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Research Paper

Effects of clove (*Syzygium aromaticum*) on productive performance, nutrients value and digestibility, blood lipid profile, antioxidant status and immune response of growing rabbits

Suliman MAE, Ahmed FG, El-Kholy KhF, Mohamed RAE and Abdel-Mawla LF.

Online J. Anim. Feed Res., 13(1): 01-09, 2023; pii: S222877012300001-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.1

Abstract
The current study evaluated the effect of feeding clove (*Syzygium aromaticum*) as a natural additive on productive performance, digestibility and nutritive value, antioxidant enzymes activities, and immune response of growing rabbits. A total of 48 New Zealand White (NZW) rabbits aged 6 weeks were randomly allocated to 4 groups (12 rabbits/group). Clove buds powder (CLP) was supplemented at 0.5, 1, and 1.5% of basal diet. Four tested diets formulated to contain basal diet without CLP (treatment 1, T1), 0.5% CLP (T2), 1% CLP (T3), and 1.5% CLP (T4). The animals were provided pelleted diets and fresh water ad libitum throughout the experimental period. The rabbits fed diets containing CLP improved FCR (P=0.007) and consumed (P<0.0001) less than those fed control group. The diet containing 1.5% CLP had the best feed conversion ratio (FCR) value (P<0.05). No significant differences were observed among experimental groups in all nutrients digestibility except CP digestibility significantly (P=0.0261) increased with 0.5 and 1% CLP groups compared to control group. Blood total lipid (TL) was significantly decreased (P<0.009) with increasing the dietary level of CLP, (being 379.17 and 361.11 mg/dl for 1% and 1.5% CLP groups vs. 470.84 for control group). The catalase and total antioxidant capacity (TAOC) concentrations significantly (P<0.0001) increased with CLP groups compared to control group. The immunoglobulins titres (IgG and IgM) improved (P>0.05) with rabbits fed CLP diets when compared to those fed the control diet. In conclusion, using CLP as an alternative feed additive in rabbit's diets up to 1.5% without any adverse effect on productive performance and vital activities. The CLP inclusion in rabbit diets decreased feed intake (FI), improved FCR and increased profitability, moreover, had a positive effect on antioxidant enzyme activity and immunity (IgG and IgM) titres.

Keywords: Antioxidant status, Clove, Immune, Performance, Rabbits

[Full text-PDF] [Scopus] [ePub] [Export from ePrint]

Research Paper

Effects of biosecurity practices on the health management system of poultry farms in Nigeria

Aderemi FA, Ayandiji A, and Adeleke GO.

Online J. Anim. Feed Res., 13(1): 10-15, 2023; pii: S222877012300002-13
DOI: https://dx.doi.org/10.51227/ojafr.2023.2

Abstract
The purpose of the study was to determine how Oyo State, Nigerian biosecurity strategies, affected the poultry health management system. The regional data were collected through a planned investigation. The 120 respondents were selected using random and purposeful sampling approaches. The analysis revealed that 43.3% of poultry farmers were between the ages of 31 and 40; the majority were men; 72.5% were married; 37.5% had been in farming for between 11 and 20 years, and 95.5% had one to six children. Most farmers (75.8%) reported that raising poultry was their main source of income; 70.8% stated they got their information from the farmers' association; 95.0% stated burning birds reduced susceptibility to infectious diseases, and nearly all (99.2%) stated keeping foot dips in place stopped the spread of infectious diseases. According to the regression analysis, there is a strong

correlation between respondents' age, sex, marital status, agricultural experience, family size, source of income, and adoption of biosecurity methods in the research region. The greatest and most affordable way of infection protection can be found in biosecurity. Without appropriate biosecurity measures, no single disease prevention program will succeed. It is can be concluded that the introduction of additional biosecurity measures could be a significant boost to the prevention and spread of poultry diseases in the study area.

Keywords: Biosecurity strategies, Commercial Farm, Health Management Practices, Infection protection, Poultry.

[Full text-PDF] [Scopus] [ePub] [Export from ePrint]

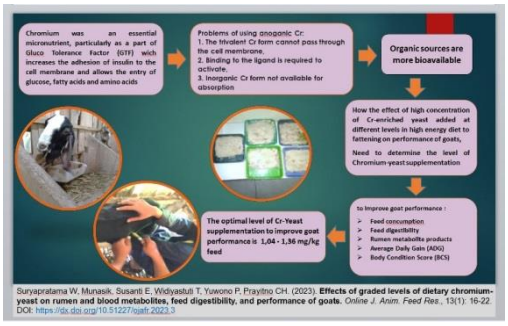
Research Paper

Effects of graded levels of dietary chromium-yeast on rumen and blood metabolites, feed digestibility, and performance of goats

Suryapratama W, Munasik, Susanti E, Widiyastuti T, Yuwono P, Prayitno CH.

Online J. Anim. Feed Res., 13(1): 16-22, 2023; pii: S222877012300003-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.3



Abstract
The study was conducted to determine the effect of dietary supplementation of Chromium-yeast minerals on consumption, feed digestibility, rumen and blood metabolites, Average Daily Gain (ADG) and body condition score (BCS). The research was conducted at Gunung Tugel Farm, Banyumas, Central Java, Indonesia. The material used was 24 male Jawarandu goats with an average initial weight of 25 ± 1.23 kg, individual cages, the feed given consisted of elephant grass silage and concentrate. The treatment feed contains chromium-yeast at levels of 0, 0.5, 1, and 1.5 mg/kg. The research method used was experimental using a completely randomized design. There were 4 treatments tested, namely T0 (70% concentrate + 30% elephant grass silage), T1 (70% concentrate + 30% elephant grass silage + 0.5 mg/kg chromium-yeast), T2 (70% concentrate + 30% elephant grass silage + 1 mg/kg chromium-yeast) and T3 (70% concentrate + 30% elephant grass silage + 1.5 mg/kg chromium-yeast). Each treatment was repeated 6 times so there were 24 trials. The further test used is polynomial orthogonal. The variables measured in this study were feed consumption, feed digestibility, rumen metabolite products, daily body weight gain and body condition score. The results of the analysis of variance showed that the treatment had a significant effect on dry matter and organic matter consumption, feed digestibility, rumen volatile fatty acids (VFA), ammonia nitrogen (NH3-N) and blood glucose 3 h post-feeding, chromium-yeast levels, and had a very significant effect on ADG and BCS. In conclusion, chromium-yeast supplementation was able to improve goat performance with optimal levels ranging from 1.04–1.36 mg/kg of feed.

Keywords: Body condition score, Chromium-yeast, Daily gain, Goat

[Full text-PDF] [Scopus] [ePub] [Export from ePrint]

Research Paper

Control of dipterids in a feedlot under construction in a forest area of center region of Cameroon

Sevidzem SL, Burinyuy KA, Mintsa Nguema R, Mavoungou JF.

Online J. Anim. Feed Res., 13(1): 23-29, 2023; pii: S222877012300004-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.4

Abstract
The forest agro-ecological zone of Cameroon is heavily infested with biting dipterids, but no control is ongoing in this part of the country. In the rainy season (May 2022) in a feedlot under construction in Ndogbea village, eight days entomological study consisting of (i) baseline fly collection using five vavoua traps set in all the sides of a one hectare feedlot yard for four days and (ii) installation of deltamethrin impregnated screens set at 1m from trap and their spraying at frequency of two days in four days. About 1368 biting and non-biting dipterids were collected and classified under five important genera namely *Musca*, *Stomoxys*, *Tabanus*, *Chrysops,* and *Glossina. Musca* spp. were frequent than other species. Only one *Glossina fuscipes fuscipes* was identified. The vavoua trap (VT4) facing the forest with canopy trees had highest

fly catches. The apparent density (ADT) of all the fly genera dropped from pre-screen installation phase (ADT=86.8 flies/trap/day (f/t/d)) to screen installation phase (ADT= 38.2 f/t/d) with overall fly population density reduction rate of 55.99%. However, there was no statistically significant difference (X^2=35.000; df =30; P=0.243) in population density reduction rates of the various fly-groups. In conclusion, five dipterid groups of veterinary and zoonotic importance constituted the fly-vector fauna of Ndogbea village. The presence of deltamethrin impregnated screens contributed to the fly population density reduction rate of 55.99%. An integrated approach including: animal spraying, herd hygiene, use of traps and screens is needed to maintain low fly numbers in this feedlot.

Keywords: Dipterids, Feedlot, Fly-vector, Forest, Parasite.

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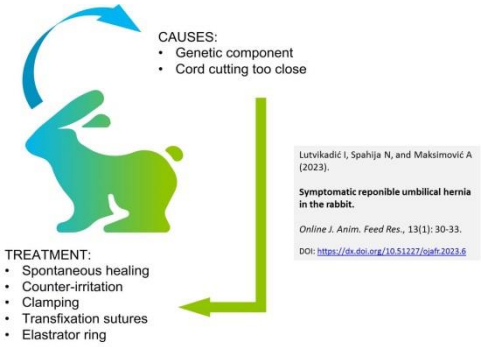
Case Report

Symptomatic reponible umbilical hernia in the rabbit

Lutvikadić I, Spahija N, and Maksimović A.

Online J. Anim. Feed Res., 13(1): 30-33, 2023; pii: S222877012300005-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.5



Abstract
A case of umbilical hernia in a 7-month-old female rabbit was presented to the Surgery Clinic of the Veterinary Faculty, University of Sarajevo. The owner noticed inappetence and lethargy four days before arrival at the clinic. Clinical parameters on physical examination were within the physiological range. Bruxism and lethargy were noted as signs of discomfort due to gas accumulation in colon. A reponible, nonpainful mass in the umbilical scar area was palpated but intestinal peristalsis was not altered. General anesthesia was induced by intramuscular administration of ketamine with medetomidine and maintained with isoflurane. Surgical treatment of hernia included the peritoneal sac dissection and amputation, repositioning of small intestines, and correction of abdominal wall defect. Intraoperative multimodal analgesia approach was used to reduce inhalant anesthesia requirements and to prevent pain-related and stress-related complications. In this case report we described a surgical and veterinary treatment of the reponible umbilical hernia in a rabbit.

Keywords: Herniorrhaphy, Rabbit, Umbilical hernia, Veterinary treatment

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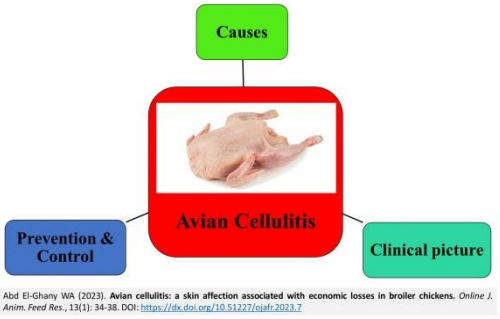
Review

Avian cellulitis: a skin affection associated with economic losses in broiler chickens

Abd El-Ghany WA.

Online J. Anim. Feed Res., 13(1): 34-38, 2023; pii: S222877012300006-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.6



Abstract
This review was designed for focusing on cellulitis condition in broiler chickens regarding causes, clinical picture, and prevention measures of this condition. Cellulitis is an acute diffuse inflammation of subcutaneous tissues and muscles especially on the skin of thighs and abdomen. This condition is more common in broilers than others and it is usually associated with economic losses. At processing, low grade chicken carcasses and high incidences of condemnation are the sequels of cellulitis. Skin integrity, stocking density, and litter conditions are predisposing factors for induction of cellulitis. However, other infectious bacterial and immunosuppressive viral pathogens are associated with cellulitis. Affected birds display areas of yellow skin along with a plaque of pus underneath the skin and the underlying muscles show hemorrhages. Presence of caseous, yellowish to green, dark red, or brown fetid gangrenous exudate could also be observed in the advanced cellulitis cases. Prevention and control of cellulitis are based on application of hygienic practices, vaccination, antibiotic therapy, genetic selection, and nutrition.

Keywords: Avian cellulitis, Broiler, Disorder, Lesions, Skin.

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Research Paper

Fermentation of blood meal absorbed by oil palm fronds with *Bacillus amyloliquefaciens* and *Lactobacillus plantarum*

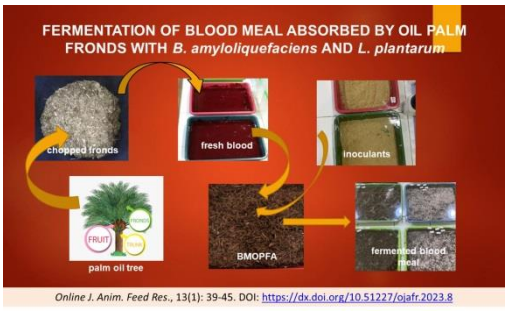
Imsya A, Riswandi, Malik B, Yakup.

Online J. Anim. Feed Res., 13(1): 39-45, 2023; pii: S222877012300007-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.7

Abstract

This study was aimed at improving the efficiency of blood meal (BM) use as feedstuff through the application of agricultural waste absorbance and fermentation technology. Blood was absorbed by oil palm fronds and fermented by using *Bacillus amyloliquefaciens* (BAF) and *Lactobacillus plantarum* (BLP) inoculants in 0, 60, and 120 hour incubation times. Quality was assessed by using Van Soest fiber analysis and *in vitro* digestibility trial on the best fermented product. Results showed that there was significant interaction effect (P<0.05) of inoculant type and fermentation times on the changes in fiber fraction of BM absorbed by oil palm fronds. Inoculant types were found to give significant effects (P<0.05) on ration digestibility rate and *in vitro* rumen condition characteristics. It was concluded that fermentation of BM absorbed with palm oil fronds with BLP in 120 hours resulted in BM with the best fiber fraction reduction, digestibility rate, and *in vitro* rumen condition characteristics.



Keywords: Agricultural waste, Feedstuff, Fiber fraction, Digestibility, Rumen condition.

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Research Paper

Effects of different processing methods on nutrient contents and acceptability of hog plum (*Spondias mombin* Linn.) leaf by West African Dwarf sheep

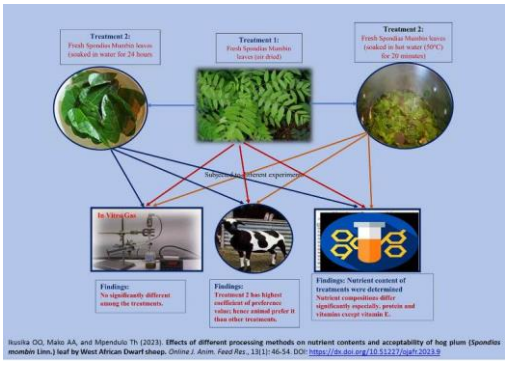
Ikusika OO, Mako AA, and Mpendulo Th.

Online J. Anim. Feed Res., 13(1): 46-54, 2023; pii: S222877012300008-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.8

Abstract

Three experiments were conducted to evaluate the effect of processing method on leaves from *Spondias mombin* tree as fodder for ruminants in the tropics. The leaves were subjected to three different physical processing methods; T1 control (fresh but air drying), T2 (fresh but soaked in ordinary water for 24 h then air drying), and T3 (fresh but soaked in water at 50°C for 20 min then air drying). Nutrient and secondary metabolites content were determined in experiment 1. In experiment II, the Coefficient of preference (CoP) was determined. In vitro gas production was used to predict metabolizable energy (ME), organic matter digestibility (OMD), short-chain fatty acid (SCFA) and methane (CH4) of *S. mombin* leave with different processing methods in experiment 111. Results revealed significant differences in the chemical composition of *S. mombin* leaf subjected to different processing methods. The dry matter value was highest in *S. mombin* leaves soaked in hot water (90.22%), and lowest in *S. mombin* leaves soaked in water at room temperature (85.05%). Crude protein was highest in leaf processed with hot water (11.25 %) and lowest in control (9.59 %). No significant variations were observed for minerals and anti-nutrients investigated. The Vitamin content of leaves of *S. mombin* tree with various processing methods differed significantly except for vitamin E. The preference coefficient value was greater in leaves soaked at 50°C for 20 mins than leaves from the other processing method and control. All leaves of *S. mombin* tree from all processing methods considered in this study were acceptable to the animals, but leaves soaked in 50°C for 20 mins were most preferred. The in vitro gas production parameters and characteristics were not significantly different. In conclusion, *S. mombin* leaf subjected to 50 °C for 20 mins is more advantageous as forage in animal nutrition than unprocessed.



Keywords: *In vitro* gas production, Nutritional value, Processing method; Ruminant, *Spondias mombin* leaf.

[Full text-PDF] [Scopus] [ePub] [Export from ePrint]

Research Paper

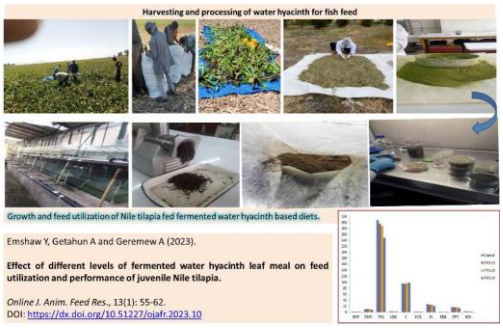
Effect of different levels of fermented water hyacinth leaf meal on feed utilization and performance of juvenile Nile tilapia

Emshaw Y, Getahun A and Geremew A.

Online J. Anim. Feed Res., 13(1): 55-62, 2023; pii: S222877012300009-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.9

Abstract



This study was conducted to evaluate the effects of different inclusion levels of water hyacinth leaf meal fermented with *Aspergillus niger* on feed utilization efficiency and growth performance of Nile tilapia (*Oreochromis Niloticus* L.). Fermented water hyacinth leaf (FWHL) at 0, 10, 20 and 30% inclusion levels were incorporated into four isonitrogenous (35% CP), and isoenergetic (18 KJ g-1 g) test diets. The fishes were stocked in 80 liters aquarium units, in a closed, recirculating indoor system. The diets were fed to triplicate groups of fish fingerlings (1.6 g average body weight) twice a day, at 6% of body weight/day, for three months. The study demonstrated that Nile tilapia fed FWHL at levels 30% had a significant negative impact ($P<0.05$) on weight gain, specific growth rate, feed utilization efficiency, and whole body composition. But, there were no significant changes between diets supplemented with 10% and 20% FWHL when compared with the control group. Therefore, supplementation of fermented water hyacinth leaf meal to diets of Nile tilapia is recommended up to 20% because it is cheaper than fish meal and corn.

Keywords: Body composition, Feed utilization, Growth performance, Nile tilapia, Water hyacinth

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Systematic Review

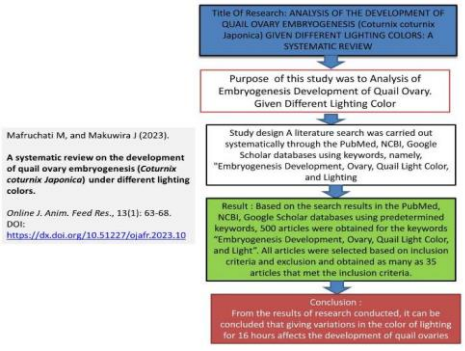
A systematic review on the development of quail ovary embryogenesis (*Coturnix coturnix Japonica*) under different lighting colors

Mafruchati M, Makuwira J and Wardhana AK.

