biological function of peptides as antioxidants and antibacterial improve intestinal function through the development and integrity of intestinal cells as well as the diversity of intestinal microorganisms so that the digestibility of feed nutrients is increasing. Supplementation of soybean meal bioactive peptides up to 6 g/kg feed markedly increased FCR and tended to escalate dry matter digestibility and intestinal villi length (Abdollahi et al., 2017). The addition of small commercial peptides as much as 4.5 g/kg feed significantly increased the diversity of microorganisms in the intestines of laying hens (Zhao et al., 2022). The aim of this study was to examine the effect of chicken claw biopeptides supplementation on carcass weight, total intestinal bacteria, dry matter (DMD) and organic matter digestibility (OMD) in broiler feed.

MATERIALS AND METHODS

This experiment was performed in accordance with regulations of University of Jenderal Soedirman number 1310/UN23/HK.03/2021. The authors complied with the ARRIVE (Animal Research: Reporting of in Vivo Experiments) guidelines 2.0.

Experimental design and treatments

In this study, 200 unsexed birds CP 707 strains of day-old chicks were raised for 35 days. The birds were grown in an open house arrangement divided into 20 pens, each with ten birds. Prepare a feeder and a drinker tube for ad libitum supply. The basal feed consists of 42% milled corn, 21% rice bran, 23% soybean meal, 10% fish meal, 3% vegetable oil, 0.8% mineral mix, 0.1% methionine and 0.1% lysine. The levels of chicken claws biopeptides were 0% (T0), 2% (T1), 4% (T2) and 6% (T3).

Table 1 - Nutritional content of feed treatments

Nutrients	T0 (%)	T1 (%)	T2 (%)	T3 (%)
Crude protein (%)	22.76	23.04	23.32	23.60
Metabolizable energy (Kcal/kg)	2969	2990	3015	3024
Crude fat (%)	6.870	7.150	7.420	7.700
Crude fibre (%)	5.840	6.200	6.570	6.930
Calcium (%)	0.720	0.880	0.880	0.880
Phosphor (%)	0.560	0.640	0.640	0.640
Methionine (%)	1.180	1.320	1.320	1.320
Lysine (%)	0.390	0.590	0.590	0.590

Production of biopeptides

A total of 100 g chicken claws were added to 500 ml of water and then extracted by autoclave for 30 min. Filtering the chicken claws to obtain extracts liquid, the extracted fat was separated using an extract solution stored in the refrigerator overnight, so that the fat was easily separated from the collagen. The protein content of the chicken claws extracts was 0.662 g/ml. Papain commercial (0.602 U/mg) was dissolved in 250 ml 0.02 M Tris-Cl pH 7.00 then added to chicken claws extracts and homogenized. The reaction mixture was incubated at 50^{0}C for 24 h. Chicken claws hydrolyzate was mixed with sterilized rice bran (4:1) as a carrier and dried in an oven at 60^{0}C for 48 h. The chicken claws biopeptides were ground and stored for in vivo treatments, it contains 14.10% protein, 13.81% fat and 18.19% crude fibre.

Data collection

Carcass traits

To assess the carcass at the end of the trial, two birds from each pen were chosen and slaughtered, the corpses were de-feathered and the giblet was hand eviscerated. The process of slaughtering followed the rules of halal according to Islamic religion that is UU No. 33/2014 and regulation of University of Jenderal Soedirman. The carcass was then divided into several components, including the breast, thigh, wings, and back weight, and the proportion of each yield weight over the carcass weight was computed.

Total bacteria

Samples for total bacteria calculation were obtained when the chickens were 35 days old. A total of 20 chickens were slaughtered and digesta samples were taken from the duodenum, jejunum and ileum and homogenized. For slaughtering chickens were fasted in a comfortable cage for 12-24 h, then each bird were taken and slaughtered

according to halal rules (cutting oesophagus, trachea and vena jugularis using a sharp knife). Digesta samples were stored in sterile vials and tightly closed and then put in ice bags and moved to the laboratory for total bacteria analysis. Total bacteria were calculated by total plate count method according to Barrow and Feltham (1993). One-gram of the digested sample was diluted in stages from 10^{-1} to 10^{-9} using sterile distilled water. A total of 100 µl of the sample was placed in a petri dish, and then sterile nutrient agar media was poured and left to solidify. All sample were incubating at 40°C for 24 h. The number of intestinal bacterial colonies was counted using the Quebec colony counter at a 10^{-5} dilution.

Determination of feed digestibility

Residual feed and fecal samples were collected for five days when chickens reached 30 days of age. The total residual feed and fecal weighing data were obtained by collecting and weighing the samples every morning before being fed. As much as 10% samples were taken, samples were dried at 105°C for 8 h. The dry matter content (%) is calculated based on the wet weight minus the moisture content of the samples. Then the dry samples were composited and analyzed for ash content. The organic matter content (%) was obtained after the dry matter content was reduced by the ash content (AOAC, 2012). The coefficient digestibility of dry matter and organic matter were measured as:

$$\text{Nutrients digestibility (\%)} = \frac{[(\%\text{ nutrient in feed x Feed Intake}) - (\%\text{ nutrient in faeces x Total Faeces})]\text{x } 100}{(\%\text{ nutrient in feed x FI})}$$

Statistical analysis

A completely randomized design (CRD) was applied with four levels of chicken claw bio-peptide supplementation viz. 0% (T0), 2% (T1), 4% (T2) and 6% (T3). Each treatment was repeated five times, so there were 20 trial units fraught with 10 birds/unit. To find out the differences between treatments, an honest significant difference test was carried out.

RESULTS AND DISCUSSION

Chicken claws that were extracted using an autoclave were solved with a fairly high protein content is 0.662 g/ml and the protein content increased to 1.005 g/ml after being precipitated by 40% ammonium sulphate. Chicken claws hydrolysate produced by commercial papain resulted in a 2.90% degree of hydrolysis and it has antioxidant activity in the amount of 92.29% and antibacterial (Table 2). Supplementation of 4% and 6% chicken claws bioactive peptides significantly affected organic matter digestibility (OMD) and carcass weight ($P<0.01$), dry matter digestibility (DMD) and total intestine bacterial ($P<0.05$). Increasing bioactive peptides level in feed significantly improved carcass weight, digestibility and total bacteria (Table 3), body weight gain and feed efficiency but it has no effect on feed intake (Table 4). I t also led to an increase in the levels of feed protein, fat and crude fibre (Table 1). The crude fibre content of feed increased due to the use of rice bran as a carrier for chicken claw peptides.

Table 2 - Biological activity of hydrolysates chicken claws

Biological activity	Type of assay	Process	Hydrolyzate	References
Antioxidant	In vitro	Hydrolysis with papain	Protein content= 0.662 g/ml	Hartoyo et al. (2022)
		Precipitated by 40% ammonium sulphate	Protein content= 1.005 g/ml, antioxidant activity= 92.29%	
Antibacterial	Animal study	Hydrolysis with papain	Protein content= 0.662 g/ml	Hakim et al. (2023)
		Precipitated by 40% ammonium sulphate	Protein content= 1.005 g/ml, intestinal *E. Coli* decreased by 67% at 1.6% supplementation	

Table 3 - Effect of chicken claw biopeptides on carcass weight, total intestine bacteria, dry matter and organic matter digestibility in broiler

Variables	T0	T1	T2	T3
Carcass weight (kg/b)	0.67 ± 0.02^{a}	0.69 ± 0.02^{ab}	0.71 ± 0.01^{bc}	0.72 ± 0.01^{c}
Total bacteria of digesta (cfu/mL)	7.20 ± 3.09^{a}	6.40 ± 2.98^{b}	6.80 ± 1.50^{c}	7.50 ± 2.03^{ac}
Dry matter digestibility (%)	76.9 ± 1.99^{a}	77.8 ± 2.74^{b}	79.2 ± 2.76^{bc}	82.0 ± 1.73^{c}
Organic matter digestibility (%)	77.3 ± 2.03^{a}	78.6 ± 1.69^{b}	80.2 ± 2.08^{bc}	81.6 ± 1.45^{c}

^{abc} superscript with different letters in the same column indicates a difference in $P<0.05$ or $P<0.01$. T0 = basal feed; T1 = basal feed + 2% biopeptides; T2 = basal feed + 4% biopeptides; T3 = basal feed + 6% biopeptides

Table 4 - Effect of chicken claw biopeptides on performance of broiler*

Variables	T0	T1	T2	T3
Body weight gain (g)	1018,97±90.63[a]	1045,47±67.27[a]	1048,83±20.25[a]	1182,07±59.50[b]
Feed efficiency (%)	42.74±3.41[a]	43.99±2.64[a]	43.74±1.22[a]	49.96±2.45[b]
Feed intake (g)	2383,86±75.42	2376,08±29.97	2398,12±21.76	2379,83±16.32
Total *E. coli* (log cfu/ml)	10.91±6.09[b]	3.64±4.98[ab]	1.82±2.50[a]	0.91±2.03[a]

[abc] superscript with different letters in the same column indicates a difference in P<0,05. T0 = basal feed; T1 = basal feed + 2% biopeptides; T2 = basal feed + 4% biopeptides; T3 = basal feed + 6% biopeptides. * Hakim et al. (2023)

Hydrolysis of chicken claw protein (collagen) by papain will produce gelatin, and further, gelatin hydrolysis generates small peptides (3-6 kDa) that are soluble and have antioxidant and antibacterial activity (Ketnawa et al., 2017). Bioactive peptides are short amino acids chain possessing biological activity, such as antibacterial, antioxidant, antihypertensive, and immunomodulatory effects (Hou et al., 2017). These bioactive peptides are typically 2-20 AA residues long; however, some may have more than 20 AA residues (Bah et al., 2016). The length of time hydrolysis by enzymes affects the amount and type of producing peptides. In this study, the hydrolysis time of chicken claws by papain was 24 hours. It was suspected that this time has resulted in many small peptides with different lengths and amino acid compositions that have various biological functions. According to Hou et al. (2017) generally, biopeptides production by enzyme hydrolysis takes 4-48 hours. Production of antioxidant peptides from casein cow's milk using pepsin and trypsin for 24-28 hours produces small-sized peptides with several amino acid residues of 2-8 kDa (Power et al., 2013).

Increasing carcass weight associated with chicken claws peptides supplementation improved the chicken's immune system, this condition directly resulted in growth rate and meat protein deposition, which got higher. Meat protein deposition is influenced by feed protein content and it will determine carcass weight (Widiyawati et al., 2020). T3 (6% chicken claws peptides) resulted in the highest carcass weight and total intestinal bacteria, which were 0.721 kg/bird and 7.2 x 10-5 cfu/ml compared to T0 (control). The addition of chicken claws peptides of as much as 6% (T3) afforded to produce meat tissue of as a carcass component. Carcass weight was directly proportional to body and linearly to weight gain (BWG). These results are consistent with the research of Hakim et al. (2023) that BWG of broiler chickens fed chicken claws peptides up to 6% improved bursa fabricius weight which has a positive impact to immunomodulators and antioxidants agent that affect the health of chickens. According to Abdelaziz et al. (2018), bursa fabricius plays an important role in cell differentiation that maintains the immune system, that is B lymphocytes and it has the ability to produce local antibodies. The addition of antimicrobial peptides (AMP) has a linear pattern, it increased the immune response and antioxidant activity of broiler chickens (Sholikin et al., 2021). Antioxidant compounds are capable of capturing and neutralizing free radicals so that oxidative stress stops and cell damage can be avoided. In this study, the effects of immunomodulatory and antioxidant compounds in chicken claws peptides were reflected in carcass weight increase. Besides that, there is a strong correlation between biopeptide with an increment of carcass weight by nitrogen metabolism pathway (Seifi et al., 2018). As we know, nitrogen is a part of protein which is needed due to its metabolism to produce amino acids.

Antibacterial peptides in chicken claws significantly (P<0.01) reduced *E. coli* population in chicken intestines (Table 4) from 10.91 x 10^{-5} cfu/ml to 0.91 x 10^{-5} cfu/ml when it supplemented up to 6% in broiler rations (Hakim et al., 2023). In this study, the addition of 2-4% chicken claws peptides was able to reduce the total intestinal bacteria (Table 2). This result agrees with Sholikin et al. (2021) who stated that increasing the level of antimicrobial peptides (AMP) linearly reduced the number of intestinal bacteria and broiler feces (coliform, *E. coli*, *Clostridium* spp., lactic acids bacteria and total aerobic bacteria), however, several other bacterial species were not affected by the addition of AMP. Batt and Lou Tortorello (2014) reported that *E. coli* and total aerobic bacteria are a group of coliforms, which are gram-negative and lactose-utilization bacteria. Coliform bacteria are reported can produce various toxins such as indole, scatole and thionine which stimulate the development of cancer and cause diarrhea (Girard and Bee, 2020). Gram-negative bacteria viz. *E. coli* was reported to be inhibited by AMP cecropin from maggot *H. illucens* and lysozyme (Park and Yoe, 2017). Papain hydrolysis on chicken claws protein presumably generates small peptides which can reduce total bacteria in the chicken intestines.

A decrease in total intestinal bacteria increased the DMD and OMD of feed (Table 2). The enhancement level of chicken claws peptides induced the value of DMD from 76.9 ± 1.99 (T0) to 82 ± 1.73 (T3) and OMD 77.3 ± 2.03 (T0) to 81.6 ± 1.45 (T3) (OMD). Karimzadeh et al. (2016) informed that the addition of Canola peptides as much as 200 and 250 mg/kg broiler feed improved villi length the duodenal and ileum and the activity of hydrolytic enzymes i.e., amylase, protease and lipase as well as DMD and OMD. The DMD value increased from 70.64% (control) to 74.60% and the OMD

from 71.5% (control) to 74.0%. According to Zhao et al. (2022), small peptides supplementation increases intestinal function through stimulation of intestinal development, integrity, barrier function and the diversity of intestinal microbiota in growing hens. In this study, chicken claw peptides presumably caused an increase in digestive enzyme activity, its boost the DMD and OMD values of feed and nutrient absorption. Improving the feed digestibility was proven by the higher carcass weight.

CONCLUSION

Performance, carcass weight, dry matter and organic matter digestibility and total intestinal bacteria of broiler chickens improved by biopeptides from chicken claw up to the level of 6%. The next research is to study the optimal level of bioactive peptides supplementation derived from chicken feet in broiler chicken or laying hen feed.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

SR came up with the idea, created the basic outline, and wrote the manuscript. SR, BH, and TW assisted with experimental coordination and data collecting, while EAR performed statistical analyses. TRP and MB wrote and proofread the manuscript, while FMS evaluated, critiqued, and edited it based on its intellectual content. The final manuscript was read and approved by all authors.

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Competing interests

The authors declare no competing interests in this research and publication.

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GENOTYPIC CHARACTERIZATION OF *Escherichia coli* ISOLATED FROM INFECTED CHICKEN IN BASRAH, IRAQ

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ABSTRACT: This study aimed to detect the presence of *Escherichia coli* in broiler and layer hens in the Basrah province, Iraq using macroscopic and microscopic diagnosis and bacterial isolation that causes infection in some internal organs (liver and heart), and by polymerase chain reaction. Randomly chosen samples were taken from different places within Basrah province for further investigation (poultry fields in Al-Qurnah and Al-Hartha). The bacteriological analysis revealed that the presence of *Escherichia coli* is responsible for causing fibrinous pericarditis and perihepatitis in birds. The macroscopic examination revealed hemorrhagic lesions and a significant buildup consisting of a white fibrous accumulation in the pericardial sac of the infected birds' hearts. The livers of infected birds exhibited significant deposition of white fibrous exudate on the liver surface, along with hepatomegaly. The afflicted heart displays a microscopic appearance marked by a notable aggregation of inflammatory cells in the pericardial sac and the release of fibrinous exudate. Additionally, there is an accumulation of edematous exudate in the cardiac muscle fibers, accompanied with congestion of blood vessels in the myocardium. The microscopic examination of the infected liver revealed the existence of a significant infiltration of inflammatory cells in the liver capsule, as well as the presence of a thick fibrinous exudate encapsulated on the liver surface and congestion of the central vein. The histological analysis of the affected heart and liver revealed a significant buildup of collagen and fibrin fibers, which exhibit a prominent dark bluish staining. This buildup is widely distinguished in the pericardial and hepatic capsules. The study indicated that fibrinous pericarditis and perihepatitis affected birds, as indicated by the examination of bacterial results. *Escherichia coli* emits endotoxins that induce vascular damage in the heart and liver, resulting in an elevated presence of fibrin exudate around the affected tissue. The histological analysis supported this conclusion.

Keywords: Fibrinous, Pericarditis, Perihepatitis, Pathology, Biological.

INTRODUCTION

Fibrinous pericarditis is inflammation of the pericardium that is accompanied by hyperemia and the deposit of fibrin within the pericardial sac (Perkins et al., 2004). Fibrinous perihepatitis is inflammation of the hepatic capsule by the accumulation of large amounts of There is a layer of fibrinous exudate covering the liver's surface. consisting of heterophils and lymphocytes (Bhalerao et al., 2013).

Fibrinous pericarditis and perihepatitis in poultry associated with colibacillosis and mycoplasmosis cause Issues pertaining to the economy and well-being of chickens. The frequent incidence of this phenomenon had detrimental impacts on both growth and health status. Termed airsacculitis or chronic respiratory disease in medical terminology, this condition leads to respiratory discomfort, stunted growth, reduced food consumption, and an increased mortality rate. *Escherichia coli* infections are often concurrent and result in exudative accumulations, adhesive fibrinous pericarditis, and fibrinous perihepatitis (Vandemaele et al., 2002).

Pneumonia and airsacculitis may eventually allow for vascular system entry. The primary source of systemic colibacillosis or colisepticemia is thought to be this aerogenic route of infection (Dho-Moulin and Fairbrother, 1999). Colisepticemia is characterized by *E. coli* in the circulation (Pourbakhsh et al., 1997). Airsacculitis, a respiratory infection that first develops in colisepticemia, is followed by a widespread infection that includes perihepatitis and pericarditis (Mellata et al., 2003).

Avian pathogenic *Escherichia coli* causes a variety of systemic or localized infections, including colisepticemia (fibrinous exudates being present in several organs), respiratory infections, airsacculitis, swollen head syndrome, peritonitis, salpingitis, yolk sack infections in newly hatched chicks, and skin infections (Nolan et al. 2013). *Escherichia coli* infections frequently occur concurrently and produce exudative accumulations, sticky pericarditis, and fibrinous perihepatitis in addition to significant air sac thickening and turbidity (Nolan et al., 2013).

Physical and biological risk factors that significantly increase the likelihood of colibacillosis in chickens include housing conditions and co-infections with other bacteria. Most diseases spread through aerosols and colonize air sacs (Lamarche et al., 2005). Dust and ammonia work together to produce harmful consequences, and the inhalation of dust

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contaminated with feces can result in respiratory illnesses caused by *E. coli*. Soon after the air in chicken houses contains high levels of *E. coli*, outbreaks of colisepticemia start to happen (Barnes and Gross, 1997).

The current study aimed to establish a relevant definition of vulnerability to colibacillosis requires more understanding of the relative severity of lesions as well as microbiological analysis of the main causative agents of fibrinous pericarditis and fibrinous perihepatitis based on confirmative methods. The primary distinctive macroscopic abnormalities of fibrinous pericarditis and perihepatitis have been recognized, and the distinctive lesions of fibrinous pericarditis and perihepatitis were examined microscopically. Specific stains were used to perform a histochemical study of fibrin deposition.

MATERIALS AND METHODS

The study concentrated on postmortem lesions, histological changes, and PCR for laboratory diagnosis confirmation. The current study lasted from October 2022 to March 2023. Out of 150 domestic bird samples were selected from several locations throughout the province of Basra (poultry fields in Al-Qurnah and Al-Hartha) according on presence of clinical cases. The postmortem lesions were prepared in processed steps according to Davis and Morishita (2001). The current study was performed under the permission of the ethical committee in the Faculty of Veterinary Medicine, University of Basrah (Ref. No. 79/2023).

Culturing

Swabs were obtained by using sterile cotton swabs from fibrin material on the liver surface and heart surface in an attempt to isolate *E. coli* then placed in test tubes containing nutrient broth and incubated at 37°C for 24 hours, culture was streaked onto plates of MacConkey agar (MC), Eosin methylene blue agar (EMB) and blood agar. The dishes were then incubated aerobically for 24 to 48 hours at 37 degrees Celsius, Subsequently; the bacteria that were cultivated on the same medium were isolated and purified. and separated and preparation for the polymerase chain reaction (PCR) process (Ali and AL-Mayah, 2015).

Genetic Identification

Genomic DNA extraction

The genomic DNA purification kit (Promega/USA) was used to extract the DNA from the ten isolated *E. coli* bacteria. The outcome was identified using electrophoresis on a gel consisting of 1.5% agarose and revealed the DNA bands under ultraviolet light were visualized (Jaber, 2019).

Polymerase chain reaction (PCR)

Detection of the DNA of *E. coli* bacteria was performed by PCR with master mix and specific primers as in Table 1 according to Corp (2005).

Purification and sequencing

The PCR products of the 16S rRNA gene were sequenced by Macrogen Company (South Korea) for comprehensive identification isolates of *E. coli* bacteria.

Histopathological analysis

Tissue sample taken from heart and liver were fixed in 10% buffered formalin to fix for 72 hours and tissue were embedded in paraffin blocks and then routine tissue procedures divided into sections with a thickness of 5 microns and then stained with Haematoxylin & Eosin. The sections were examined under a light microscope according to Suvarna *et al*. (2018) and Ahmed (2020). Masson's trichrome stain was also used to detect fibrin in tissue sections (Khismatullin et al. 2020).

Table 1 - Universal 16S rRNA primer used in PCR amplification of *E. coli* bacreria

Gene	PCR Primers	Nucleotide sequence (5' → 3')	Base pairs
16S rRNA	Forward	AGAGTTTGATCMTGGCTCAG	1500
	Reverse	CCGTCAATTCCTTTRAGTTT	

RESULTS

Bacteriological results

Isolation of *E. coli* bacteria that cause fibrinous pericarditis and perihepatitis in birds. Based on the culturing of heart and liver swaps on MacConkey agar, eosin methylene blue agar, and the use of blood agar. The distribution of the *E. coli* bacterial isolation results is as follows: out of a total of 150 samples, from each heart and liver sample that were collected from the infected broiler 80/90 (88.88%), while the cultured and isolated samples from the infected layer 30/60 (50%) as in Table 2.

PCR-based molecular identification

Extraction and detection of DNA

Ten isolated *E. coli* The DNA of the bacteria was isolated, separated using electrophoresis with a 1.5% agarose gel, and seen using UV light. Subsequently, the 16S rRNA was amplified using the polymerase chain reaction (PCR) technique, resulting in the identification of a clearly distinguishable gene band of 1500 base pairs, as depicted in Figure 1.

Sequencing of 16s rRNA and *E. coli* bacterial identification

Ten *E. coli* were isolated from chicken liver and heart tissue during the bacteriological examination of fibrinous pericarditis and perihepatitis. The results of 16S ribosomal RNA nucleotide sequencing of all investigated *E. coli* strains isolates were registered in NCBI-BLAST under sequence ID as in Table 3.

Table 2 - The data provided pertains to the quantity and proportion of avian specimens that have tested positive for infection, namely in heart and liver samples obtained from both broiler and layer birds.

Birds	No. of birds	No. of infected birds	% of infected
Broiler	90	80	88.88%
Layer	60	30	50%

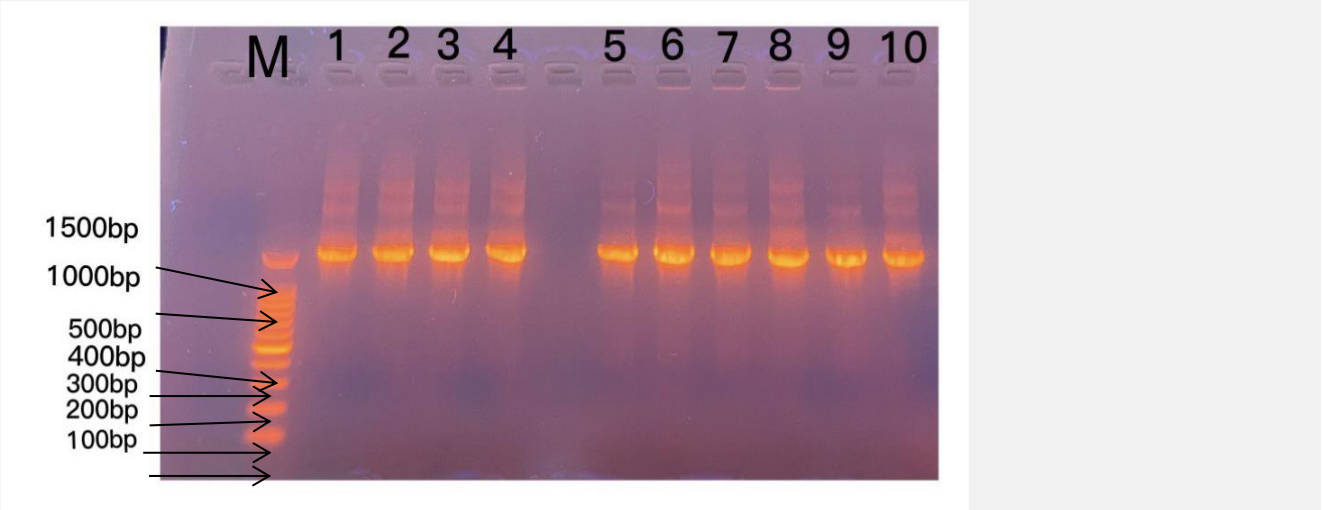

Figure 1 - Using agarose gel electrophoresis, the PCR product analysis of 16S rRNA amplification in the 1500 bp region may be seen.

Table 3 - Identified *Escherichia coli* strains by16S rRNA gene sequencing

Number	Source	Sequence ID with Submission	Sequence ID with compare	Identities
1	*Escherichia coli* Strain Bu.Ji.Ha.1.IRAQ	ID: OQ954793.1	ID: OP630887.1	99%
2	*Escherichia coli* Strain Bu.Ji.Ha.2.IRAQ	ID: OQ954794.1	ID: OP630887.1	99%
3	*Escherichia coli* Strain Bu.Ji.Ha.3.IRAQ	ID: OQ954795.1	ID: OP630887.1	99%
4	*Escherichia coli* Strain Bu.Ji.Ha.4.IRAQ	ID: OQ954796.1	ID: OP630887.1	99%
5	*Escherichia coli* Strain Bu.Ji.Ha.5.IRAQ	ID: OQ954797.1	ID: OP630887.1	99%
6	*Escherichia coli* Strain Bu.Ji.Ha.6.IRAQ	ID: OQ954798.1	ID: OP630887.1	100%
7	*Escherichia coli* O104:H4 Strain Bu.Ji.Ha.7.IRAQ	ID: OR082830.1	ID: CP031902.1	99%
8	*Escherichia coli* Strain Bu.Ji.Ha.8.IRAQ	ID: OR082831.1	ID: OQ891229.1	99%
9	*Escherichia coli* O157:H7 Strain Bu.Ji.Ha.9.IRAQ	ID: OR082832.1	ID: CP039834.1	99%
10	*Escherichia coli* Strain Bu.Ji.Ha.10.IRAQ	ID: OR082833.1	ID: OQ753150.1	99%

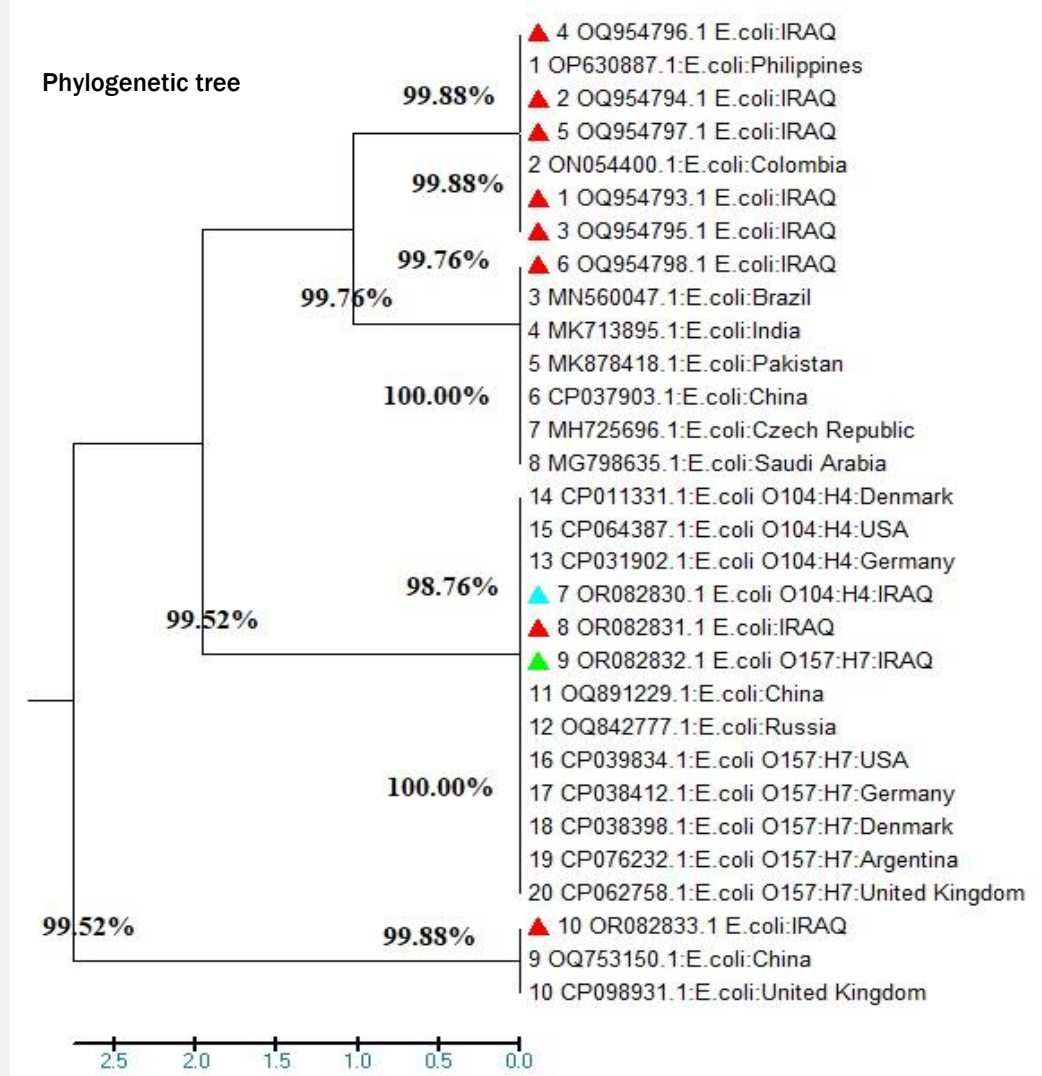

Figure 2 - A maximum likelihood tree illustrates the evolutionary relationship between Escherichia coli's 16S rRNA sequence and 16S rRNA sequences of closely related bacterial species. *Escherichia coli* were isolated from a local chicken heart and liver in Iraq. Their accession numbers are used to express them in international nucleotide databases. The phylogenetic tree was produced using Mega 6 sequencing, version 6.5 software.

Pathological manifestations

Macroscopic findings

The pericardial sac of the sick chicken showed a notable buildup of white fibrinous exudate within its internal organs, indicating a serious case of fibrinous pericarditis. Additionally, the liver showed enlargement and congestion (hepatomegaly), with visible white fibrinous exudate on the outer surface, indicating fibrinous perihepatitis as shown in Figure 3. The hearts of hens affected by the illness exhibited substantial accumulation of white fibrinous debris in the sac surrounding the heart, indicating the presence of severe fibrinous pericarditis. Additionally, there was a hemorrhagic lesion on the surface of the pericardium, indicative of hemorrhagic pericarditis as depicted in Figure 4. The liver of the diseased chicken had a pronounced buildup of white fibrinous exudate on its surface, indicating severe fibrinous perihepatitis. Additionally, the liver was enlarged, known as hepatomegaly, as depicted in Figure 5.

Microscopic results

The histopathological examination of the chicken's infected heart shows fibrinous exudate in the pericardial sac and a significant infiltration of inflammatory cells with fibrinous exudation in the pericardium. Furthermore, there is a necrotic region in the myocardial muscle fibers (Figure 6). In addition to demonstrating the presence of many active micro-abscesses in the pericardium, the pericardium also exhibits a notable infiltration of inflammatory cells with fibrinous exudation, as well as congestion of blood vessels in the myocardium (Figure 7). The histological examination of the chicken's liver reveals a significant presence of inflammatory cells in the hepatic capsular region. Additionally, there is a thick layer of fibrinous exudate encapsulated on the surface of the liver, as depicted in Figure 8. In addition to the central vein being congested with active inflammatory exudate (shown by the black arrow), there is also extensive perivascular necrosis of the hepatocytes (Figure 9).

Histochemical findings

The examination of the heart tissue from the infected chicken revealed a notable accumulation of pericardial collagen and fibrin, indicating the presence of active fibrinous pericarditis. These fibers stained dark bluish in color. Additionally, the myocardial muscle fibers appeared pinky-red when stained with Masson's trichrome (Figure 10). The liver section of the infected chicken exhibits a significant buildup of collagen and fibrin fibers in the capsular hepatic region, indicating active fibrinous perihepatitis. This is evident from the light bluish positive staining observed in the histochemical analysis. Additionally, the hepatic parenchyma appears pinky-red in color when stained with Masson's trichrome, (Figure 11).

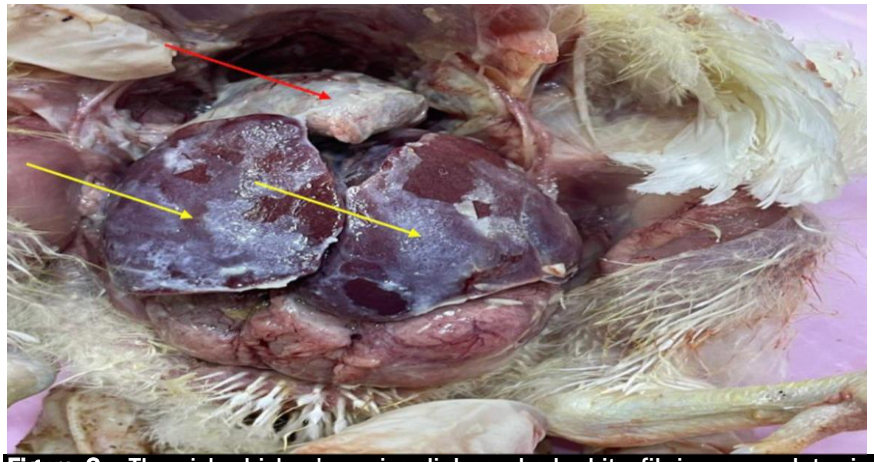

Figure 3 - The sick chicken's pericardial sac had white fibrinous exudate, indicating severe fibrinous pericarditis (red arrow). The enlarged liver (hepatomegaly) has white fibrinous exudate on its outer membrane, indicating fibrinous perihepatitis (yellow arrow).

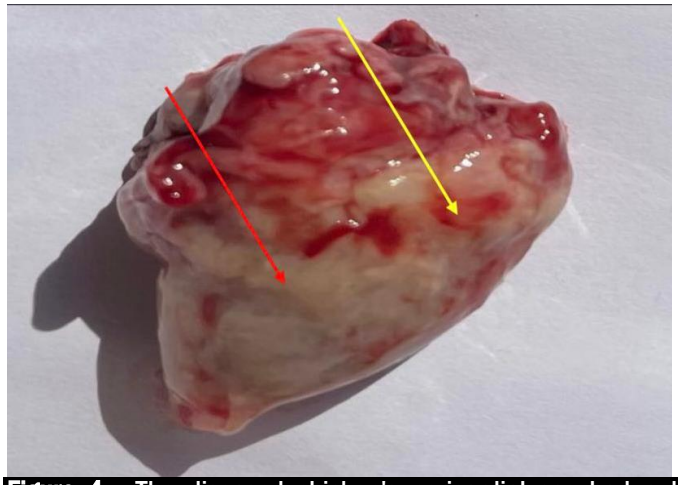

Figure 4 - The diseased chicken's pericardial sac had a lot of white fibrinous exudate, indicating severe fibrinous pericarditis (red arrow). The liver was enlarged (hepatomegaly) and had white fibrinous exudate on the outside, indicating fibrinous perihepatitis (yellow arrow).

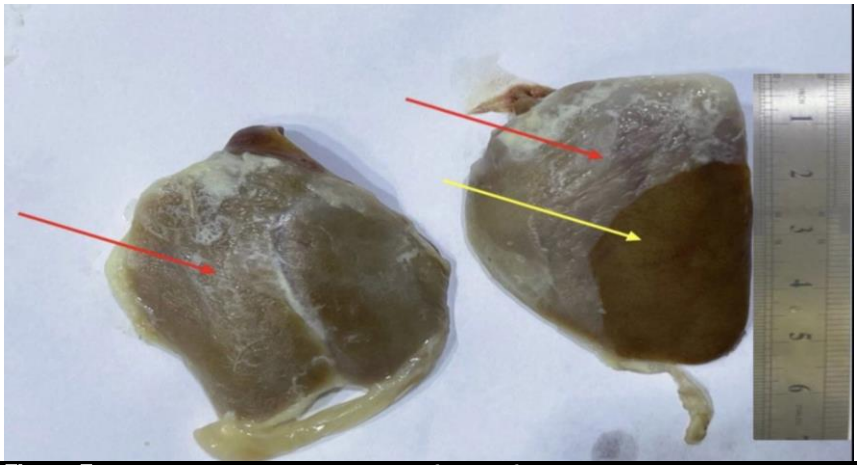

Figure 5 - The chicken's liver had a lot of white fibrinous exudate, indicating severe fibrinous perihepatitis (red arrow). Liver enlargement indicated hepatomegaly (yellow arrow).

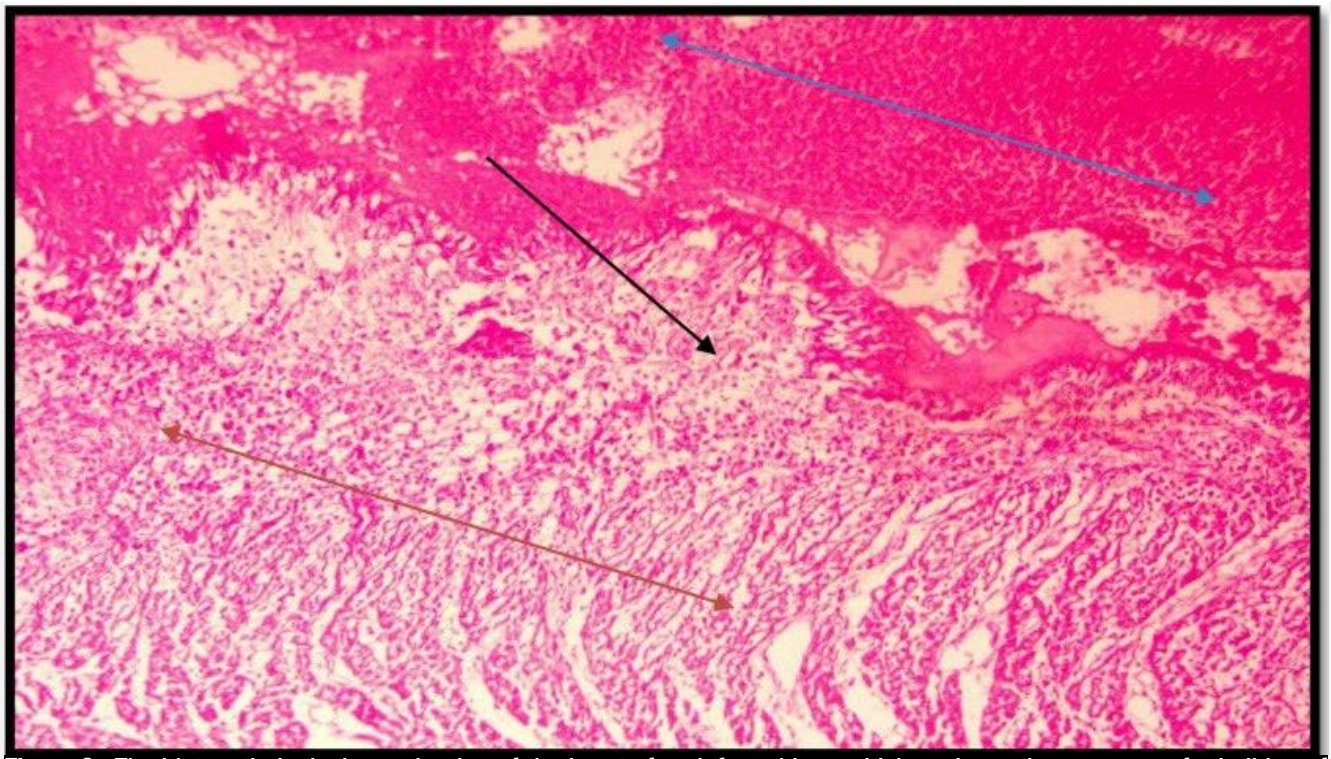

Figure 6 - The histopathological examination of the heart of an infected layer chicken shows the presence of a buildup of fibrinous exudate in the pericardial sac (shown by a double-headed blue arrow), along with a significant infiltration of inflammatory cells in the pericardium and the presence of fibrinous exudation. The presence of a black arrow indicates the existence of a necrotic region within the cardiac muscle fibers, as indicated by the double-headed red arrows. Hematoxylin and eosin stain. One hundred times.

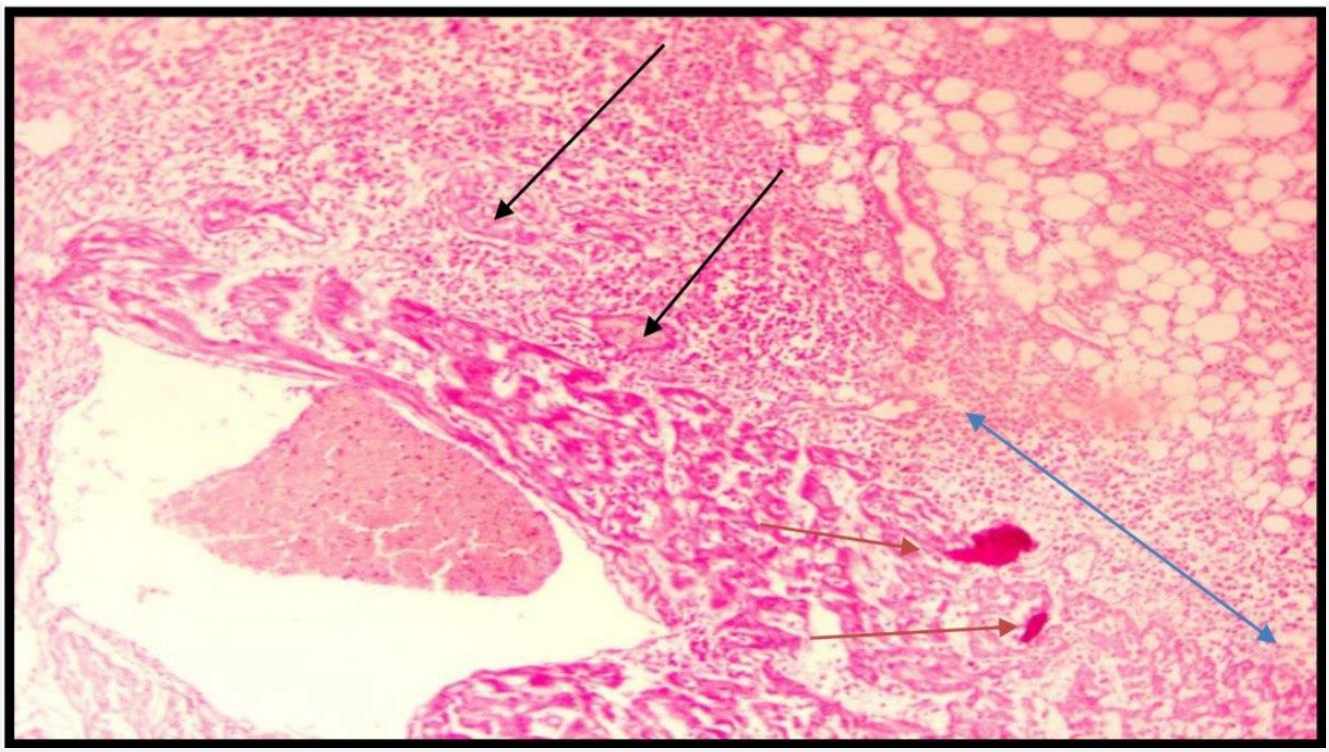

Figure 7 - The histopathological image of the chicken's heart reveals the presence of numerous active micro-abscesses in the pericardium (black arrows). Furthermore, the pericardium displays infiltration of inflammatory cells and the release of fibrinous exudate (indicated by a double-headed blue arrow), along with blood vessel congestion in the myocardium (red arrows). Hematoxylin and eosin stain at a magnification of 100X.

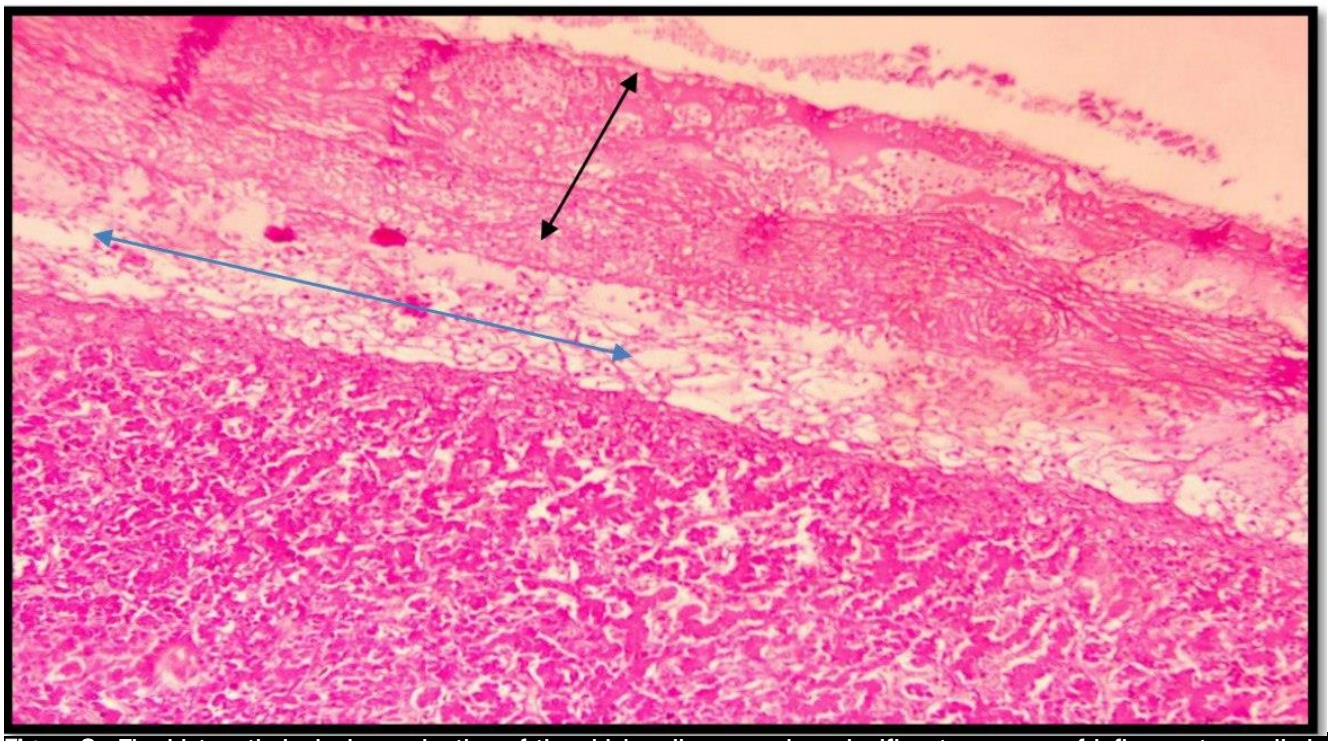

Figure 8 - The histopathological examination of the chicken liver reveals a significant presence of inflammatory cells in the outer layer of the liver (double-headed blue arrow). Additionally, there is a thick layer of fibrinous exudate covering the surface of the liver (double-headed black arrows). Hematoxylin and eosin stain. Magnification of 100 X.

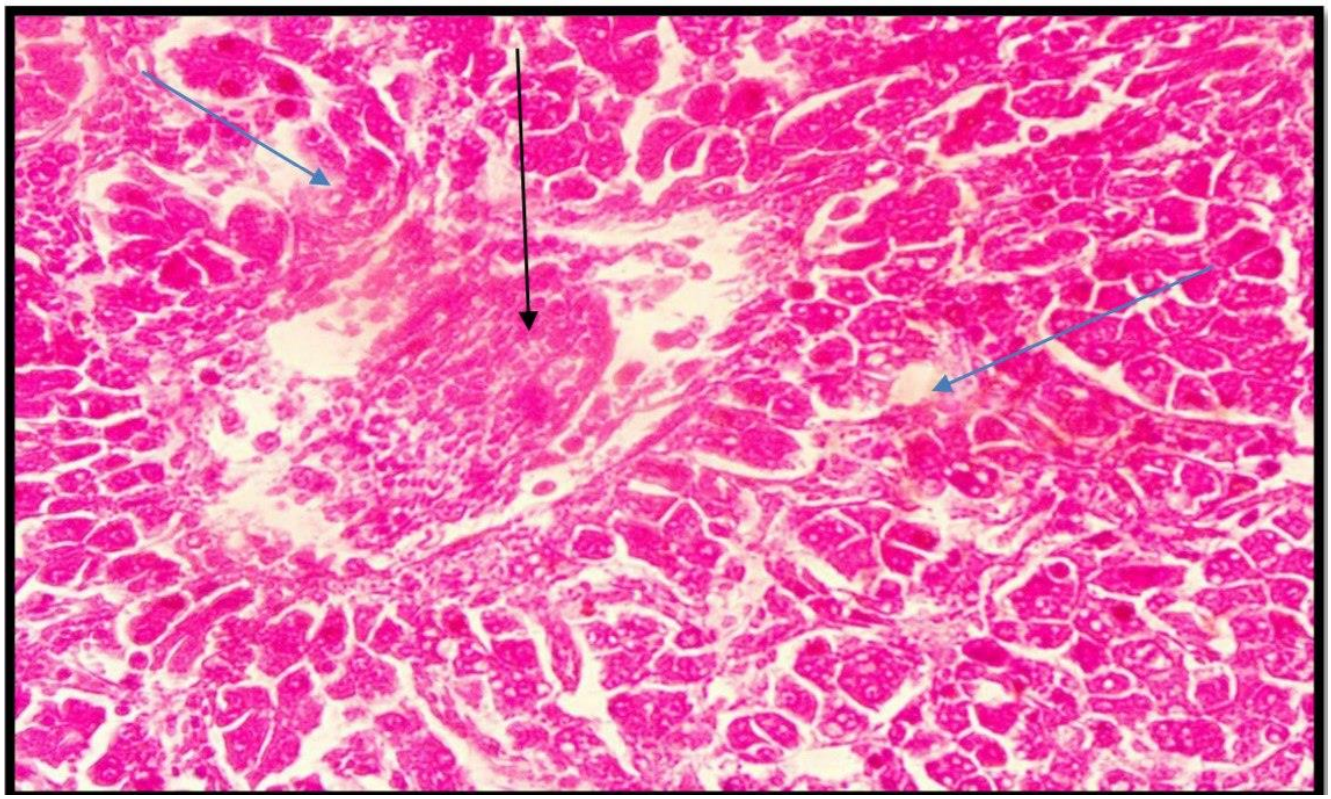

Figure 9 - Microscopic image depicting the liver tissue of a chicken that has been infected. reveals congestion of the central vein with active inflammatory exudate (black arrow), also there is severe perivascular necrosis of the hepatocytes (blue arrows). H&E stain. 40X

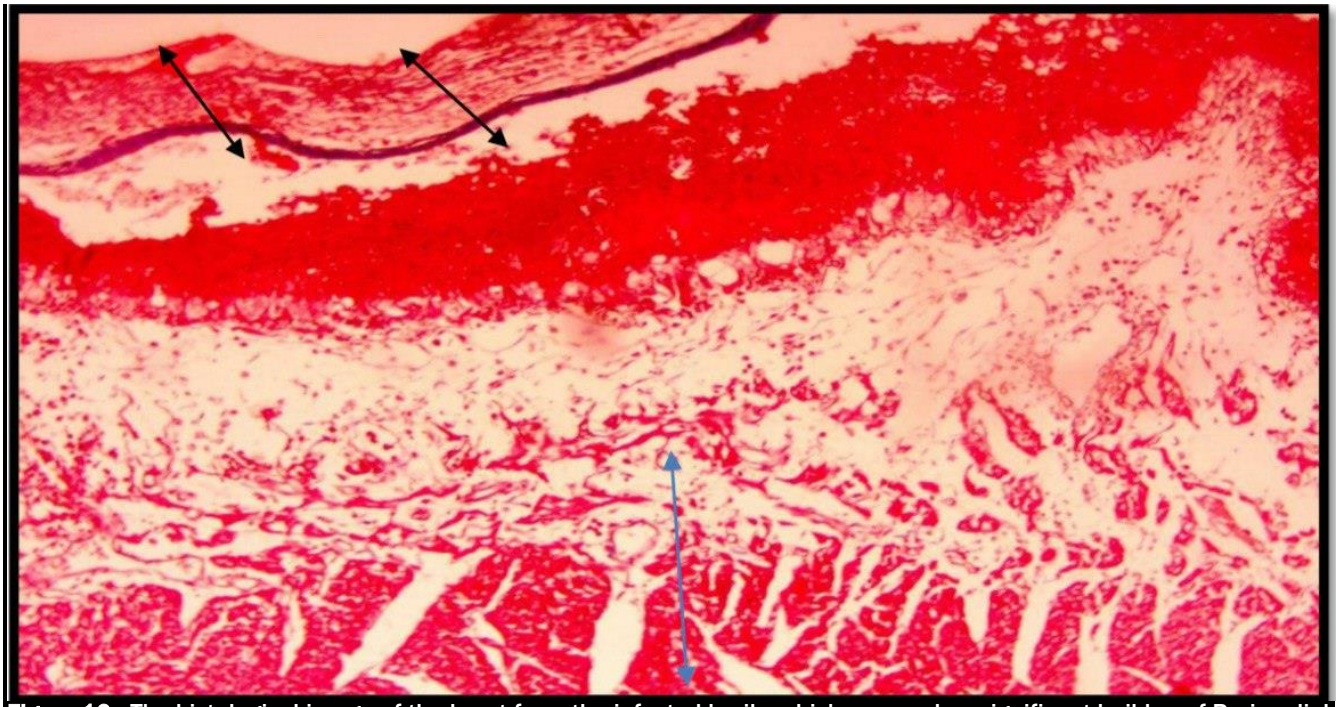

Figure 10 - The histological image of the heart from the infected broiler chicken reveals a significant buildup of Pericardial collagen and fibrin, indicating the presence of active fibrinous pericarditis. These fibers are stained dark bluish. In contrast, the myocardial muscle fibers are observed in a pinky-red color. Masson's trichrome stain is a histological staining technique 100X.