Online J. Anim. Feed Res., 13(1): 63-68, 2023; pii: S222877012300010-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.10

Abstract



Quail (*Coturnix coturnix Japonica*) is one of the commercial poultry that is being developed and produced more frequently. Various lighting programs (pre-hatching) on Aves have been carried out to provide an increased biological response, including growth, reproduction, and productivity. The addition of light or the lighting program is also a factor in the growth of Aves which directly plays a role in controlling various physiological processes. The purpose of this study was to review embryogenesis development of the Quail ovary under various lighting conditions. A literature search was carried out systematically through the PubMed, NCBI, and Google Scholar databases using keywords, namely, "embryogenesis development, ovary, quail light color, and lighting". The articles obtained were selected based on these keywords by setting several inclusion criteria. Papers that do not meet the inclusion criteria are eliminated, and articles that meet the criteria will be analyzed to obtain data. Based on the search results in the databases using predetermined keywords, 500 articles were obtained. All articles were selected based on inclusion criteria and exclusion and obtained as many as 35 articles that met the inclusion criteria. From the results of the research, it can be concluded that giving variations in the colour of lighting for 16 hours affects the development of quail ovaries. Because of the significant effect of lighting and its colour on embryo development, pre-hatch lighting programs should be considered in future studies.

Keywords: Egg, Embryogenesis, Incubation, Lighting, Quail.

[Full text-PDF] [Scopus] [ePub] [Export from ePrint]

Research Paper

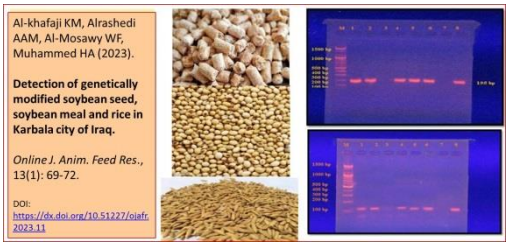
Detection of genetically modified soybean seed, soybean meal and rice in Karbala city of Iraq

Al-khafaji KM, Alrashedi AAM, Al-Mosawy WF, Muhammed HA.

Online J. Anim. Feed Res., 13(1): 69-72, 2023; pii: S222877012300011-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.11

Abstract



Rice and soybean are two high-demanded grains for human foods and animal feeds. The current study aimed as first time in one of Iraqi region to find genetically modified soybean seed, soybean meal and rice grain samples utilizing the *Cauliflower mosaic virus* (CaMV) 35S promoter and nopaline synthase (NOS) terminator catalyst like *Agrobacterium tumefaciens* NOS (ANOS) terminator, in PCR tests. A total of 55 samples of soybeans seed, soybean meal, and rice cereal were collected from the market in Karbala, Iraq. The samples were collected from markets in Karbala city during January-March 2021, and evaluated in the Food Laboratory, College of Al Safwa University of Karbala, Iraq. DNA was isolated from dry vegetable samples. Two genes, including CaMV-35S and NOS terminator, that are routinely used in genetic engineering were employed to evaluate genetically modified crops. The present study revealed CaMV-35S and NOS genes in soybean meals. In conclusion, the obtained results indicated that all rice samples tested with the same primers were genetically unaltered. Whereas, there is genetically alternations in soybean seeds and soybean meal.

Keywords: Genetically modified seeds, Feedstuff, Soybean meal, Rice, CaMV-35S, NOS terminator.

[Full text-PDF] [Scopus] [ePub] [Export from ePrint]

Research Paper

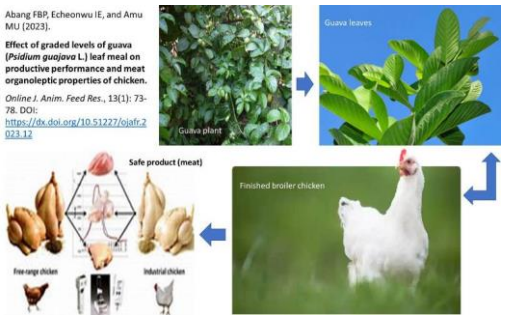
Effect of graded levels of guava (*Psidium guajava* L.) leaf meal on productive performance and meat organoleptic properties of chicken

Abang FBP, Echeonwu IE, and Amu MU.

Online J. Anim. Feed Res., 13(1): 73-78, 2023; pii: S222877012300012-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.12

Abstract



A study was carried out to determine the productive performance and meat organoleptic properties of finisher broiler fed diets supplemented with graded levels of dried guava leaf meal (DGLM) as a phytogenic feed additive. The study was conducted at the livestock experimental unit of National Veterinary Research Institute Vom, Nigeria. Two hundred and forty unsexed 5-weeks-old broilers of similar mean live weight were randomly assigned to 1 of 4 dietary groups with 3 replicates (0, 150g, 300g and 450g of DGLM per 100kg basal diets) over a four weeks finisher period, in completely randomized design. All the diets of iso-nitrogenous and iso-caloric and water were served to the birds ad libitum. The results of the finisher phase of the experiment showed that, though the average daily feed intakes of all the treatments were the same, the final body weights of birds fed T4 diets were significantly ($P<0.05$) higher than those fed other diets. Feed conversion ratio (FCR), protein efficiency ratio (PER) and feed cost/weight gain followed similar trend as in body weight gain. The organoleptic properties (colour, appearance, texture, taste and aroma) of all the treatment groups revealed that DGLM had no adverse effect on broiler meat. The study concluded that the supplementation of DGLM at 300g and 450g/ 100kg enhanced utilization of nutrients in the diets resulting in impressive growth performance, reduced feed cost/weight gain, and high survivability without influencing the organoleptic properties of finisher broiler chickens.

Keywords: Broiler Chickens, Guava Leaf Meal, Herbal additive, Productive Performance, Organoleptic Properties.

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EFFECTS OF CLOVE (*Syzygium aromaticum*) ON PRODUCTIVE PERFORMANCE, NUTRIENTS VALUE AND DIGESTIBILITY, BLOOD LIPID PROFILE, ANTIOXIDANT STATUS AND IMMUNE RESPONSE OF GROWING RABBITS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The current study evaluated the effect of feeding clove (*Syzygium aromaticum*) as a natural additive on productive performance, digestibility and nutritive value, antioxidant enzymes activities, and immune response of growing rabbits. A total of 48 New Zealand White (NZW) rabbits aged 6 weeks were randomly allocated to 4 groups (12 rabbits/group). Clove buds powder (CLP) was supplemented at 0.5, 1, and 1.5% of basal diet. Four tested diets formulated to contain basal diet without CLP (treatment 1, T1), 0.5% CLP (T2), 1% CLP (T3), and 1.5% CLP (T4). The animals were provided pelleted diets and fresh water *ad libitum* throughout the experimental period. The rabbits fed diets containing CLP improved FCR (P=0.007) and consumed (P<0.0001) less than those fed control group. The diet containing 1.5% CLP had the best feed conversion ratio (FCR) value (P<0.05). No significant differences were observed among experimental groups in all nutrients digestibility except CP digestibility significantly (P=0.0261) increased with 0.5 and 1% CLP groups compared to control group. Blood total lipid (TL) was significantly decreased (P<0.009) with increasing the dietary level of CLP, (being 379.17 and 361.11 mg/dl for 1% and 1.5% CLP groups vs. 470.84 for the control group). The catalase and total antioxidant capacity (TAOC) concentrations significantly (P<0.0001) increased with CLP groups compared to control group. The immunoglobulins titres (IgG and IgM) improved (P>0.05) with rabbits fed CLP diets when compared to those fed the control diet. In conclusion, using CLP as an alternative feed additive in rabbit's diets up to 1.5% without any adverse effect on productive performance and vital activities. The CLP inclusion in rabbit diets decreased feed intake (FI), improved FCR and increased profitability, moreover, had a positive effect on antioxidant enzyme activity and immunity (IgG and IgM) titres.

Keywords: Antioxidant status, Clove, Immune, Performance, Rabbits

INTRODUCTION

Phytogenic as herbs or spices is a natural growth promoters or non-antibiotics growth promoters which are used as feed additives in rabbit diets to improve the productive performance, health status and meat quality (Christaki et al., 2012; Ingweye et al., 2020; Nwachukwu et al., 2021). Moreover, phytogenic help to improving immune system performance in critical situation due to increase the intestinal availability of essential nutrients for absorption, therefore, helping animals to grow better within the framework of their genetic potential (Windisch et al., 2008).

The ban of using antibiotics in livestock nutrition as feed additives attributed to its residual effect which found in final products and increased the consumer's awareness about the health hazards occurs (Anadón et al., 2006; Silveira et al., 2021). Moreover, due to the use of antibiotics in livestock nutrition triggered searching for alternative natural and safe healthy for animals and human when used as feed additives, especially a source of antibiotic (Khamisabadi et. al., 2016). Like, herbs are often preferred because they are natural and do not put harmful chemicals into the body (Agrawal et al., 2014). Herbs considered as an alternative feed additive of antibiotics and drug that using in poultry diets to avoid the residual cumulative effect in final poultry products, which negatively affects human health (Ragab, 2012). Clove (*Syzygium aromaticum*) has potent as antioxidant and antimicrobial activities standing out among the other spices (Shan et al., 2005). The clove powder supplementation in broilers diet at 0.5% improved body weight gain and feed conversion ratio (Mahrous et al., 2017). Also, supplementation of 0.50% clove buds and aloe vera leaves improved dressing percentage and breast weight of Japanese quails (Tariq et al., 2015). In rabbit diets added clove a combination with onion, garlic, caraway, fennel, gentian, melissa, peppermint, anise, and oak bark decreased post-weaning mortality rate, improved feed utilization, and enhanced animal performance (Krieg et al., 2009). Sulieman et al. (2007) used clove (*Syzygium aromaticum*) as an antimicrobial, antiseptic, and preservative agent. Furthermore, clove essential oil exhibits a

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wide range of pharmacological and biological activities such as antioxidant (Gülçin et al., 2012), antifungicidal (Omidbeygi et al., 2007), and antiprotozoal effects (Machado et al., 2011).

Current study investigated effect of clove (CLP) supplementation in growing rabbit diets on productive performance, nutritive value, blood lipid profile, antioxidant enzymes activity, and immune response.

MATERIALS AND METHODS

The experiment was conducted in Borg El-Arab experiment station, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Giza, Egypt. The laboratory works were carried out at Utilization of By-products Research Department, APRI, Giza, Egypt. Feed mixing and pelleting processing were prepared at Nobaria feed manufactory, Nobaria experiment station, APRI, Alexandria, Egypt.

Ethical approval

This study was carried out after approved ethically from the APRI, Giza, Egypt under code No. 432429-21-6.

Diets and animals management

Clove buds (*Syzygium aromaticum*) grinded by hammer mill then was taken sample from clove buds powder (CLP) to determine the chemical analysis composition (A.O.A.C., 2000) and total antioxidant capacity (TAOC; Prieto et al., 1999) assayed by spectrophotometer (JENWAY 3600). Rabbit basal diet supplemented with 0.0, 0.5, 1.0 and 1.5% clove buds powder (CLP). Basal diet without CLP supplementation (T1) which considered as a control group, basal diet with 0.5% CLP (T2), 1.0% CLP (T3) and 1.50% CLP (T4). The experimental diets (Table 1) were meet the nutrients requirement of growing rabbits (Lebas, 2004) also, it were to be isonitrogenous and isocaloric.

The experimental New Zealand White (NZW) rabbits were randomly allocated to 4 groups, 12 rabbits for each group. Rabbits weighting averaged 652.81±33.43 g. the experiment lasted 8 weeks (6-14 weeks of rabbit age). The growing rabbits housed in metal battery cage (30 × 35 × 40 cm). The pelleted feed and fresh water provide *ad-libitum* access to separated feeders and automatic nipple fresh water throughout the tested period. The experimental rabbits were kept under the hygienic condition, vaccine program, and management.

Table 1 - Formulation and chemical composition of tested diets

Ingredients	Control diet	Clove buds powder addition levels		
		0.5%	1.0%	1.5%
Soybean meal (44% CP)	17.00	17.00	17.00	17.00
Yellow corn	13.00	13.00	13.00	13.00
Barley	12.95	12.95	12.95	12.95
Wheat bran	16.00	16.00	16.00	16.00
Clover hay	35.00	34.50	34.00	33.50
Clove buds powder (CLP)	0.00	0.50	1.00	1.50
Dl-methionine	0.20	0.20	0.20	0.20
Dicalcium phosphate	2.00	2.00	2.00	2.00
Salt (NaCl)	0.35	0.35	0.35	0.35
Vitamins and minerals mixture[1]	0.30	0.30	0.30	0.30
Anti-coccidia and fungi	0.20	0.20	0.20	0.20
Molasses	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00
Chemical analysis on DM basis [2]				
Dry matter (DM, %)	82.92	82.93	82.95	82.96
Organic matter (OM, %)	85.94	85.93	85.91	85.90
Crude protein (CP, %)	17.73	17.67	17.60	17.54
Crude fiber (CF, %)	14.12	14.08	14.04	14.00
Ether extract (EE, %)	2.13	2.19	2.25	2.31
Nitrogen free extract (NFE, %)	54.95	55.00	55.05	55.11
Ash (%)	5.14	5.13	5.13	5.12
Digestible energy (DE, kcal/kg)[3]	2605.44	2607.98	2610.54	2613.09

[1] Commercial vitamin and mineral premix contained (per 3 kg premix) vit. A 12000 000 IU, vit. D3 3000 000 IU, vit. E 10 000 mg, vit. K3 2000 mg, vit. B1 1000 mg, vit. B2 5000 mg, vit. B6 1500mg, vit. B12 10 mg, pantothenic acid 10 000 mg, nicotinic acid 30 000 mg, folic acid 1000 mg, biotin 75 mg, copper 4000 mg, manganese 80 000 mg, zinc 50 000 mg, iron 30 000 mg, iodine 500 mg, selenium 100 mg and cobalt 100 mg. [2]NRC (1977). [3]Digestible Energy (kcal/kg) = 4.36-0.049 × [28.924 + 0.657 (CF %)] according to Cheeke, (1987).

Productive performance measurements

Live body weight (BW, g/rabbit/day) of rabbits and feed intake (FI, g/rabbit/day) were recorded weekly. Then the feed conversion ratio (FCR, g feed: g gain) were calculated over an experimental period. The BW, FI, and FCR were calculated on per cage basis and then average by treatment.

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Digestion trial

According Perenze et al. (1995), 20 rabbits divided randomly into 4 groups (5 rabbits/group) to execution the digestion trial. Rabbits were allocated in metabolic cages (56 × 38 × 28 cm). The feces were collected daily before the morning meal. The fresh feces were weighed then dried in air-dry oven at 60 ºC for 24 hour. The diets and dried feces ground samples used to estimate the moisture, ash, nitrogen, ether extract, and crude fiber (A.O.A.C., 2000). Those data were used to calculate the digestion coefficient of nutrients, nutritive value (Fekete, 1985), and digestible energy (Schneider and Flatt, 1975) for each tested diets.

Blood lipid profile, antioxidant enzymes activity, and immune response

The blood samples were collected during slaughtering time from five rabbits which randomly selected from each treatment at the end of growing period. The samples were collected in heparinized tubes and centrifuged at 3000 rpm for 20 minute, then transferred the plasma to tubes and stored at -20 ºC till biochemical analysis. Plasma total lipids was determined according to Frings and Dunn (1970), cholesterol was estimated according to Young (1997), LDL cholesterol was determined according to Assmann et al. (1984) and HDL cholesterol was determined according to Lopez et al. (1977). The antioxidant enzymes as catalase concentration and total antioxidant capacity were determined according to Góth (1991) and Fischer et al. (2006). Immunoglobulin (IgG and IgM) responses were estimated according to Van der Zipp et al. (1983). All measurements were assayed by colorimetric methods. All kits were purchased from Bio-diagnostic Co, Egypt

Economic profit

Economic efficiency was calculated as a ratio between the return of weight gain and the cost of feed intake. The price of ingredients and selling of one kg live weight of rabbits ($2.55/kg) was calculated according to the price in local market at the time of experiment.

Statistical analysis

All data were subject to one-way analysis of variance (ANOVA). The data obtained herein were analyzed by the GLM procedure of SAS (2004, USA). Duncan's multiple range test (1955) was performed to separate means and significance accepted at P≤0.05.

RESULTS AND DISCUSSION

Nutritional analysis and total antioxidant of CLP

The nutritional analysis and total antioxidant capacity of CLP are presented in Table 2. The DM, OM, CP, CF, EE, NFE, ash, and DE values of CLP were 91.40, 94.36, 9.17, 13.50, 6.40, 64.80, 6.17%, and 2508.12kcal/kg, respectively. In contrary, the chemical analysis of clove buds were 90, 1.20, 20.10, 12.10 and 5.4% for DM, CP, CF, EE and ash, respectively (Sulieman et al., 2007); 85.20, 12.40, 17.50, 16.20 and 12.60%, respectively (Sulaiman and Anas, 2017).

The total antioxidant capacity (TAC) content of CLP was 1069.20 mg/100g (ascorbic acid equivalent). This TAC value is higher than those obtained by Ahmed et al. (2022) who found the TAC as form 2,2-diphenyl-1-picrylhydrazyl (DPPH%) radical scavenging activity in CLP was 83.90 mg/100g. However, Anita et al. (2015) who found the oxidized, reduced, and total ascorbate were 8084.40, 8014, and 6098.50 mg/100g dry wt., respectively. The DPPH scavenging of antioxidant content of CLP was 13660 mg/100mg according to Turgay and Esen (2015). The polyphenols and antioxidant content of CLP is higher than those in other spices (Pérez-Jiménez et al., 2010). The TAC in clove or their extract can promote health (Abo El-maati et al., 2016). Furthermore, it prevents the oxidation of lipids by chelating metal ions or inhibits the propagation reaction being hydrogen / electron donor (Shobana and Naidu, 2000). The active ingredients of clove buds and lemon balm extracts are able to scavenge the free radicals in *in vitro* trail (Petrovic et al., 2012).

Table 2 - Chemical analysis and total antioxidant capacity of clove powder

Items	Clove buds powder (CLP)
Chemical analysis on DM basis	
Dry matter (DM, %)	91.40
Organic matter (OM, %)	94.36
Crude protein (CP, %)	9.17
Crude fiber (CF, %)	13.50
Ether extract (EE, %)	6.40
Nitrogen free extract (NFE, %)	64.80
Ash (%)	6.17
Digestible energy (DE, kcal/kg)[1]	2508.12
Total antioxidant capacity mg/100 g (ascorbic acid equivalent)	
TAOC	1069.20

TAC: Total antioxidant capacity; [1]Digestible Energy (kcal/kg) = 4.36-0.049 × [28.924 + 0.657 (CF %)] according to Cheeke, (1987).