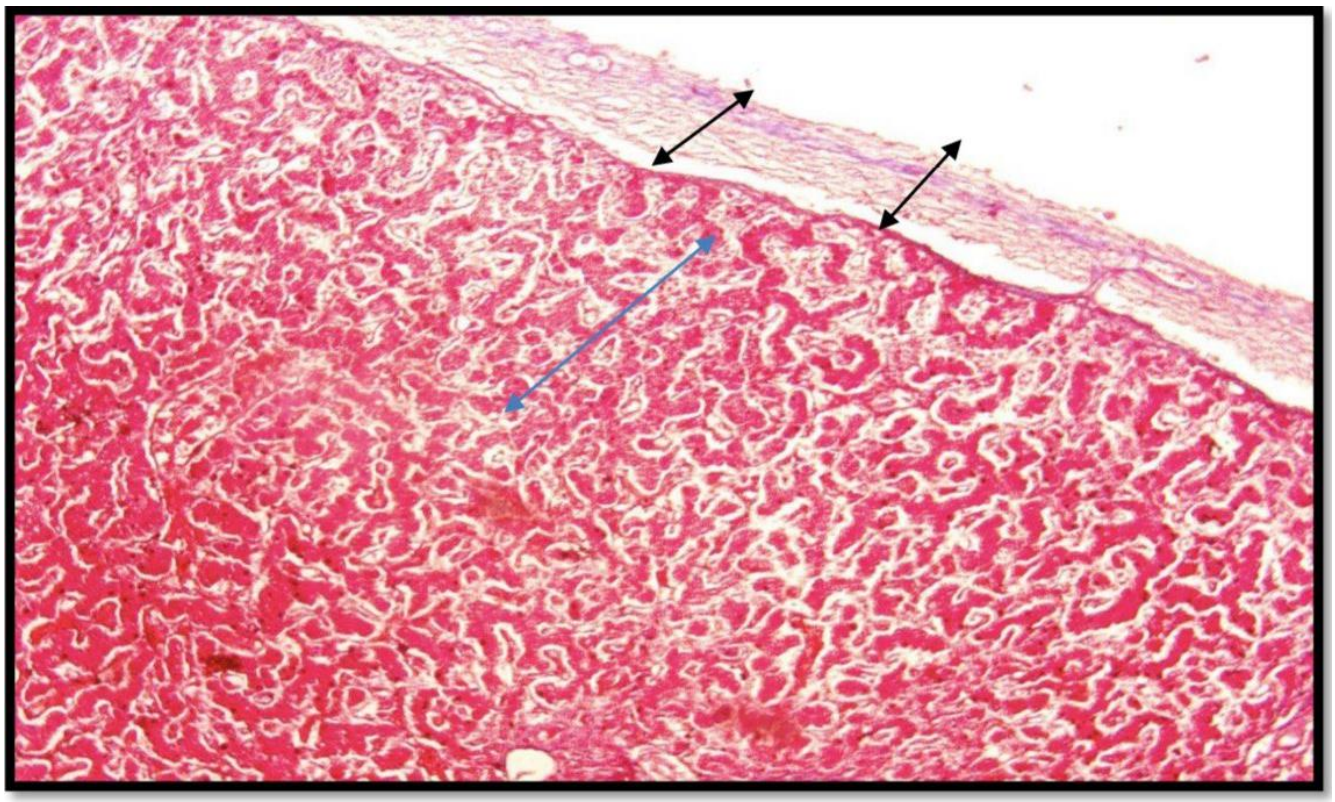

Figure 11 - The ill broiler chicken's hepatocyte cells revealed light bluish positive staining of collagen and fibrin fibers that build up in the capsular hepatic region, indicating active fibrinous perihepatitis. The hepatic parenchyma was pinky-red (double-headed blue arrow). Masson trichrome 100X

DISCUSSION

The results of bacterial culture on MacConkey, EMB, and blood agar revealed that the heart and liver samples contracted an infection with *E. coli,* our results are in agreement with research by Wani et al. (2020) which discovered that *E. coli* colonies showed a metallic sheen on EMB agar and appeared pink when cultured on MacConkey agar plates, Due to its ability to ferment lactose and the formation of an amide linkage between eosin and methylene, *E. coli* colonies are pink in color and show a metallic sheen on EMB. Their findings revealed the presence of *E. coli* bacteria, which cause pericarditis and perihepatitis.

According to the results, the broiler had a larger percentage of E. coli bacterial isolation than the layer, with infection rates of 88.88% and 50%, respectively. The current study's findings concurred with those of Mohanty et al. (1979) and Ezz El-Deen et al. (2010), who found the *E. coli* bacteria in infected broilers and layers, respectively, with an incidence of 88.8% and 75%. Furthermore, these results align with the research conducted by Dho-Moulin and Fairbrother (1999), which found that fibrinous pericarditis and fibrinous perihepatitis are caused by *E. coli* epithelium penetrating the mucosa of the respiratory organs and multiplying in the bloodstream and internal organs (liver and heart).

As indicated in Table 3 and Figure 2, which depicts the distribution of Iraqi samples, the current work employed neighbor-joining analysis of the 16S rRNA gene to generate a phylogenetic tree in order to investigate the relationship between local samples and the higher query cover (99%) of national samples, in this Figure 2 indicates the phylogenetic tree for *E. coli* (No. 1- 6) display related similarity (99.88%) with the sample from the Philippines, while *E. coli* (No.7) display related similarity (99.52%) with the sample from Germany; while *E.coli* (No.8) indicates maximum similarity (100%) with the sample from China. Also, *E. coli* (No.9) indicates maximum similarity (100%) with the sample from the USA, while *E. coli* (No.10) display related similarity (99.88%) with the sample from China, according to (Zhang and Sun, 2008). The majority of isolates of the genetically heterogeneous species *Escherichia coli* are commensal digestive system organisms. However, some isolates are opportunistic pathogens that infect a range of hosts' extra intestinal and gastrointestinal systems (Denamur et al., 2021).

The macroscopic analysis revealed that diseased birds with fibrinous pericarditis and perihepatitis exhibited a significant buildup of white, characteristic fibrinous exudate in the pericardial sac, together with hemorrhagic lesions on the surface of the pericardium. *E. coli* infection of the heart causes damage to the heart blood vessels resulting in hemorrhage. This result agrees with Pruthi et al. (2012); Bhalerao et al. (2013) who mentioned that the fibrinous layer on the pericardium and hemorrhagic due to adhesions of the heart with the chest cavity.

Also, severe accumulation of white typical fibrinous exudate in the liver surface and enlargement of the liver. This in line with Dutta et al. (2013) who found that the deposition of large amounts of fibrinous exudate on the liver, a bacterial infection of the liver causes inflammation with a large number of heterophils over the hepatic capsule due to enlargement of the liver (hepatomegaly).

The microscopical observation in the heart changes appeared including fibrinous exudation and congestion of blood arteries in the myocardium, pericardial sac infiltration of inflammatory cells, edematous exudate in the cardiac muscle fibers, and numerous active micro-abscesses in the pericardium. Regarding the further alterations, they include a significant polymorphonuclear inflammatory cell infiltration in the region that lies between the cardiac muscle fibers and the pericardium. Damage to the pericardium brought on by bacterial infections results in the release of fibrin, an inflammatory cell. This lesion is consistent with the observations made by Snyder et al. (2014), who reported that pericardial inflammation results in a serous or purulent discharge, inflammatory exudate, and heterophil inflow, which causes a fibrinous reaction with adhesions and fluid accumulation.

The contaminated chicken's liver exhibits severe inflammatory cell infiltration in the capsular hepatic region, as well as thick fibrinous exudate capsulated on the liver surface, inflammatory cell infiltration of the liver parenchyma, abscesses in the hepatic parenchyma, and dilation of the sinusoids. Also, there are foci of necrosis in the hepatocytes. *E. coli* release beta hemolysin toxin that causes increased vascular permeability and escape of inflammatory cell and fibrinogen due to accumulation of fibrinous exudate in the surrounding tissue, and due to lack of blood supply to tissues and cell death (necrosis). This result was in lined with Dutta et al. (2013), Kadhim and Ahmed (2020) and IRufaei and Alwan (2023) who noted that the liver showed localized necrosis in the hepatocytes as well as a thick layer of fibrinous exudates covering the hepatic capsule and a large number of heterophils.

The heart histochemical section of the infected chicken in this study, stained with Masson's trichrome stain, revealed a significant accumulation of collagen and fibrin fibers in the pericardial sac, indicating active fibrinous pericarditis. The myocardial muscle fibers showed up pinky-red in color. These findings were similar to that of Franca et al. (2010) who described the accumulation of collagen fibers in the heart (pericardium and myocardium) that appear in different colors when stained with Masson's trichrome stain.

The histological analysis of the liver using Masson's trichrome stain revealed a significant accumulation of collagen and fibrin fibers in the capsular hepatic region, indicating the presence of active fibrinous perihepatitis. These fibers were stained pale bluish. In contrast, the hepatic parenchyma appeared pinky-red in color. This observation was also documented by Krishna (2013), who discovered that the stain imparts a blue hue to collagen in contrast to the red color of hepatocytes and other structures.

CONCLUSION

Overall, our study revealed fibrinous pericarditis and perihepatitis were higher in broilers than in layers, as well as the bacteriological study showed the of *Escherichia coli* which cause fibrinous pericarditis and perihepatitis, as well as white fibrinous exudate accumulated in the pericardial sac and on the liver's surface, as well as microscopic examination of fibrinous pericarditis revealed infiltration of inflammatory cells with fibrinous exudation, edematous exudate congestion of blood vessels in the myocardial muscle fibers with the presence of a necrotic area, and multiple active micro-abscesses in the pericardi, as well as microscopical examination of fibrinous perihepatitis revealed infiltration of inflammatory cells and thick fibrinous exudate in the liver capsule, as well as congestion of the central vein with active inflammatory exudate also there is severe perivascular necrosis of the hepatocytes, as well as the histochemical section of fibrinous pericarditis and perihepatitis revealed the proliferation of collagen and fibrin fibers in the pericardial sac and capsular hepatic region.

DECLARATIONS

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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution
B.M. Lateif performed genetic analysis of the results and the manuscript's writing.
J.A. Ahmed performed molecular and pathological detection.
H.A. Najem contributed to the design of the research and field diagnosis of the disease.

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Consent to publish
The authors agree to the publication of this manuscript.

Competing interests
The authors declare no competing interests.

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TEXTURE PROFILE, WATER HOLDING CAPACITY, ANTIOXIDANT ACTIVITY AND LIPID OXIDATION OF BEEF DURING RETAIL DISPLAY FROM CATTLE FED TOTAL MIXED RATION SUPPLEMENTED WITH *Capsicum frutescens* L. AND *Curcuma longa* L. POWDERS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study aimed to elucidate the effects of supplementation of *Capsicum frutescens* L. or chili pepper (ChP) and *Curcuma longa* L. or turmeric (T) powders combination in total mixed ration (TMR) on texture profile, water holding capacity (WHC) and oxidative stability of beef during days 0, 5 and 10 of retail display. The experiment was carried out on 16 crossbred bulls (Brahman and Charolais) of about 2 years in age. The bulls were randomly assigned to 4 dietary treatment groups as follows: 1) TMR as control, 2) TMR + 1%ChP powder, 3) TMR + 1%T powder, and 4) TMR supplemented with a mixed powder of 1%ChP + 1%T, over a 6 months feeding period. The results revealed that the hardness and gumminess of control beef were higher than other groups, and the cohesiveness of beef from cattle fed a mixed powder of 1%ChP + 1%T was lower than other groups ($P<0.05$). Regarding WHC, the results showed that, on days 0 and 5 of storage, the control group meat had higher cooking losses than either the 1%T or a mixture of 1%ChP + 1%T groups ($P<0.05$). Also, on 0 and 5 days of retail display, the 1%ChP + 1%T group showed the highest antioxidant activity when compared to other groups ($P<0.05$). As for the lipid oxidation in beef, on day 5 of storage MDA level in control beef was higher than the 1%T or a mixture of 1%ChP + 1%T groups ($P<0.05$). It can be concluded that the combination of chili pepper and turmeric powder in TMR can improve texture, water holding capacity, and oxidative stability of beef during refrigerated storage.

Keywords: Beef, Chili pepper, Cooking loss, Oxidative stability, Texture profile, Turmeric powder.

INTRODUCTION

In livestock production, antibiotics play an important role in the protection of animals against infectious diseases, and in the treatment of diseases. In addition, it is well known that the antibiotics supplemented in animal feeds can serve also as antimicrobial growth promoters (AGPs) that enhance productivity and profitability (Cheng et al., 2014). However, excessive use of antibiotics may have detrimental impacts on both livestock and human health, so many countries have banned the use AGPs in animal feed (Brown et al., 2017). This leads to considering medicinal herbs that could improve animal productivity as alternatives to antibiotics. Previously, countless studies have demonstrated the use of medical plants as feed additives to improve livestock production (Hashemi and Davoodi, 2011; Hanczakowska et al., 2015; Kuralkar and Kuralkar, 2021). Moreover, there have been recent studies aiming to use combinations of different natural bioactive substances in farm animals, to benefit from the synergistic effects of multi-herbal feed additives (Giannenas et al., 2018). In this regard, chili pepper (*Capsicum frutescens* L.) and turmeric (*Curcuma longa* L.) seem to be good candidates to substitute for antibiotics in animal feed, due to their bioactivities and medicinal properties, and the fact that these are commonly used in cuisines all over the world corroborates the absence of safety concerns (Gurnani et al., 2016; Verma et al., 2018; Akbar et al., 2019).

Chili pepper, also known as Bird's eye chili, is widely cultivated throughout the tropical countries, especially in Thailand. It is a beneficial source of nutrients, minerals, and phytochemicals, and has substantial prospects for developing food additives (Otunola et al., 2010). The phytochemicals in chili pepper, especially capsaicinoids, carotenoids, and saponins, contribute its antibacterial, antidiabetic, antifungal, anticancer, and antioxidant activities (Chinnkar and Jadhav, 2023). Turmeric is an herbal plant that belongs to the Zingiberaceae (ginger) family. It is widely used as a spice, food preservative, and coloring agent in tropical areas, especially in south-east Asia. Moreover, it has been used extensively by traditional medicine all over the world. It is a major source of curcumin, a yellow bioactive component with many biological actions, including anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility,

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antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive, and hypocholesteremia activities (Verma et al., 2018).

Previously, several studies have shown synergistic effects of chili pepper and turmeric powder in animal feed on production performance and health of animals, especially in poultry (Adegoke et al., 2018; Sanwo et al., 2020). However, whether there is such synergy in ruminants is still largely unknown. This study is a continuation of prior work that assessed the effects of chili pepper and turmeric powder supplementation in total mixed ration (TMR) in beef cattle, on growth performance and fresh meat quality, and this study aimed to investigate the beef during refrigerated storage for effects on texture, water holding capacity (WHC), and oxidative stability.

MATERIALS AND METHODS

Experimental animals, treatments and feed

The 16 crossbreds of beef cattle used in this experiment had approximately 450 kg body weights and were about 2 years of age. The cattle were mixed breeds of Brahman and Charolais (60% or more of Charolais bloodline). Before starting the experiment, the cattle were raised from about 250 kg weight by feeding with concentrate and roughage from the local area for 6-8 months, until the body weight reached 450 kg. All 16 bulls were castrated and randomly assigned to 4 experimental groups (4 replicates in each group) according to a Completely Randomized Design (CRD) as follows: 1) TMR without herbal supplements (control), 2) TMR supplemented with 1%Chili pepper (ChP) powder, 3) TMR supplemented with 1% turmeric (T) powder, and 4) TMR supplemented with 1%ChP + 1%T powder. Chili pepper and turmeric powders were produced with quality control of the herbal raw materials by the Ban Khao Na Nai community enterprise group (herbal production group) in Ton Yuan sub-district, Phanom district, Surat Thani province, Thailand. The feeding trial was carried out at Phatthalung College of Agriculture and Technology, Phatthalung, Thailand. The cattle were fed with TMR according to NRC (1984) as shown in Table 1. The trial period was 6 months, during which all the beef cattle were fed *ad libitum* 2 times daily (at 8.00 am and 3.30 pm) and had free access to water.

Table 1 - Ingredients of total mixed ration (TMR) in the experiment

Ingredients	%
Acacia leaves	2.4
Napier grass	24
Cassava	23.6
Molasses mixed with yeast	7.1
Soybean meal	11.5
Salt	1.0
Sea shell powder	1.5
Oil palm meal	28.4
Urea	0.5
Chemical composition (% of dry matter)	
Crude protein	10.8
Total digestible nutrients (TDN)	69.0

Meat samples

After the 6 months of feeding trial, the cattle were transported to an abattoir in Phatthalung province, Thailand. The animals were fasted before transport and slaughtered according to common practices, i.e. stunning, bleeding, de-hiding, eviscerating, and cooling. Determination of texture profile, antioxidant activity, and lipid oxidation was performed on the *Longissimus dorsi* (LD) muscles and a simulation of retail display was conducted by wrapping the meat samples with oxygen permeable foil and displaying them at 4°C under fluorescent light for 24 h per day, with sampling at 0, 5, and 10 days.

Analysis of water holding capacity (WHC)

The WHC of the beef from LD was determined at 24 h *post mortem* in terms of the cooking loss. The beef samples were cooked in a water bath controlled at 90 °C for 15 minutes. Each sample was weighed before and after cooking. The amount of cooking loss was calculated using the difference between the weight before and after cooking, then expressed as percentage of the pre-cooked weight.

Texture profile analysis (TPA)

The texture profile of beef from LD was measured at 24 h *post mortem* by using a texture analyzer, Brookfield model CT3, with a capacity of 10 kg. The beef LD muscles were cooked in a water bath at 90°C for 15 minutes, then they were cut into 1x1x1 cm sample size. The measurement was performed in 3 replicates for each type of sample. The texture profile of the beef was summarized in terms of hardness, cohesiveness, springiness, gumminess, and chewiness.

Antioxidant activity analysis

Antioxidant activity of beef was analyzed at 0, 5, and 10 days of display at 4°C by using a 2, 2–diphenyl–1–picrylhydrazyl (DPPH) assay according to the method of Wang et al. (2019) based on the principle that DPPH is a free radical generator. Briefly, 2.5 g of beef was mixed and homogenized with 7.5 ml of ethanol. The homogenates were extracted on a shaker for about 10 minutes at room temperature and then centrifuged at 1,800 rpm for 10 minutes. Subsequently, the supernatants were collected. Then 0.5 ml of supernatant from each sample was added to 3.5 ml of 0.1 mM DPPH in ethanol, mixed and stored in dark at room temperature for 20 minutes. The absorbance was measured at 517 nm by using a spectrophotometer. Ethanol was used as a blank. DPPH scavenging activity was calculated as follows:

DPPH Scavenging activity (%) = (1-As/Ac) × 100

Here Ac is the absorbance of the control (DPPH in ethanol solution without sample) and As is the absorbance of the sample.

Lipid oxidation analysis

Lipid oxidation was evaluated in LD muscle samples on days 0, 5 and 10 of display during cold storage. The lipid oxidation in beef was assessed from the thiobarbituric acid reactive substances (TBARS) determined according to the standard method of Tarladgis et al. (1960). The secondary product of lipid oxidation, malondialdehyde (MDA), was measured at 532 nm by using a spectrophotometer and expressed as µg MDA per g meat.

Statistical analysis

The data were analyzed in SPSS Statistics program using the general linear model procedure to evaluate the influences of experimental treatments. Means were compared using Duncan's multiple range test and differences were considered significant at $P<0.05$.

Ethical statement

All animal procedures in this study were approved by the Institutional Animal Care and Use Committee, Prince of Songkla University (Approval project no. 2564–15–090).

RESULTS AND DISCUSSION

Meat texture profile and cooking loss

Effects of chili pepper and turmeric powder supplementation in TMR on texture profile of beef LD are shown in Table 2. The results show that the hardness and gumminess of the control group were higher than of the groups supplemented with herbal powders in TMR ($P<0.05$). Moreover, the cohesiveness of LD muscle from beef fed with 1%ChP + 1%T powder was lower than those of the others ($P<0.05$). However, the springiness and chewiness of beef among all treatment groups had no significant differences ($P>0.05$).

The influences of herbal powder in TMR on WHC in terms of cooking loss (%) of beef from LD are indicated in Figure 1. The results reveal that on days 0 and 5 of cold storage the control group had a higher cooking loss from meat than either 1%T or 1%ChP + 1%T groups ($P<0.05$). However, on day 10 of display, there was no longer any significant difference in cooking loss among the dietary treatment groups ($P>0.05$).

Eating quality or palatability of meat comprises 3 main properties, which are texture, juiciness and flavor/odor (Warriss, 2010). Juiciness is related to water retention or WHC, which is determined as the ability of meat to retain its own water and can be described in drip, purge, weep, and exudate or cook losses. Juicy meat may be perceived as more tender than a less juicy meat (Warriss, 2010). Bejerholm and Aaslyng (2004) stated that the cooking loss is well correlated with juiciness of meat, and depends on the cooking temperature. In addition, the study of Hughes et al. (2014) shows a strong positive correlation between the cooked meat tenderness and meat juiciness, while this may vary between different muscles. These agree with the present study, in which the meat tenderness was related to the cooking loss. In this study, the results showed that the herbal supplementation resulted in a lower cooking loss and more meat tenderness in accordance with Li et al. (2022), who found that herbal tea residue improves meat quality by reducing cooking loss and shear force in finishing steers.

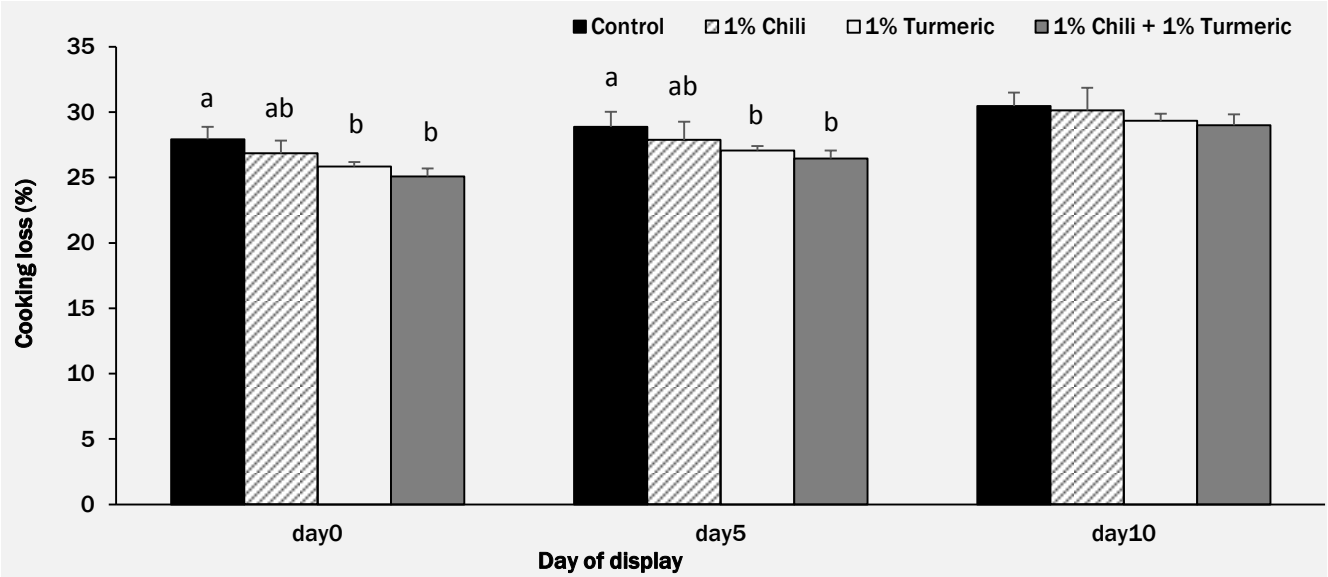

Figure 1 - Mean values of cooking loss (%) from beef during cold storage, compared by dietary treatment. Error bars represent standard deviations. [a,b:] Means with different superscripts are significantly different at P<0.05, compared for the same sampling day.

Table 2 - Effects of chili pepper and turmeric powders supplementation in total mixed ration (TMR) on meat texture profile of beef cattle

Items \ Level of herbal powder in TMR	0	1% chili pepper	1% turmeric	1% chili pepper +1% turmeric	SEM	P-value
Hardness (g)	4590.3[a]	2557.5[b]	2123.0[b]	1624.3[b]	375.37	0.01
Cohesiveness (%)	0.51[b]	0.51[b]	0.59[a]	0.41[c]	0.02	<0.01
Springiness (mm)	4.75	8.83	4.23	4.78	0.86	0.21
Gumminess (g)	2317.50[a]	1222.50[b]	1248.25[b]	656.75[b]	201.60	0.01
Chewiness (mJ)	104.90	107.68	51.80	29.80	13.05	0.06

[a,b,c:] Means within a row with different superscripts differ significantly (P<0.05).

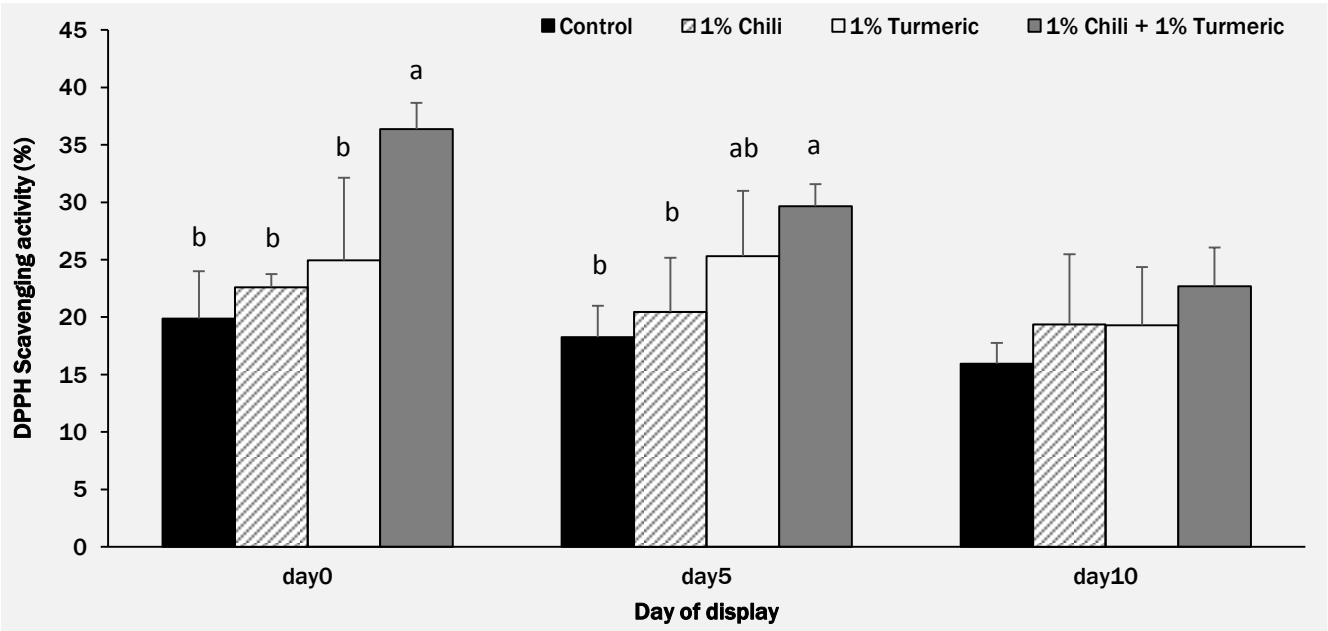

Figure 2 - Mean values of DPPH scavenging activity (%) in beef during cold storage, compared by dietary treatment. Error bars represent standard deviations. [a,b:] Means with different superscripts are significantly different at P<0.05, compared for the same sampling day.

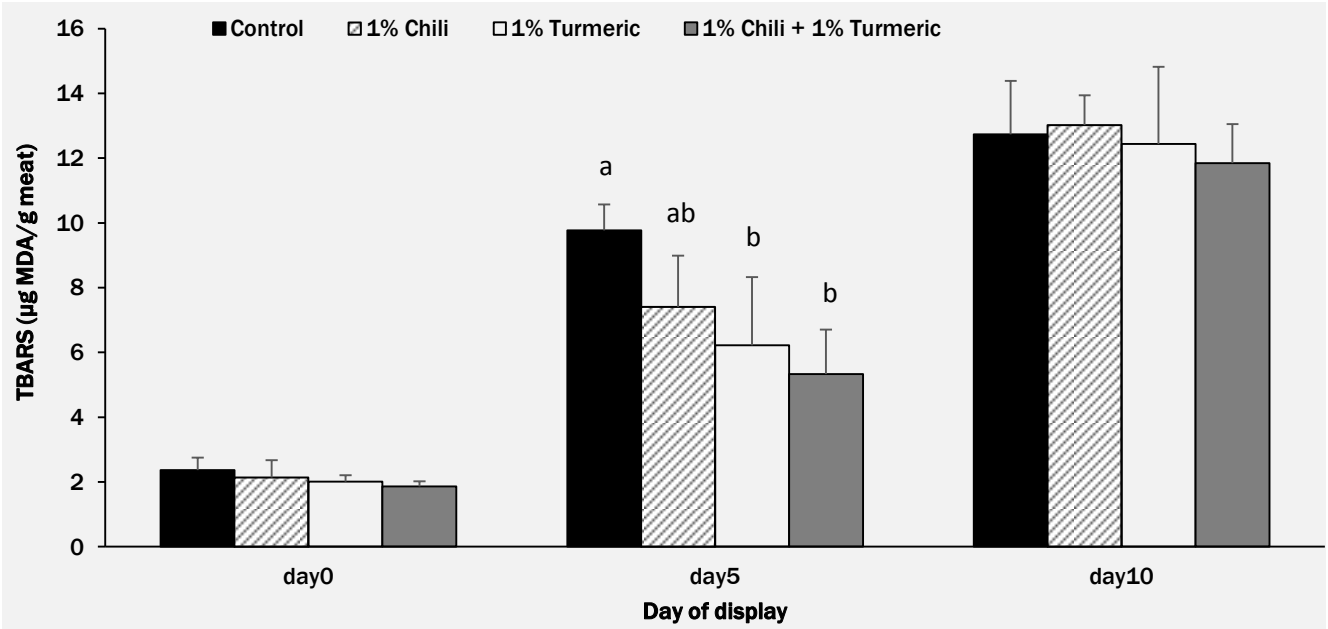

Figure 3- Mean values of lipid oxidation (µg MDA per g meat) in beef during cold storage, compared by dietary treatment. **Error bars represent standard deviations.** [a,b:] Means with different superscripts are significantly different at P<0.05, compared for the same sampling day.

Antioxidant activity and lipid oxidation in meat

Effects of chili pepper and turmeric powder in TMR on antioxidant activity in beef during cold storage are shown in Figure 2. On days 0 and 5 of retail display, it was observed that the experimental group with the highest antioxidant activity was that supplemented with 1%ChP along with 1%T powder, when compared to other groups (P<0.05). However, antioxidant activity in beef on day 10 of cold storage did not significantly differ across the experimental groups (P>0.05).

Results of lipid oxidation in beef during cold storage are indicated in Figure 3. From the experiment, it was found that on day 5 of retail display the amount of MDA (µg MDA/g Meat) in beef without herbal supplementation was higher than in either the group supplemented with 1%T or that supplemented with 1%ChP + 1%T in TMR (P<0.05). However, on days 0 or 10 of display there were no significant differences among the groups (P>0.05).

Lipid oxidation is a major cause of deterioration in meat quality. Meat tends to be susceptible to oxidation due to its high concentration of fats. Oxidative deterioration in meat results in discoloration, off flavor/odor, formation of toxic compounds, poor shelf life stability, and nutrient and drip losses (Morrissey et al., 1998; Contini et al., 2014). The current study found that beef from animals that received herbal plants, especially mixed herbs, had more antioxidant activity and less lipid oxidation. This is in line with Giannenas et al. (2018) who found that a combination of different herbal extracts could have synergistic effects to improve antioxidant activity and reduce lipid oxidation in meat of broilers, attributing this to the phenolic compounds. Also, Hanczakowska et al. (2015) indicated that the herbal extracts significantly improved meat oxidative stability. This may suggest that phytochemicals from plants were absorbed by the animals, consequently increasing the antioxidant activity of tissues, and could prevent lipid oxidation through quenching free radicals or through activation of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (Frankič et al., 2009).

CONCLUSION

The dietary supplementation of 1%chili pepper and 1%turmeric powders, especially their synergistic combination, had a positive influence on meat palatability, in terms of juiciness and tenderness, antioxidant activity, and lipid oxidation in beef, during refrigerated storage.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

This work was performed with contribution of all authors. O. Pimpa designed the experimental procedures. F. Sresomjit and R. Bochuai performed the experiments. U. Pastsart interpreted the data and prepared the manuscript. All authors read and approved the final manuscript.

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Conflict of interests

The authors declare that they have no conflicts of interest.

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SOLID STATE FERMENTATION CHARACTERISTIC OF RICE STRAW USING HERBIVORE'S CECUM MICROBES

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ABSTRACT: This study was aimed at identifying highly capable lignolytic microbes from nature for use in Solid State Fermentation (SSF) of rice straw. The SSF silage was prepared in laboratory scale, as the following treatments: uninoculated (control), *Lactobacillus plantarum* FCC 123 (LP), fiber-degrading fungi (*Aspergillus* sp.) from horse cecum (FF), and fiber-degrading bacteria (*Enterococcus casseliflavus*) from buffalo cecum (FB). Incubation was carried out for a month at room temperature. The observed parameters were: organic acids, water-soluble carbohydrate (WSC), microorganism and nutrient composition. Rice straw SSF that was inoculated with LP showed the highest quality of fermentation, indicated by significant highest lactic acid bacteria (LAB) population, and has the lowest of poor bacteria indicators (coliform, aerobic bacteria, and bacilli). The LP treatment also has the highest LAB content and lowest WSC. Among treatments, FB treatment seems to have given a similar result with LP followed by FF. While the chemical composition seems unaffected by treatments. Compared with the fresh material, all fermentation with and without inoculants has reduced neutral detergent fiber (NDF) and increased acid detergent fiber (ADF), but there were no differences among all treatments. Inoculation of both LP and FB could improve rice straw SSF silage quality, but this system could not improve fiber degradation as well as in liquid state fermentation (LSF).

Keywords: Microorganism, Organic acid, Silage, Straw, Water soluble carbohydrate

INTRODUCTION

Fluctuating forage production as feed fiber sources in tropical could give a negative impact to forage availability for livestock. On the other hand, there are large quantities of rice straw that have challenges for disposal (Rathod et al., 2017) which can provide bulk animal feed, and is one of the major feed sources for the livestock production in some rice-producing countries in Southeast Asia (Tang, 2018). Moreover, feeding straw to livestock could be an alternative forage scarcity in the dry season (Ansah and Issaka, 2018) that leads to unoptimized livestock productivity in arid, semi-arid, and tropical areas (Sheikh et al., 2017) and an environmentally friendly way of utilizing straw as a by-product (Aquino et al., 2020). The use of by-products of agro-industry as SSF raw materials is very useful, they play an important role in the production of high-value animal feed because; 1) the quantity is abundant, 2) less competition between humans and livestock, 3) it is available at minimal cost for further processing, 4) it has an appropriate nutrient composition, and 5) it can aid microbial development during SSF (Oladapo et al., 2019). Meanwhile, SSF has the potential to be a candidate for antibiotic replacement strategies in animal feed (Yang et al., 2021). Using rice straw as livestock feed has been reported by some previous studies (Asmare and Yayeh, 2018; Shahryari et al., 2018), both in raw bulk (Rathod et al., 2017) or processed through various physical, chemical, or biological processes (Shrinivasa and Maski, 2017).

In the past, expansive studies have been done to improve nutritional value of rice straw by chemical and enzymatic pre-treatments. Chemical treatment of rice straw has been studied and documented very adequately, but this pretreatment was corrosive and poison potential to workers, meanwhile enzymatic treatment was expensive (Nurjana et al., 2016; Mutmainna et al., 2021). Inoculants can be applied to increase the nutritional value of rice straw, but they are considered impractical and the process has not been optimized especially under field conditions (Mahesh and Mohini, 2013). Implementation of inoculant from various rice paddy (Oryzae sativa) to total mixed ration silage microbial composition have also been studied (Wahyudi et al., 2022). Fresh rice straw with a DM content of 250 g/kg has a high concentration of water-soluble carbohydrates (WSC; 9.79% DM), which is suitable for silage (Lia et al., 2016). Because rice straw contains high fiber, the inoculant needed must be able to degrade complex carbohydrate bonds. Therefore, one of the possible future technologies for rice straw fermentation should focus on isolating and identifying highly capable lignolytic microbes from nature, and multiplying them for the production of lignolytic enzymes.

In previous study, both lignolytics fungi and bacteria have been isolated from Herbivore's lower gut. The study on rumen liquid state fermentation (LSF) showed that lignolytic fungi has improved 6.69% rice straw crude fiber digestion

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(Wahyudi et al., 2010a), and the lignolytic bacteria has increased 53.77% of feed crude fiber digestibility (Wahyudi et al., 2012), but their role in solid state fermentation (SSF) silage using Herbivore's Cecum Microbes has not been determined. Due to the minimal humidity required, only a limited number of microorganisms such as yeast and filamentous fungi can grow well under SSF conditions (Oladapo, et al., 2019). Among others, starter cultures that are widely used for Solid state fermented-feed are namely; Lactobacillus, yeast, bacillus, and mold (Yang, et al., 2021). This study was addressed to confirm the effect of fiber-degrading fungi and bacteria particularly in SSF silage to identify rice straw fermentation characteristics. Nutritional studies are encouraged to study plant by-products, especially rice straw as feed.

This study focuses to develop a simple technology that may partially solve the livestock's forage problem, particularly in small and mixed farming systems in developing countries.

MATERIALS AND METHODS

Ethical regulations

This study was accepted by health research ethics committee at the University of Muhammadiyah Malang according to seven WHO 2011 standard that was referred to by the guidance of CIOMS 2016.

Study area

Rice straw (*Oryzae sativa*) was obtained from rice field. The condition contained no seed, cut about 10cm from the soil surface and withered, chopped, then put in a plastic bag as soon as possible. The inoculants used in this study were *Lactobacillus Plantarum* (LP) FCC 123; FF, fiber-degrading fungi (*Aspergillus* sp.); and FB, fiber-degrading bacteria (*Enterococcus casseliflavus*). The *L. plantarum* (LP) FCC 123 was a typical strain that isolated from plant materials, a commercial LAB the strain has been known for silage making for decades years. The FF was *Aspergillus* sp., fiber-degrading fungi that has been isolated from horse cecum (Wahyudi et al., 2010a). *Aspergillus* sp. has also been used by Sidauruk et al., (2019) to ferment fiber in banana peel. The FB was *E. casseliflavus*, a fiber-degrading bacteria has been isolated from buffalo cecum (Wahyudi et al., 2010b).

Solid state fermentation silage preparation

The SSF silages were prepared on laboratory scale fermentation system treated with 50 µL LP, FF, and FB inoculants and un-treated control (F0). Approximately 100g of wilted rice chopped on the approximate length of 20mm, put on a plastic bag (KRIS BR 2205 type, 22cm × 500cm) and then the bags sealed with a vacuum sealer machine (KRIS VS200). The bag SSF were left for 30 days incubation at room temperature (average 25°C).

Microbial analysis

A-10g sample was merged into 90ml of sterilized water and diluted serially until 10-9. Agar of deMan Rogosa Sharpe (MRS; Difco) was used for total lactic acid bacillus (LAB) incubated under anaerobic conditions at 30°C for 48h (Pazla et al., 2021). Meanwhile, blue light broth (BLB; Nissui Ltd) and Potato Dextrose Agar (PDA; P2182 Sigma-Aldrich) were used for counting Coliform bacteria and mold respectively. Both were incubated at 30°C for 24h. For distinguishing yeast from molds, the appearance of the morphological colony and cell forming were observed through microscopic observation. Incubating at 75°C for 15 minutes before cultivating in nutrient agar (NA; Nissui Ltd.) would differentiate Bacilli from aerobic bacteria. Incubation would last at 30°C for 24h under aerobic conditions. Viable numbers of microorganisms in colony-forming units per gram of fresh matter (FM) were then counted.

Water soluble carbohydrate analysis

The HPLC was used to measure the WSC content, which included glucose, fructose, and sucrose. The analysis was set up: column, shodex sugar SC1011 (8.0mm x 30cm, Shoko); oven temperature 80°C; mobile phase, water; detector, 1,0ml/min; detector, (Jasco RI-1530).

Organic acid analysis

Cold water extract was used to determine fermentation products of SSF by homogenizing a 10g wet sample in 90ml sterile distilled water. Organic acid contents then were measured by HPLC (Jasco) as described method. The pH meter (Echem E-512 ex GR Scientific) was used to find out the sample pH. Meanwhile, the determination of ammonia-N was measured by steam distillation of filtrates.

Nutrient composition

Rice straw was dried in forced-air oven at 65°C for 48 h and ground to pass a 1 mm screen with a Willey mill (ZM200, Retsch GmbH and Co.). Contents of DM, OM, CP, and EE were analyzed according to methods 934.01, 942.05, 976.05, and 920.39 respectively, of AOAC by reference (William, and George, 2005). NDF was analyzed with a thermo stable amylase and sodium sulfite and ADF was analyzed none sequentially; results were expressed without residual ash (Van Soest et al., 2018).

Data analysis

Data obtained were analyzed by one-way analysis of variance (ANOVA). The differences between pairs of means were measured using Duncan's Multiple Range test (DMRT) ($P < 0.05$). All statistical analyses were performed in SPSS version 16 software.

RESULTS AND DISCUSSION

Microorganism and nutrient composition

The treatment was effect on the microorganism composition (Table 1). Lactic acid bacteria were higher in LP and FB compared to FF and F0 ($P<0.05$) otherwise, coliform was higher in FF and F0 compared to LP and FB ($P<0.05$). Aerobic bacteria were highest in F0, FF, and FB compared to LP ($P<0.05$). Bacilli was highest in FF compared to F0, LP ($P<0.05$) however FB was similar with both. Yeast was highest in FF, followed by LP and the lowest was F0 ($P<0.05$) while FB was similar with LP, FF, and F0. Otherwise, the result of mold was on the contrary with yeast ($P<0.05$). The nutrient composition is shown in Table 2. The result showed that the nutrients composition was not significantly different among treatments.

Generally, in this study ensilage with or without inoculants has increased LAB, meanwhile inoculant reduce aerobic bacteria and mold (Ridwan et al. 2019). LAB showed a significant role in silage fermentation. Species, population, and characteristics of LAB could be suggested as a factor to predict the sufficiency of silage fermentation. Silage can be well-preserved when LAB, especially lactobacilli, reaches at least 105 CFU/g of FM. In all treatments, the LAB population counts 107 (Table 1), suggesting high quality silage may be fermented well by all inoculant. Even though the number of coli-form unexpectedly still high, inoculant addition only cause a slight decreasing.

It has stated that addition of *Aspergillus oryzae* or *Saccharomyces cerevisiae* in SSF influence bacterial composition, but not affected to fungi population. Inoculation of *L. plantarum* FCC 123 (LP) in this study has anaerobic bacteria, coliform, bacilli, and mold compared with control (Yuan et al., 2015). It has ensured that LP could well prepare to rice straw ensilage. Furthermore, inoculation of FF and FB also increased LAB and yeast population, decrease aerobic bacteria, coliform, and mold, but slightly increased bacilli. Meanwhile FB was able to inhibit the pathogen bacteria as well as LP (Mutmainna et al., 2021).

In contrast to Zayed (2018), almost all nutrient parameters in this study were not significantly affected by the addition of inoculant. ADF content of silages were higher than raw material content, the increasing ADF content was caused by reducing of NDF by microorganisms. Yanti et al. (2014) and Zhang et al. (2015) reported that increasing of ADF content in fiber fermentation, is caused hemicellulolytic fraction (NDF) has been hydrolyzed by *Aspergillus niger*. Comparing with fresh material, all fermentation with and without inoculants has reduced NDF (Nurjana et al., 2016) and increased ADF, but there were not differences between all treatments. It could be stated the using of fiber degrading bacteria and fungi in SSF could not improve hydrolyzed NDF and ADF fraction, so in this case the inoculants have not needed.

Table 1 - Microorganism's composition (log cfu/g FM)

Parameters \ Treatments	Raw	F0	LP	FF	FB	P Value
Lactic acid bacteria	nd	7.647^{a}	7.929^{b}	7.668^{a}	7.929^{b}	<0.0001
Coliform	6.699	7.398^{c}	5.407^{a}	7.204^{c}	6.301^{b}	<0.0001
Aerobic bacteria	8.398	8.000^{b}	6.146^{a}	7.954^{b}	7.903^{b}	<0.0001
Bacilli	5.531	5.477^{a}	5.342^{a}	5.740^{b}	5.580^{ab}	<0.002
Clostridia	nd	nd	nd	nd	nd	–
Yeast	7.653	6.699^{a}	7.398^{b}	7.602^{c}	7.477^{bc}	<0.0001
Mold	6.699	7.699^{c}	3.544^{b}	3.000^{a}	3.394^{b}	<0.0001

CFU, colony forming unit; FM, fresh matter; F0, Control; LP, *L. plantarum* FCC 123; FF, fiber-degrading fungi/ *Aspergillus* sp; FB, fiber-degrading bacteria/ *E. casseliflavus*; nd, not detected.

Table 2 - Nutrient composition

Parameters		Raw	F0	LP	FF	FB	P Value
DM (%)		68.92	60.33	59.53	60.04	59.73	0.24
NC (%DM)	OM	83.49	83.41	83.25	83.52	83.51	0.94
	CP	4.49	7.70	7.82	7.56	7.48	0.24
	EE	0.03	0.04	0.04	0.04	0.04	0.06
	ADF	37.78	40.25	39.69	39.94	40.33	0.85
	NDF	62.22	59.75	60.31	60.06	59.67	0.85
	OM	83.49	83.41	83.25	83.52	83.51	0.94

DM, dry matter; NC, nutrient composition; OM, organic matter; CP, crude protein; EE, ether extract; ADF, acid detergent fiber; NDF, neutral detergent fiber; F0, Control; LP, *L. plantarum* FCC 123; FF, fiber-degrading fungi (*Aspergillus sp.*); FB, fiber-degrading bacteria (*E. Casseliflavus*)

Table 3 - Water soluble carbohydrate content

Parameters \ Treatments		Raw	F0	LP	FF	FB	P Value
DM (%)		68.92	60.33	59.53	60.04	59.73	0.24
WSC	Glucose	0.1910	0.0512^{a}	0.0630^{b}	0.0600^{b}	0.0630^{b}	<0.0001
	Sucrose	1.1298	1.0912^{d}	0.5206^{a}	0.7144^{c}	0.6337^{b}	<0.0001
	Fructose	0.1990	0.1380^{a}	0.1610^{b}	0.1410^{a}	0.1440^{a}	<0.0001
Total		1.5198	1.2804^{d}	0.7446^{a}	0.9114^{c}	0.8347^{b}	<0.0001

WSC, water soluble carbohydrate; F0, Control; LP, *L. plantarum* FCC 123; FF, fiber-degrading fungi (*Aspergillus* sp.); FB, fiber-degrading bacteria (*E. Casseliflavus*).

Table 4 - pH value and organic acid content

Parameters \ Treatments	Raw	F0	LP	FF	FB	P Value
Moisture (%)	31.08	39.70	40.53.00	39.96	40.97	0.24
pH	6.39	6.06	5.77	6.05	5.97	0.38
Lactic acid (%FM)	Nd	0.31^{a}	1.22^{c}	1.04^{b}	1.31^{c}	<0.0001
Acetic acid (%FM)	1.53	1.53^{b}	0.73^{a}	0.71^{a}	0.67^{a}	<0.0001
Propionic acid (%FM)	ND	ND	ND	ND	ND	–
n-butyric acid (%FM)	ND	ND	ND	ND	ND	–
VBN (g/kg FM)	0.10	0.15	0.14	0.14	0.14	0.84

F0, Control; LP, *L. plantarum* FCC 123; FF, fiber-degrading fungi (*Aspergillus* sp.); FB, fiber-degrading bacteria (*E. Casseliflavus*); nd, not detected; VBN, volatile inoculantse nitrogen.

Water soluble carbohydrate

Water soluble carbohydrate total was significantly different among treatments (Table 3), the highest was in F0 followed by FB and FF, and the lowest was LP ($P<0.05$). The glucose was similar among LP, FF, and FB but it was higher than F0 ($P<0.05$). The sucrose was highest in F0 followed by FB, and FF, and the lowest was in LP ($P<0.05$). The fructose was highest in LP than those of F0, FF, and FB ($P<0.05$). Water soluble carbohydrate content of materials is crucial factors for good silage preparation. Rice straw in this study contain less than 2% sugar and more than 65% dry matter, so with this condition may FF can grow better than LP or FB. Reported that low content of WSC would result a poor fermentation quality of silage. So, actually rice straw need more WSC and water for silage making, but in this study inoculation of FF has been expected to solve the problem. FF would hydrolyze crude fiber for producing sugar as energy source for LAB, and then will be converted into lactic acid or another organic acid such as acetic acid, propionic acid and butyric acid, but in this case, they could not work well same as previous study silage quality of sorghum that mixed legumes (Ardiansyah et al., 2016).

Organic acid content

The characteristic fermentation and the quality of rice straw during silage fermentation in 30 days are shown in Table 4. The result showed moisture, pH, and VBN were not significantly different among treatments, meanwhile, propionic acid and n-butyric acid were not detected. Lactic acid was highest in LP and FB than those of FF, and F0 was the lowest ($P<0.05$). Acetic acid was highest in F0 compared to LP, FF, and FB ($P<0.05$).

Inoculation of with and without inoculant could reduce pH value lower than raw material, but the pH value of the silages was not low enough. LAB presented on surface of plant residue, they will responsible for silage fermentation and influence the fermentation quality. The data proved that inoculant was important to improve a fermentation process. The reducing pH value was followed by increasing of lactic acid and acetic acid production, on the other hand volatile base nitrogen (VBN) content was decreased. But, if the concentration of acetic acid in the SSF is too high, the palatability of the feed will be greatly reduced (Yang et al., 2021). In this case, LP, FF, and FB have similar pattern for producing all organic acids and VBN in silages. The fermentation quality could be evaluated from lactic acid, organic acid, and VBN content in silage. The low level of VBN indicated the silage was well preserved and has a good quality. Several studies that has been reported (Wahyudi et al., 2017) inoculation of LAB could increase silage quality. Base on Table 4, the pH value is relatively high (5.77 – 6.06). But the control had higher pH value compared to the inoculant as well as previous study (Santoso et al., 2014), indicated that the rice straw contains less WSC, so the lactic acids production not enough reducing pH value. Material should have to contain more than 2% WSC to make good quality silage. Different with LP, FB was hetero-fermentative, cocci form, isolated from buffalo's digestive tract, also has ability to produce lactic acid. Lactococcus generally work in early of silage fermentation process before lactobacilli take over their role in lactic acid production (Santoso et al., 2014). Acetic acid and VBN content tend to decrease on this rice straw silage, propionic acid and butyric

acid even not detected. Rice straw silage in this study only contain 31,08% moisture showed that raw material needed 60-65% moisture in material for good silage making, so rice straw actually need water to make a good silage.

CONCLUSION

The present study demonstrated that the use of different inoculant affected the characteristics of rice straw silage of Solid State Fermentation (SSF). Inoculation of both *Lactobacillus plantarum* FCC 123 and *Enterococcus casseliflavus* could improve rice straw solid state fermentation silage quality, but this system could not improve fiber degradation as well as in liquid state fermentation. Making solid state fermentations on a big scale is required for further investigation.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

Wahyudi and Hendraningsih contribute on design the study. Mahmud, Mulatmi and Prima contribute on data collection and data analysis. All authors were contributing to write the manuscript.

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Conflict of interests

The authors declare that there are no conflicts of interest in this study.

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EFFECTS OF PROBIOTIC *Enterococcus faecium* AND RAW, SPROUTED AND FERMENTED PEARL MILLET BASED DIETS ON PERFORMANCES, CARCASS TRAITS, HEMATOLOGICAL AND BIOCHEMICAL INDICES OF BROILER CHICKENS

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RESEARCH ARTICLE

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ABSTRACT: Probiotics, recognized as a safe substitute for antibiotics in the animal industry, have been acknowledged for their growth-enhancing properties. This study assessed the impact of *Enterococcus faecium* strain NCIMB 11181 and diets incorporating Raw, Sprouted, and Fermented pearl millet on the performance, carcass traits, organ weights, and blood parameters of broiler chickens. In a randomized design, 120 one-day-old Arbor Acre broiler chickens were assigned to five groups: 1) No supplement, negative control (N-con); 2) Control + antibiotics, positive control (P-con); 3) Raw pearl millet + probiotics in drinking water (RPM + PRO); 4) Sprouted pearl millet + probiotics in drinking water (SPM+PRO); 5) Fermented pearl millet + probiotics in drinking water (FPM + PRO). Probiotic supplementation did not significantly impact body weight gain (BWG) but influenced feed intake (FI) ($P<0.05$). FPM+PRO increased feed conversion ratio (FCR), thigh yield, and drumstick yield. Thymus weight is reduced in the RPM+PRO and SPM+PRO groups compared to the control groups. Serum high-density lipoprotein (HDL) levels decreased ($P<0.01$) in the P-con and FPM+PRO groups. No treatment effect ($P>0.05$) was observed on hematological indices. Overall, pearl millet diets supplemented with probiotics demonstrated no adverse effects on the health status of broiler chickens, suggesting their potential as viable alternatives to antibiotics.

Keywords: Pearl millet, Broiler chickens, Blood, Carcass traits, Growth performance, Probiotics.

INTRODUCTION

Antibiotics gained popularity in the poultry industry for promoting growth and maintaining poultry health. However, their extensive use led to concerns about residues in livestock products, antibiotic-resistant gene transfer, and negative effects on human health and safety (Ronquillo and Hernandez, 2017; Vieco-Siaz et al., 2019). Consequently, antibiotic use in food animals faced restrictions (Vieco-Siaz et al., 2019).

Probiotics, non-pathogenic microorganisms in the intestinal flora, offer an alternative by benefiting host physiology and health. They stabilize intestinal microbiota, enhance carcass traits, intestinal morphology, gut microbial population, modulate the immune response, and strengthen the mucosal barrier (Attia et al., 2017; Wu et al., 2019; Zhang et al., 2021). Probiotics may replace antibiotics as growth promoters in poultry (Suryadi and Prasetyo, 2018). Notably, different probiotic strains within the same genus and species can have varying clinical effects (Vieira et al., 2013). The European Food Safety Authority (EFSA) approved *Enterococcus faecium* strain NCIMB 11181 as an animal feed additive to improve growth performance (EFSA, 2012).

Enterococcus faecium, a lactic acid bacterium, is a natural intestinal inhabitant resistant to acidic conditions and bile salts. It produces enterocins, antimicrobial substances serving as poultry probiotics (Zommiti et al., 2018). The *E. faecium* strain 11181 has demonstrated efficacy in improving feed conversion ratio, daily weight gain, and gut health, inhibiting gut pathogen proliferation, and stimulating the immune system (Wu et al., 2019; Shao et al., 2022). Adding *E. faecium* to broiler feed or drinking water enhances intestinal morphology, modulates microflora, and inhibits pathogen proliferation, including Salmonella (Cao et al., 2013; Zheng et al., 2016; Wu et al., 2019; Shao et al., 2022).