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Growth performance

The effect of CLP supplementation on growing rabbit's performance is presented in Table 3. No significant differences in FBW (P=0.7092) and BWG (P=0.6400) were found between the control group (T1) and treatment groups (T2-T4). Final body weight (FBW, g) and body weight gain (BWG, g/day/rabbit) increased with groups fed CLP more than 0.5% level addition (P>0.05). However, FI (g/rabbit/day) and FCR (g, feed: g, gain) were significantly (P<0.0001 and P=0.007, respectively) affected by inclusion of dietary CLP. Rabbits in dietary CLP treatments (T2, T3, and T4) decreased (P<0.0001) the FI consuming and improved (P<0.0078) FCR during the growing period as compared with control treatment (T1). The dietary 1.5% CLP group had the lowest FCR value (T4, 3.89), which was comparable to the control (T1, 4.70) and better than the 0.5% (T2, 4.15) and 1.0% (T3, 4.28) dietary CLP. The FCR of broiler at finisher period was improved (P<0.05) with clove essential oil (455ppm) addition (Mehr et al., 2014). Similarly with Petrovic et al. (2012) who showed slightly improvement (P>0.05) in broilers performance (BW, FI and FCR) which fed diets supplemented 1% clove bubs with 0.2% lemon balm extract. Mahrous et al. (2017) observed no significant differences in broilers performance indices (FBW, BWG, FI, and FCR) fed diets supplemented 0.5, 1.0, and 1.5% clove buds.

In present study, the improvements in FBW (P=0.7092), BWG (P=0.6400) and FCR (P<0.05) with increasing level of CLP than 0.5% due to increase the diets content of growth promoters properties such as antimicrobial (Dorman et al., 2000). Many studies confirmed the positive effect of spices or their active components on the digestion process, wherein they activate bile salts secretion and digestive enzyme activities in the intestinal mucosa and pancreas (Hernández et al., 2004) which reflect to broilers productive performance (Jang et al., 2007). In contrary, Al-Mufarrej (2019) who reported that negative effect of final live body (P<0.05) for broiler fed clove supplemented more than 2%. Hussein et al. (2019) added 1.5 ml clove oil/kg of Japanese quails diet, increased (P<0.05) performance in terms of BWG and FI, with no improvement (P>0.05) in FCR.

Table 3 - Effect of clove buds powder addition on growing rabbit's performance

Items	IBW (g)	FBW (g)	BWG (g/rabbit/day)	FI (g/rabbit/day)	FCR (g.feed : g.gain)
T1 (0.0% CLP)	650.83±35.17	2251.67±53.66	28.60±1.11	134.28 ±1.86 [a]	4.70±0.18 [a]
T2 (0.5% CLP)	655.42±32.40	2240.42±58.37	28.30±0.77	117.44±2.37 [c]	4.15±0.11 [b]
T3 (1.0% CLP)	651.67±32.90	2307.50±47.02	29.57±0.67	126.48±1.62 [b]	4.28±0.10 [b]
T4 (1.5% CLP)	653.33±33.25	2344.17±109.23	30.19±1.78	117.64±2.53 [c]	3.89±0.20 [b]
P-value	0.9997	0.7092	0.6400	<0.0001	0.0078

Mean values with different superscript letters in the same column are significantly different (P<0.05); CLP: clove buds powder, IBW: initial body weight, FBW: final body weight, BWG: body weight gain, FI: feed intake, FCR: feed conversion ratio

In vitro digestibility and nutritive values

The effect of CLP feeding on digestibility and nutritive values are presented in Table 4. There were slight increase (P>0.05) in digestion coefficients percentage of dry matter (DM; P=0.3476), organic matter (OM; P=0.2883), crude fiber (CF; P=0.1507), ether extract (EE; P=0.4753), nitrogen free extract (NFE; P=0.1507), also, nutritive value as a total digestible nutrients (TDN%, P=0.1107) and digestible energy (DE, Kcal/kg, P=0.1106) with 0.5 and 1.0% CLP groups compared to control group. Percentage of crude protein (CP) digestibility significantly (P=0.0261) increased with 0.5 and 1.0% CLP groups compared to control group. The diet containing 0.5% CLP recorded the best digestibility for all nutrients except % of NFE digestibility. Percentage of digestible crude protein (DCP) had significantly (P<0.0001) affected by dietary CLP addition. Percentage of DCP significantly (P<0.05) improved by 9.39% with 1.5%CLP addition, but 1.0% CLP inclusion significantly (P<0.05) decreased DCP% by 8.23% with relative control group. No significant difference in DCP% was found between 0.50% CLP group and the control group.

The results agreed with Dalkiliç and Güler (2009) who found DM, CP, and EE digestibilities significantly (P<0.05) improved by clove extract level up to 400 ppm in in broiler diets. Generally, when looking at either rabbit's performance (Table 3), the FCR improved and FI decreased or nutrient digestibility (Table 4), CP% and DCP% enhanced by dietary CLP addition. This refers to herbs bioactive substances that help to regulate the FI in animals by improving the flavor and regulate the functioning of digestive system (Mirzaei -Aghsaghali, 2012). Moreover, inhibit or enhance metabolism, shape of the sensory, and dietary properties of animal products (Meineri et al., 2010). The spices such as pepper, cinnamon, and clove stimulate the secretion of pancreatic enzymes (lipases, amylases, and proteases), and increase the activity of digestive enzymes of gastric (Srinivasan, 2005).

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Table 4 - Digestibility and nutritive values of growing rabbits

Items	Digestion coefficients (%)						Nutritive value		
	DM	OM	CP	CF	EE	NFE	DCP %	TDN %	DE Kcal/kg[1]
T1 (0.0% CLP)	68.05±1.25	71.17±1.32	67.66±0.71 [b]	40.57±1.69	89.32±1.35	72.17±1.24	12.46±0.13 [b]	68.43±0.86	3031.30±38.47
T2 (0.5% CLP)	69.90±3.91	72.74±3.59	75.71±2.47 [a]	58.60±5.27	91.45±1.01	71.31±3.67	12.29±0.14 [b]	68.63±2.61	3040.26±115.78
T3 (1.0% CLP)	68.14±3.97	72.62±3.13	73.26±0.91 [a]	42.54±7.93	90.34±3.31	74.12±2.68	11.33±0.08 [c]	69.08±2.52	3060.18±111.79
T4 (1.5% CLP)	61.43±3.34	64.77±3.67	71.62±1.08[ab]	47.85±4.32	85.88±3.39	59.98±4.76	13.63±0.21 [a]	60.66±3.09	2687.35±136.70
P-value	0.3476	0.2883	0.0261	0.1507	0.4753	0.1507	<0.0001	0.1107	0.1106

Mean values with different superscript letters in the same column are significantly different (P<0.05); CLP: clove buds powder, DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fiber, EE: ether extract, NFE: nitrogen free extract, DCP: digestible crude protein, TDN: total digestible nitrogen, DE: digestible energy. [1]DE (kcal/kg) =TDN x 44.3 (Schneider and Flatt, 1975).

Table 5 – Blood lipid profile, antioxidant enzymes activity, and immunoglobulin titres of growing rabbits

Items	Blood lipid profile				Antioxidant enzymes activity		Immunoglobulin titres	
	TL (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Catalase (U/L)	TAOC (mM/L)	IgG	IgM
T1 (0.0% CLP)	470.84±8.82 [a]	138.35±5.40	25.95±0.55	98.775±4.23	134.55±3.55 [c]	1.62±0.01 [d]	296.00±30.60	55.50±8.37
T2 (0.5% CLP)	425.00±25.66 [ab]	174.75±7.01	28.65±1.65	74.40±4.00	146.90±3.58 [c]	1.68±0.01 [c]	453.50±34.92	58.50±21.65
T3 (1.0% CLP)	379.17±8.82 [bc]	151.40±16.17	30.05±1.47	73.52±8.41	162.95±2.86 [b]	1.78±0.01 [b]	476.50±28.58	65.50±10.68
T4 (1.5% CLP)	361.11±20.85 [c]	157.95±7.01	31.00±3.00	69.84±17.14	218.50±6.41 [a]	1.83±0.01 [a]	719.00±192.26	76.50±10.10
P-value	<0.0090	0.1400	0.3220	0.2320	<0.0001	<0.0001	0.0910	0.7170

Mean values with different superscript letters in the same column are significantly different (P<0.05). CLP: clove buds powder, TL: total lipids, HDL: high density lipoprotein, LDL: low density lipoprotein, TAOC: total antioxidant capacity, IgG: immunoglobulin G, IgM: immunoglobulin M.

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Blood lipid profile, antioxidant enzymes activity and immune response in plasma

Blood lipid profile is shown in Table 5. Total lipid (TL) concentrations significantly (P=0.009) decreased with increasing the levels of CLP supplementation. However, no significant differences were observed between tested groups in cholesterol (P=0.1400), HDL (P=0.3220), and LDL (P=0.2320) values. Both cholesterol and HDL concentrations increased (P>0.05) with CLP diets, while LDL values were insignificantly lower (P>0.05) with rabbits fed CLP diets than control group. Addition of CLP by 0.5, 1.0 and 1.5% increased (P>0.05) HDL concentrations (10.40, 15.80, and 19.46%, respectively), however, decreased LDL concentrations (24.68, 25.60 and 29.30%, respectively). As the same trend, by increasing clove essential oil levels in Japanese quail diets, the concentrations of HDL increased (P<0.05) and LDL decreased (P<0.05) in plasma (Hussein et al., 2019). The decreasing of LDL in blood may due to bioactive substrate (Eugenol) in CLP which plays a vital role in reducing LDL concentrations (Harb et al., 2019). In broilers plasma, no significant differences (P>0.05) in concentrations of TL, total cholesterol, LDL and HDL due to feeding on 1% clove buds in diet and 0.2% lemon balm extract in drinking water (Petrovic et al., 2012). In contrast, total cholesterol significantly (P<0.05) decreased with supplementation 0.5, 1.0 and 1.5 g clove bud /kg broiler diets (Mahrous et al., 2017).

Antioxidant enzymes activity are presented in Table 5. Dietary CLP supplementation significantly increased (P<0.05) catalase and TAOC concentrations in growing rabbits plasma when compared with those in the control group. Rabbits fed on diet including 1.5% CLP gave the highest (P<0.05) catalase and TAOC concentrations (218.50 and 1.83, respectively) with compared to rabbits in control diet (134.55 and 1.62, respectively). Similarly, catalase concentration in quails blood significantly (P<0.05) increased with diets containing clove essential oils (Hussein et al., 2019). The improvement of catalase and TAOC concentrations in blood are attributed to eugenol (bioactive substrate) that forms iron-oxygen chelate complex through its allyl group and maintains iron and copper in their reduced forms (Ito et al., 2005). Moreover, clove supplementation can prevent hydroxyl radical's synthesis (the secondary products of lipid peroxidation; like clove oil may serve as an effective antioxidant even at the later stages of lipid peroxidation during beta-oxidation process (Jirovetz et al., 2006). In this study, antioxidant status improved (P<0.0001) by feeding growing rabbits on highest CLP level (1.5%), this improvement is due to clove active substances with the antioxidant properties (Dragland et al., 2003). A slightly increased in antioxidants (superoxide dismutase and glutathione peroxidase) due to feeding broilers on 1% clove buds and 0.2% lemon balm extract in drinking water (Petrovic et al., 2012).

Immunoglobulin responses are illustrated in Table 5. The immunoglobulin titres (IgG and IgM) recorded insignificantly (P=0.0910 and P=0.7170, respectively) improvement with CLP rabbit groups when compared to those in control group. Rabbits fed 1.5%CLP recorded the highest IgG and IgM values compared to the other tested groups including the control group. Furthermore, the concentrations of plasma IgG and IgM significantly (P<0.05) increased in broiler chickens (5 weeks of age) fed diets supplemented with 1.0 and 1.5% CLP (Mahrous et al., 2017). The improvement of IgG and IgM may be due to that clove act as additional bonds with immunoglobulin molecules at the Fc receptors, which stimulated the immune response (Ahmed et al., 2013).

Economic profit

Profitability and economic efficiency of tested diets are showed in Table 6. According the productive performance of rabbits (Table 3) showed improving in FBW (g) for rabbit's groups fed 1.0 and 1.5% CLP supplementation also, FCR improved with all CLP supplementation groups. The incoming selling price per rabbit recorded increasing with 1.5% CLP ($5.98) followed by 1.0% CLP ($5.88) supplementation. The net revenue and economic efficiency showed improving with all CLP supplementation levels. The best economic efficiency of tested diets showed with 1.5%CLP (270.63). Monsi and Onicihi (1991) found that addition of chamomile powder to chicken diets reduced the cost of diets. Karangiya et al. (2016) found that the spices (ginger) supplementation in broiler diets significantly increased incoming from birds selling and feed cost during whole test period while, decreasing the return over feed cost.

Table 6 - The economic profit for growing rabbit tested diets

Items \ Clove buds powder levels	0.0%	0.5%	1.0%	1.5%
Total average weight (kg)	2.25	2.24	2.31	2.34
Price of one kg body weight ($)	2.55	2.55	2.55	2.55
Selling price/rabbit ($)	5.74	5.71	5.88	5.98
Total feed intake (kg)	7.52	6.58	7.08	6.60
Price/kg feed ($)	0.36	0.32	0.28	0.24
Total feed cost/rabbit ($)	2.70	2.10	1.98	1.60
Net revenue ($)[1]	3.04	3.61	3.90	4.38
Economic efficiency[2]	112.91	174.23	196.10	270.63

[1]Net revenue = selling price/rabbit ($) – total feed cost/rabbit ($); [2]Economical efficiency (%) = (Net revenue / total feed cost/rabbit ($)) x 100

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CONCLUSION

It can conclude that using clove buds powder (CLP) as natural supplement in rabbit diets up to 1.5% without any adverse effect on productive performance and vital activities during growing period from 6-14 weeks of age. The supplementation of CLP in growing rabbit diets improved FCR and decreased FI, moreover, had been positive effect on antioxidant enzymes activity and immunity (IgG and IgM titres). Finally, using the CLP supplementation improved the net revenue and economic efficiency, especially at level of 1.5% CLP.

DECLARATIONS

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Authors' contribution

M. A. Suliman designed the experiment and drafted the manuscript; F.G. Ahmed collaborated the statistical analysis and participated in manuscript review; Kh. F. El-Kholy participated in manuscript review; R. A. Mohamed performed the practical part and laboratory analysis; L. Abdel-Mawla collaborated the laboratory analysis. All authors read and approved the final manuscript.

Conflicts of interests

The authors have declared that no competing interest exists.

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EFFECTS OF BIOSECURITY PRACTICES ON THE HEALTH MANAGEMENT SYSTEM OF POULTRY FARMS IN NIGERIA

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The purpose of the study was to determine how Oyo State, Nigerian biosecurity strategies, affected the poultry health management system. The regional data were collected through a planned investigation. The 120 respondents were selected using random and purposeful sampling approaches. The analysis revealed that 43.3% of poultry farmers were between the ages of 31 and 40; the majority were men; 72.5% were married; 37.5% had been in farming for between 11 and 20 years, and 95.5% had one to six children. Most farmers (75.8%) reported that raising poultry was their main source of income; 70.8% stated they got their information from the farmers' association; 95.0% stated burning birds reduced susceptibility to infectious diseases, and nearly all (99.2%) stated keeping foot dips in place stopped the spread of infectious diseases. According to the regression analysis, there is a strong correlation between respondents' age, sex, marital status, agricultural experience, family size, source of income, and adoption of biosecurity methods in the research region. The greatest and most affordable way of infection protection can be found in biosecurity. Without appropriate biosecurity measures, no single disease prevention program will succeed. It is can be concluded that the introduction of additional biosecurity measures could be a significant boost to the prevention and spread of poultry diseases in the study area.

Keywords: Biosecurity strategies, Commercial Farm, Health Management Practices, Infection protection, Poultry.

INTRODUCTION

Poultry occupies a sizable portion of the creature kingdom, with little commerce and sometimes expansive legal frameworks that overwhelm the industry, especially in agricultural countries according to Conan et al. (2012). Adene and Oguntade (2006) reported that about 70% of grills cultivated globally are grown in indoor, severe cultivation frameworks that are extremely similar to one another, in poultry breeding. Furthermore, broilers and occasionally egg-laying birds are commonly raised using deep litter systems with wood shavings to contain the chicken excrement (Riber et al., 2018; Prasai et al., 2018). If not promptly and securely managed by the farmers, this typically results in respiratory illnesses in chickens, sores, and zoonotic diseases (Munir et al., 2019; Bello and Oriola, 2020)

Consequently, the measures taken to prevent the emergence and spread of disease-causing natural agents in flocks of poultry is refers to biosecurity. Poultry producers should practice daily biosecurity measures due to the concentration of poultry surges in energy business creation projects in terms of size and area as well as the typical disorder risks associated with this type of creation (Maduka et al., 2016). According to Cunningham and Fairchild (2020), routine biosecurity measures can reduce the likelihood of birds being exposed to transmissible diseases like Avian Flu and Colorful Newcastle on poultry farms as best management practices.

In addition, modern bird health programs include biosecurity measures to reduce the spread of infectious illnesses across farms and inside individual farms (Dorea et al., 2010). According to Abdelqader et al., (2007), weak biosecurity measures and poor disease control techniques lead to high baseline death rates from infectious illnesses. In addition to assisting in the elimination of management risks brought on by environmental factors like human and animal contact with the poultry environment, biosecurity can prevent financial losses in the poultry farm. Executing biosecurity involves planning, attention, resources, and a perspective of heightened risk and loss of reward (Conan et al., 2012).

However, preventing the spread of infectious illnesses also helps farmers make less money by lowering the expense of treating the infections and the losses brought on by bird death. Additionally, failure to get biosecurity estimates from small-business poultry boards will unquestionably jeopardize biosecurity standards in the modern poultry sector (Negro-calduch et al., 2013). Biosecurity measures are a combination of frameworks and procedures to lessen the presence of

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any disease-deliverers on ranches and so prevent the negative effects of diseases on farms according to Ajewole and Akinwumi (2014).

Despite the industry's strengths and prospects, issues that jeopardize the sustainability of the sector and continued expansion are encountered by poultry producers. It is impossible to ignore the connection between these difficulties and biosecurity measures, which endanger the viability of the poultry business. In light of this context, the following objectives of this study include identifying the personality traits of commercial poultry farmers, figuring out the respondents' sources of information regarding the use of biosecurity practices, determining the respondents' benefits derived from and effectiveness of biosecurity measures, and figuring out the variables influencing the respondents' use of biosecurity in the study area.

MATERIALS AND METHODS

Registered poultry farmers made up the study's population in Nigeria, which was conducted in the state of Oyo. The farmers were contacted to seek their consent before going ahead with the administration of the questionnaire for ensuring ethical allowance of study.