This study focuses on Pearl Millet (PM), a nutrient-rich grain abundant in the Sahel region, especially Nigeria. PM boasts protein, amino acids, vitamins, minerals, fiber, fat, energy, ash, and antioxidants, with fewer anti-nutritional factors than other cereals (Uppal et al., 2015; Weckwerth et al., 2020). Furthermore, PM includes fewer anti-nutritional factors, compared to other cereals (Kaushik and Grewal, 2017; Boncompagni et al., 2018; Punia, 2020).

Different processing techniques, including sprouting and fermentation, enhance nutrient availability in PM (Gowda et al., 2022). Our prior study confirmed that processed PM did not negatively impact the physiology and welfare of broiler chickens (Olasehinde and Aderemi, 2023). Yet, the potential benefits of supplementing processed PM diets with probiotics on broiler chicken growth performance and blood metabolites remain unexplored. This study aimed to address

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this gap by investigating the effects of unprocessed and processed PM, supplemented with probiotic *E. faecium*, on various aspects health and performance of broiler chickens.

MATERIALS AND METHODS

All experimental procedures were approved by Bowen University's committee for research and ethics. The birds were managed and handled following standard guidelines of the University and ARRIVE 2.0 and National Research Council (NRC) Committee guidelines (du Sert et al., 2020), which reduced pain and discomfort on the birds.

Source of probiotics supplementation

The *E. faecium* NCIMB 11181 strain (Protexin) used in the study was a commercial product from Probiotics International Ltd (Lopen Head Somerset, UK) which contained a total bacteria count ≥ 2.0 x 10 11 CFU/kg. The probiotic product was added to drinking water on daily basis for 42 d according to manufacturer instruction. Broilers on antibiotics treated group receive colistin (as sulphate, 4,800,000 IU, Kepro, Holland) daily through drinking water for 42 d.

Animal, design, and diets

A total of 120 one-day-old Arbor Acres chicks were individually weighed, labelled, and randomly allocated, following a completely randomized design, to 5 dietary treatment groups each comprising 4 replicate cages with 6 birds per cage. The basal diet was isocaloric and met or exceeded NRC (1994) nutrient requirements for starter (day 1 to 21) and finisher (day 22 to 42) phases. Pear millet replaced 25% maize in the basal diet. The composition and nutrient levels of the basal diet of maize or PM + soybean meal-based is presented in Table 1. The treatments consisted of the following: 1) basal diet without supplementation, negative control (N-con); 2) basal diet with antibiotics supplementation, positive control (P-con); 3) Raw pearl millet + probiotics supplementation (RPM + Pro); 4) Sprouted pearl millet + probiotics supplementation (SPM+Pro); 5) Fermented pearl millet + probiotics supplementation (FPM+Pro). Antibiotics and probiotics were administered according to the manufacturer's recommendations through drinking water. The broiler chickens on N-con, P-con and probiotics were placed in separate rooms to prevent contamination. The rooms were identical in environmental configuration throughout the study. The lighting programs during the study were 1 hour of darkness (0 - 7 days after hatch) and 4 hours of darkness (8 - 42 days, experimental period). The ambient temperature ranged between 25^{o}C and 33^{o}C during the experimental period. Temperature within the pen was regulated and was carried out through use of natural and mechanical means within the pen.

Table 1 - Basal diet formulation and composition

Ingredient (%)	Starter (1 to 21 d)					Finisher (22 – 42 d)				
	N-con	P-con	RPM+PRO	SPM+PRO	FPM+PRO	N-con	P-con	RPM+PRO	SPM+PRO	FPM+PRO
Maize	52.10	52.10	39.78	39.60	39.28	59.78	59.78	46.97	45.49	45.77
Pearl millet	-	-	14.42	14.29	14.81	-	-	14.94	16.14	16.20
Soybean meal	40.89	40.89	39.24	39.66	39.52	34.00	34.00	32.33	32.61	32.50
Soybean oil	2.68	2.68	2.20	2.26	2.03	2.17	2.17	1.68	1.69	1.46
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	2.16	2.16	2.16	2.15	2.14	1.95	1.95	1.95	1.93	1.92
Limestone	1.49	1.49	1.50	1.51	1.52	1.39	1.39	1.40	1.41	1.42
Vit-Min Premix [1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine		-	0.03	0.03	0.03	0.05	0.05	0.08	0.08	0.08
Methionine	0.18	0.18	0.17	0. 17	0.17	0.16	0.16	0.15	0.15	0.15
Calculated composition (%)										
ME (kcal/kg)	2970	2970	2970	2970	2970	3004	3004	3004	3004	3004
Protein	22.5	22.5	22.5	22.5	22.5	20.00	20.00	20.00	20.00	20.00
Methionine	0.53	0.53	0.53	0.53	0.53	0.45	0.45	0.45	0.45	0.45
Lysine	1.21	1.21	1.21	1.21	1.21	1.10	1.10	1.10	1.10	1.10
Calcium	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Phosphorus	0.50	0.50	0.50	0.50	0.50	0.45	0.45	0.45	0.45	0.45

N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; ME = Metabolizable energy; DCP = Dicalcium phosphate; Vitamin-Mineral Premix supplied per kilogram of diet: vitamin A, 30,000 IU; vitamin D3, 6,250 IU; vitamin K, 5 mg; Vitamin E, 75 mg; vitamin B1, 5.63 mg; vitamin B2, 15 mg; vitamin B6, 11.25 mg; vitamin B12, 0.0375 mcg; Niacin, 100 mg; Pantothenic acid, 37.5 mg; Folic acid, 3.75 mg; choline chloride, 750 mg; manganese, 200mg; biotin, 0.125 mcg; zinc, 125 mg; iodine, 2.5 mg; copper, 12.5 mg; selenium, 0.5 mg; cobalt, 1.25 mg; iron, 50 mg; antioxidant, 312.5 mg.

Growth performance, carcass traits and organ measurements

Body weight (BW) was recorded daily to calculate body weight gain (BWG) for starter (1 to 21 days), finisher (22 to 42 days) and for the overall (1 to 42 days) growth period. Feed intake (FI) per cage was measured daily to calculate FI for each growth period. Feed conversion ratio (FCR) was determined for the starter, finisher, and overall growth period. At the end of starter and finisher growth period, carcass and organ variables were sampled and measured as we previously described (Olasehinde and Aderemi, 2023).

Blood analysis

At the end of finisher growth period, blood samples were drawn from wing 8 birds for each treatment and analyzed as described by Olasehinde and Aderemi (2023).

Statistical analysis

Data were analyzed as a one-way analysis of variance (ANOVA) using the general analysis of variance model of statistical software GenStat version 21 (VSN international). Where there are significant differences at the P<0.05 level, the treatment means were assessed using Tukey's test. Data are presented as means and pooled standard error of mean (SEM).

RESULTS

The BWG by broilers was not altered (P>0.05) by dietary treatments in the starter, finisher, and overall growth periods (Table 2). Broilers in FPM+PRO group consumed most amount of feed during the starter period, while addition of antibiotic decreased (P<0.001) feed intake. During the finisher phase, lowest FI was observed for SPM+PRO group while highest FI was recorded for broiler chickens in the FPM+PRO group (Table 2). There was no difference in the FI of broilers chickens in the N-con, P-con and RPM+PRO groups. When considering the entire experimental period, FI reduced significantly in the SPM+PRO and P-con group compared with the N-con and RPM+PRO group. The highest FI was obtained in broiler chickens in the FPM+PRO group (Table 2). Dietary treatments had no significant effect on FCR during the starter period (Table 2). However, during the finisher period, FCR was significantly higher in the FPM+PRO group (P=0.034) compared to the rest of the treatment groups. In the overall experiment period, FCR of broiler chickens in the FPM+PRO group was significantly higher than the N-con, P-con and SPM+PRO groups (Table 2).

Table 2 - Effect of dietary treatments on performance of broiler chickens

Parameters	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
BWG (g/bird)							
Starter phase	649	652	633	659	667	15.300	0.839
Finisher phase	1080	1056	1056	1011	1002	11.400	0.124
Overall phase	1729	1708	1689	1670	1669	21.100	0.688
FI (g/bird)							
Starter phase	545^{b}	512^{a}	552^{b}	552^{b}	589^{c}	7.110	<.001
Finisher phase	1435^{b}	1434^{b}	1446^{b}	1366^{a}	1494^{c}	11.900	0.001
Overall phase	1980^{bc}	1946^{ab}	1999^{c}	1918^{a}	2083^{d}	15.200	<.001
FCR							
Starter phase	0.84	0.80	0.89	0.85	0.89	0.025	0.392
Finisher phase	1.33^{a}	1.36^{a}	1.37^{a}	1.35^{a}	1.49^{b}	0.018	0.034
Overall phase	1.15^{a}	1.14^{a}	1.19^{ab}	1.15^{a}	1.25^{b}	0.016	0.047

Means with different letters in the same row differ significantly (p≤0.05, 0.001); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; BWG = Body weight gain; FI = Feed intake; FCR = Feed conversion ratio; g = gram; SEM = Standard error of the mean.

There were no significant differences between treatments for all carcass traits, except abdominal fat, at the end of the starter period (Table 3). Abdominal fat in the RPM+PRO group increased significantly compared with the N-con and the SPM+PRO treatment groups. FPM+PRO increased drumstick and thigh weights of broiler chickens on finisher diets while the addition of antibiotic decreased (P<0.01) drumstick weight. However, there was no effect (P>0.05) of dietary treatments on carcass yield, breast, wing, and abdominal fat at the end of the finisher period (Table 3). There was no significant effect of dietary treatments on digestive and immune organ weights at the end of the starter period (Table 4).

Similarly, dietary treatments did not influence the relative weights of the pancreas, gizzard, proventriculus, liver, bursa, and spleen at the finisher period. However, relative weight of thymus of broiler chickens in the SPM + PRO group decreased ($P<0.05$) compared with the control groups (Table 4).

The hematology indicators presented in Table 5 were not affected by dietary treatments. Similarly, there were no significant treatment effects on albumin, AST, globulin, serum protein, LDL-cholesterol, triglycerides (Table 6). Glucose concentration in PM-based diets supplemented with probiotic showed a decreased trend ($P<0.05$) in contrast to the control groups. However, HDL-cholesterol concentration increased ($P<0.01$) in the SPM+PRO group compared with the control groups. HDL of broiler chickens in the RPM+PRO also increased compared to broilers chickens in the P-con group (Table 6).

Table 3 - Effect of dietary treatments on carcass characteristics of broiler chickens.

Parameters (%)	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
Starter phase							
Carcass yield	53.70	54.40	56.20	54.90	50.70	1.380	0.685
Breast	19.24	14.97	19.10	18.82	18.58	0.560	0.069
Drumstick	9.44	8.73	10.11	8.96	8.76	0.240	0.393
Thigh	9.77	9.05	10.29	9.92	8.88	0.230	0.313
Wing	6.61	5.66	6.74	6.15	6.16	0.160	0.124
Abdominal fat	0.24^{a}	0.75^{ab}	1.40^{b}	0.60^{a}	0.76^{ab}	0.110	0.018
Finisher phase							
Carcass yield	60.52	60.11	61.79	60.16	60.24	0.480	0.790
Breast	25.20	25.50	25.50	24.10	23.10	0.490	0.401
Drumstick	10.38^{b}	9.65^{a}	10.03^{ab}	10.41^{b}	11.14^{c}	0.140	0.007
Thigh	10.39^{a}	10.73^{a}	10.92^{ab}	10.69^{a}	11.39^{b}	0.099	0.011
Wing	6.48	6.33	6.37	6.50	6.52	0.056	0.779
Abdominal fat	0.35	0.57	0.54	0.67	0.57	0.110	0.908

Means with different letters in the same row differ significantly ($p\leq0.05$, 0.01); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; BWG = Body weight gain; FI = Feed intake; FCR = Feed conversion ratio; g = gram; SEM = Standard error of the mean.

Table 4 - Effect of dietary treatments on digestive organ weights of broiler chickens.

Parameters (%)	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
Starter phase							
Pancreas	0.40	0.30	0.31	0.32	0.33	0.013	0.076
Gizzard	2.07	1.81	1.83	1.97	1.90	0.055	0.647
Proventriculus	0.54	0.50	0.49	0.47	0.41	0.019	0.345
Liver	2.81	3.00	3.10	2.62	2.61	0.083	0.250
Bursa	0.26	0.24	0.20	0.23	0.30	0.016	0.062
Spleen	0.10	0.10	0.11	0.07	0.10	0.009	0.698
Thymus	0.41	0.44	0.41	0.42	0.44	0.020	0.992
Finisher phase							
Pancreas	0.18	0.17	0.20	0.16	0.15	0.007	0.170
Gizzard	1.27	1.19	1.19	1.28	1.21	0.030	0.716
Proventriculus	0.26	0.23	0.24	0.24	0.23	0.009	0.785
Liver	1.76	1.64	1.75	1.84	1.70	0.036	0.587
Bursa	0.15	0.12	0.07	0.06	0.09	0.011	0.068
Spleen	0.07	0.08	0.07	0.07	0.05	0.006	0.636
Thymus	0.29^{c}	0.23^{bc}	0.14^{ab}	0.11^{a}	0.19^{abc}	0.020	0.030

Means with different letters in the same row differ significantly ($p\leq0.05$); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; SEM = Standard error of the mean.

Table 5 - Effect of dietary treatments on hematological profile of broiler chickens.

Parameters	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
Hemoglobin (g/dl)	9.47	9.18	10.10	9.85	9.25	0.270	0.848
Heterophil (%)	31.80	34.20	29.50	32.20	33.00	1.300	0.894
Lymphocytes (%)	61.00	58.80	62.00	61.00	59.80	1.310	0.969
Monocytes (%)	3.50	3.25	2.75	3.25	3.00	0.200	0.846
PCV (%)	29.75	28.00	30.50	30.00	28.00	0.850	0.877
RBC (x 10^6/µL)	3.00	2.84	2.82	2.95	2.93	0.082	0.969
WBC (x 10^3/µL)	15675	14888	16100	14975	15738	331	0.780

Means with different letters in the same row differ significantly (p≤0.05); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; SEM = Standard error of the mean; PCV = Packed cell volume; RBC = Red blood cell; WBC = White blood cell; µL= microliter; g = gram; mg = milligram; dl = deciliter.

Table 6 - Effect of treatments on serum biochemical indices of broiler chickens.

Parameters	N-con	P-con	RPM+ PRO	SPM+ PRO	FPM+ PRO	SEM	ANOVA
Albumin (g/dl)	1.57	1.57	1.64	1.50	1.59	0.052	0.949
AST (µ/L)	81.90	91.60	103.10	92.40	82.80	3.250	0.228
Globulin (g/dl)	1.25	0.90	1.06	1.38	1.24	0.088	0.538
Glucose (mg/dl)	235.20	253.80	228.10	203.60	217.00	6.220	0.087
HDL (mg/dl)	32.40^{ab}	29.63^{a}	33.26^{b}	35.04^{b}	29.96^{a}	0.690	0.008
LDL (mg/dl)	16.32	15.38	16.66	16.77	16.29	0.450	0.892
Triglycerides (mg/dl)	39.60	61.80	74.50	54.10	69.20	6.220	0.377
Total Protein (g/dl)	2.81	2.46	2.75	2.88	2.82	0.096	0.755

Means with different letters in the same row differ significantly (p≤0.05, 0.01); Data represent the mean of 8 replicates; N-con = Negative control (birds received control diet only); P-con = positive control (birds received control diet + antibiotics); PRO = birds on pearl millet diet + probiotics; RPM = Raw pearl millet; SPM = Sprouted pearl millet; FPM = Fermented pearl millet; SEM = Standard error of the mean; AST = Aspartate Transaminase; HDL = High density lipoprotein; LDL = Low density lipoprotein; µL= microliter; g = gram; mg = milligram; dl = deciliter.

DISCUSSION

Growth performance

Probiotics, known for maintaining gut health and enhancing productivity, were studied in broiler chicken diets. Previous research showed no performance impact on chickens fed processed PM (Olasehinde and Aderemi, 2023). In our study, PM with probiotics did not affect BWG, aligning with findings by Shao et al. (2022) who reported no significant change in BWG with probiotic *E. faecium* NCIMB 11181. During the starter period, PM diets with probiotics had no effect on FCR. However, raw and sprouted PM diets with probiotics influenced FCR in the finisher and overall growth phases, consistent with previous reports (Marcato et al., 2023; Awad et al., 2015).

Probiotics influenced FI in our study, in line with existing literature (Rehman et al., 2020; Zhang et al., 2021). However, disparities in FI effects may stem from feed type, probiotic characteristics, and environmental factors. Fermented PM increased FI, but when supplemented with probiotics, it adversely impacted overall performance. In contrast, sprouted PM with probiotics reduced feed consumption without negative effects on FCR and BWG, suggesting potential economic advantages. The impact of probiotic supplementation on broiler growth performance varies across studies (Rehman et al., 2020; Zou et al., 2022). Factors like probiotic type, dosage, diet composition, and animal health status contribute to these discrepancies. Further exploration is needed to clarify these influences and enhance our understanding of probiotics' role in broiler diets.

Carcass traits

Assessing carcass traits is crucial for evaluating broiler chicken quality. In this study, the starter diet showed no significant impact on carcass traits. Olasehinde and Aderemi (2023) indicated that sprouted PM had no effect on broiler

chicken carcass traits. Similarly, fermented PM did not increase carcass yield, though a dose effect was noted in broilers on finisher diets (Olasehinde and Aderemi, 2023). However, adding probiotics to the finisher's diet with fermented PM increased drumstick and thigh weights, suggesting a positive effect on carcass traits of broiler chickens. This aligns with studies by Ghasemi-Sadabadi et al. (2019) and Salehizadeh et al. (2019), demonstrating that probiotics improved carcass and thigh yield. On the contrary, Pelicano et al. (2003) reported that probiotics did not enhance weights of carcass, thigh, breast, and liver in broiler chickens.

Abdominal fat is a key indicator of lipid accumulation in broiler chickens. Our previous work (Olasehinde and Aderemi, 2023) demonstrated that sprouted or fermented PM in diets did not affect abdominal fat of broiler chicken. In this study, broiler chickens on processed PM with probiotics had similar abdominal fat levels to those on control diets, while raw PM with probiotics increased abdominal fat. In contrast, studies by Agboola et al. (2015) reported that probiotic supplementation could reduce abdominal fat in broiler chickens, similar to findings with *E. faecium* as a probiotic supplement (Demeterova, 2009; Weis, 2011). Variations in these results may stem from differences in basal diet, bacterial type and strain, chicken breed, and environmental conditions.

Organ weights

The thymus, spleen, and bursa of Fabricius play crucial roles in coordinating immune functions. Chen et al. (2020) suggested that increased weight of these organs in broilers may indicate the development and proliferation of immune cells. However, our results showed no impact of supplementation on the weight of the spleen and bursa of Fabricius. While various studies have reported positive effects of probiotic supplementation on poultry immune organ weight (Hidayat et al., 2020; Zhang et al., 2021), our study observed a reduction in thymus weight in broilers fed sprouted PM diets with probiotics compared to the control groups.

The thymus, a key lymphoid organ, is essential for the development and maturation of T-lymphocytes that regulate immune protection (Farley et al., 2013). A decrease in thymus weight may indicate a suppression of adaptive immunity (Sharma and Moroni, 2021). Notably, the reduction in thymus weight in broilers fed sprouted PM with probiotics was accompanied by an increase in HDL concentration without adversely affecting performance. HDL's role in removing excess cholesterol from peripheral cells has implications for immune cell activation (Pradhan et al., 2021). The interaction between HDL and immune cells may influence immune cell development and response. Therefore, our results suggest that probiotic supplementation in sprouted PM diets may impact

Hematological and biochemical content

No significant differences were observed among dietary treatments for the studied hematological indices in broiler chickens. This aligns with findings from Alkhalf et al. (2010) and Abdel-Hafeez et al. (2017), who reported positive effects of probiotics on packed cell volume and hemoglobin concentration. However, Beski and Al-Sardary (2015) noted a significant increase in hemoglobin concentration and a reduction in the heterophil to lymphocyte ratio, differing from our results. The variation might be attributed to differences in probiotic bacteria, basal diet, and the birds' physiological and nutritional status (Etim et al., 2014).

In broiler chickens, serum HDL-cholesterol concentration was higher in those on the negative control diet, raw PM with probiotics, and sprouted PM with probiotics compared to the control diet with antibiotics. HDL plays a crucial role in transporting free cholesterol for disposal in the liver (Zannis et al., 2015). Additionally, HDL is involved in glucose homeostasis through insulin secretion, direct glucose uptake in muscles, and potentially increased insulin sensitivity (Han et al., 2007; Drew et al., 2012; Haase et al., 2015). Despite probiotic supplementation, a decreasing trend in glucose concentration in PM diets may be related to pearl millet's intrinsic property of lowering blood glucose due to its low glycemic index (Dias-Martins et al., 2018). These findings suggest that probiotic supplementation may not have influenced energy metabolism in broiler chickens.

CONCLUSION

In our study, diets containing raw PM and sprouted PM, both supplemented with probiotics *Enterococcus faecium*, showed no significant impact on the performance, HDL-cholesterol concentration, or blood indices of broiler chickens. However, fermented PM with probiotics improved carcass traits, thigh and drumstick yields but decreased HDL concentration. Despite these variations, PM diets with probiotics did not adversely affect the overall health of broiler chickens, possibly due to the non-pathological conditions of the birds in our study. Our findings suggest the importance of exploring these treatments under pathological conditions for a comprehensive understanding.

DECLARATION

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Author's contribution

O. Olasehinde conceived, designed, conducted the experiment, analyzed, and wrote the manuscript for publication. O. Olasehinde and F. Aderemi reviewed and approved the manuscript.

Consent to publish

Not applicable.

Competing interest

The authors declare that there is no conflict of interests.

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PROCESSING OF SLAUGHTERHOUSE BLOOD FOR ANTIANEMIC FOOD PRODUCTS

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ABSTRACT: Currently, rational processing of blood from slaughterhouses remains as a waste fluid in many regions. Traditional approaches to use the blood for food are significantly limited because of specific and non-favorable organoleptic characteristics. Present study provides a comparison of various methods for modifying the red blood cell (RBC) mass of animals and a more in-depth study of acid hemolysis. The solution of ascorbic acid has been proposed as a hemolyzing agent. There has been established experimentally that the addition of equal volumes of RBC and a solution of an ascorbic acid with a concentration of 0.75 mol/dm^3 can effectively destroy the stroma more up 90% of red blood cells within 15 minutes. By this, the hemoglobin oxidation degree to methemoglobin is about 50%, which forms the desired color of the resulting hydrolysate. The dry semi-finished product has a neutral odor and brown color with high functional and technological properties. It also contains 0.9% organic iron with good biological value. Thus, the study shows that blood products can effectively use in various foods such as meat products, and also as a dietary supplement for various proposes. Consumption of these products has potent positive effect on hemoglobin levels and it is recommending for people with iron deficiency anemia.

Keywords: Antianemia products, By-products, Heme iron, Farm animals, Slaughterhouse.

INTRODUCTION

Slaughterhouse blood and blood products are using for various food products production (Bah et al., 2013; Chiroque et al., 2023). Currently, the food industry uses about 30% of the slaughterhouses' blood; Majority of this blood going to the meat industry for use as a gelling agent and natural coloring agent (Álvarez-Castillo et al., 2023; Chiroque et al., 2023). Various methods have been developed for obtaining dyes from blood to stabilize the color of meat products (Wismer-Pedersen, 1988; Ofori and Hsieh, 2012).

Clarified blood and formed elements in dry, liquid and frozen form are using together with soy protein or sodium caseinate (Lynch et al., 2017; Damba, 2017). One of the ways for using blood cells is as raw materials for protein hydrolysates, produced in the form of coagulate or dried form (Lynch et al., 2017).

The slaughtered animals' blood is a significant resource of highly digestible organic iron (Hertrampf et al., 2000; Alao et al., 2017). Consumption of foods with heme iron is an effective means for the prevention and treatment of iron deficiency anemia (Bak et al., 2018; Siti et al., 2021), which, according to WHO, affects more up 500 million people worldwide in 2020. The limiting factor to use the blood and its products is the hemoglobin heme component. It gives the final product an undesirable dark brown color, a specific blood odor, and a metallic taste (Lynch et al., 2017; Ofori and Hsieh, 2012). There are of existing approaches for reducing these undesirable characteristics boil down to separate the heme and globin protein. That is complicates the technological process and impairs the bioavailability of nutrients.

It can achieve discoloring blood formed elements by treating them with a strong oxidizing agent, which is a well-known and effective technique (Ofori and Hsieh, 2014). The resulting bleached blood is a highly effective emulsifier with good foaming properties, a source of essential amino acids, and can also be used as a fat substitute in foods (Kikafunda and Sserumaga, 2005). Strong oxidizing agents like acetone and hydrogen peroxide damage the heme part, affecting iron absorption. This makes it promising to use more gentle approaches to the treatment of blood and its formed elements.

Enzymatic hydrolysis breaks down erythrocyte membranes without harming the heme part. This method of hydrolysis neutralizes the bloody odor and increases the digestibility of heme iron by up to 30% (Musa and Idrus, 2021). The presence of low molecular weight peptides in the hydrolysate decreases the raw material properties by a third (Sanchez-Reinoso et al., 2021). Also, the red color of low oxidized hemoglobin restricts the use of this product in food technology.

One way to eliminate the characteristic color of blood can be considered the transformation of hemoglobin into methemoglobin, which occurs at a certain heme iron oxidation degree (Velusamy et al., 2022). This will allow the blood to

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turn brown, allowing it to be used not only in meat products but also in imitation confectionery products. To achieve this effect, it can use solutions of food organic acids. They will be able to cause as blood cells destruction and the iron oxidation with methemoglobin formation, without destroying the structure of the metallo-protein.

Thus, the aim of present study was to study the effectiveness of the use of acid hydrolysis in the modification of erythrocyte mass for its use in food technologies. The study objectives are to choose the acid and its quantity. Also, to assess the rate and hemolysis completeness and heme iron oxidation degree and the formed organoleptic and functional-technological characteristics.

MATERIALS AND METHODS

The study is aimed to investigate the effectiveness of hemolysis various methods for modifying the qualitative characteristics of the slaughtered animals' RBC mass. The explanatory research design was based on mixed methods and a constructivist view of veterinary and medical therapies. Use of bovine blood from animals were examined by a veterinarian and found to be healthy. The slaughter of animals was in accordance with the veterinary rules for the slaughter of animals approved by the Ministry of Agriculture of the Russian Federation (order No. 269 of April 28, 2022; ethical regulation). The slaughter was carried out in a modular slaughterhouse, which is a complex of technological lines equipped with units and devices that ensure humane slaughter. Animals were stunned mechanically, using a pneumatic pistol. The animals on the way of bleeding were an unconscious state. Blood collection carried out in a closed manner, by a hollow knife into a closed reservoir under a slight vacuum (according to GOST 33674-2015). A mixture of 4% sodium citrate solution and 0.75% disubstituted sodium phosphate solution in equal proportions was used as a stabilizer. The dose of stabilizer is 1:9. There are carried out the experimental studies in scientific laboratories of the Department of Technology of Production and Processing of Agricultural Products of the Stavropol State Agrarian University in the period 2022-2023. The main studied indicators were the rate and completeness of hemolysis, hemoglobin oxidation degree and the hemolysate resulting organoleptic assessment.

The study objects were hemolysates of erythrocyte mass (ascorbic acid). Materials were included of 1) cattle blood (pH 7.4, viscosity - 5.5 N-s/m^2, stabilizer - sodium pyrophosphate) and ascorbic acid (mass fraction of the main substance 99.00%).