Due to the significant number of commercial farmers in Oyo State's Ido, Oluyole, Akinyele, and Egbeda local governments who are registered with the Nigerian Poultry Association, a purposive sample of these four local governments was chosen. From among the Poultry Association of Nigeria members in each of the local government areas, thirty poultry producers were chosen at random, totaling one hundred and twenty responses. A structured interview schedule was used to gather data from the farmers. Both descriptive statistics (frequency counts, percentages, and averages) and inferential statistics were used to examine the acquired data (regression). Regression analysis is a statistical method used to estimate relationships between dependent variables and one or more independent variables. It can be used to evaluate the strength of the relationship between variables and for forecasting the future relationship between them.

The general equation of each type of regression model is:

Linear regression equation:

$Y=a+bX+bX1+bX2+bX3+ bX4+..... u$

Where,

a = constant; *u*= error term; Y= Biosecurity; X1 = AGE; X2 = Sex; X3 = Marital Status; X4 = Farming Experience; X5 = Size of family; X6 = Source of income; X7 = Membership of Association.

RESULTS AND DISCUSSION

According to Table 1, 43.3% of respondents are between the ages of 31 and 40, 24.2% are between the ages of 41 and 50, and just 10.8% are between the ages of 51 and 60. The majority of responders (70.0%) were men, while just 30.0% were women. Additionally, 10.0% of respondents were divorced, 17.5% were single, and 72.5% of respondents were married. Only 3.3% of respondents had between 41 and 50 years of agricultural experience, compared to 59.2% who had between 1 and 10 years of farming experience and 37.5% who had between 11 and 20 years. Less than half (48%) of the respondents had families with 1-3 members, 47.5% had families with 4-6 members, and 6.7% had 7-9 members per home.

The respondents' age range is deemed to be young, and as a result, they still possess the strength to operate their farms successfully. Poultry farming is a serious endeavour that calls for young farmers to become accustomed to strict biosecurity procedures. This finding agrees with Akpan (2010) argument that youth participation in broiler production has to be increased. In the study area, men are more involved in commercial poultry production, and the majority of them are married, which comes with more responsibilities than being single. As a result, they need to diversify their sources of income to meet household expenses.

However, the average family size was just four people. As a consequence of their modest family sizes, the respondents entered the lucrative commercialized poultry farm production industry and improved their quality of life by providing a healthy diet for their families. This concurs with Arthur (2009) observation that smaller families have better economic and social circumstances, which has a significant impact on a better knowledge of farming. Table 1's data showed that 75.8% of the respondents exclusively farmed poultry as a source of income, whereas 24.2% of the respondents also earned a wage. While 6.7% of farmers and 15.0% of Nigerian Association of Animal Health and Husbandry Technologists members were members of the farmers' association, 23.3% of cooperative society members were (NAAHHT). This suggests that most responders could maximize their output since they were generally dependable.

According to Adene and Oguntade (2006) research, respondents' participation in one or more social organizations helped their farming businesses grow. Being a member of a cooperative or farmers' association gave farmers more access to training sessions offered by those organizations, a channel for requesting government assistance, and chances to raise their quality of life.

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Table 1 - Distribution of respondents according to socio-economics characteristics of poultry farmers.

Variables		Frequency	Percentage	Mean
Age	Below 20	3	2.5	36
	21 – 30	23	19.2	
	31 – 40	52	43.3	
	41 – 50	29	24.2	
	51 – 60	13	10.8	
	Above 60	-	-	
Sex	Male	84	70.0	
	Female	36	30.0	
Religion	Christianity	96	80.0	
	Islam	24	20.0	
Marital status	Married	87	72.5	
	Single	21	17.5	
	Divorced	12	10.0	
	Widow	-	-	
Farming experience	1 – 10	71	59.2	6
	11 – 20	45	37.5	
	21 – 30	0	0.0	
	31 – 40	0	0.0	
	41 – 50	4	3.3	
Family size	1 – 3	55	45.8	4
	4 – 6	47	47.5	
	7 – 9	8	6.7	
Source of income	Salary	91	24.2	
	Self employed	29	75.8	
Member of association	Cooperative	28	23.3	
	Farmers' Association	8	6.7	
	NAAHHT	18	15.0	
	NIAS	12	10.0	
	Poultry Association of Nigeria	14	11.7	

*NAAHHT- Nigerian Association of Animal Health and Husbandry Technologists- NIAS-Nigeria Institute of Animal Science

Table 2 - Respondents' distribution according to sources of information of poultry farmers

Variable	Yes (%)	Frequency of use	
		Regular (%)	Occasional (%)
Veterinarian	69.2	60.0	9.2
Farmer's association	70.8	65.0	5.8
Friends and relatives	59.2	56.2	3.0
Internet	58.3	43.3	15.0
Research institutes/universities	59.2	44.0	15.2
Television	58.3	40.0	18.8

Source of Information

Table 2 data shows that 69.2% of respondents and 70.8% of respondents, respectively, obtained their information from veterinarians and farmers' groups, respectively. Moreover half (59.2%) of the respondents stated that they acquired their knowledge from research institutions and friends/family, respectively. However, 58.3% stated they acquired their knowledge via the internet, while 58.3% watched television to get their information.

Given their proximity to and regular attendance at farmers' associations, where important information and concerns relevant to their poultry company are being addressed, it may be inferred that the majority of respondents obtained their knowledge from these organizations. This strengthens the rationale for why joining friendly organizations has a significant influence on how biosecurity is used. Maningas et al. (2005) contest the notion that having data in farmers' possession entails control over their resources and dynamic cycles.

Table 2 shows the respondents' distribution according to the source of information and its benefits derived from the use of biosecurity measures. Table 3 demonstrates that the majority of respondents adhered to nearly all biosecurity measures, including restricting access to the farm for people and equipment, using disinfectants, administering vaccinations when necessary, and fencing poultry farms among others. Burning dead birds lowers susceptibility to

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infectious diseases. Therefore, biosecurity measures enable farmer's easy-to-use, cost-effective ways to secure their farms, while extension services should be improved to provide enough training to promote output and increase food supply.

Additionally, the majority of respondents exercised biosecurity, such as burning dead birds to lower susceptibility to infectious illnesses and using disinfectants to lower the transmission of infections. Farmers are urged to check their flocks daily if they exhibit any of the following symptoms: a lack of appetite, green or yellow diarrhoea, and watery manure, birds who are struggling to breathe, coughing or sneezing, have decreased egg production.

In filthy conditions, parasitic, bacterial, and viral infections can persist for weeks or even months, but the application of disinfectants reduces mortality and increases the survival rate of the supplied birds. These steps will help to keep infections from spreading and to preserve a thriving and successful chicken industry. Cleanliness and effective biosecurity go hand in hand. This shows that more respondents believe using disinfectants to be extremely useful, which is consistent with Sharma (2010) results.

Table 3 shows the distribution of respondents' benefits derived from the use of biosecurity measures. According to Table 4, the majority of respondents (99.2%, 93.3%, 95%, 88.3%, 85%, 89.1%, and 94.1%, respectively) agreed that "maintenance of foot dip, provision of quality feed," "regular disinfection of poultry equipment and tools," "regular medication/vaccination of birds," and "quarantine of new stock" are efficient ways to ensure biosecurity measures and have reduced the spread of infectious diseases. Given the research conducted by Alhaji and Odetokun (2011) and Henning et al. (2011). According to Sharma (2010), visitors to poultry barns may exhibit severe diseases.

Therefore, a foot dip should be available. In order to eliminate disease specialists from chicken buildings, Mccrea and Bradley (2008) argue that disinfection is essential. Sanitizer footbaths could help reduce the amount of life on boots, but to maintain effectiveness, the sanitizer has to be changed on a regular basis. These have significant effects on reducing the spread of contagious poultry diseases by humans and are important for general health in relation to a few poultry diseases. However, Fasina et al. (2012) observed that failure to implement these biosecurity measures may be due to ignorance, exposure, lack of knowledge, and a lack of equipment.

Table 4 shows the effectiveness of biosecurity measures employed by the respondents. Table 5 shows the outcome demonstrates the importance of age ($P<0.000$), sex ($P<0.013$), married status ($P<0.042$), farming experience ($P<0.000$), family size ($P<0.001$), and financial source ($P<0.032$) to biosecurity measures in chicken production. Farmers of a certain age will benefit more from biosecurity, and Langy and Mekura (2005) found that older farmers had larger wealth accumulation. Additionally, it is believed that older farmers are superior to younger ones since they have more knowledge and experience.

Farmer's experience gained through learning by doing among farmers themselves or by observation or training from different organizations is essential because it will make farmers more effective and efficient because they will have a better understanding of biosecurity practices (Oluwatayo et al., 2008). In this regard, Table 5 shows the factors influencing the use of biosecurity among poultry farmers. Furthermore, financial accessibility ensures that poultry producers have access to practice the majority of these biosecurity measures.

Table 3 - Distribution of respondents' benefits derived from the use of biosecurity measures among farmers

Benefits	Yes	Highly Beneficial	Beneficial	Not Beneficial
Burning of dead birds reduce susceptibility to infectious disease	114 (95%)	98 (81.7%)	10 (8.3%)	6 (5.0%)
Use of disinfectants reduces the spread of infectious diseases	108 (90.0%)	90 (75.0%)	18(15.0%)	-
Regular washing of overalls and boot for field workers and visitors reduced susceptibility to infectious diseases	98 (81.7%)	91(75.8 %)	7(5.8%)	-
Regular cleaning and draining of slaughter house limits the spread of disease	112 (93.3%)	97 (80.8%)	15(12.5%)	-
Fenced poultry farm limits entrance of pests reduces evasion of poultry farm	109 (90.8%)	92 (76.7%)	17(14.2%)	-
Provision of quarantine pen reduces the spread of diseases	103 (85.8%)	91 (75.8%)	8(6.7%)	4 (3.3%)
Use of disinfectants suppress mortality	108 (90%)	105 (87.5%)	3(2.5%)	
Burying of dead animals reduce the spread of infectious diseases	76 (63.3%)	56(46.7%)	8 (6.7%)	12 (10.0%)

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Table 4 - The effectiveness of biosecurity measures employed by the respondents

Biosecurity	Yes (%)	Very effective	Not effective
Maintenance of foot dip	119 (99.2%)	119 (99.2%)	-
Provision of good quality water	75 (62.5)	75 (62.5)	-
Provision of quality feed	112 (93.3%)	112 (93.3%)	-
Regular disinfection of poultry equipment and tools	114 (95.0%)	112 (93.3)	2 (1.7)
Distance disposal lacking of litter/poultry waste.	87 (72.5%)	85(70.8)	-
Regular medication/Vaccination of birds	106 (88.3%)	106 (88.3%)	-
Restriction of movement of customer's vehicles.	98 (81.7%)	91 (75.8%)	7(5.8)
Regular culling of sick birds	106 (88.3%)	106 (88.3%)	-
Regular washing of disinfection of protective clothing's	85 (70.8%)	75 (62.5%)	10.0 (8.3%)
Regular sanitation of the pen.	102(85.0%)	98 (81.7%)	4 (3.3)
Quarantine of new stock	107 (89.1%)	105 (87.5%)	2 (1.7)
Physical examination of birds against deformities	113 (94.1%)	111 (92.5%)	2 (1.7)

Table 5 - The factors influencing the use of biosecurity among poultry farmers

Variable	Coefficient	Std. Error	T-value	Significant
(Constant)	1.697	0.174	9.761	0.0001
Age	-0.126	0.034	-3.688	0.0001[a]
Sex	-0.136	0.054	-2.538	0.013[a]
Marital status	-0.148	0.072	-2.071	0.042[a]
Farming experience	0.040	0.006	6.784	0.000[a]
Size of family	-0.061	0.017	-3.646	0.001[a]
Source of income	0.207	0.094	2.193	0.032[a]
Membership of association	-0.018	0.014	-1.266	0.210
Adjusted R^2	0.557			
P-Value	0.000			

*Note x^a – superscript a means significant

CONCLUSION

According to the study's conclusions, the majority of respondents were married (72.2%), young men (43.3%), with an average family size of four, and had an average amount of agricultural experience of six years. They were also self-employed. The findings imply that most farmers do follow biosecurity regulations which assisted them to prevent and combat the spread of diseases, however, it is still important to take the required procedures to manage arriving animals and people on and around the farm. Because any biosecurity flaw will be disastrous, it is important to promote ongoing training in current biosecurity practices. Therefore, provincial collaboration may streamline the feasibility of a biosecurity program. Any level of biosecurity is beneficial, but the program will be more effective overall if optimal management practices are used by all poultry producers in a particular area. Thus, consistently practicing strong biosecurity measures as part of the best management program can help to reduce the likelihood of contracting a disease, slow the spread of infection in the event of an epidemic, and inevitably boost farmer profits.

DECLARATIONS

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Author's contribution
This work is a project work by G.O. Adeleke supervised by Prof. F.A. Aderemi, co supervised by Dr. Adebamiji Ayandiji. Adebamiji Ayandiji drafted the manuscript while Prof. Aderemi proofed read it.

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There is no conflict of interest

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EFFECTS OF GRADED LEVELS OF DIETARY CHROMIUM-YEAST ON RUMEN AND BLOOD METABOLITES, FEED DIGESTIBILITY, AND PERFORMANCE OF GOATS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The study was conducted to determine the effect of dietary supplementation of Chromium-yeast minerals on consumption, feed digestibility, rumen and blood metabolites, Average Daily Gain (ADG) and body condition score (BCS). The research was conducted at Gunung Tugel Farm, Banyumas, Central Java, Indonesia. The material used was 24 male Jawarandu goats with an average initial weight of 25 ± 1.23 kg, individual cages, the feed given consisted of elephant grass silage and concentrate. The treatment feed contains chromium-yeast at levels of 0, 0.5, 1, and 1.5 mg/kg. The research method used was experimental using a completely randomized design. There were 4 treatments tested, namely T0 (70% concentrate + 30% elephant grass silage), T1 (70% concentrate + 30% elephant grass silage + 0.5 mg/kg chromium-yeast), T2 (70% concentrate + 30% elephant grass silage + 1 mg/kg chromium-yeast) and T3 (70% concentrate + 30% elephant grass silage + 1.5 mg/kg chromium-yeast). Each treatment was repeated 6 times so there were 24 trials. The further test used is polynomial orthogonal. The variables measured in this study were feed consumption, feed digestibility, rumen metabolite products, daily body weight gain and body condition score. The results of the analysis of variance showed that the treatment had a significant effect on dry matter and organic matter consumption, feed digestibility, rumen volatile fatty acids (VFA), ammonia nitrogen (NH3-N) and blood glucose 3 h post-feeding, chromium-yeast levels, and had a very significant effect on ADG and BCS. In conclusion, chromium-yeast supplementation was able to improve goat performance with optimal levels ranging from 1.04–1.36 mg/kg of feed.

Keywords: Body condition score, Chromium-yeast, Daily gain, Goat

INTRODUCTION

Chromium (Cr) was an essential micronutrient. This mineral was an integral part of Gluco Tolerance Factor (GTF) which increases the adhesion of insulin to the cell membrane surface and allows the entry of glucose, fatty acids and amino acids into cells (Lashkari et al., 2018; Mohanty et al., 2022), due to the binding of these (chromodulin) to the insulin receptor transmembrane (Leiva et al., 2017, 2018; Rode, 2017) specifically to the a subunit, converting it to autokinase via phosphorylation of the b subunit (Vincent and Brown, 2019). Although Cr was essential for insulin function and efficient nutrient metabolism (Assis, 2021) in humans and livestock, consumption of Cr in feed was often less than the recommended dose (0.2 mg Cr/kg DM) (National Research Council, 2005).

Growth hormone (GH) and insulin growth factor (IGF) were involved in Cr metabolism (Lashkari et al., 2018; Moreira et al., 2020) in ruminants, Cr has an impact on growth (Moreno-Camarena et al., 2020; Setyaningrum et al., 2022) Cr increased the response capacity of the immune system (Karaulov et al., 2019); In poultry and swine, Cr supplementation had a better positive effect on metabolic responses, nutrient distribution and carcass characteristics, possibly due to increased sensitivity to insulin and glucose utilization efficiency (Mohanty et al., 2022). The degree of this effect was depend on the dietary intake, as well as the chemical form and concentration of Cr (Genchi et al., 2021; Maret, 2019; Zarczynska and Krzebietke, 2020). The trivalent form of Cr cannot pass the cell membrane, hence its activation requires attachment to a ligand (Sánchez-Mendoza et al., 2015); also some inorganic forms of Cr were not available for uptake (Piray and Foroutanifar, 2022), whereas organic sources were more bioavailable. In sheep and cattle administration rates of 0.2–1.2 mg Cr/kg DM have been tested and doses equal to or greater than 0.35 mg Cr/kg DM has an effect primarily on blood glucose levels. As such, positive effects such as increased growth of lean tissue in ruminants, may be expected from using Cr-enriched yeast. There seems a lack of knowledge on the effects of Cr-yeast on goat fattening, hence further study is required.

Citation: Suryapratama W, Munasik, Susanti E, Widiyastuti T, Yuwono P, Prayitno CH. (2023). Effects of graded levels of dietary chromium-yeast on rumen and blood metabolites, feed digestibility, and performance of goats. *Online J. Anim. Feed Res.*, 13(1): 16-22. DOI: https://dx.doi.org/10.51227/ojafr.2023.3

The aim of this study was to evaluate the effect of high concentrations of Chromium-enriched yeast added at different levels in a high-energy diet fed to fattening goats on rumen and blood metabolites, feed digestibility, and growth performance.

MATERIALS AND METHODS

Ethical approval

Care and treatment of animals in this study were conducted in full compliance with the code of ethics for animal experiments, as outlined in Indonesian Law No. 41 of 2014 concerning Amendments to Law Number 18 of 2009 concerning Animal Husbandry and Animal Health.

Material

The material used was twenty-four male Jawarandu goats aged 8-10 months (25 ± 0.17 kg), the goats were dewormed, multivitamins and placed in individual cages (1.5 × 1.2 m) for an adaptation period of 15 days. Goats were fed basal feed (BF) (Table 1) formulated according to the requirements of finishing goats (National Research Council 2007). The basic feed given was recorded every day and feed samples were taken every week, dried at 55 °C for 48 hours to determine the chemical composition (Table 1). Nutrient level was determined by the Association of Official Analytical Chemists (AOAC) method (2012), dry matter (method 967.03), ash (method 942.05) and crude protein (Kjeldahl procedure, method 976.06, N_6.25), metabolic energy was calculated based on composition of feed ingredients (National Research Council, 2007). Chromium was given every day and mixed with feed which was served at 08.00. Feed was given ad libitum twice at 08.00 and 16.00. The feed given was recorded every day. Body weight was recorded at the start of fattening and every two weeks during maintenance. On days 14, 35, 49, 63, 77, and 91, three hours after the morning feeding, blood samples (7 mL) were collected from the jugular vein in a vacutainer tube with potassium oxalate. Blood samples were centrifuged and stored at -20°C to glucose analysis (Kitchalong et al., 1995) with semi-automated equipment (Vitros DT60II-Johnson and Johnson Co.). Besides, chromium-yeast levels were also analyzed to reveal Average Daily Gain (ADG). Rumen fluid was collected by ribs between 3 and 4 by a veterinarian 4 hours after feeding at the end of the study period.