Tools and techniques

- Blood fractionation into formed elements and plasma was carried out on a laboratory centrifuge Bios Neofuge 15 (Heal Force, China);
- The completeness of hemolysis was determined by determining the optical density of a mixture of 0.25 cm^3 of hemolysate and saline solution (84 times dilution) at a wavelength of 670 nm on a Unico S-1200 spectrophotometer (USA);
- Determination of the content of hemoglobin derivatives was carried out according to the Austin and Drabkin method based on the analysis of the absorption spectra of the two-component system of oxyhemoglobin and methemoglobin;
- Organoleptic assessment was carried out in accordance with GOST ISO 13299-2015;
- The mass fraction of moisture/dry substances was determined by drying a sample to constant weight at a temperature of 105 ºC;
- The mass fraction of protein was determined by the Kjeldahl method (GOST 25011-2017);
- Active acidity was determined by the potentiometric method on a pH meter / millivoltmeter pH-410 (Russia) (according to GOST R 51478-99);
- The mass fraction of ash was determined according to GOST 31727-2012 by the method of mineralization of a sample followed by its combustion in a muffle furnace SNOL 6-10 (Russia) at a temperature of 500 ºC;
- The mass fraction of iron was determined according to GOST 26928-86.

Procedure

Stabilized blood was centrifuged at 8000 rpm for 10 minutes in order to isolate the formed elements. Hemolysate were prepared by adding ascorbic acid to 50 ml of red blood cells with concentrations of 0.25; 0.5; 0.75 or 1.5 mol/dm^3. The ratio of PE and acid solution varied 1:0.5; 1:1 and 1:1.5. The completeness of hemolysis was assessed every 60 seconds by pipetting a sample from the sample volume. The study of the effect of ascorbic acid included the addition of its solution with concentrations of 0.25, 0.75, 1.0 and 1.5 mol/dm^3 in 3 ratios: 1:0.5; 1:1; 1:1.5. Organoleptic studies included a descriptive assessment of the consistency, color and smell of the resulting hemolysate in liquid form.

Data analysis

Experiments and analytical determinations were carried out in triplicate. Only representative, reproducible data from each experiment are discussed. Statistical processing of experimental data performed by STATISTICA Base (Statsoft

products) and included the determination of the following values: arithmetic mean, quadratic dispersion, standard deviation of a single result, standard deviation of an average result, adequacy degree, confidence interval (P<0.05).

RESULTS AND DISCUSSION

Given the known strengths and weaknesses of RBC hemolysis methods, it was decided to conduct an in-depth study of acid hemolysis using organic acid used in the food industry. For these purposes, it was decided to use ascorbic acid, the addition of which will also provide fortification of the product.

The major criteria of hemolysis effectiveness are the speed and completeness of the process. For this, primarily, will be influence the ratio of volumes formed elements to the acid solution in the system and its concentration. In order to establish the optimal values of these parameters, a number of studies have been carried out. Working solutions of ascorbic acid had the following concentrations: 0.25 mol/dm^3, 0.5 mol/dm^3, 0.75 mol/dm^3 and 1.5 mol/dm^3.

Using an acid with a molar concentration of 0.25 mol/dm^3 showed a low efficiency of hemolysis over the studied time period (Figure 1), while a concentration of 1.5 mol/dm^3 causes the fastest and most complete destruction of red blood cells (Figure 4). Intermediate options for adding ascorbic acid with a concentration of 0.75-1 mol/dm^3 also causes almost complete hemolysis in a given period, but the intensity of its occurrence is lower (Figures 2-4).

Analysis of the experimental data obtained led to the conclusion that the optimal concentration of ascorbic acid solution in terms of the speed and completeness of hemolysis is 0.75 mol/dm^3. An increase in concentration leads to active acidity values below 5.0 units, the formation of a sour odor and astringent taste, which is unacceptable for a number of food technologies.

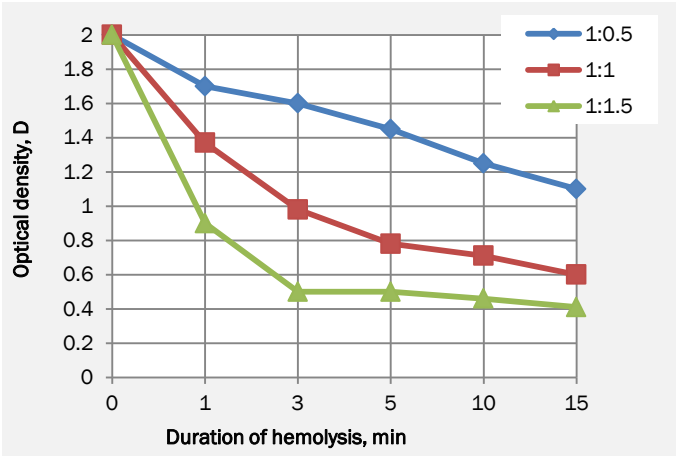

Figure 1 – The hemolysis effectiveness by ascorbic acid (concentration 0.25 mol/dm^3)

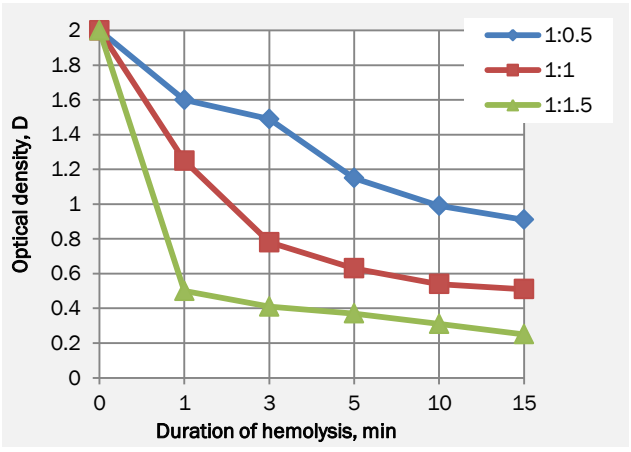

Figure 2 – The hemolysis effectiveness by ascorbic acid (concentration 0.5 mol/dm^3)

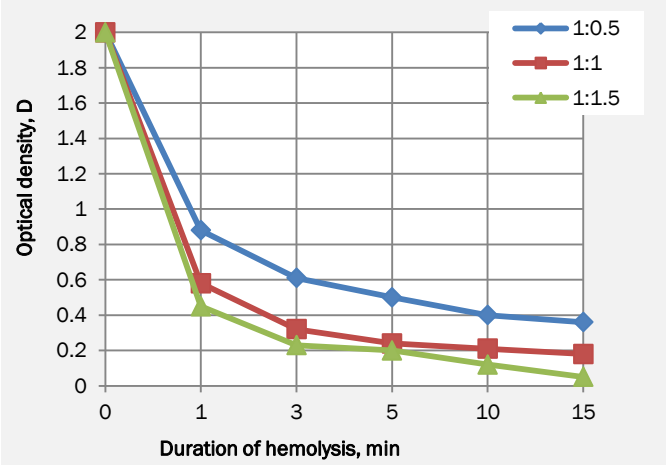

Figure 3 – The hemolysis effectiveness by ascorbic acid (concentration 0.75 mol/dm^3)

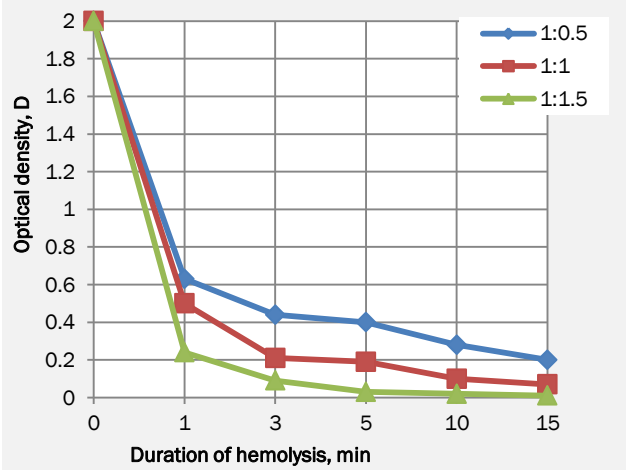

Figure 4 – The hemolysis effectiveness by ascorbic acid (concentration 1.5 mol/dm^3)

An assessment of the significance of the ratio of formed elements and the hemolyzing agent showed that, with an increase in the proportion of acid solution, the completeness of hemolysis increases. However, taking into account the undesirability of excessive dilution of the system, the optimal ratio of components is 1:1. Another factor confirming the optimal ratio of formed elements and ascorbic acid with a concentration of 0.75 mol/dm^3 was the study of the hemoglobin oxidation degree when varying the ratios of components and concentrations. Under the influence of ascorbic acid, hemoglobin is oxidized to methemoglobin, which significantly changes the color of the product from red to brown. Therefore, it is important to control the concentration of methemoglobin, as this shapes the organoleptic characteristics of the hemolysate (Figure 5).

The optimal color for hemolysate, from the point of view of its use in the formulations of meat products, as well as confectionery products, is chocolate brown. This color is achieved when about 50% of hemoglobin is oxidized to methemoglobin. The results presented in Figure 5 showed that to achieve the desired color, it is formed at an acid concentration of 0.75 mol/dm^3 and its equal volumetric ratio with formed elements. An increase in the concentration of methemoglobin leads to the formation of an excessively dark color, and a decrease in the concentration give the hemolysate a red tint.

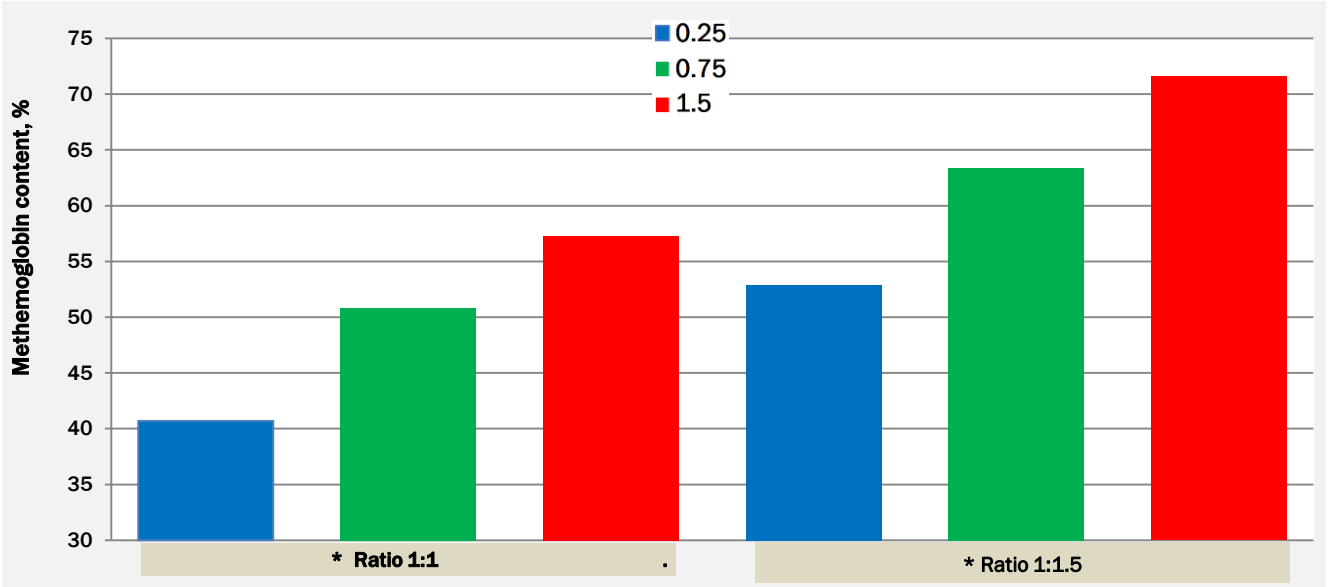

Figure 5 – Accumulation of methemoglobin in hemolysate when treated with ascorbic acid. * The ratio 1:0.5 had a very low effectiveness, so there are only 2 items for comparison in the graph.

Izgarishev et al. (2013) studied the use of acetic and citric acid for the hydrolysis of red blood cells. The authors suggest adding acids with a concentration of 5 to 10% in a ratio of an erythrocyte mass of 10:1. The process takes 12 hours at a temperature of 50 ℃, and the degree of erythrocyte hydrolysis does not exceed 32%. The authors did not study the oxidation degree of hemoglobin. A certain amount of blood plasma is always present in the separated mass of formed elements. The acid will affect globular proteins in the blood plasma, destroying their structure and causing irreversible transformation into the fibrillar type of proteins (Xing et al., 2022). The presence of modified blood plasma proteins in the hemolysate increases its structuring and binding properties, which are valuable in the development of polydisperse food systems.

Using the stronger citric and acetic acids will cause a decrease in acidity below 5 pH units, which will negatively affect the structuring properties of the hemolysate protein system. And the accumulation of many amines low molecular weight peptides will cause the formation of a bitter taste (Song et al., 2018; Bak et al., 2018). In addition to the destruction of red blood cells, hemolysis is accompanied by the destruction of hemoglobin molecules, which leads to the accumulation of free heme iron in the solution. This fact will help improve the bioavailability of iron and emphasize the antianemia nature of the resulting food component (Song et al., 2018). The resulting liquid hemolysate has a neutral odor and is brown (Powers and Buchanan, 2019; Evlash et al., 2022). However, in liquid form, it is a suitable nutrient medium for the development of microorganisms, which is due to the presence in the system of a significant amount of protein in hydrolyzed form. To solve this problem, it was decided to dry it in order to remove free moisture as much as possible. The hemolysate was dried at an air temperature of 120°C. The qualitative characteristics of the resulting dry product are presented in tables 1 and 2.

The dry semi-finished product has satisfactory organoleptic characteristics, as well as high levels of organic iron and animal protein. An assessment of the quality of the protein according to the profile of essential amino acids showed its

usefulness, and the limitation in threonine, methionine and lysine can be successfully leveled through the principles of food combinatorics, since the product is planned to be used mainly as an antianemia additive. It has also been established that dry hemolysate is well compatible with fats, forming with them fairly stable dispersion systems like emulsions. This makes its use promising in the formulations of emulsion meat products, as well as imitation confectionery products. Good solubility allows the hemolysate to be used in injection brines for injection of whole-piece meat products (Coker et al., 2002; Irinmwinuwa et al., 2023).

Table 1 - Qualitative indicators of dry ascorbic acid hemolysate (P <0.05)

Indicators	Value
Dry substances, %	80.3±1.2
Moisture, %	8.4±0.1
Protein, %	74.6±1.1
Fat, %	2.9±0.1
Iron, %	0.9±0.04
Ash,%	4.4±0.06
Active acidity, units	5.8±0.07
Emulsifying capacity, %	69.3%±0.9%
Emulsion stability, %	78.9%±0.85%
Solubility degree, %	95.8±1.2
Structure	Powdery, lumps break down easily
Color	Light brown, uniform throughout the product
Smell	Without smell

Table 2 - Results of studying the quality of the amino acid composition of dry hemolysate

Indicators / Amino acids	The content of amino acids in the protein of dry hemolysate, g/100 g of protein	Amino acid rate, %	Reference protein (indispensable amino acids), g/100 g of protein	SEM	P-value
Phenylalanine + Tyrosine	6.88	114.7	6.0	0.067	<0.01
Valyn	5.74	114.8	5.0	0.047	<0.01
Threonine	2.47	61.7	4.0	0.032	0.012
Leucine + Isoleucine	11.57	105.2	11.0	0.1157	0.01
Methionine + Cystine	1.58	45.1	3.5	0.0177	0.011
Lysine	4.96	90.2	5.5	0.059	0.12

Some of studies about of preparation of red blood cells for food use (Rossi et al., 2019; Chiroque et al., 2023), proposed adding lactic acid bacteria Enterococcus faecalis and Lactobacillus salivarius. This method allows you to suppress the development of pathogenic microorganisms and stabilize the level of hemolysis for 48 hours. However, maintaining red blood cells in their native state impairs the digestibility of the product, since the cell stroma is resistant to the action of digestive enzymes. In addition, the hemoglobin oxidation degree will be minimal, which will limit the use of the product because of its specific color and odor.

Heme iron is one of the most easily absorbed forms of iron in organic form, without the undesirable effects that are inherent in metallic iron and inorganic forms of iron (poor tolerance, organoleptic defects). The bioavailability of heme iron is almost 4 times greater than that of ferrous sulfate. This can be explained by the presence of two absorption pathways - binding to two types of receptors. The first type of DMT1 receptor, located in the duodenum, is for iron salts. A second type of receptor, PEPT1, located throughout the small intestine, is designed to bind peptides, resulting in significantly increased absorption (Ofori and Hsieh, 2012). It is important to keep in mind that the absorption of iron from a fortified product is largely determined by the composition of the food. In particular, ascorbic acid enhances the absorption of iron. Based on the quantitative content of iron in the resulting dry hemolysate, the level of its inclusion in the product for the prevention of nutritional anemia should be 1.5-2%.

CONCLUSION

Based on the studies conducted, it was concluded that acid hemolysis of red blood cells is the most rational way of processing it in preparation for use for food purposes. The use of ascorbic acid with a molar concentration of 0.75 mol/dm^3 can effectively destroy the stroma of more up 90% of red blood cells within 15 minutes. In this case, hemolysis

under the influence of acetic and citric acids ensures the destruction of no more than 32% of red blood cells and takes significantly more time. The resulting additive has acceptable organoleptic characteristics, good functional and technological properties and high nutrient status due to the high content of protein and organic iron. The brown color of the additive comes from a transformation of 50% of hemoglobin into methemoglobin. That makes it useful for anti-anemic meat products and chocolate-like confectionery products. 100 g of dry hemolysate contains 0.9 g of iron; therefore, to achieve a preventive effect, the dosage added to the product should be 1.5-2%. The high content of iron in organic form allows us to recommend blood products in the diets of children, pregnant women, and people with various types of anemia.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' Contribution

Both authors designed the study and manuscript writing. S. Shlykov collected samples and data. R. Omarov analyzed data and wrote manuscript. All authors drafted and revised the manuscript and approved the final manuscript.

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Competing interests

The authors have not declared any competing interests.

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EFFICIENCY OF VACUUM DRIED METHOD ON PHYSICAL, ORGANOLEPTIC AND VIABILITY PROPERTIES OF LACTIC ACID BACTERIA SYNBIOTICS

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ABSTRACT: Vacuum drying storage is a more efficient storage method for synbiotic feeds, compared to fresh storage. The current study aimed to examine the effect of vacuum drying on the physical, organoleptic, and microbiological qualities of synbiotics made from cabbage and Chinese cabbage greens. The study was conducted using a completely randomized design with a 5x3 factorial pattern with two replications consisting of two factors, namely five levels of drying time (24, 48, 72, 96, and 120 hours) and three levels of storage time (4, 8 and 16 weeks). The variables observed were physical-organoleptic quality in water content, color, odor, and texture, and microbiological quality in the form of total lactic acid bacteria. The results showed no interaction between the two treatments in terms of vacuum drying method, drying time, and storage time. The recommended treatment is drying for 48 hours, as evidenced by the moisture content factor supporting the viability of the lactic acid bacteria and maintaining the sensory properties. This study suggests a more efficient storage method of synbiotics for food applications.

Keywords: Lactic Acid Bacteria, Orgoleptic, Storage, Synbiotic, Vacuum Drying.

INTRODUCTION

The use of antibiotic growth promoters (AGP) can enhance livestock growth by suppressing bacterial catabolism and improving intestinal flora. However, the consequential issues of resistance and water pollution are potential threats to food security and public health (Corpet, 2000). The use of AGP has been banned since 2005 in the European Union (Wu et al., 2020). In Indonesia, the ban on the use of AGP officially began in January 2018 (Prasetyo et al., 2020). One of the alternative products to replace AGP is the provision of feed additives with synbiotics, which are mixed products of probiotics and prebiotics (Hartono et al., 2016). Fermented vegetable extracts in cabbage and Chinese cabbage meet the standards of probiotics since they contain *Lactobacillus Brevis, Lactobacillus Plantarum, Saccharomyces cerevisiae,* and *Rhizopus Oryza* (Utama et al., 2018). The lactic acid bacteria (LAB) content in the fermented vegetable extract is 2.1 x 10^{10} CFU/ml (Utama et al., 2013).

In order to enhance the function of Lactic acid bacteria in animals, it is necessary to supplement the diet with prebiotics. The prebiotics provide essential nutrients to the bacteria, allowing them to function more effectively as probiotics (Andriani et al., 2017). Among the prebiotics suitable for livestock are fructooligosaccharide (FOS), galactooligosaccharide (GOS), mannan oligosaccharides (MOS), and inulin (Allahdo et al., 2018). Cassava waste and soybean meal can serve as prebiotics due to their distinct properties. Cassava waste contains high carbohydrates in the form of polysaccharides, acting as a substrate for LAB. Soybean meal contains feed fiber Jenin soybean oligosaccharides (SOS) which is used as nutrition for LAB (Renschler et al., 2020). Moreover, amino acids and high protein in soybean meal make it a valuable nutritional provider for LAB (Wu et al., 2020).

Synbiotics made from cabbage and fermented Chinese cabbage greens are prone to quality deterioration during prolonged storage due to their high moisture content. Therefore, implementing a method to extend the shelf life while maintaining the quality, is of utmost importance (Solihin et al., 2015). Common techniques for the long-term preservation of bacterial cultures involve various drying applications, such as ordinary ovens, vacuum ovens, spray drying, drying with fluid flows, freeze-drying, and sublimating liquids from frozen products known as lyophilization (Soro-Yao et al., 2014). Regular drying ovens are frequently used; however, spray drying, which typically involves high temperatures ranging from 110 to 180°C at the inlet and 85 to 105°C at the outlet during culture preservation, may potentially lead to damage of synbiotic products.

While the use of vacuum drying in synbiotic feeds has been studied by Yang et al. (2020) and Goh et al. (2022), it has not been widely applied in Indonesia despite its ability to operate at lower temperatures and pressures. The

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fundamental principle of vacuum drying involves optimizing the vacuum pressure in the drying room to facilitate drying at low temperatures. This drying is used to obtain products with high quality, minimize the waste of odor and active and volatile substances (volatile), and minimize nutrient damage to ingredients, such as protein denaturation, browning (browning of ingredients), and enzymatic reactions (Widyanti et al., 2019).

Synbiotics made from fermented cabbage and mustard greens can maintain their properties when stored fresh with a temperature range of 5-10°C. However, this method is considered less efficient since it requires a large container, susceptibility to damage, and substantial energy demands to maintain ideal environmental conditions. The dry storage method is an effective choice as it can overcome these problems and ensure that the synbiotic remains in optimal condition. Drying can extend shelf life, decrease the costs of logistics due to the decrease in water activity, and provide unique physical properties as a dried product (Omolola et al., 2017). The drying process will reduce the water content, resulting in extended preservation and shelf life. However, this process also causes various changes in the physical, chemical, sensory and nutritional properties of the products. Therefore, it is important to consider the potential impact of the drying process on the overall quality and characteristics of the product (Khan et al., 2016). The quality of the dried products is affected by the drying method and physicochemical changes (Ramírez, et al., 2011). Sensory evaluation, an integral part of the quality evaluation (Noorbakhsh et al., 2013), is initiated after the samples are stored (Zhu et al., 2022). Preserving the characteristics of probiotics during the drying process is of utmost importance, considering the numerous benefits they provide. The viability and activity of LAB must be carefully considered as they should be abundant and viable at the time of consumption. Moreover, the survival of these microorganisms is critical to maintaining the product functionality (Sfakianakis and Tzia, 2004).

Given the above studies, it is evident that vacuum drying does not cause significant damage to the active compound of the dried material. Therefore, observations conducted on the odor, color, and texture of the synbiotic in the current study aimed to demonstrate that the chemical reactions leading to changes in color and texture during the drying process do not significantly affect the active compound. In addition, the study also confirmed that the LAB of the synbiotics dried using the vacuum drying method could remain viable even after storage.

MATERIALS AND METHODS

Materials

The cabbage, Chinese cabbage, glutinous rice tuning, chili, garlic, molasses, cassava waste, soybean meal, NaCl, and MRS were purchased from the local market, city of Semarang-Indonesia. The tools used were blenders (Miyako®, Indonesia), basins, jars, sieves, spoons, plastic sealers, spatulas, label paper, trays, digital scales (SF-400, Indonesia), beaker glass (Boro3.3, Germany), sealers (Arashi AIS-300, Indonesia), autoclave (Autoclave All American 75X, U.S.A), oven binder (ED 53 UL, U.S.A), petri dish (Pyrex 3160-120, U.S.A), analytical scales (balance kern ABJ-220, Germany), silica gel (Merck, Germany), desiccator (Normax, Portugal), vacuum machine (Araki, Indonesia), Thermohygrometer (HTC-2®, Switzerland), litmus paper (Merck®, Germany), the vacuum tube (GP, China), and petri dish (Iwaki®, Indonesia).

Method

The prerequisite to performing a completely randomized design (CRD) is that the materials and components used in the experiment are relatively homogeneous, except for the effect of the treatment given to the object (Steel and Torrie, 1980). The materials used in the current study had relatively consistent LAB content and organoleptic physical characteristics. This homogeneity facilitated the evaluation of the effect of drying and storage treatments on the object under study. Therefore, the experimental design used in this study was a CRD with a factorial pattern of 5 x 3 with two factors. The first factor (F1) was drying time at five levels, of 24, 48, 72, 96, and 120 hours and the second factor was storage time using three levels, namely 4, 8, and 16 weeks. The treatment combinations were as follows:

CA1.0 = Fermented vegetable liquid extract + carrier 100%
CA2.0 = Fermented vegetable liquid extract + chilli + carrier 100%
PA1.0 = Fermented vegetable solid extract + carrier 50%
PA2.0 = Fermented vegetable liquid extract + chilli + carrier 50%
CA1.1 = Fermented vegetable liquid extract + carrier 100% + MRS 1 ml
CA2.1 = Fermented vegetable liquid extract + chilli + carrier 100% + MRS 1 ml
PA1.1 = Fermented vegetable solid extract + carrier 50% + MRS 1 ml
PA2.1 = Fermented vegetable liquid extract + chilli + carrier 50% + MRS 1 ml

Each sample was repeated according to the drying time and storage time level. In order to maintain the functional properties of synbiotics, it is necessary to consider the sensory properties that change during drying and the viability of LAB (Khan and Karim, 2016; Sfakianakis and Tzia, 2004). Therefore, the parameters observed to ensure product quality included color, odor, texture, physical form, and microbiological content in the form of total LAB. The determination of

total microbial LAB was conducted following the methodology outlined in a study by Sulistiyanto et al. (2019). The research method consisted of four stages of activity, including the fermentation, mixing fermented extracts with prebiotics, drying process, and storage.

Fermentation of vegetable

The fermentation process for the fermented vegetable mixture followed the procedure outlined by Sulistiyanto et al. (2019). To create a fermented mixture of cabbage, white mustard greens, garlic, and chilies, all ingredients were thoroughly washed, cut into small pieces, and finely ground. The finely ground mixture was weighed according to a specific formula and homogenized. Then, the homogeneous mixture was placed into fermenter containers and tightly sealed to create an anaerobic environment. A hole in the lid of the fermenter tube was connected to a hose and thermometer, which was covered with plasticine to maintain an aerobic atmosphere and monitor changes in temperature and pressure in the fermentors. The fermentation process lasted 5 days, with the mixture kept in an anaerobic environment at room temperature. After the fermentation process, the liquid and solid extracts were separated through mechanical extraction.

The soybean meal and cassava waste were sterilized first by autoclaving at 121°C for 15 minutes before being used as prebiotics. In the next step, the cassava waste and soybean meal were homogenized with 60% of soybean meal and 40% of cassava waste. The extracted liquid and solids were supplemented with prebiotics according to the treatment and then placed in a petri dish to be dried.

The petri dish was filled and the sample was then dried in the drying stage using vacuum drying. The vacuum engine was turned on one time per 24 hours, with the duration of the engine running for 15 minutes. In the storage stage, the dried samples were put into a vacuum tube to be stored according to the treatment for 4, 8, and 16 weeks.