To ensure consumption of the planned dose, the total daily dose of Cr from each treatment was mixed with 30 g of rejected bread flour and given in the morning using individual feeds. The experiment lasted 105 days. Goats were weighed one by one in the morning (07:00) before feeding. The research design used was a completely randomized design (CRD) with 4 treatments and 6 replications so there were 24 experimental units. The treatment in this study were T0 = basal feed (70% concentrate + 30% elephant grass silage, 15.33% CP and 69.44% TDN); T1 = T0 + 0.5 mg/kg chromium-yeast; T2 = T0 + 1 mg/kg chromium-yeast; T3 = T0 + 1.5 mg of chromium-yeast.

Goats were given feed 4.5% of body weight. Basal feed formulations were presented in Table 1.

Table 1 - Nutrient Level of Basal Feed

Feedstuff	g/1000 g DM
Wheat Pollard	308
Rice hull	37.8
Seaweed	25.2
Palm oil meal	105
Soybean Meal	21
Coconut meal	105
CGF	77
Soya shell	35
Mineral mix	10.5
Salt	7
Dolomites	3.5
Kinggrass Silage	300
Nutrients	
DM (%)	67.12
Ash (%)	19.94
Crude Fiber (%)	20.76
Crude Protein (%)	15.06
TDN (%)	66.74
Calcium (Ca, %)	0.6
Phosphorus (P, %)	0.4

CGM: Corn gluten feed, DM: Dry matter, TDN: Total digestible nutrients, Ca: Calcium, P: phosphorus.

Parameter measurement

Daily body weight gain was obtained by weighing the animals every 2 weeks during the investigation period from the beginning to the end of the study before the animals were given feed. The final result of ADG was obtained by the

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difference between the final weight minus the initial weight then divided by the length of maintenance expressed in units of grams/head/day. The formula that was utilized could be explained as follows:

$$\text{ADG} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Days (D)}} = \text{g/head/day}$$

Assessment of the body condition score (BCS) was carried out by the palpation method and observations were made by 3 people as raters. The BCS assessment was carried out by touching the back, base of the tail and hips which were then compared with the BCS table to find out the value.

RESULTS

The results of this study showed that Chromium yeast supplementation has an effect on the consumption of dry matter and organic matter. The consumption value of dry matter and organic matter was highest in the supplementation of 1 mg/kg dry matter. The results of this study indicated that in the T2 treatment where Jawarandu goats were given basal feed supplemented with 1 mg/kg of chromium-yeast mineral, it was able to produce the highest average value of dry matter and organic matter consumption, namely 1130.23±121.38 g/head/day and 542.28±58.03 g/head/day compared to treatment T0, T1, and T3. The results of this study have lower average consumption of dry matter and organic matter compared to the Moreno-Camarena et al. (2020) who conducted a study adding Chromium yeast up to 0.6 mg Cr-yeast.

Other researchers who conducted research on sheep showed that Chromium-methionine supplementation of up to 3 mg/kg would reduce feed consumption (Seifalinasab et al., 2022), while Kargar et al. (2019) highlight increased feed consumption with chromium supplementation in Holstein male calves. Dry matter consumption was the difference between the dry matter level of the feed given and the dry matter level of the rest of the feed, so that the dry matter consumption of livestock was strongly influenced by the dry matter level of the feed. Consumption of dry matter may also be influenced by the crude fiber level in the ration. The high level of crude fiber in the feed would be difficult to be degraded by rumen microbes so that it has an impact on the digestibility of the feed.

Table 2 - Effects of chromium-yeast added at different levels in a high-energy diet fed to fattening goats on rumen and blood metabolites, feed digestibility, and growth performance.

Level of Cr-yeast (ppm) / Variables	T0 (Control)	T1 (0.5 mg/kg)	T2 (1.0 mg/kg)	T3 (1.5 mg/kg)	P-value
DMI (g/d)	1014.47±47.30^{ab}	918±99.31^{a}	1130.23±121.38^{b}	1010.28±78.52^{ab}	0.017
DMI/BW (%)	2.99	2.68	3.07	2.88	
OMI (g/d)	486.98±23.61^{ab}	440.55±46.49^{a}	542.28±58.03^{b}	485.35±37.74^{ab}	0.016
OMI/BW (%)	1.44	1.29	1.47	1.38	
CPI (g/d)	141.67±12.43	157.79±20.85	167.90±21.33	157.80±17.68	0.199
CFI (g/d)	183.84±19.36	196.24±23.77	217.93±29.03	188.07±19.71	0.138
DMD (%)	75.37±2.90^{ab}	71.07±1.47^{a}	76.56±2.69^{b}	73.21±2.63^{ab}	0.015
OMD (%)	75.69±3.44^{ab}	71.21±1.59^{a}	76.83±2.84^{b}	73.00±2.38^{ab}	0.022
CPD (%)	82.88±2.34	80.48±3.21	83.98±2.62	82.02±2.51	0.251
CFDs (%)	59.72±6.01	57.20±4.51	66.43±5.18	58.96±4.96	0.360
ADG (g/d)	131.53±10.48^{a}	119.19±16.57^{a}	162.65±3.76^{b}	177.19±20.78^{b}	0.0002
BCS	2.5±0.24^{a}	2.8±0.16^{ab}	2.61±0.18^{a}	3.0±0.22^{bc}	0.0195
VFA (mMol)	73.2±4.72	86.0±9.57	97.4±12.81	99.6±13.15	0.002
NH3-N (mMol)	27.68±1.5	28.72±1.1	27.08±1.67	30.14±1.98	0.09
Glucose (mg/dL)	57.60±8.12	63.43±12.39	60.57±11.36	54.29±8.48	0.067

^{a,b,} Means with different supercripts row-wise very sigificantly. DMI: Dry matter intake, BW : body weight, OMI : organic matter intake, CPI: Crude protein intake, CFI : Crufe fober intake. DMD: Dry matter digestibility, OMD : organic matter digestibility, CPD : Crude protein digestibility, CFD : Crude fiber digestibility. T0 = basal feed (70% concentrate + 30% elephant grass silage, 15.33% CP and 69.44% TDN); T1 = T0 + 0.5 mg/kg chromium-yeast; T2 = T0 + 1 mg/kg chromium-yeast; T3 = T0 + 1.5 mg of chromium-yeast.

Meanwhile, the consumption of protein and crude fiber in all treatments with the addition of Chromium-yeast showed the same results, which ranged from 141.67±12.43 g/d (T0) to 167.90±21.33 g/d (T2) on protein consumption and 183.84±19.36 g/d to 217.93±29.03 g/d on crude fiber consumption. Even though the effect was not statistically significant, there was a similar trend with the consumption of DMI and OMI in that the T2 treatment (addition of 1 mg/kg) showed the highest consumption of protein and crude fiber. Feed containing chromium could increase the fermentation

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of the ration in the rumen because chromium was an essential mineral for the growth, performance and population of rumen microbes so that the fermentability in the rumen increases (Prayitno et al., 2014; Setyaningrum et al., 2022).

Based on the average value of the digestibility of dry matter and organic matter in Table 3, it showed that the treatment has a significant effect on the digestibility of dry matter and organic matter. The results of this study indicated that in the 1 mg/kg Chromium-yeast supplementation treatment where Jawarandu goats were given basal feed supplemented with organic chromium mineral 1.0 mg/kg of feed was able to produce the highest average digestibility of dry matter and organic matter, namely 76.56±2.69% and 76.83±2.84% compared to the 0, 0.5 and 1.5 mg/kg Chromium-yeast treatments. Digestibility of dry matter was the difference between the amount of dry matter consumption minus the dry matter of faeces divided by the amount of dry matter consumption multiplied by one hundred percent, while the digestibility of organic matter was the difference between the amount of consumption of organic matter minus the organic matter of faeces divided by the amount of organic matter consumption multiplied by one hundred percent.

The results indicated that chromium-yeast supplementation has an effect on daily gain, body condition score, rumen volatile fatty acids, ammonia nitrogen and blood glucose. Chromium as a glucose tolerance factor was able to stimulate the hormone insulin to carry glucose to target organs. It was characterized by the rise and fall of glucose concentrations. Chromium-yeast supplementation increased the concentration of total rumen Volatile Fatty Acids. Rumen volatile fatty acids was the main source of energy in ruminants, thus increase in VFA has a positive impact on growth. Chromium-yeast supplementation 1–1.5 mg/kg gives the most response on daily gain. It was believed that the availability of energy in the form of VFA was also balanced with the optimal availability of N sources (in the form of NH3-N). The fatty acid component of VFA which greatly influences growth was propionic acid. Propionic acid has glucogenic properties which in the liver would be converted into blood glucose. Blood glucose would enter the cells and be used to support the body's fat and protein synthesis as well as a source of ATP (He, 2022). The function of organic chromium added to the feed may help speed up the process of glucose transportation in the livestock body. Organic chromium could increase the activity potential of the insulin hormone which plays an important role in the transport of glucose and amino acids. Increased use of glucose by insulin would then be used by livestock for organ formation, namely the formation of muscle and adipose tissue.

Chromium level result was different with as already mentioned. As such, it can be seen that chromium level was affected by the treatment performed. These results showed that Chromium had normal and higher total VFA levels compared to goats not given organic Chromium.

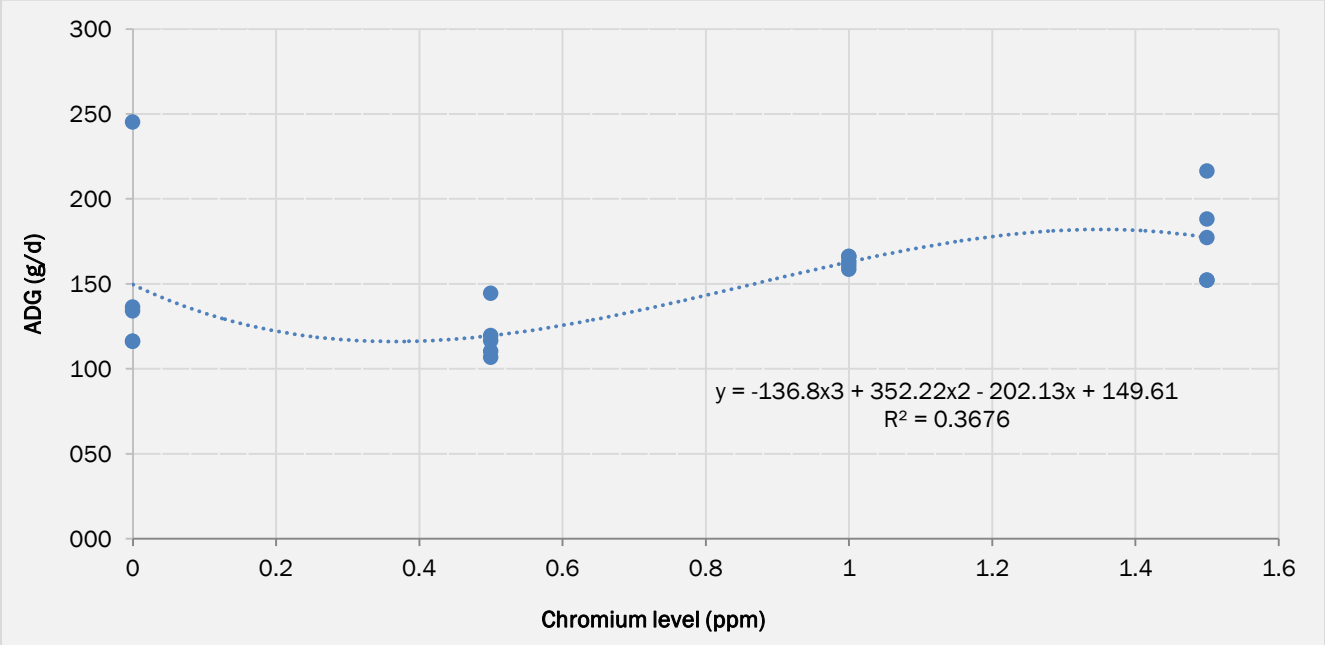

Figure 1 - Average daily gain (ADG) result.

DISCUSSION

Based on the polynomial orthogonal further test results that the use of chromium levels to produce the maximum average daily gain (ADG) was achieved at the level of 1.36 mg/kg. Using chromium at this level results in ADG which was at the top of the curve (Figure 1). The high ADG of goats was supported by the age of the goats used. Goats used as research material have ages ranging from 8-10 months, at this age there was rapid growth, before sexual maturity and slow growth occurs when the body was mature.

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The high ADG in the T3 treatment, which was 177.19±20.78 g/head/day, was due to the provision of good quality feed. The feed given was in the form of 30% forage and 70% concentrate. The feed produced a CP of 15.33% and a TDN of 69.44%. Based on the SNI table for fattening goat feed, the feed given was according to the standard. The high ADG results was supported by chromium-yeast supplementation at a level of 1.5 mg/kg feed. Giving chromium-yeast in the feed would accelerate the digestibility of the feed so that the absorption of feed nutrients for the formation of meat becomes more optimal. The use of Cr-yeast was thought to increase the proportion of propionate. the addition of organic chromium increased the production of ammonia (NH3), total VFA and the proportion of propionate in the rumen (Abdel-Raheem and Hassan, 2021). Propionic acid has glucogenic properties which in the liver would be converted into blood glucose. In this case, blood glucose would enter the cells and be utilized to support the body's fat and protein synthesis as well as a source of ATP (He, 2022; Sultana et al., 2022). The function of chromium added to the feed may help speed up the process of glucose transportation in the livestock body. Chromium-yeast could increase the activity potential of the insulin hormone which contributed in the transport of glucose and amino acids. Increased use of glucose by insulin would be used by livestock for tissue formation, namely the formation of muscle and adipose tissue (Setyaningrum et al., 2022; van der Kolk et al., 2017).

The results of this study also indicated that the treatment given has a very significant effect on the goat's BCS. The highest BCS was achieved in the T3 treatment which was 3.00±0.19. This is was when compared to the study of Ahreza et al. (2020), which has a BCS of 2.64 ± 0.37. These results highlight that the study goats showed ideal body conditions. The ideal BCS indicated that the feed given was of good quality and the management applied was appropriate. The lowest BCS value was achieved in the T0 treatment with an average of 2.5±0.24. This indicated that the T0 feed in the form of elephant grass silage + concentrate has not been able to increase BCS to the fullest. However, this figure was still within the normal/ideal BCS range. BCS value in the T1 treatment (2.8±0.26) higher than the T2 treatment (2.6±0.15). However, this was inversely proportional to the ADG. Treatment on T2 resulted in greater ADG than T1 (162.89±3.87 vs 119.50±17.12). The difference in BCS was caused by many factors, one of which was age. According to Ghosh et al. (2019), as goats get older, goats may lose body fat quickly. This was in line with the fact that the goats used as research material had ages in the range of 8-10 months. The BCS value in the T2 treatment (1 mg/kg) was lower than the T1 treatment (0.5 mg/kg) which may also be suspected because of the lower fat level in T2 goats.

The mean values of BCS T0, T1 and T2 were in the range of 2.5. This figure was still within the normal range and gets the ideal predicate. Visualization that appears in goats was a slightly bony appearance, the backbone was quite visible, the spinous bones look slightly protruding and one third of the transverse bones were visible. When palpating, the transverse bones could be easily felt by the fingers with pressure. The ribs could be felt, the intercostal spaces were smooth but permeable, the muscle areas were of moderate depth and little overlying fat. Factors that affect the BCS of goats include genetics, feed, and applied management.

The mean value of the BCS T3 treatment would be the pressure required to feel the spine. The transverse and spinous bones may be felt by the fingers and there was moderate muscle and fat tissue when pressure was applied. The visual appearance of the goat was somewhat rounded, the spine was not very prominent, the ribs were not clearly visible and the intercostal space could still be felt with a little pressure. Based on the results of the BCS assessment, goats in the T3 treatment received an average rating.

Based on Table 4, treatment T0 where goats were fed basal diet without Chromium resulted in total volatile fatty acids (VFA) levels with an average lower than normal levels, namely below 80 mM, whereas in treatments T1, T2, and T3 where goats were given feed supplemented with organic Chromium produced higher levels Total volatile fatty acids (VFA) with an average that was within normal levels, namely between 80-160 mM. These results showed that in this study goats fed organic chromium had normal and higher total VFA levels compared to goats not given organic Chromium. This was in accordance with the research of Jayanegara et al. (2006) that Chromium supplementation in both inorganic and organic forms resulted in total VFA production within the optimal and proper range for the survival of ruminants, namely between 80–160 mM.

Normal levels of total VFA in goats fed chromium-yeast at T1, T2, and T3 with successive doses of 0.5, 1 and 1.5 mg/kg indicated that the administration of Chromium- Yeast at this dose was safe for the feed fermentation process by rumen microbes. Normal volatile fatty acids (VFA) levels indicated that the feed fermentation process that occurs in the rumen was going well (Sutaryo et al., 2019). This was supported by statements by Prayitno et al. (2014) that the better the growth of rumen microbes may cause the population of carbohydrates digesting microbes to be higher, this causes the process of carbohydrate fermentation in the rumen to run better.

Ammonia nitrogen (NH3-N) in the rumen fluid was the result of the degradation process of protein and non-protein nitrogen (NPN) in the rumen which plays a role in the synthesis of rumen microbial protein. Ammonia nitrogen was used by rumen microbes as the main source of nitrogen to synthesize protein. The concentration of NH3-N in the rumen was an indicator to determine feed fermentability which was related to feed protein digestibility, activity and rumen microbial population. Based on the results of all treatments in this study, the lowest average concentration of NH3-N was 29.2 mM and the highest total NH3-N level was equal to 30.4 mM. This figure was higher than Jayanegara et al. (2006) with NH3-N concentrations of 9.97-13.28 mM in in vitro feeding supplemented with Chromium in organic and inorganic forms. According to Evvyernie et al. (2019), the optimal NH3-N concentration that supports rumen microbial protein synthesis

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was 6-21 mM. The high concentration of NH3-N indicated that some of the feed protein sources were easily degraded, however, concentrations up to 30.15 mM were still safe for the growth of rumen microbes and hosts.

CONCLUSION

From the results of the study, it was concluded that Chromium supplementation in goat feed affects feed consumption, feed digestibility, rumen and blood metabolites, average daily gain and body condition score. The best Chromium-yeast supplementation in optimal fattening goat feed at the level of 1.04-1.36 mg/kg feed.

DECLARATIONS

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Authors' contribution

W.Suryapratama, Munasik, T.Widiyastuti, E.Susanti performed conceptualization, data curation, formal analysis, investigation, methodology, software, validation, writing original draft; P.Yuwono performed conceptualization, methodology, supervision, writing original draft, review and editing of the manuscript for important academic contents.

Conflict to Interest

The authors have not declared any conflict of interest

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CONTROL OF DIPTERIDS IN A FEEDLOT UNDER CONSTRUCTION IN A FOREST AREA OF CENTER REGION OF CAMEROON

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RESEARCH ARTICLE

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ABSTRACT

The forest agro-ecological zone of Cameroon is heavily infested with biting dipterids, but no control is ongoing in this part of the country. In the rainy season (May 2022) in a feedlot under construction in Ndogbea village, eight days entomological study consisting of (i) baseline fly collection using five vavoua traps set in all the sides of a one hectare feedlot yard for four days and (ii) installation of deltamethrin impregnated screens set at 1m from trap and their spraying at frequency of two days in four days. About 1368 biting and non-biting dipterids were collected and classified under five important genera namely *Musca*, *Stomoxys*, *Tabanus*, *Chrysops,* and *Glossina. Musca* spp. Were more frequent than other species. Only one *Glossina fuscipes fuscipes* was identified. The vavoua trap (VT4) facing the forest with canopy trees had the highest fly catches. The apparent density (ADT) of all the fly genera dropped from pre-screen installation phase (ADT=86.8flies/trap/day (f/t/d)) to screen installation phase (ADT= 38.2 f/t/d) with overall fly population density reduction rate of 55.99%. However, there was no statistically significant difference (X^2=35.000; df =30; P=0.243) in population density reduction rates of the various fly-groups. In conclusion, five dipterid groups of veterinary and zoonotic importance constituted the fly-vector fauna of Ndogbea village. The presence of deltamethrin impregnated screens contributed to the fly population density reduction rate of 55.99%. An integrated approach including: animal spraying, herd hygiene, use of traps and screens is needed to maintain low fly numbers in this feedlot.

Keywords: Dipterids, Feedlot, Fly-vector, Forest, Parasite.

INTRODUCTION

Dipterans are common pests of cattle in feedlots in the tropics (Shety et al., 2022; Walker, 2022). Apart from the transmission of some dangerous diseases during blood meals, their irritating painful bites stress the animals leading to behaviour change that result in poor body condition, low milk production and overall poor performance of the farm. Naturally, cattle uses several defensive mechanisms to drive landing insects and some of them include foot stamping, head tossing, tail switching, skin twitching and general aggregation to dilute the frequency of attack (Lendzele et al., 2019). A study conducted in Thailand reported a significant loss in live body weight gain of cattle exposed to insects, estimated at 8.0±1.5 Kg/month (Boonsaen et al., 2021). They also reported that dipterans were responsible for 10-11% loss of live body weight during the main grazing season of feeder-cattle.

Apart from tsetse flies, little is known about other stable dipterous insects in the Center forest region of Cameroon. However, in a rangeland in Yoko, the presence of *Stomoxys* spp. and *Tabanus* spp. alongside tsetse flies have already been reported (Simo et al., 2020). The reverse is true for the Guinean/Sudano-saheian savannas of the Northern regions where these flies have been well studied (Sevidzem et al., 2016; Lendzele et al., 2017; Lendzele et al., 2019). Some common dipterous insect pests of livestock already identified in rangelands of Cameroon include *Musca*, *Glossina*, *Tabanus*, *Haematopota*, *Haematopota*, *Haematobia*, *Stomoxys*, *Culex*, *Anopheles*, *Simulium*, and *Culicoides* (Hiol et al., 2019; Sevidzem et al., 2019).

The control of biting insect pests remains problematic to farmers in developing and developed countries. The frequent use of insecticides in farms has contributed to increase selection of resistant genes in fly populations against some commonly used families of insecticides as already reported in Europe and USA (Olafson et al., 2019). Despite the

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recent reports on the increase resistance of some stable flies population against insecticides, there is no doubt that the use of insecticides remains an important component of control strategies of vector-borne diseases of man and animals (Gratz and Jany, 1994). Several approaches have been devised to control stable flies and a review on some of them have already been published (Cook, 2020). In the Northern region of Cameroon, screens and traps were reported to effectively reduce the population of tsetse flies and improve the body condition score of cattle (Mamoudou et al., 2017). Also, in the tsetse free zone of Ngaoundere, screens and traps were reported to reduce the population of biting flies (Sevidzem et al., 2019). In the Democratic Republic of Congo, the use of tiny screens or targets impregnated with insecticides resulted in a >85% tsetse reduction rate (Tirados et al., 2020).

There is need to test the effectiveness of this control tool against forest biting flies in the Center forest region. To improve farm performance for small scale farmers and ranchers in the Center region, there is need to free their environment from tsetse flies and other mechanical vectors.

The current study aimed to set a control mechanism to reduce the population of biting flies in a cattle fattening pen.

MATERIALS AND METHODS

Description of study area

An entomological survey in the rainy season from 19 to 26 of May 2022 was conducted in Ndogbea village found in the Nyong and Kelle division of the Center region. This village falls within latitude 03.74231 north and longitude 011.23158 east. It is a fast growing village with several ongoing agricultural projects such as cocoa (Figure 1 A), palm oil (Figure 1 A) and cassava plantations (Figure 1 B) as well as livestock projects such as aquaculture and cattle rearing. The study site was a 1 hectare feedlot under construction (Figure 1C), located beside >40 hectares of palm oil and cocoa plantation. This agricultural and livestock site had about 20 workers staying in wooden houses beside the farm (Figure 1D). The vegetation of this site is entirely forest as this area falls within the mosaic-forest agro-ecological zone of Cameroon. A river network, palm trees and forest canopies provide favourable breeding grounds for fly-vectors. During the study month, the average climatic variables were: mean temperature of 30°C, precipitation of 39.3mm, humidity of 85%, and wind speed of 4 km/hr. The four sides of the feedlot yard were ecologically heterogenous and traps were set facing the different sides. The characteristics of the different trap-sites are described in table 1.

Figure 1 - Images of study site. Palm tree and cocoa mix farm (A); cassava farm (B); feedlot under construction (C) and house of workers (D).

Citation: Sevidzem SL, Burinyuy KA, Mintsa Nguema R, Mavoungou JF (2023). Control of dipterids in a feedlot under construction in a forest area of center region of Cameroon. *Online J. Anim. Feed Res.*, 13(1): 23-29. DOI: https://dx.doi.org/10.51227/ojafr.2023.4

Table 1 - Characteristics of trap-points.

Trap code	Site	North	East	Altitude (m)
VT1	Entrance of ranch facing open grass vegetation	03.74216	011.23115	722
VT2	Side of ranch facing resident of palm oil and cocoa plantation workers	03.74237	011.23155	731
VT3	Side of ranch facing resident of palm oil and cocoa plantation workers	03.74224	011.23184	720
VT4	Side of ranch facing the forest with canopy trees	03.74173	011.23200	725
VT5	Side of ranch facing the palm oil plantation	03.74174	011.23142	720

VT: Vavoua trap

Entomological survey

The entomological study comprised of two phases notably (i) pre-intervention and (ii) intervention.

Phase I-Pre-intervention

Baseline data collection on biting dipterids

This phase consisted of collecting biting flies in the feedlot using a blue-black cloth trap known as the Vavoua that has been shown to be effective in the collection of biting and non-biting dipterids in a rangeland in North Cameroon (Sevidzem et al., 2016). Five vavoua traps were set around the fence of the feedlot at an average distance of 40m from each other. All the five Vavoua traps were coded as VT1, VT2, VT3, VT4, and VT5. The emptying of traps was made after every 24hrs for consecutively four days. During each collection session, the following information was documented : date, GPS coordinates and trap code.

Phase II-Intervention

The activities of this phase included the preparation of screens, manual treatment of screens with insecticide and installation in the 1 hectare feedlot yard.

Sewing of screens

Blue-black polyester fabrics were purchased from Ngaoundere main market. The blue fabric (100% polyester, Vestergaard Frandsen, Denmark) had reflectance of around 460 nm. Screens of 150 cm length × 50 cm width in size were prepared by sewing two pieces of 50 cm × 50 cm blue to one piece of 50 cm × 50 cm black (Figure 2).

Figure 2 - A 150 cm length × 50 cm width screen.

Manual treatment of screens with deltamethrin

The insecticide used for the treatment of screens was deltamethrin (DECTROL EC 50; MEDIVET). In the field, the insecticide solution was prepared by diluting 5ml of insecticide in 10L of H_2O as instructed by the manufacturer (Figure 3A). The screens were soaked in the insecticide solution for at least 30 minutes to allow maximum absorption of the product.

Installation of screens

The screens were set atleast 1 m from the vavoua traps (Figure 3B). Flies were collected from traps every 24hours with the screens in place for consecutively four days. Additional 15 screens were set in riverine areas and rangeland beside the pen. Since the study was conducted during the rainy season that was characterised by heavy rains, they were sprayed (Figure 3C) after two days post-installation.

Figure 2 - Preparation, installation and replenishment of screens and traps. A) dilution of insecticides to soak screens; B) installation of screens 100 cm from traps ; and C) spraying of screens after two days post-installation.

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Fly identification

The genus *Chrysops* was identified using the published taxonomic keys of Oldroyd (1957). The *Musca* genus was identified using the morphological key of Gregor et al. (2002). *Stomoxys* spp. were identified using the identification key of Zumpt (1973) and a local color key referring to the landmarks on their abdomen (Sevidzem et al., 2016). The identification of *Glossina* species was carried out using the morphological key prepared by CIRDES (2001).

Data analysis

The abundance of flies was defined as the number of flies per trap per day (f/t/d) from Sevidzem et al. (2022b) as follows:

$$\mathrm{ADT} = \frac{\mathrm{NTC}}{\mathrm{NT\ x\ NTD}}$$

Where, ADT: Apparent density; NTC: Number of tabanids captured; NT: Number of traps; NTD: Number of trapping days

The fly density reduction rate (FDRR) was calculated using the formula from Sevidzem et al. (2019) as follows:

$$\mathrm{FDRR} = \frac{\mathrm{ADTi-ADTf}}{\mathrm{ADTi}}$$

Where, ADTi: initial apparent density; ADTf: final apparent density

The FDRR of the different fly-groups was compared using the Chi-square test. Data was analysed using the JASP 0.13.0.0 statistical software.

RESULTS

Genus composition

The study led to the collection of 1368 dipterans belonging to five genera (*Musca*, *Stomoxys*, *Tabanus*, *Chrysops*, and *Glossina*) of biting insects of medical, veterinary and zoonotic importance (Figure 4). Concerning the five important genera identified in the current study, the population of *Stomoxys* and *Musca* was five times that of *Tabanus*. *Chrysops* and *Glossina* were rarely caught by the vavoua during the study period.

Mean catches of dipterids by trap and period

High catches were made with the VT4 that was set facing the forest with canopy trees. Tsetse fly was only collected by VT1 that was set at the entrance of the ranch facing open grass. For the five important genera, their population dropped post-installation of screens.

Trend of fly catches

For most fly-groups, their population dwindled after the application of a booster spray on screens at day 6 except for *S. omega* that had a delay in their population reduction (day 8).

Fly density

The non-biting muscids *Musca* spp. had highest density, followed by *S. omega, T. fasciatus* but that with lowest density was *G. fuscipes fuscipes*. The densities of the different fly-species dropped tremendously post-screen installation (Table 2).

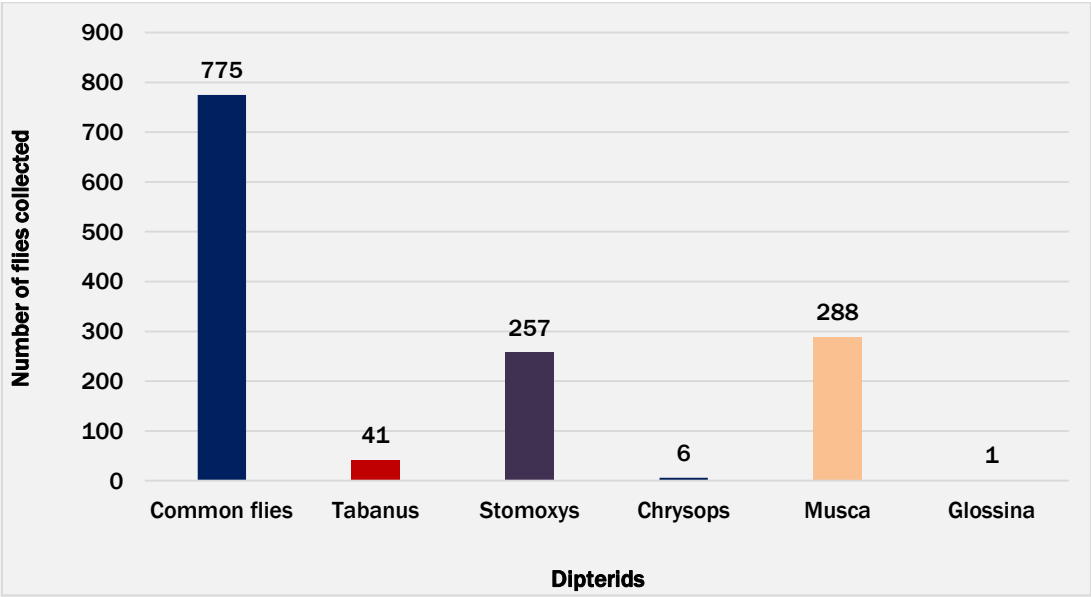

Figure 3 - Composition of dipterids.

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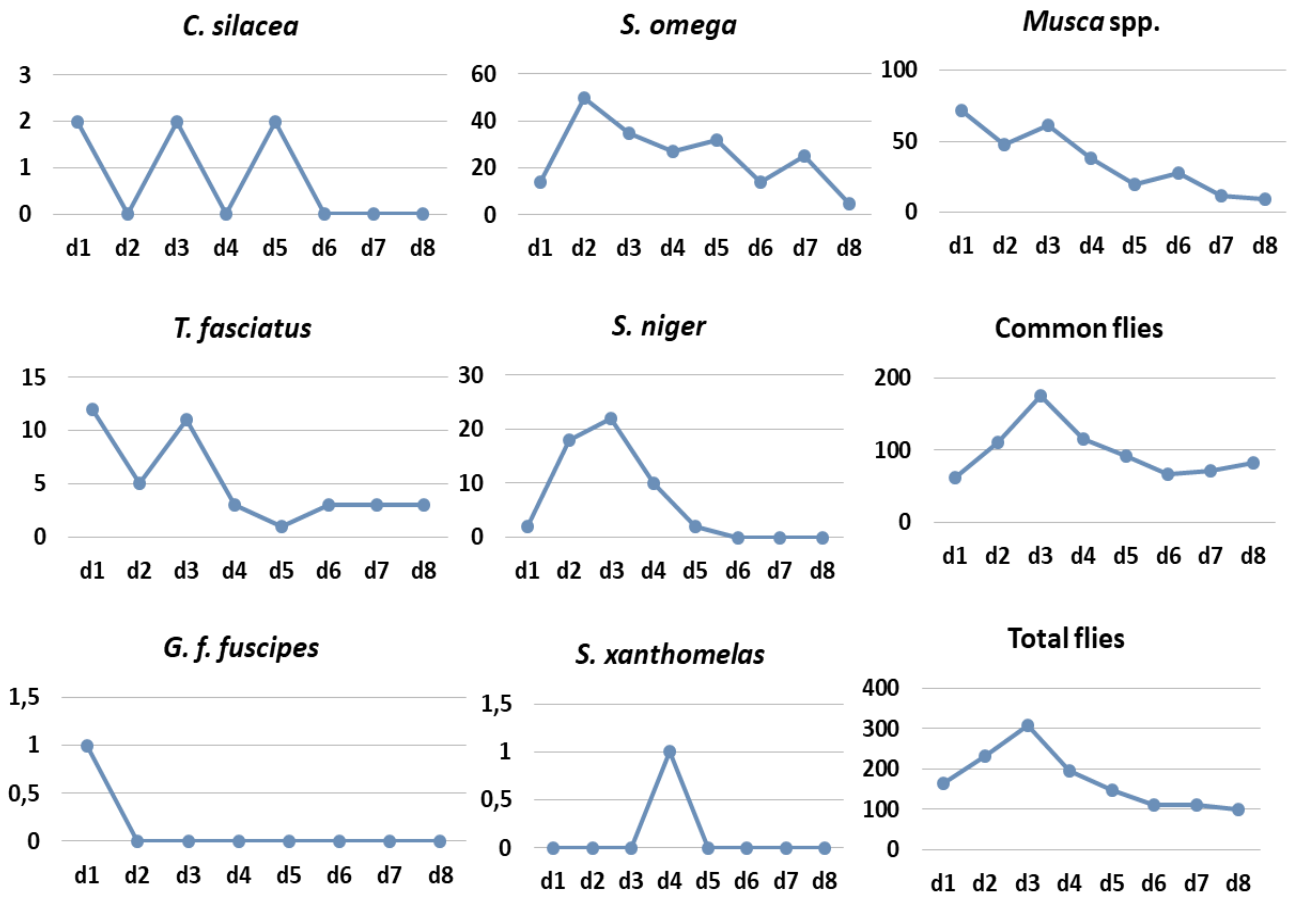

Figure 4 - Trend of fly catches during study period.

Table 2 - The density of dipterids by collection phases.

Phase	*T. fasciatus*	*S. xanthomelas*	*S. omega*	*S. niger*	*S. silacea*	*G. f. fuscipes*	*Musca spp.*
	n (ADT)	n (ADT)	n (ADT)	n (ADT)	n (ADT)	n (ADT)	n (ADT)
Pre-installation of screens	12(2.4)	0	14(2.8)	2(0.4)	2(0.4)	1(0.2)	72 (14.4)
	5(1.0)	0	50(10)	18(3.6)	0	0	48(9.6)
	11(2.2)	0	35(7)	22(4.4)	2(0.4)	0	61(12.2)
	3(0.6)	1(0.2)	27(5.4)	10(2)	0	0	38(7.6)
	21(6.2)	1(0.2)	126(25.2)	52(10.4)	4(0.8)	1(0.2)	219(43.8)
Post-installation of screens	1(0.2)	0	32(6.4)	2(0.4)	2(0.4)	0	20(4)
	3(0.6)	0	14(2.8)	0	0	0	28(5.6)
	3(0.6)	0	25(5)	0	0	0	12(2.4)
	3(0.6)	0	5(1)	0	0	0	9(8.2)
	10(2)	0	76(15.2)	2(0.4)	2(0.4)	0	69(20.2)

n: number of fly catches; ADT: trap apparent density

Table 3 - Density reduction rates for fly species.

Species	ADTi	ADTf	%RR
	X^2=35.000; df =30; P=0.243		
T. fasciatus	6.2	2	67.74[a]
S. xanthomelas	0.2	0	100[a]
S. omega	25.2	15.2	39.68[b]
S. niger	10.4	0.4	96.15[a]
C. silacea	0.8	0.4	50 [b]
G. f. fuscipes	0.2	0	100 [a]
Musca spp.	43.8	20.2	53.88[b]
Total	86.8	38.2	55.99

ADTi: initial apparent density; ADTf: final apparent density; RR : reduction rate. %RRs with similar superscript letters are not statistically significantly different (P>0.05) and %RRs with different superscript letters are statistically significantly different (P<0.05).