Testing parameters

During the analysis stage, comprehensive testing was conducted, including organoleptic quality evaluation in terms of color, odor, and texture, as well as microbiological assessment considering LAB and water content analysis. Physical-organoleptic testing was conducted by distributing questionnaires among 15 semi-trained panelists, who were selected from adept student who worked in the Feed Technology Laboratory but were not members of the Team. These judges were chosen based on their excellent health, normal sense of smell and taste, and absence of color blindness, ensuring they possessed the necessary skills for accurate assessments. To guarantee unbiased and precise evaluations, the judges underwent thorough training, including in-depth instruction on the assessment of the subtle nuances of odor, color, and texture, which was conducted three times to ensure that the judges were fully prepared for the task. The normal values, according to Sulistiyanto et al. (2019), Kurniawan (2020), and Anggraeni et al. (2021), are as follows:

- Color index on a scale ranging from 1 to 3 as 1 = dark brown, 2 = light brown, 3 = cream
- Texture index on a scale ranging from 1 to 3 as 1 = lumpy and smooth, 2 = slightly lumpy and smooth, 3 = no lumps and smooth
- Odor index on a scale ranging from 1 to 3 as 1 = rotten, 2 = odorless, 3 = acid

Data processing was performed using descriptive methods. The research data were arranged in tabular form, facilitating the arrangement of the data and then interpreted according to the existing observations. For the analysis of moisture content, a drying method was employed using an oven set at 105°C for 4 hours. The total LAB count was carried out using the total plate count (TPC) method (Nadliroh et al., 2019).

The total plate count can be calculated by = colonies × 1/dilution factor × $1/\sum$inoculum.

Data analysis

The obtained data were analyzed using analysis of variance (ANOVA) through SPSS Statistic 26 (IBM, 2018). In cases where the analysis indicated a significant effect, further analysis was carried out through Duncan's difference test at the 5% significance level. For the graphical presentation of the data, Figure Pad Prims 9.4.1 was used.

RESULTS AND DISCUSSIONS

Synbiotic physical-organileptic test

The organoleptic values of synbiotics dried by the vacuum drying method are presented in Table 1. Physical-organoleptic testing is one way to test synbiotic quality using the five senses and can be measured quantitatively. The observed physical-organoleptic features include color, texture, and odor. The quantitative data was carried out by a scoring method involving 15 semi-trained panelists. As can be seen in Table 1, extended drying resulted in a lighter color, smoother texture with fewer lumps, and a drier, sour odor in the synbiotic. Organoleptic test before and after storage shown there was not change the synbiotics' color, texture, and odor.

Color

The results of the ANOVA test showed that the interaction of drying time had a significant effect on synbiotic color ($P<0.05$). The analysis of data in Table 1 revealed that synbiotics dried by the vacuum drying method affected synbiotic color. Synbiotics dried for 24 hours were dark brown, while synbiotics dried for 120 hours were cream, changing color from dark to light as the length of synbiotic drying increased. The change of color in synbiotics was due to the water content, meaning that a decrease in the water content correlated with a brighter synbiotic color. According to Bora et al. (2018), water content significantly affects a product's color. Horváth (2016) stated that adding color to a product is influenced by several factors, including its particle size, oil content, and water content, as well as the content of color agents. The light color represents the high quality of synbiotics, while the brownish color indicates the lower quality synbiotics (Utama et al., 2020). Changes in color may result from the presence of mold and causing the color to shift from green to dark brown. Recognizing color as an important quality attribute, Rather and Rajain (2019) emphasize that it plays a crucial role in the product's overall attractiveness.

Texture

The results of the ANOVA test showed that the interaction of drying time had a significant effect on the synbiotic texture ($P<0.05$). The scores ranged from the lowest at 1.00 to the highest at 3.00 (Table 1). There was a noticeable change in texture from clumping to non-clumping, corresponding to the increased duration of synbiotic drying. The texture in the sample dried for 24 and 48 hours had a lumpy and smooth texture due to the high moisture content in synbiotics, making them prone to clumping. The moisture content can play a significant role in synbiotic texture. According to Zainuddin et al. (2014), moisture content can affect the physical properties and texture of the feed. The higher moisture content tends to promote quicker coagulation of powder products, while lower moisture content enhances smoothness and reduces clumping. The clumping observed in synbiotics due to high water content suggests a potential limitation in shelf life. An increase in the moisture content of a powder product leads to clumping (caking), which is a sign of poor quality and safety (Kurniawan, 2020).

Table 1 - Organoleptic score of dry synbiotics at different drying time

Parameter	Sample	Drying Time				
		24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
Colour	CA 1.0	1±0	1.67±0.49	2.67±0.49	3±0	3±0
	CA 1.1	1±0	1±0	2±0	3±0	3±0
	CA 2.0	1±0	1.67±0.49	2±0	3±0	3±0
	CA 2.1	1±0	1±0	2±0	2.67±0.49	3±0
	PA 1.0	1±0	1±0	2±0	2.67±0.49	3±0
	PA 1.1	1±0	1±0	2±0	2.33±0.49	3±0
	PA 2.0	1±0	1±0	2±0	2.33±0.49	3±0
	PA 2.1	1±0	1±0	2±0	3±0.00	3±0
	Average	1±0	1.17±0.31	2.08±0.24	2.75±0.30	3±0
Texture	CA 1.0	1±0	1.60±0.50	2.60±0.50	3 ±0	3±0
	CA 1.1	1±0	1.00±0	2±0	3±0	3±0
	CA 2.0	1±0	1.73±0.46	2±0	3±0	3±0
	CA 2.1	1±0	1±0	2±0	2.60±0.51	3±0
	PA 1.0	1±0	1±0	2±0	2.60±0.51	3±0
	PA 1.1	1±0	1±0	2±0	2.70±0.46	3±0
	PA 2.0	1±0	1±0	2±0	2.47±0.51	3±0
	PA 2.1	1±0	1±0	2±0	3±0.00	3±0
	Average	1±0	1.17±0.31	2.08±0.21	2.78±0.26	3±0
Odour	CA 1.0	3±0	3±0	3±0	3±0	3±0
	CA 1.1	3±0	3±0	3±0	3±0	3±0
	CA 2.0	3±0	3±0	3±0	3±0	3±0
	CA 2.1	3±0	3±0	3±0	3±0	3±0
	PA 1.0	3±0	3±0	3±0	3±0	3±0
	PA 1.1	3±0	3±0	3±0	3±0	3±0
	PA 2.0	3±0	3±0	3±0	3±0	3±0
	PA 2.1	3±0	3±0	3±0	3±0	3±0
	Average	3±0	3±0	3±0	3±0	3±0

CA 1.0= Fermented vegetable liquid extract+carrier 100%; CA 2.0= Fermented vegetable liquid extract +chilli+ carrier 100%; PA 1.0= Fermented vegetable solid extract+carrier 50%; PA 2.0= Fermented vegetable liquid extract + chilli+carrier 50%; CA1.1= Fermented vegetable liquid extract+carrier100%+MRS 1 ml; CA2.1= Fermented vegetable liquid extract+chilli+carrier 100%+MRS 1 ml; PA1.1= Fermented vegetable solid extract+carrier 50%+MRS 1 ml; PA2.1= Fermented vegetable liquid extract+chilli+carrier 50%+MRS 1 ml

Odor

The results of the ANOVA test showed that there was no significant interaction between drying time and synbiotic odors ($P<0.05$). The odor values obtained were all 3.00, indicating a sour odor. The process of vacuum drying has been proven to be effective in maintaining the favor and odor of synbiotics. This method ensures that the aroma of synbiotics remains consistent throughout all stages of treatment. Hence, vacuum drying is considered a reliable and efficient for preserving the distinctive odor of synbiotics. According to Zhang et al. (2019), vacuum drying produces products with an odor similar to fresh products. Odor is often used to determine the quality of the products produced as good or bad. The resulting odor indicates the level of microorganisms contained in synbiotics, with a sour odor suggesting a lower level of microorganisms (Utama et al., 2020). According to Kurniawan et al. (2020), changes in odor are also caused by bacteria that change complex compounds to be more straightforward.

The sour odor in synbiotics comes from the fermentation process that produces lactic acid bacteria. Gonzalez et al. (2011) stated that the acid odor is caused by the presence of lactic acid, acetaldehyde, propionic acid, butyric acid, and other volatile compounds produced by starter cultures due to fermentation. Setiarto et al. (2017) added that the odor of acid comes from the activity and growth of LAB, which decomposes lactose into lactic acid, resulting in a decrease in the pH value. A good synbiotic odor is an odor that resembles the raw materials used in its production, presenting a fresh and non-rancid aroma (Utama et al., 2020).

Synbiotic moisture test

The ANOVA test showed an interaction between drying time and synbiotic moisture content ($P<0.005$). The average values ranged from 48.4 to 8.2 (Table 1). The findings indicated that the decrease in water content was accompanied by an increase in the drying time of the synbiotic (Figure 1). The moisture content was tested using proximate analysis, revealing optimal moisture content of 12% for well-dried synbiotics. The high level of water content (above 15%) led to a decrease in synbiotic quality by rendering it susceptible to bacterial and fungal contamination, while also facilitating coagulation of synbiotic texture. As indicated in Figure 1, the duration of drying was inversely correlated with synbiotic moisture content. The synbiotic moisture content after storage remained stable.

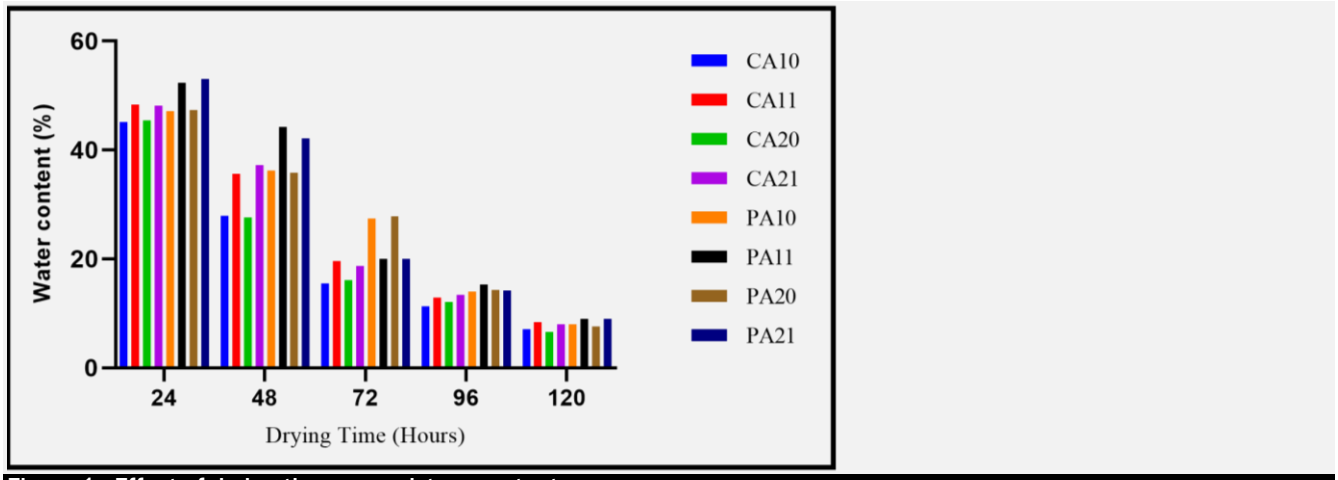

Figure 1 - Effect of drying time on moisture content

Figure 1 shows an overall decline in moisture content levels throughout the drying process. Longer drying time resulted in lower moisture content, with a notable reduction to 7.2% in CA10 and CA20 samples during 120-hour drying. According to Balzarini et al. (2018), the drying time is the time used in reducing the water content of the material; the longer the drying time, the more water evaporates from the dried material, so the water content obtained is lower. The decrease in water content for each synbiotic can be seen in Figure 1, and this occurs depending on the level of the water content of each synbiotic. According to Wirawan et al. (2020), the composition of water in feed ingredients, such as free water and bound water, can affect the material's drying rate or duration.

The grinding process before 24 hours was a constant rate phase, where in this phase, the drying process of the moisture content rate in the material moved to the surface of the material. Between 24 and 96 hours, there was a sharp decrease as substantial water evaporation occurred on the surface, resulting in dryness. After 96 hours, the moisture content decreased slowly indicating that the moisture content in the material was already low. Xu et al. (2022) stated that the vacuum drying process is divided into three different phases, the first is the initial transient period and the continued constant rate period, where the water extract evaporates and exits the material to form a layer of water on the surface of the porous medium, in the period of the rate of decline where the loss of moisture and water extracts (turbid particles and macromolecules) gradually becomes a porous medium. The remaining bound water diffuses as a layer on the material's surface. The grinding process before 48 hours was at a constant rate phase, where in this phase, the drying process of the

moisture content rate in the material moved to the surface of the material. The vacuum drying process was carried out by applying air pressure to the synbiotics, facilitating the diffusible removal of water towards the material's surface and subsequent conversion from the synbiotic surface to free air. According to Balzarini et al. (2018), vacuum drying is an alternative method for processing heat-sensitive products by maintaining air pressure so that the material releases moisture in the drying chamber. The longer the drying time and the pressure applied to the synbiotics result in greater moisture release, leading to lower water content. Xu et al. (2022) emphasize that an increase in drying time and temperature contributes to increased evaporation of water molecules from the dried material and lower water content in the final product.

Microbiological (*Lactic acid* bacteria validity) test

The validity test was conducted to determine the LAB's viability during drying and shelf-life. The results of the analysis indicated that the interaction of drying time with storage time did not yield a significant difference in LAB viability ($P<0.05$). Since the study aimed to find out which group had the highest LAB, further analysis was carried out using Duncan's test. Based on the results of further tests using Duncan's test (Table 2), it is known that simultaneously drying time and storage time had no significant effect on LAB. However, it has been found that drying time and storage time individually have a significant effect on LAB. Based on the results of the analysis, the best sample was shown in CA1.1 with the highest LAB value. This can be related to the water content and growth phase of LAB. Lower water content leads to increased water evaporation, resulting in a lower water activity value and suppressing the growth rate of microorganisms. Rolfe et al. (2012) proposed that the LAB growth phase comprises four phases. The first phase is the lag phase, during which bacterial growth is slow as they activate to the environmental conditions. The second phase is the exponential phase, where bacterial growth occurs at a rapid rate. The third phase is the stationary phase, where bacterial growth ceases due to the depletion of nutrients in the media and energy reserves. Finally, the fourth phase is the death phase, where the bacteria population decreases over time.

Table 2 - Results of dry synbiotic lactic acid bacteria at different drying and storage times

Storage time	Sample	Drying time (Hours) —— × 10^6 CFU/ml ——					Average
		24	48	72	96	120	
4 weeks	CA 1.0	0	6.25±7.42	1.0±7.64	5.00±3.27	0.26±0.35	10.39±25.31[a]
	CA 1.1	44.85±62.44	113.50±77.07	10.0±1.41	18.50±2.97	92.15±35.14	
	CA 2.0	0.50±0.71	2.00±1.41	0	0	0	
	CA 2.1	5.10±7.21	0.50±0.71	2.50±5.94	0	0.25±0.35	
	PA 1.0	0.53±0.74	0.29±0.29	0.50±0.16	1.50±1.17	1.75±0.35	
	PA 1.1	62.04±77.73	4.75±5.30	1.00±0	0	1.00±0	
	PA 2.0	0	0	0	0	0	
	PA 2.1	0.05±0.07	38.75±54.80	1.00±0.70	0	0.25±0.35	
8 weeks	CA 1.0	2.45±3.46	1.77±2.45	0.50±0.28	0.50±0.35	5.40±0.57	6.26±14.25[ab]
	CA 1.1	43.15±6.58	8.50±2.83	0	0	12.50±17.68	
	CA 2.0	0.25±0.35	5.00±7.07	0	0	0.01±0.01	
	CA 2.1	32.20±41.44	42.70±59.82	0	0	0.06±0.08	
	PA 1.0	1.00±1.41	1.20±1.13	1.00±0.70	0	0	
	PA 1.1	32.85±14.35	58.20±61.94	0	0	0.25±0.35	
	PA 2.0	0	0.75±1.06	0	0	0	
	PA 2.1	0	0.25±0.35	0	0	0	
16 weeks	CA 1.0	3.50±0	0	1.00±0	0	0.50±0	1.18±2.18[b]
	CA 1.1	4.00±0	0	0.50±0	0	0	
	CA 2.0	8.00±0	0.50±0	0	0	0	
	CA 2.1	5.00±0	0	0	0	0.50±0	
	PA 1.0	1.50±0	0	1.00±0	0	5.50±0	
	PA 1.1	0.50±0	0	0.50±0	0.50±0	0.00±0	
	PA 2.0	0.50±0	0	9.00±0	0	1.00±0	
	PA 2.1	1.00±0	0	2.00±0	0	0.50±0	
Average		10.37±17.95[a]	11.87±26.69[a]	1.31±2.61[b]	1.08±3.86[b]	5.08±18.76[ab]	

[a.b.] Means within a column with different superscripts differ significantly ($P<0.05$); CA 1.0= Fermented vegetable liquid extract+carrier 100%; CA 2.0= Fermented vegetable liquid extract+chilli+carrier 100%; PA 1.0= Fermented vegetable solid extract+carrier 50%; PA 2.0= Fermented vegetable liquid extract+chilli+carrier 50%; CA1.1= Fermented vegetable liquid extract+carrier100%+MRS 1 ml; CA2.1= Fermented vegetable liquid extract+chilli+carrier 100%+MRS 1 ml; PA1.1= Fermented vegetable solid extract+carrier 50%+MRS 1 ml; PA2.1= Fermented vegetable liquid extract+chilli+carrier 50%+MRS 1 ml

The results of the current study indicated that vacuum drying was less effective for synbiotic drying since the longer the drying process, the lower the water content and activity of water, leading to a lower LAB growth rate. The LAB value significantly decreased during the drying period from 48 hours to 96 hours due to the pressure exerted by the vacuum machine, resulting in a reduced water activity value. Consequently, bacterial cells were damaged and there was a decrease in LAB in synbiotics. As mentioned by Juniawati et al. (2019), when the capsule wall is not strong enough to withstand the pressure of the microcapsule particles, the wall breaks, and the particles collapse so that the cell is damaged. Damage to the bacterial membrane in the vacuum process is due to dehydration, thereby reducing the production of lactic acid bacteria (Romano et al., 2021).

The most optimal storage of synbiotics is four weeks of storage as the water content of the dried synbiotic for 48 hours is an average of 35.9%. Therefore, higher water content results in the shorter shelf life of the synbiotic. Utama et al. (2020) reported that the storage of a product can be affected by the water content of the product. Nurhidajah et al. (2020) added that high and low water content affect the shelf life meaning that lower water content results in longer shelf life.

CONCLUSION

Based on the experiment results, it can be concluded that using the 48-hour vacuum drying method is effective in preserving synbiotics in dry form without affecting their organoleptic and physical properties or the viability of lactic acid bacteria. Further research is required to investigate the impact of using synbiotic products dried through the vacuum method on the growth and health of animals.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' Contribution

B. Sulistiyanto is responsible for coordinating research activities, data processing, and finalization of scientific articles; C.S. UTAMA provide suggestions and finalization of scientific articles; K.U. Albab is responsible for research, preparation of tools and materials, research data processing.

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Competing interests

The authors declare that they have no competing interests.

Animal Ethical regulation

The treatment of experimental animals in accordance with the "Guidelines for the Care and Use of Animal Laboratory" from the Diponegoro University. All procedures carried out in this study involving animals have been in accordance with ethical standards and approved by the Feed Technology Laboratory, Faculty of Animal and Agriculture Sciences, Diponegoro University.

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EFFECTS OF FERMENTED PALM KERNEL CAKE IN HUMIC ACID AND LIMESTONE SOLUTIONS ON THE PERFORMANCE OF BROILER CHICKENS

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RESEARCH ARTICLE

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ABSTRACT: Palm kernel meal is a by-product used very sparingly in poultry feed, due to its low nutritional value and gravelly appearance which could be improved by physical or chemical treatments. The aim of this study was to assess the effect of palm-kernel meal fermentation period on its nutritional value and growth performances of broiler chickens. The treatment consisted of fermenting palm kernel meal in a solution of humic acid (HA) or limestone, for 0, 2, 4 and 6 days. A control ration without palm kernel meal (R0) was compared to rations containing 15% unfermented palm kernel cake (R0+) and 15% fermented palm kernel cake in humic acid and limestone solutions. Each experimental ration was randomly assigned to 8 chicks in 4 experimental units of 02 chicks each, repeated 4 times per a 2×3 factorial design (2 fermentation modes and 3 fermentation period). The main results showed that fat content (13.04%) and metabolizable energy (5314 Kcal/kg DM) of palm kernel meal were higher when fermented in humic acid for 6 days. Fermentation in the basic solution for the same period (6 days) increased protein (13.52%) and cellulose (24.21%) contents. Whatever the fermentation mode, the digestive utilization coefficient of dry matter, organic matter, crude protein and crude cellulose increased with the fermentation period. Fermentation mode and period had no significant effect on growth performance. However, growth characteristics tended to improve with fermentation period. In conclusion, fermentation of palm kernel in humic acid and limestone solutions improved significantly ($P<0.05$) the digestibility of all feed components, enabling chickens to take advantage of the nutrients for better growth performances.

Keywords: Chemical composition, Digestibility, Fermentation, Humic acid, Metabolizable energy, Palm kernel meal.

INTRODUCTION

The nutrition policy of most states in the world relies on eggs and poultry meat to cover the protein needs of populations due to their availability, affordable price and lack of taboo about poultry products. The focus on poultry requires the intensification of production systems, which in turn leads to a high demand for feed resources capable of covering the protein and energy requirements of broilers (Alshelmani et al., 2021). Poultry farming relies heavily on conventional feed resources such as corn and soybean meal, which respectively account for 60-70% and 15-30% of broilers rations (Kana et al., 2015). This dependence on conventional resources comes up against several crucial problems such as, fluctuating prices and above all competition between animals, agri-food industries and humans for corn and soybean in particular. Cameroon produces a wide range of agro-industrial by-products such as wheat bran, rice bran and palm kernel meal, which are used in very limited quantities in poultry feed. This limitation is due to the presence of a multitude of compounds such as non-starch polysaccharides (NSPs) like xylan and mannan found in palm kernel meal, which at a certain level depress bird growth (Aftab and Bedford, 2018). The non-starch polysaccharides increased viscosity of the contents of the birds' small intestine, which induced the excessive dehydration of the bird, materialized by the increased water content of the droppings (Aftab and Bedford, 2018; Ramiah et al., 2019; Alshelmani et al., 2021).

Palm kernel meal is a by-product derived from the extraction of oil from palm kernels. This by-product (Aderolu et al., 2006; Faridah et al., 2020; Prasetya et al., 2021), widely available in Cameroon and inexpensive, is used in very large quantities in pig feed, and rather sparingly in poultry feed due to its high fiber content (24.25%) and low protein content (17-22%) and very low biological value of its protein (Yemdjie et al., 2020). This meal is an important source of crude energy (4939 kcal/kg DM) and metabolizable energy (2570 Kcal/Kg DM; Meffeja et al., 2003). It has a gravelly appearance and coarse texture (Alshelmani et al., 2016; 2017; Zamani et al., 2017; Mirnawati et al., 2018; 2019). These characteristics limit its use in poultry feed in general and broiler feed in particular.

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The addition of amino acids (Yemdjie et al., 2020), exogenous enzymes (Sharmila et al., 2014) and solid-state fermentation by cellulolytic microorganisms (Alshelmani et al., 2017) or organic compounds such as humic acid (Mirnawati et al., 2019) could improve the nutritional value and digestibility of palm kernel meal. Mirnawati et al. (2018) reported that mushroom-acid combination could enable this by-product to be better valorized in poultry feed. Humic acid stimulates the growth of microbes that facilitate the conversion of fiber into glucose accessible to enzymes in the digestive tract of birds (Sukaryana et al., 2010). The present study was designed to test the hypothesis that certain organic compounds improve the nutritional value of poor feed resources. The aim was to assess the effect of humic acid (HA) and limestone fermentation period on palm kernel meal nutritional value and growth performances of broiler chickens.

MATERIALS AND METHODS

Study area

The study was done at the Teaching and Research Farm (TRF) of the University of Dschang. The TRF is located at 05°26' North latitude, 10°26' East longitude and at an altitude of 1420 m. The prevailing climate is equatorial characterized by two seasons; a rainy season that goes from mid-March to mid-November and a dry season that covers the rest of the year. Precipitation varies between 1500 and 2000 mm per year. The average temperature is around 21°C, and the average relative humidity is 76.8%.

Ethical considerations

The present study has been performed in agreement with the guidelines of ethical standards from the Department of Animal Science of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, Cameroon.

Processing of palm kernel meal

The palm kernel cake was soaked in an acidic and basic solution in the proportions 1/2 (1 kg of meal for 2 liters of solution) then fermented for 2, 4 and 6 days. The acidic solution consisted of dissolving 2 g of humic acid (HA) in one liter of water, and the basic solution consisted of dissolving 30 g of limestone in one liter of water. The fermented meal from these solutions was sun-dried for 5 days, and samples were taken for the analysis organic matter, crude cellulose and crude protein content.

Birds and experimental rations

A total of 64 chicks (32 males and 32 females), 21 days old, with an average weight of 758.31 ± 40.6g were housed in 32 wire cages, with 8 chicks per cage for 4 weeks trial. Eight experimental rations were formulated and each was randomly assigned to 4 experimental units consisting of 08 birds (4 males and 4 females) each in a 2×3 factorial design (2 fermentation modes and 3 fermentation period). An antistress (Tetracoli®) was administered to the chicks for three consecutive days through drinking water as soon as they arrived at the farm. Anticoccidial (Vetacox®) and vitamins (Amintotal®) were administered in drinking water for three consecutive days each week. Two nutrient-balanced control rations were formulated, one without palm kernel meal (R0) and the other contained 15% unfermented palm kernel meal (R0+). The other 06 rations contained 15% palm kernel meal fermented in acidic and basic solution for 2, 4 and 6 days respectively (Table 1).

Feed component digestibility

Feed was weighed and served each morning to the birds, the refusals and faeces from each experimental unit were collected and weighed the next day at the same time during 4 days. The faeces samples were dried in an oven at 50°C until constant weight for dry matter (DM), organic matter (OM), crude cellulose (CC), Neutral Detergent Fiber (NDF) and crude protein (CP) were analysed as described by AOAC (1990). The apparent digestive utilization coefficients (aDUC) of DM, OM, CC, NDF and CP of the experimental rations were calculated according to the following formula:

$$\mathbf{aDUC}\ (\%) = \frac{\text{Quantity ingested (g)} - \text{Quantity excreted (g)}}{\text{Quantity } ingested\ (g)} \times 100$$

Growth performances

Feed intake was determined by the difference between the amount of feed served and leftovers at the end of the week. Broilers were weighed at the beginning of the experiment and a weekly basis thereafter using an electric scale at 0.1 g sensitivity. Average weekly weight gain was obtained by doing the difference between two consecutive weekly weights. Feed conversion ratio was obtained by dividing the amount of feed intake by the weight gain of the same week.

Statistical analysis

Collected data were submitted to a 2-ways analysis of variance (ANOVA) (2 fermentation modes and 3 fermentation periods). Duncan's test was used to separate treatments means a 5% significance when there was a significant difference level using SPSS 22.0 (Statistical Package for Social Sciences). Results were expressed as mean ± standard deviation.

$x_{ijk} = \mu + a_i + \beta_j + e_{ijk}$

Which, x_{ij} = observation on animal j having received treatment I; μ = General average of the observation; a_i = Effect of fermentation mode; β_j = Effect of fermentation duration of palm kernel cake in the ration; e_{ij} = Residual error due to the animal having received treatment i.