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DISCUSSION

The present study with aim to set a mechanism to control biting insects, vectors of dangerous diseases of animals and humans in a 1 hectare feedlot yard under construction in the forest Center region of Cameroon, led to the identification of five dipterid genera notably- *Tabanus*, *Stomoxys*, *Chrysops*, *Musca* and *Glossina*. The different biting fly-groups identified in this study have already been reported in the forest area of Sanaga Maritime of Cameroon by Hiol et al. (2019) and in Peninsular Malaysia (Ola-Fadunsin et al., 2020). Non-biting muscid *Musca* spp. were highly frequent than other fly-groups and this could be linked to favourable conditions for their reproduction and survival during the study period. Non-biting muscids have already been reported to be most abundant in rangelands of the sahel savanna of the North region of Cameroon (Sevidzem et al., 2016). It has been reported that these nuisible dipterids represents biological and mechanical vectors of major cattle diseases in Cameroon (Sevidzem et al., 2021; 2022 a,b). Apart from being vectors of animal diseases, some species like *C. silacea* and *G. f. fuscipes* are biological vectors of *Loa loa* filariasis and *T. brucei gambiense* respectively. During trapping, *C. dimidiata* was observed biting researchers and workers but were never caught by the stationary Vavoua traps. This could possibly be due to their less attractiveness to this trap type and limited trapping days. Similarly, we experienced mosquito bites and observed *Anopheles* temporary breeding sites (stagnant water in tracks created by tires of trucks), but never had a trap to collect and identify adults. Mosquitoes have already been observed biting cattle at night time in a semi-extensive cattle farm in Ngaoundere of Cameroon (Lendzele et al., 2019) and in feedlots in Thailand where they were reported as frequent nocturnal dipterids (Boonsaen et al., 2021). According to Boonsaen et al. (2021), the significant loss in live body weight gain of cattle exposed to these insects was estimated at 8.0±1.5 Kg/month.

The density of fly-groups reduced after the installation of screens with an overall reduction rate of 55.99%. The percentage density reduction rate for *S. xanthomelas* and *G. f. fuscipes* was 100%. This could be due to the low population of the two species during the study period as in the case of *G. f. fuscipes* that was only caught by one trap at day 1 during the pre-screen installation phase. However, in the Democratic Republic of Congo, tiny targets impregnated with insecticide resulted in >85% tsetse reduction rate (Tirados et al., 2020). Similarly, ZeroFly® screen significantly reduced tsetse population in Tanzania (Nagagi et al., 2017). It is important to know that screens or targets were developed to control tsetse flies in Africa and have been reported to be effective in clearing stable flies in farms in USA. Furthermore, the effective treatment frequency of 2 to 3 times insecticide spray per week is required to control tabanids and *Stomoxys* (Bruce and Decker, 1951; Mullens et al., 2006). However, an integrated control approach is most preferred for the control of stable flies (Cook, 2020). Although the current study was only conducted for eight days in the rainy season in one location, there is need to conduct a seasonal, diurnal/nocturnal study in different feedlots to know the fauna of all the biting fly-groups as well as evaluate their economic impact.

CONCLUSIONS AND RECOMMENDATIONS

1368 dipterids were collected and classified under five important genera namely *Musca*, *Stomoxys*, *Tabanus*, *Chrysops,* and *Glossina.* The presence of deltamethrin impregnated screens contributed in fly population density reduction rate of 55.99%. There is need for a seasonal entomological study in this area to understand the population dynamics of these important genera. An integrated approach including animal spraying, herd hygiene, use of traps and screens is needed to maintain low fly numbers in the feedlot. Fly control authorities of Cameroon should extend their control activities to the forest part of the country to enable safe and productive feedlot husbandry practices in this agroecological zone.

DECLARATIONS

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Authors' contribution

S.S. Lendzele performed conceptualization, project administration, data collection, data analysis and preparation of initial draft. K.A. Burinyuy – Data collection, R. Mintsa Nguema – preparation of initial draft, J.F. Mavoungou –finalization of the draft. All authors read and approved the final draft.

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Conflict of interest

None

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SYMPTOMATIC REPONIBLE UMBILICAL HERNIA IN THE RABBIT

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Supporting Information

CASE REPORT

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ABSTRACT: A case of umbilical hernia in a 7-month-old female rabbit was presented to the Surgery Clinic of the Veterinary Faculty, University of Sarajevo. The owner noticed inappetence and lethargy four days before arrival at the clinic. Clinical parameters on physical examination were within the physiological range. Bruxism and lethargy were noted as signs of discomfort due to gas accumulation in colon. A reponible, nonpainful mass in the umbilical scar area was palpated but intestinal peristalsis was not altered. General anesthesia was induced by intramuscular administration of ketamine with medetomidine and maintained with isoflurane. Surgical treatment of hernia included the peritoneal sac dissection and amputation, repositioning of small intestines, and correction of abdominal wall defect. Intraoperative multimodal analgesia approach was used to reduce inhalant anesthesia requirements and to prevent pain-related and stress-related complications. In this case report we described a surgical and veterinary treatment of the reponible umbilical hernia in a rabbit.

Keywords: Herniorrhaphy, Rabbit, Umbilical hernia, Veterinary treatment

INTRODUCTION

Hernias are uncommon pathological findings in laboratory animals. It frequently occurs in domestic animals, such as foals, pigs, calves, and dogs (Monsang et al., 2014). Hernia represents a protrusion of abdominal content through the defect caused by tearing in the abdominal wall or natural abdominal opening (Lassandro et al., 2011, Monsang et al., 2014, Temple, 2018, Yang et al., 2019). Umbilical hernias in animals have a genetic component that can lead to improper closure of the umbilicus at birth or weakened supportive muscles around the umbilicus. Furthermore, it can occur due to cutting the umbilical cord too close to the abdominal wall. Its size varies depending on the umbilical defect and hernia content (Sutradhar et al., 2009, Monsang et al., 2014). During the clinical examination, umbilical hernias are usually represented as a soft mass ventral on the abdomen at the umbilical scar. The umbilical ring can be palpated by deep palpation but not necessarily (Fossum, 2013). Besides clinical examination, radiographs and ultrasound should be used for definitive diagnosis and hernia content determination (Grunkemeyer et al., 2010, Fossum, 2013). In cases where intestine segments are part of the hernia content, the formation of adhesions is most common due to the stimulative adhesive effect in its contact with the skin. That can lead to abnormal digestion primarily due to intestinal strangulation or obstruction. In such cases, the hernia is usually warm and painful, and the content is irreducible (Grunkemeyer et al., 2010, Fossum, 2013, Monsang et al., 2014). Signs of gastrointestinal pain in rabbits are anorexia, weight loss, improper defecation, and depression. In such cases, dehydration usually leads to generalized ileus and aggressive medical treatment is recommended (Reusch, 2005).

Many umbilical hernias can be spontaneously resolved at an early age. Otherwise, it is essential to correct any hernia as soon as possible (Fossum, 2013, Monsang et al., 2014), and herniorrhaphy is recommended corrective method (Grunkemeyer et al., 2010). The most common postoperative complications are hernia recurrence and surgical wound infection. The presence of any abdominal swellings should be used as a differential diagnosis of umbilical hernia including hematomas, seromas, abscesses, and neoplasia (Fossum, 2013). Our case report describes a surgical and medical treatment of the reponible umbilical hernia in the rabbit.

CASE PRESENTATION

A 7-months-old intact female, a mixed breed lop-eared rabbit, was presented to the Surgery Clinic of the Veterinary Faculty, University of Sarajevo, with a 4-day history of inappetence and lethargy. The owner also noticed smaller fecal particles. The rabbit was being housed indoors and fed with commercial rabbit food. Written informed consent was obtained from the owner for the procedures undertaken.

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A patient was 1.2 kg body weight and had a body score condition 3 of 5 by recommended scoring system. During the clinical examination, all clinical parameters were within the physiological range with no obvious signs of ileus. Reponible, nonpainful, soft abdominal mass at the umbilical scar area was noticed. Bruxism was presented and interpreted as a sign of pain. Right lateral abdominal radiography revealed a mass corresponding to the soft tissue opacity in the umbilical area with unclear content. Gas accumulation in the colon was also presented (Figure 1).

Prior to the surgery rabbit received tramadol (TramadolSTADA, 100mg/2ml, AG, Germany) at a dose of 10 mg/kg, SC, meloxicam (Meloxidolor, 5 mg/ml, Dechra, UK) at a dose of 0.3 mg/kg, SC, and enrofloxacin (Enroxil 50mg/ml, Krka, Slovenia) at a dose of 5 mg/kg, SC diluted in sterile saline. Anesthesia induction was achieved with 5 mg/kg of ketamine (Ketaminol 10, Intervet International BV, Netherland) in combination with 0.1 mg/kg of medetomidine (Sedastart 1mg/ml, Dechra, UK) intramuscularly. A 26G intravenous catheter was placed in a marginal auricular vein (*Vena auricularis caudalis*). The combination mentioned above was sufficient to allow endotracheal intubation and anesthesia maintenance was achieved with isoflurane. After the surgical preparation of the ventral abdomen, the patient was positioned in dorsal recumbency with a slight elevation of the head and thorax. Sterile saline (0.9% NaCl) was used intravenously in a dose of 10 ml/kg/h during the surgery and an electric heater was placed to prevent hypothermia.

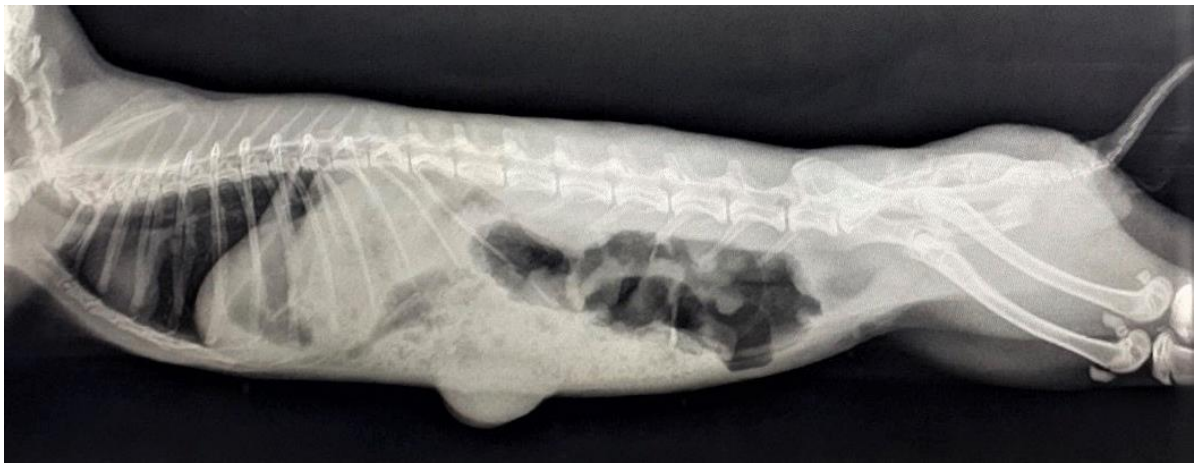

Figure 1 - Right lateral abdominal radiography in the rabbit with umbilical hernia and gas accumulation in the colon

Surgical and medical treatment

After the hernia skin incision, the hernial sac 2 cm in size was identified and dissected. The content of the hernia consisted of small intestine segments with no visible tissue changes, which were repositioned into the abdominal cavity (Figure 2). The hernial ring was corrected and freshened before suturing and gentle organ omentalization was performed. The muscle defect was sutured using a simple continuous suture pattern with 3-0 polydioxanone suture material (PDO). The subcutaneous layer and skin were closed with 3-0 polyglycolide acid suture material (PGA) by a simple continuous and intradermal suture pattern. Postoperative treatment consisted of meloxicam (0.2 mg/kg, PO, q24h) for 5 days, enrofloxacin (5 mg/kg, PO, q24h) and commercially available probiotics for the next 7 days. Promptly after anesthesia recovery, the rabbit showed great interest in eating. The owner was advised to monitor the rabbit closely and restrict all excessive activities. Also, fiber-enriched food was recommended. The fecal output was regular and the healing process was clinically evaluated and completed after 14 days. No complications or reoccurrences were recorded.

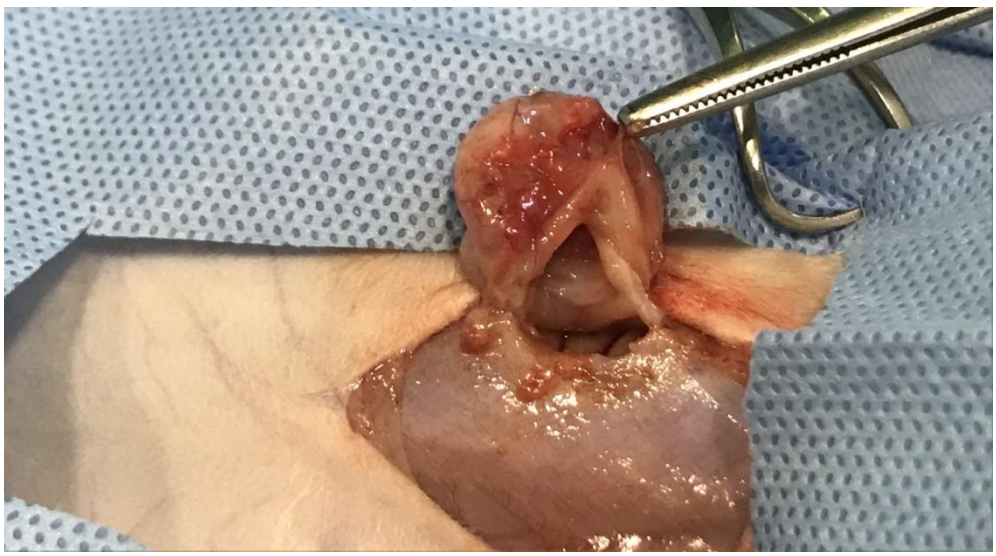

Figure 2 - Peritoneal sac after dissection and repositioning of the small intestine segments

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DISCUSSION AND CONCLUSION

An incidence of umbilical hernia in rabbits is unknown and congenital hernias are the most common, especially in young calves (Al-Sobayil and Ahmed, 2007, Monsang et al., 2014). Clinical examination of suspected hernia can be difficult to perform in cases where pain, obesity or excessive scar formation is present. It can lead to misinterpretation and diagnostic imaging is very important for accurate diagnosis (Lassandro et al., 2011). In veterinary medicine, small umbilical hernias very often heal spontaneously. Contrary, large or persistent hernias usually require surgical intervention such as counter-irritation, clamping, transfixation sutures, safety pins, rubber bands, and elastrator ring (Al-Sobayil and Ahmed, 2007, Pollicino et al., 2007, Monsang et al., 2014). In human medicine, giant hernias are considered if a defect is larger than 12 cm, but in rabbits it is considered as such if the size is between 2.8 and 3.5 cm. It corresponds to higher mortality, morbidity and recurrence rate and usually applies primarily to abdominal hernias (Yang et al., 2019). Even the size of hernia in our case did not required immediate surgical correction, presence of the symptoms could not be ignored and surgery was proceeded. Because the exact cause of umbilical hernia and its occurrence in rabbits is still unknown, intraoperative assessment and surgical reparation must be performed cautiously. In our case, hernial sac was not adhered to herniated visceral content which simplified procedure significantly. The suturing material and pattern used to correct the hernial ring should be carefully chosen. In rabbits, mattress pattern with black braided silk suture material was previously described (Monsang et al., 2014). The use of the same suture material was also reported on sheep and goats and recommended for animals older than 2 years (Al-Sobayil and Ahmed, 2007). As it's described in previous studies, monofilament sutures are more appropriate so we used PDO in our case for hernial ring correction to prevent complications from encapsulating fibrous connective tissue. Unlike PDO, polyglactin 910 can create tissue reaction for an extensive time period (Temple, 2018). Prior to muscle layer suturing, the omentum was carefully positioned over the viscera, preventing direct contact of the viscera with the incision line. Selection of adequate suturing material and visceral organs omentalization prevent potential gastrointestinal stasis, visceral adhesions, granuloma formation, and promote faster wound healing (Engelsman et al., 2007, Mourad et al., 2021).

Common postsurgical complications are heat loss, anorexia, gastrointestinal stasis, visceral adhesion formation, inadvertent organ penetration, peritonitis, abscessation, wound infection, seroma, and surgical wound dehiscence. These complications can interfere with gut motility and cause discomfort and pain in the rabbit. Therefore, it is imperative to maintain sterility during the surgery, and abdominal organs should be kept moist and handled gently during whole procedure (Szabo et al., 2016).

In the presence of any type of stress or pain in rabbits, gut stasis often occurs. It is very important to monitor potential stasis which is considered as an emergency condition. Postoperative care of rabbits should occur parallel with treatment for gut stasis (Varga, 2015). If untreated, the pain will cause gastrointestinal hypomotility, and gas accumulation will lead to more pronounced pain (Oglesbee and Lord, 2020). Because of the presence of reponible and nonpainful abdominal mass, intestinal incarceration was not suspected. Anyhow, bruxism as a sign of discomfort or pain was probably result of the gas accumulation in colon which can lead to the presented clinical signs, but can be result of the beginning of impairment of the intestinal blood supply as well. In already metabolically unstable animals such are those with gastrointestinal disorders, anesthesia and surgery are high-risk procedures. Aggressive medical treatment is necessary especially in prolonged ileus where hepatic lipidosis is a common complication (Reusch, 2005). To maintain gut motility, adequate feeding and hydration of the animal, as well as analgesia and prokinetics are crucial. Some studies showed that high doses of meloxicam in rabbits are well tolerated (Varga, 2015). Meloxicam as a NSAID is effective mainly against somatic and integumentary pain, but also play a role in reduction of adhesion formation in rats (Temple, 2018). In our case, multimodal approach to analgesia, intraoperative gentle and careful manipulation with internal organs, and appropriate postoperative medical and feeding support resulted in the fast recovery of the patient without gastrointestinal syndrome occurrence. Postoperative meloxicam usage will prevent adhesion formation in rabbits that are certainly predisposed to it. Because fecal output was not significantly impaired, absence of prokinetics in our treatment plan did not impact clinical outcome and adequate pain management was sufficient.

In conclusion, it is essential to provide sufficient analgesia and reduce stress in rabbits, parallel with treating any other condition. Good medical care will significantly improve the outcome and survival rate of the patient so stress-related disorders play a huge role in preventive medicine.

DECLARATION

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Authors' contribution

Dr. Ismar Lutvikadić was the veterinarian on patient admission and anesthesia, and was included in treatment plan and manuscript preparation. Dr. Nermina Spahija was included in surgical treatment and manuscript preparation. Dr. Alan Maksimović was included in surgical treatment and manuscript preparation and revision.

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Conflict of interest

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article.