Table 1 - Composition of experimental rations

Experimental rations / Ingredients	Control		Acid solution			Basic solution		
	R0	R0+	A2	A4	A6	B2	B4	B6
Maize	68	59	59	59	59	59	59	59
Soybean meal	14	6.8	6.8	6.8	6.8	6.8	6.8	6.8
Palm kernel meal	0	15	15	15	15	15	15	15
Fish meal	5	6	6	6	6	6	6	6
Blood meal	5	4	4	4	4	4	4	4
Palm oil	2	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Premix 5%	5	5	5	5	5	5	5	5
Lysine	0	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Methionine	0	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Oyster shell	1	1	1	1	1	1	1	1
Calculated chemical composition								
Metabolizable energy (Kcal/Kg)	3164.92	2949.23	3024.73	3021.01	3012.18	2937.44	3084.35	3083.89
Crude protein (%)	20.41	18.79	19.00	19.24	19.20	19.13	19.18	19.28
Crude cellulose (%)	2.16	6.77	6.15	6.00	6.4	6.46	5.39	5.35
Lipid (%)	4.93	6.68	7.00	6.8	7.19	6.45	7.09	7.02
Calcium (%)	1.63	1.24	1.24	1.24	1.24	1.24	1.24	1.24

*MNVC5%: Mineral, nitrogen and vitamin complex: crude protein =40%, Lysine=3.3%, Methionine=2.4%, Calcium=8%, Phosphorus=2.05%, Metabolisable Energy=2078 Kcal/Kg. ME =Metabolisable Energy; R0= Control ration without palm kernel meal; R0+ =Ration containing 15% untreated palm kernel meal; A2= Ration containing 15% palm kernel meal fermented in acidic medium for 2 days; A4= Ration containing 15% palm kernel meal fermented in acidic medium for 4 days; A6= Ration containing 15% palm kernel meal fermented in acidic medium for 6 days;B2= Ration containing 15% palm kernel meal fermented in basic medium for 2 days; B4= Ration containing 15% palm kernel meal fermented in basic medium for 4 days;B6= Ration containing 15% palm kernel meal fermented in limestone medium for 6 days.

RESULTS

Effects of fermentation mode and period on the chemical composition of palm kernel cake

Table 2 summarizes the chemical composition of palm kernel cake fermented in acidic and basic solutions for 2, 4 and 6 days. Concerning the fermentation period, the fermentation mode had no significant ($P>0.05$) effect on the proximate composition of palm kernel cake. Irrespective of fermentation mode, crude protein, ash, lipid, crude cellulose and metabolizable energy content of palm kernel meal increased significantly ($P<0.05$) with increasing fermentation period. Crude cellulose and NDF content of palm kernel meal decreases significantly ($P<0.05$) with increasing fermentation period irrespective of the fermentation solution.

Figure 1, showing the regression of crude protein, crude cellulose and NDF as a function of fermentation time, revealed that in humic acidic solution, the increase in crude protein content and the significant linear decrease ($p=0.0001$) in crude cellulose and NDF are essentially linked to fermentation period (96%; 99%; 97% respectively). Similarly, the same figure shows that under basic solution, the linear increase in crude protein content and the decrease in crude cellulose and NDF content are essentially linked (96%; 90%; 99%) to the increase in fermentation period.

Effects of the fermentation mode and period of palm kernel meal on the feed component digestibility

The effects of the mode and period of palm kernel meal fermentation on the apparent digestibility of feed components are summarized in Table 3. Fermentation had an improving effect on the digestibility of feed components, regardless of the solution or medium used. Although not significant ($P>0.05$), fermentation tended to increase the digestibility of crude protein and NDF with increasing fermentation period. Apparent digestibility of dry matter, organic matter and crude cellulose increased linearly and significantly ($P<0.05$) with the fermentation period. With respect to the fermentation period, there was no significant ($P>0.05$) difference between fermentation modes.

Figure 2 shows the regression of feed components digestibility on the fermentation period of palm kernel meal. It is appear that the improvement in digestibility of crude protein, crude cellulose and NDF depends at 97.91%, 95.07% and 99.12% respectively on the fermentation period in humic acid solution, and respectively by 95.35%, 78.17% and 95.04% on the duration in limestone solution.

Effects of palm kernel meal fermentation mode and period on broiler growth performances

Table 4 summarizes the effects of palm kernel meal fermentation mode and duration on broiler growth performances. The fermentation mode and period did not have a significant effect ($P>0.05$) on feed intake, weight gain and feed conversion ratio of broilers at the finisher phase. However, though not significant, with respect to the control

ration containing unfermented palm kernel, feed intake, weight gain and live weight increased linearly with the fermentation period, while feed conversion ratio moved in the opposite direction. Irrespective of fermentation mode, growth parameters recorded with palm kernel meal fermented for 6 days were almost similar to those recorded with the control ration without palm kernel (R0). For the same duration of fermentation, the fermentation mode had no significant (P>0.05) effect on broiler growth parameters.

Table 2 - Variation in the chemical composition of palm kernel meal according to the fermentation mode and period

Fermentation mode \ Fermentation period (days)		0	2	4	6	P-value
Dry matter (%)	Acid	89.34 ± 0.87^{bc}	88.59 ± 0.17^{c}	91.27 ± 0.02^{a}	90.16 ± 0.32^{b}	0.001**
	Basic	89.34 ± 0.87	89.88 ± 0.04	88.56 ± 0.31	88.80 ± 0.50	0.059 ^{NS}
	P-value	1.000	0.211	0.135	0.577	
Organic matter (%DM)	Acid	96.88 ± 0.13^{a}	96.38 ± 0.01^{b}	96.88 ± 0.09^{a}	97.1 ± 0.01^{a}	0.0001***
	Basic	96.88 ± 0.13^{a}	92.67 ± 0.01^{d}	94.63 ± 0.08^{c}	94.80 ± 0.06^{b}	0.0001***
	P-value	1	0.561	0.863	0.176	
Crude protein (%DM)	Acid	10.28 ± 0.10^{d}	11.63 ± 0.02^{c}	13.23 ± 0.00^{a}	13.01 ± 0.05^{b}	0.0001***
	Basic	10.28 ± 0.10^{d}	12.49 ±0.01^{c}	12.86 ± 0.05^{b}	13.52 ± 0.08^{a}	0.0001***
	P-value	1.000	0.259	0.089	0.510	
Crude cellulose (%DM)	Acid	33.68 ± 0.33^{a}	31.19 ± 0.11^{b}	29.53±0.06^{c}	28.49 ± 0.01^{d}	0.0001***
	Basic	33.68 ± 0.33^{a}	31.58 ±0.02^{b}	24.48 ± 0.09^{c}	24.21 ± 0.14^{c}	0.0001***
	P-value	1	0.167	0.627	0.130	
Neutral Detergent Fibre (%DM)	Acid	78.99 ± 0.04^{a}	72.03 ± 0.14^{b}	70.65 ± 0.02^{c}	69.70 ± 0.68^{d}	0.0001***
	Basic	78.99 ± 0.04^{a}	76.33 ±0.27^{b}	74.48 ± 0.49^{c}	71.1 ± 0.40^{d}	0.0001***
	P-value	1	0.425	0.123	0.528	
Ash (%DM)	Acid	3.12 ± 0.13	2.99 ± 0.01	3.63 ± 0.01	4.47 ± 2.31	0.457^{NS}
	Basic	3.12 ± 0.13	5.20 ± 0.06	5.38 ± 0.08	5.33 ± 1.99	0.066^{NS}
	P-value	1	0.176	0.123	0.606	
Fat (%DM)	Acid	9.64 ± 0.09^{d}	10.50 ± 0.01^{c}	11.68 ± 0.02^{b}	13.04 ± 0.05^{a}	0.0001***
	Basic	9.64 ± 0.09^{c}	8.16 ± 0.01^{d}	11.92 ± 0.07^{b}	12.42 ± 0.05^{a}	0.0001***
	P-value	1	0.259	0.129	0.620	
Gross energy (Kcal/Kg DM)	Acid	5090.60 ± 7.38^{c}	5090.98 ± 0.25^{c}	5224.67 ± 0.09^{b}	5314.42 ± 2.52^{a}	0.0001***
	Basic	5090.60 ± 7.38^{c}	4811.94 ± 0.39^{d}	5117.01 ± 0.73^{b}	5140.14 ± 1.89^{a}	0.0001***
	P-value	1	0.581	0.157	0.709	
Metabolizable energy (Kcal/kg DM)	Acid	1423 ± 34.18^{c}	1843 ± 6.29^{b}	1902 ± 0.22^{a}	1927 ± 4.45^{a}	0.0001***
	Basic	1423 ± 34.18^{b}	1344 ± 0.90^{c}	2321 ± 9.69^{a}	2324 ± 4.55^{a}	0.0001***
	P-value	1	0.165	0.122	0.976	

^{a,b,c,d}: the means with the different letters in the same row are significantly different (P<0.05). Day 0 = 0 day of fermentation of the palm kernel meal; Days 2; 4 and 6 = correspond to the different numbers of days of fermentation of the palm meal both in the humic acid and in the limestone solution. DM = Dry matter; P: Probability. NS= Non-significant (P>0.05); *=P<0.05; **= P<0.01; ***= P<0.001.

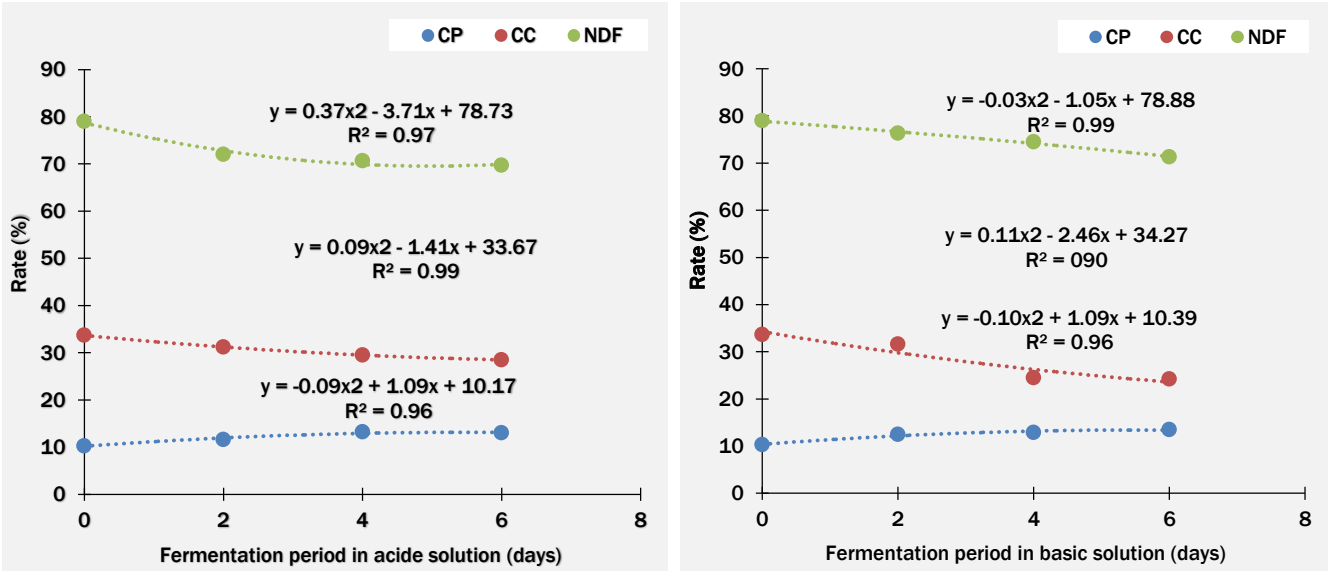

Figure 1 - Regression of crude protein (CP), crude cellulose (CC) and Neutral Detergent Fiber (NDF) with respect to the fermentation period of palm kernel cake.

Table 3 - Effects of palm kernel meal fermentation mode and period on feed digestibility of broiler chickens

Type of fermentation		Control		Fermentation period (days)			P-value
		R0	R0+	2	4	6	
aDUC - Dry matter (%)							
	Acid	63.79 ± 3.40^{a}	49.48 ± 3.36^{c}	54.60 ± 1.56^{bc}	54.45 ± 4.64^{bc}	56.93 ± 2.25^{b}	0.004**
	Basic	63.79 ± 3.40^{a}	49.48 ± 3.36^{b}	50.83 ± 3.65^{b}	52.53 ± 4.55^{b}	54.94 ± 2.58^{b}	0.004**
	P-value	1.000	1.000	0.131	0.153	0.248	
aDUC -Organic matter (%)							
	Acid	72.70 ± 3.04^{a}	62.23 ± 2.89^{c}	65.54 ± 1.15^{ab}	66.35 ± 0.20^{ab}	66.86 ± 2.25^{b}	0.002**
	Basic	72.70 ± 3.04^{a}	62.23 ± 2.89^{c}	65.49 ± 0.69^{bc}	65.18 ± 3.50^{bc}	67.93 ± 1.39^{b}	0.006**
	P-value	1.000	1.000	0.272	0.370	0.276	
aDUC - Protein (%)							
	Acid	47.73 ± 6.20	42.19 ± 4.34	47.69 ± 9.48	52.24 ± 3.57	53.12 ± 5.76	0.279^{NS}
	Basic	47.73 ± 6.20	42.19 ± 4.34	44.70 ± 7.10	48.49 ± 3.58	48.43 ± 5.04	0.556^{NS}
	P-value	1.000	1.000	0.230	0.922	0.558	
aDUC -NDF (%)							
	Acid	59.35 ± 9.47	40.491 ± 5.69	43.32 ± 17.05	50.69 ± 9.49	57.15 ± 1.65	0.242^{NS}
	Basic	59.35 ± 9.47	40.491 ± 5.69	43.44 ± 15.65	51.05 ± 7.58	53.00 ± 3.15	0.457^{NS}
	P-value	1.000	1.000	0.244	0.496	0.689	
aDUC -Cellulose (%)							
	Acid	15.94 ± 1.14^{c}	14.19 ± 0.91^{c}	23.74 ± 1.67^{b}	24.16 ± 2.36^{ab}	26.83 ± 3.22^{a}	0.007**
	Basic	15.94 ± 1.14^{c}	14.19 ± 0.1^{c}	21.34 ± 4.39^{b}	23.80 ± 2.31^{b}	28.75 ± 0.95^{a}	0.0001***
	P-value	1.000	1.000	0.690	0.196	0.482	

$^{a, b, c, d}$: the means with the different letters in the same row are significantly different (P<0.05). R0 = treatment without palm kernel meal; R0+ = treatment with unfermented palm kernel meal; 2 = Rations with meal fermented for two days with the humic acid and limestone solution; 4 = Rations with meal fermented for four days with the humic acid and limestone solution; 6 = Rations with meal fermented for six days with the acid and limestone solution; aDUC: apparent digestive utilization coefficients; NDF: Neutral detergent fiber. NS= non-significant (P>0.05); *=P<0.05; **= P<0.01; ***= P<0.001

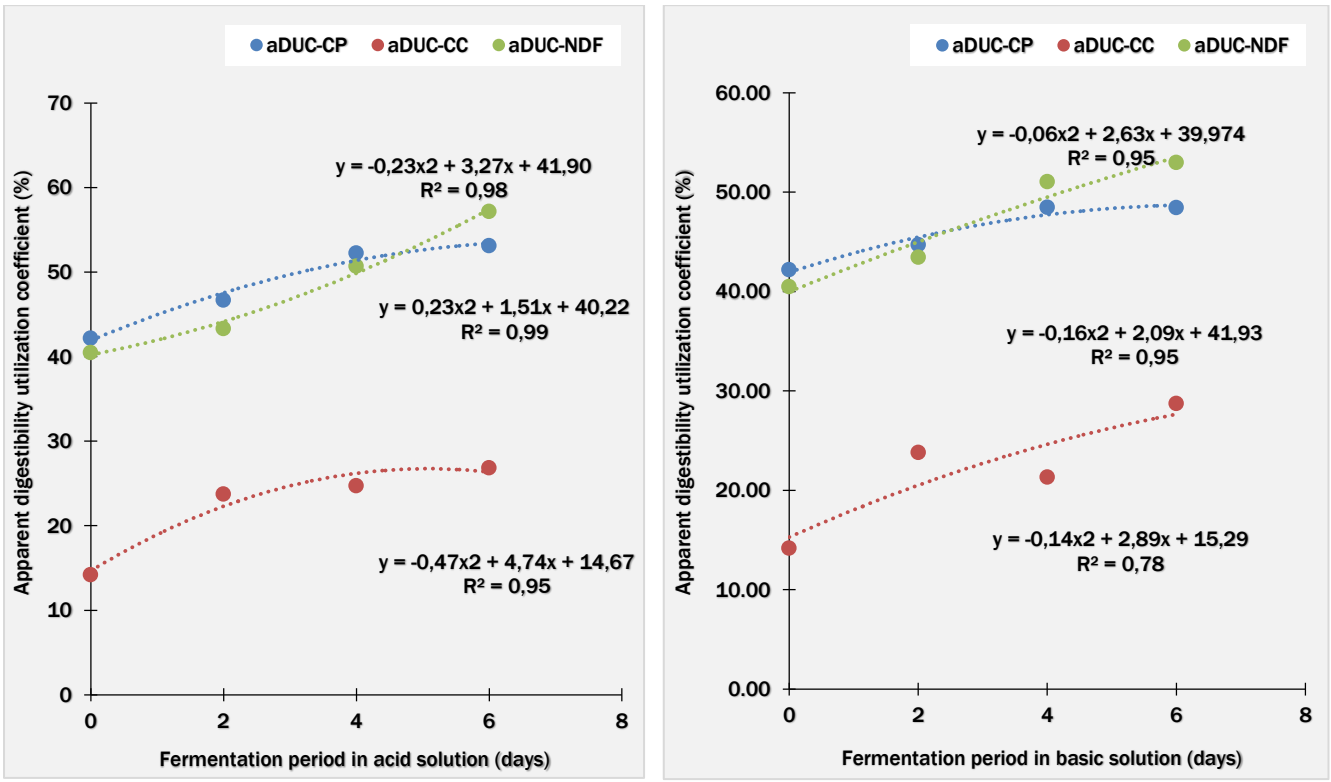

Figure 2 - Regressions curves of the apparent digestive coefficients of crude protein (aDUC –CP), crude cellulose (aDUC-CC) and Neutral Detergent Fiber (aDUC-NDF) with respect to the fermentation period of palm kernel meal.

Table 4 - Effects of palm kernel meal fermentation mode and duration on broiler growth performances

Fermentation mode		Control		Fermentation time (days)			P-value
		R0	R0+	2	4	6	
Feed intake (g)							
	Acid	4530.62 ± 237.41	4340.25 ± 316.91	4479.87 ± 240.59	4523.37 ± 151.47	4544.62 ± 413.74	0.374^{NS}
	Basic	4530.62 ± 237.41	4340.25 ± 316.91	4671.25 ± 129.96	4432.25 ± 264.38	4562.37 ± 377.26	0.327^{NS}
	P-value	1^{NS}	1^{NS}	0.901^{NS}	0.681^{NS}	0.417^{NS}	
Live weight (g)							
	Acid	2705.62 ± 275.07	2474.87 ± 125.73	2599.50 ± 208.04	2658.25 ± 228.88	2698.25 ± 45.76	0.413^{NS}
	Basic	2705.62 ± 275.07	2474.87 ± 125.73	2662.62 ± 55.26	2658.37 ± 171.12	2686.12 ± 176.83	0.487^{NS}
	P-value	1.000^{NS}	1.000^{NS}	0.807^{NS}	0.060^{NS}	0.133^{NS}	
Weight gain (g)							
	Acid	1947.31 ± 275.08	1716.56 ± 125.73	1841.19 ± 208.04	1899.94 ± 228.88	1939.94 ± 45,76	0.137^{NS}
	Basic	1947.31 ± 275.08	1716.56 ± 125.73	1904.31 ± 55.26	1900.06 ± 171.12	1927.81 ± 176,83	0.487^{NS}
	P-value	1.000^{NS}	1.000^{NS}	0.404^{NS}	0.060^{NS}	0.133^{NS}	
Feed conversion ratio							
	Acid	2.32 ± 1.967	2.52 ± 0.20	2.43 ± 0.06	2.38 ± 0.03	2.34 ± 0.03	0.254^{NS}
	Basic	2.32 ± 1.967	2.52 ± 0.20	2.45 ± 0.06	2.33 ± 0.09	2.37 ± 0.,24	0.269^{NS}
	P-value	1.000^{NS}	1.000^{NS}	0.745^{NS}	0.101^{NS}	0.978^{NS}	

R0: treatment without palm kernel meal; R0+ = treatment with unfermented palm kernel meal; 2 = Rations with meal fermented for two days with the humic acid solution and with the limestone solution; 4 = Rations with meal fermented for four days with the humic acid solution and with the limestone solution; 6 = Rations with meal fermented for six days with the acid solution and with the limestone solution. P: probability. NS= non-significant (P>0.05); NS= non significant.

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DISCUSSION

Regardless of the fermentation mode, the chemical composition of palm kernel meal improved with increasing fermentation period in humic acid and limestone solution. Thus, the lowest cellulose content and the highest lipid, organic matter, crude protein and energy contents were recorded after 6 days of fermentation. This result is in agreement with those of Mirnawati et al. (2017, 2019) who reported that the fermentation of palm kernel meal with humic acid + *Sclerotium rolfsii* and *Bacillus subtilis* respectively improved the chemical composition of palm kernel meal.

The cellulose content of palm kernel meal decreased significantly with increasing fermentation period. In fact, the longer the fermentation period, the lower the crude cellulose content. This could be justified by the fact that, the longer the fermentation period, the more fungi grow and the more cellulase is produced to break down cellulose (Sudharmono et al., 2016). Similarly, the longer the fermentation period, the more cellulase produce which converts cellulose into glucose, and thus explains the lower cellulose content of this meal. This result is similar to those of Mirnawati et al. (2017, 2019), who reported an improvement in the chemical composition of palm kernel meal with increasing fermentation period in organic solutions.

The longest fermentation period (6 days) resulted in significantly higher protein content than that obtained with the other fermentation period. This result corroborates the findings of Mirnawati et al. (2019), who reported that a longer fermentation period of palm kernel meal an increase protein content. This can be explained by the fact that the longer the fermentation period, the greater the number of microbes that proliferate. Thus, the increase in microbial growth contributes to the increase in protein content due to microbial proteins. The increase in crude protein content could also be due to the presence of enzymes produced by the microbes, as the greater the number of microbes in the fermentation process, the more enzymes, which are proteins, will be produced (Mirnawati et al., 2010; 2012, 2013).

Whatever the fermentation mode, the energy level of palm kernel meal increased significantly with the fermentation period. This can be due to the decrease in crude cellulose content, converted into glucose by cellulolytic microbes, on one hand, and the increase in fat content on the other. Through this process, fermentation transforms feed ingredients containing proteins, fats and carbohydrates that are difficult to digest for the benefit of chickens (Mirnawati et al., 2019). Irrespective of the fermentation period, NDF digestibility was not significantly affected by the fermentation mode. This could be explained by limestone's ability to soften cellulose walls, as well as the enzymatic activity of the micro-organisms produced thanks to humic acid. This result is in line with the findings of José da Silva (2020), as an anti-acid and alkalizing agent, limestone reduces flatulence, thus improving digestibility. In the same line, Mirnawati et al. (2010) reported that humic acid (HA) provides energy that favors the development of cellulolytic micro-organisms, leading to improved digestibility.

Irrespective of fermentation mode, crude cellulose digestibility increased significantly with the fermentation period. The high crude cellulose digestibility of the ration containing the meal fermented for 6 days could be related to the low crude cellulose content of the ration. This is in line with the work of Mirnawati et al. (2017, 2019), who reported that crude fiber digestibility of ration depends on the crude fiber content of the ingredients that are in the feed. The higher the crude fiber content, the lower the digestibility of the feed due to the poultry's limitations in digesting cellulose. Digestion is also influenced by several other factors, such as crude fiber composition and the activity of micro-organisms (Maynard et al., 2005). According to Walugembe et al. (2014), fermentation promotes the multiplication of catabolic micro-organisms, which break down complex components into simpler ones, making them easier to digest.

Irrespective of the fermentation mode, feed intake, weight gain, live weight and feed conversion ratio of broilers fed diets containing palm kernel meal fermented for 2, 4 and 6 days were comparable to those fed the control ration without palm kernel meal. This result can be attributed to fermentation, which improved the digestibility of all feed components, enabling the chickens to take advantage of the nutrients contained in the palm kernel meal. Fermentation is said to have improved the palatability of the feed through the quality of its aroma and taste, which was appreciated by broilers. This is in accordance with the findings of Sukaryana et al. (2010) and Mirnawati et al. (2018) who reported that fermentation, improves the aroma and taste of fermented feed, which thereby increases boiler's feed intake.

CONCLUSION

Crude protein, lipid and energy content of palm kernel meal increase with its fermentation period in humic acid and limestone solution, while crude cellulose and NDF content decrease. Improving the nutritional value of this resource through fermentation enables broilers to have growth performances comparable to those produced with a ration containing only soybean meal as the main source of plant-derived protein. Fermenting this meal for a period longer than 6 days and increasing its incorporation level in the ration will be necessary to better assess its effects on broiler growth.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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All authors of present research work were contributed equally.

Consent to publish

All the authors agree to publish this manuscript in this journal.

Competing interests

The authors have no competing interests (none).

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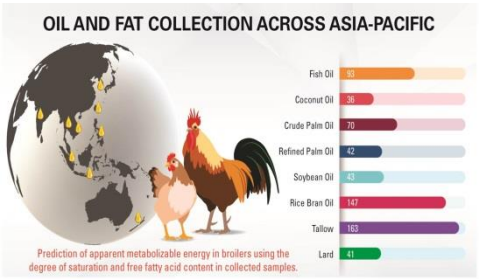

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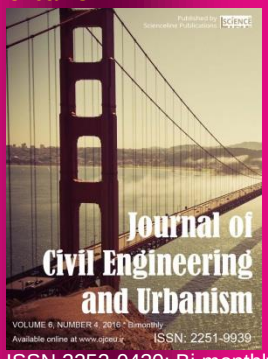

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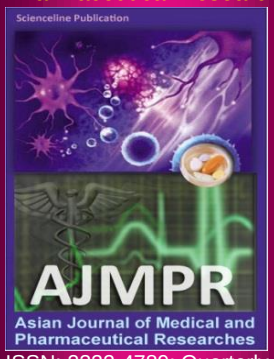

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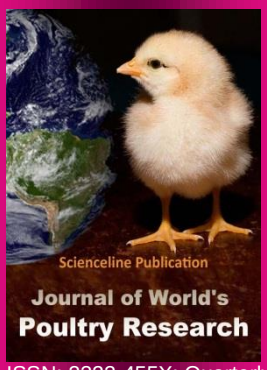

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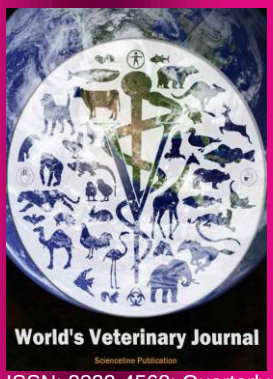

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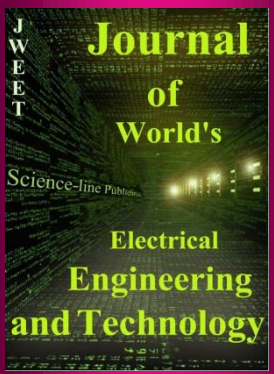

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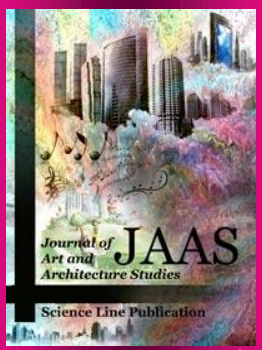

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