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AVIAN CELLULITIS: A SKIN AFFECTION ASSOCIATED WITH ECONOMIC LOSSES IN BROILER CHICKENS

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Supporting Information

ABSTRACT: This review was designed for focusing on cellulitis condition in broiler chickens regarding causes, clinical picture, and prevention measures of this condition. Cellulitis is an acute diffuse inflammation of subcutaneous tissues and muscles especially on the skin of thighs and abdomen. This condition is more common in broilers than others and it is usually associated with economic losses. At processing, low grade chicken carcasses and high incidences of condemnation are the sequels of cellulitis. Skin integrity, stocking density, and litter conditions are predisposing factors for induction of cellulitis. However, other infectious bacterial and immunosuppressive viral pathogens are associated with cellulitis. Affected birds display areas of yellow skin along with a plaque of pus underneath the skin and the underlying muscles show hemorrhages. Presence of caseous, yellowish to green, dark red, or brown fetid gangrenous exudate could also be observed in the advanced cellulitis cases. Prevention and control of cellulitis are based on application of hygienic practices, vaccination, antibiotic therapy, genetic selection, and nutrition.

REVIEW ARTICLE

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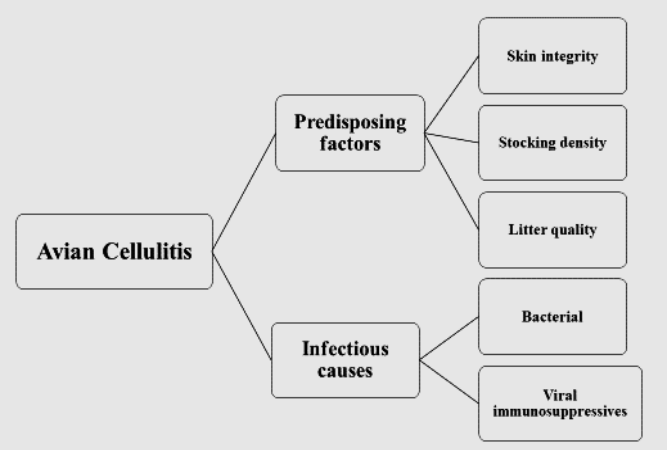
INTRODUCTION

Skin affection is one of the main reasons for broiler carcass condemnation in slaughterhouses (Bergmann et al., 1995). Cellulitis is a skin affection which was described for the first time in Great Britain as necrotic dermatitis, inflammatory process, or infectious process (Randall et al., 1984). Since that time, cellulitis has been established as condemnation category in North America. It can be referred as a consequence of overpopulation and poor flock hygiene rather than a specific disease (Glünder, 1990). From the dermato-pathological point of view, cellulitis can be defined as an acute diffuse and suppurative inflammation of the deep subcutaneous tissues and sometimes the muscles especially on skin of thighs and abdomen (Ghanbarpour et al., 2010). This condition leads to an asymmetrical change of skin texture with uneven discoloration.

Avian cellulitis is a serious problem that has a great economic influence on broiler production system. Cellulitis induces down grading and carcass condemnation at processing with high labor costs to process affected flocks (Salines et al., 2017; Radwan et al., 2018; Silva et al., 2021; Schulze Bernd et al., 2022). Aguiar et al. (2020) noted that cellulitis is one of the most important causes of carcass rejection in slaughter plants. Low grade and rejected carcasses associated with cellulitis have been estimated to cost $30 million to $40 million annually (Norton, 1997). In Germany, the total condemnation ratio was 1.4%, while condemnation due to cellulitis was 0.52%, representing 36.77% of all condemnations (Schulze Bernd et al., 2020).

Skin scratches or abrasions enhances the bacterial colonization of subcutaneous tissues (Norton et al., 1999), resulting in typical cellulitis lesions (Jeffrey et al., 2004). Other predisposing factors including inadequate management and nutrition, cannibalism, biting insects, bad litter quality, bad ventilation, high stocking density, and poor feed quality may induce cellulitis (Schrader et al., 2004).

Accordingly, this review article was designed for focusing on cellulitis regarding the causes, clinical picture, and prevention and control measures in broiler chickens.



Causes

The possible causes of avian cellulitis is represented in Figure 1.

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Predisposing non-infectious factors

Scratched, injured, or traumatized skin is an important predisposing factor for cellulitis induced by *Escherichia coli (E. coli)* (Macklin et al., 1999). The alkaline pH of the skin surface and fragility of the dermal layer produced loose extracellular matrix components. Outbreaks of cellulitis are common in broiler flocks with high stocking density. Rising of the flock density is more likely to result in increasing of birds' nervousness, cannibalism, skin injuries and subsequently entrance of organisms to induce cellulitis (Glünder, 1990). Bird's abdomens are the most contact areas to wet or caked litter, therefore, skin abrasions and the possibility of cellulitis are common in these sites (Marrow, 2008). Besides, wet litter may lead to heavy dirty contaminated nails and consequently increasing the susceptibility to skin abrasions.

Immunosuppressive viruses

Presence of immunosuppressive viral diseases increasing the susceptibility to cellulitis in chickens. Alves et al. (2007) pointed out that cellulitis had been observed in infectious bursal disease virus (IBDV) infected broilers which appeared as yellowish plaques under the skin and subcutaneous tissues. The early study of Rosenberger et al. (1975) revealed that gangrenous dermatitis could be detected following early infection with IBDV and adenoviruses in hemorrhagic-aplastic-anemia syndrome affected broilers. However, cases of staphylococcal gangrenous dermatitis were observed in 17-day-old broiler chicks secondary to IBDV infection (Cervantes et al., 1988).

Bacterial infections

Cellulitis is a necrotizing soft-tissue infection caused by a single microbial or a mixture of aerobic and anaerobic bacteria that act synergistically (Gunderson, 2011).

Escherichia coli

During the past 15 years, the condemnation rates due coliform cellulitis has been increased (Umar et al., 2015). *E. coli* is regarded as the predominant pathogen isolated from cellulitis lesions (Silva et al., 2021) as it has been usually associated with litter contamination (Schrader et al., 2004). Clinical signs of cellulitis are probably observed in case of minimum infection pressure of avian pathogenic *E. coli* (APEC) and possibly also seen with other predisposing factors in the flocks (Barbieri et al., 2013). Moreover, strains of *E. coli* which isolated from cellulitis lesions may produce cytotoxins, causing intense cytoplasmic vacuolization of cells and termed as *E. coli* vacuolating factor (Quel, 2013). About 91.8% of cellulitis cases were associated with APEC infection, while the remainder cases of broiler chickens showed mixed infection with *S. aureus* and other bacteria (Derakhshanfar and Ghanbarpour, 2002). de Brito et al. (2002) isolated *E. coli* from broiler chickens with cervical cellulitis, while de Brito et al. (2003) detected strains of *E. coli* originating from cellulitis lesions in 52 batches of broilers from the southern states of Brazil. In the study of Barros et al. (2013), the results indicated isolation of *E. coli* in 82.5% of cases with cellulitis.

Despite it is not clear which are the specific serotypes of *E. coli* can cause cellulitis in broilers (Jeffrey et al., 2004), *E. coli* induced cellulitis in broilers share a close genetic relationship (Poulsen et al., 2018). Several different of *E. coli* were serogrouped from cellulitis cases, serogroups O78, O1, and O2 were prevalent ones (Fard et al., 2007). An early study of Ngeleka et al. (1996) revealed isolation of 39 *E. coli* associated cellulitis isolates and 38.4% of the isolates were serogroups O25 and O78. Radwan et al. (2018) demonstrated that *E. coli* serogroups recovered from cellulitis lesions were O125; 32%, followed by O158, O55, and O78 as 24%, 12%, and 1%, respectively, then both O1 and O8; 6% for each, and finally O15; 4%. However, large groups of non-serotyped *E. coli* have been also isolated from cellulitis lesions (Macklin et al., 1999). Asadi et al. (2018) demonstrated 34 *E. coli* isolates from broiler carcasses with cellulitis during processing which were belonged to phylogroups; A (55.88%), B1 (5.88%), and D (38.24%).

Staphylococci species

Cases of vesicular dermatitis in chickens have been early reported due to *Staphylococcus epidermidis* (*S. epidermidis*) (Shimizu et al., 1967). However, *S. aureus* was frequently isolated from broiler chicken carcasses with cellulitis (Derakhshanfar and Ghanbarpour, 2002). Radwan et al. (2018) demonstrated that out of 157 bacterial isolates, 8 isolates of *S. aureus* (5.1%) were found in the muscles, liver and heart blood of broilers with cellulitis.

Proteus species

Proteus species were isolated from turkeys had cellulitis in slaughter houses (Olkowski et al., 1999). In the study of Radwan et al. (2018), 9 out of 157 bacterial isolates (5.7%) were identified as *Proteus* species from cellulitis. *Proteus vulgaris* (*P. vulgaris*) was identified in the subcutaneous tissues of cellulitis lesions (Gomis et al., 2002), while *P. mirabilis* was found in 40% of chickens with swollen head (Shawki et al., 2017; Sanches et al., 2020).

Other bacterial species

It has been documented that *Aeromonas* species can colonize the subcutaneous tissues of broiler chickens with cellulitis (Gomis et al., 2002), as well as turkey carcasses in slaughter houses (Olkowski et al., 1999). *Pseudomonas* species were detected in turkey carcasses with cellulitis lesions during processing (Olkowski et al., 1999), particularly, *Pseudomonas aeruginosa* (*P. aeruginosa*) (Gomis et al., 2002). Besides, Shawki et al. (2017) isolated *P. aeruginosa* from 25% of chickens with swollen head syndrome. A mixture of *Clostridium colinum* (*C. colinum*), *C. septicum*, *C. perfringens*, and *C. sordelli* could induce cellulitis (Umar et al., 2015). For the first time, *Salmonella kossen* was isolated from broiler chickens with cellulitis in Egypt (Radwan et al., 2018). *Streptococcus dysagalactae* was also identified from broilers

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chicken carcasses (Vaillancourt et al., 1992). Other bacteria such as, *Pasteurella multocida*, *Enterobacter agglomerans*, *Citrobacter ferundi*, and *Aerobacter* were isolated from cases of cellulitis (Radwan et al., 2018). Some bacteria with public health importance such as *Trueperella* (formerly *Actinomyces*) *pyogenes* and *Erysipelothrix rhusiopathiae* were incriminated as the causes of cellulitis (Derakhshanfar et al., 2004).

Clinical picture

The severity of cellulitis condition is related to some factors such as genetic condition, breed, sex, chick quality, immune status, and skin integrity of the affected bird, the surrounding environment and management conditions, and the feeding quantity and quality (Fard et al., 2007). Affected birds usually do not show any signs, however the lesions are only detected at the slaughterhouses. Usually cellulitis lesions are in apparent and difficult to be detected in the affected birds due to covering of the lesions with feathers (Gomis et al., 2001). The clinical picture of cellulitis could be observed if the infection occurs in the head region as swollen head (Morley and Thomson, 1984). The mortality rate has been recorded especially in cases with septicemia (Ghanbarpour et al., 2003).

Cellulitis could be observed as yellow and thickened skin over the lower abdomen and thigh along with spreading of edematous plaques of pus in the deep subcutaneous tissue, and the underlying muscles may display hemorrhages. Caseated sheets of fibrinous exudates adjacent to the lower abdomen and thigh may be also observed. The severity and the size of the lesions vary from one bird to another, some show localized and well-demarcated pea-size lesions, while others exhibit an extensive purulent inflammation covering most of the abdomen and breast muscles.

Oozing of exudate over the skin "waffle or honeycombed skin" could be also observed over the thigh and muscles, legs, abdomen, head, neck, back, and cloacal area (Alves et al., 2007). Deskinning showed presence of the characteristic plaques, 'flakes' or caseous, yellowish to green, dark red, or brown exudate that it can be fetid if gangrenous (Bianco et al., 2016).

Affected birds with cellulitis may occasionally show concurrent lesions of systemic affections, suggesting that cellulitis may result from systemic spread or, equally, the localized skin lesions may be a source for systemic disease. After experimental cellulitis, the lesions could appear away from the inoculation site that migrated from the dorsal to the abdominal area (Norton, 1997). Silva et al. (2021) demonstrated the relationship between *E. coli* in cellulitis of broiler chickens and the liver lesions as well as the possible systemic infection. Hepatitis, airsacculitis, and pericarditis were frequently associated lesions with cellulitis (Gomis et al., 2001; Silva et al., 2021). Cellulitis-struck broilers may have also lesions in the heart, bones, and joints (Gomis et al., 2001).

Subcutaneous inoculation of 25-day-old broiler chickens with *E. coli* serogroup O78 induced cellulitis lesions in 98% of the inoculated birds and the pathogen was isolated from more than 75% of cellulitis lesions (Gomis et al., 2001). Johnson et al. (2001) experimentally inoculated *E. coli* in broilers to enhance the induction of cellulitis lesions and to detect the other clinical picture of colibacillosis, particularly perihepatitis, pericarditis, and airsacculitis. Moreover, cellulitis and myositis lesions were developed in cage-reared broilers after infection with a mixture of *C. perfringens*, *C. septicum*, and *S. aureus*, following vaccination with IBDV and chicken anaemia virus (CAV) vaccines (Wang et al., 2005).

Microscopically, hyperkeratosis, thickening of the dermis, infiltration of mononuclear cells and heterophils along with fibrinocaseous exudates were associated with cellulitis (Vieira et al., 2006). Moreover, Bianco et al. (2016) defined cellulitis as severe, subacute to chronic, focal to locally extensive fibrinoheterophilic, granulomatous to necrotizing cellulitis, panniculitis, dermatitis, and myositis with irregular hyperkeratosis.

Prevention and control

Cellulitis must be approached as a multi factorial problem as which there is no one solution, therefore it must be multi-levels to deal with the problem. There is no specific preventive measures or available vaccines against cellulites, therefore, reducing the incidence of skin injuries can reduce the incidence rate at processing. Adoption of appropriate farm-specific cloths, changing of shoes, and an adequate cleaning of the broiler houses after each grow out period could improve outcomes regarding condemnation ratios due to cellulitis (Schulze Bernd et al., 2022). Avoid hot environment especially between 2 to 4 weeks of age to stimulate the feathers growth and consequently minimize cellulitis occurrence. Reducing stocking density and overcrowding can reduce the possibility of skin abrasions or bruising and subsequently cellulitis. Increasing drinkers and feeders to reduce the possibility of fighting and skin wounds. Removal of wet litter and replace it by clean and dry one is important. Decreasing slaughtering age and improving the welfare and management policies of poultry flocks can lead to less carcass condemnations due to cellulitis (Fard et al., 2007).

Introduction of genetic lines with a slow growth of feather increases the occurrence of cellulitis. Modern broilers breeds have large chest and abdomen that exposing them to more skin injuries. Therefore, supporting the feathers growth and selection of genetic lines of broilers with a rapid growth of feathers are important to reduce cellulitis.

Regular adoption of vaccination protocols against some viral diseases such as IBDV and CAV may reduce the incidence of cellulitis. Early protection from IBDV can help in the prevention of staphylococcosis (Santivatr et al., 1981). Moreover, trials to produce autogenous vaccines against APEC can help in reducing the incidence of *E. coli* associated cellulites. Treatment with a specific antimicrobials is one of the primary control measures for reducing cellulitis caused by APEC and other bacterial infections. Supplementation with nutrient substances such as vitamin E, zinc, copper, selenium, and manganese can improve the feather development (Macklin et al., 2000). Moreover, the incidence of cellulitis and the

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carcass condemnation rate were significantly reduced in broiler chickens treated with *Bifidobacterium bifidum* (Estrada et al., 2001).

CONCLUSION

Cellulitis represents a serious problem for broilers industry causing great financial losses as a result of low grade chicken carcasses and high incidences of condemnation at processing. Prevention of such condition are difficult due to the multifactorial non-infectious and infectious causes. Bad management practices associated with infectious pathogens increase the incidence of cellulitis. Accordingly, prevention of predisposing management factors through adoption of biosecurity measures is critical. Moreover, treatment of suspected bacterial causes of cellulitis may reduce the severity of this condition.

DECLARATIONS

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Author's contribution
Abd El-Ghany WA has collected and drafted the manuscript, formatted it, and approved the final manuscript.

Conflict of interests
The author has not declared any conflict of interest.

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FERMENTATION OF BLOOD MEAL ABSORBED BY OIL PALM FRONDS WITH *Bacillus amyloliquefaciens* AND *Lactobacillus plantarum*

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study was aimed at improving the efficiency of blood meal (BM) use as feedstuff through the application of agricultural waste absorbance and fermentation technology. Blood was absorbed by oil palm fronds and fermented by using *Bacillus amyloliquefaciens* (BAF) and *Lactobacillus plantarum* (BLP) inoculants in 0, 60, and 120 hour incubation times. Quality was assessed by using Van Soest fiber analysis and *in vitro* digestibility trial on the best fermented product. Results showed that there was significant interaction effect (P<0.05) of inoculant type and fermentation times on the changes in fiber fraction of BM absorbed by oil palm fronds. Inoculant types were found to give significant effects (P<0.05) on ration digestibility rate and *in vitro* rumen condition characteristics. It was concluded that fermentation of BM absorbed with palm oil fronds with BLP in 120 hours resulted in BM with the best fiber fraction reduction, digestibility rate, and *in vitro* rumen condition characteristics.

Keywords: Agricultural waste, Feedstuff, Fiber fraction, Digestibility, Rumen condition.

INTRODUCTION

Agricultural wastes including oil palm fronds have been extensively used as animal feedstuff (Ooi et al., 2017). Blood meal, an animal farming waste, is also a potential source of protein and its availability for animal feed is plenty. It is high in protein (80-95%) and amino acid contents particularly lysine, arginine, methionine, cystine, and leucine but it is lacking in isoleucine and methionine (Odukwe and Njoku, 1987; Kerr et al., 2019). Fresh blood can be obtained from slaughtered cattle by 7-9% of body weight (BW) (Santoso, 1989) and contains 80% water while BM contains 16.5% water (Ramadhan et al., 2015). This indicates that fresh blood may contain 36.5% dry matter (DM). Meanwhile, Setyani and Soenarno (2020) found that cattle weighed 449 kg produced about 58.63 kg fresh blood (13.06% BW) which could be further be processed to produce about 11.73 kg BM (20%) (Ridla, 2014).

Time consuming drying process and low digestibility rate due to high iron content are the constraints found in the utilization of BM. Several processing methods including drying, absorption or mixing, and fermentation have been applied to overcome these constraints. Corn waste (Makinde and Sonaiya, 2011) and cassava peels as a substitute for 50% soybean cake meal (Onyimonyi and Ugwu, 2007) were used in BM processing by using a mixing method. Absorption or mixing method was found to be better than the drying method as the former could improve the utilization of BM up to 15% in poultry ration. This might be attributed to the findings that the absorption method accelerated drying process and improved nutrient quality. Mixing corn waste and BM shortened the drying process to less than 4 days, increased protein content, and reduced environmental pollution (Makinde and Sonaiya, 2007).

Common BM processing by using a heat drying method taking relatively longer time often results in protein denaturation. The use of absorbents is believed to accelerate BM drying process without destructing protein content. Oil palm frond, like other agricultural wastes, is potential to be used as an absorbent material as it contains high fiber (32.87-57.43%) and low protein (5.3%) (Imsya et al., 2013). Wide cell surface of fiber in oil palm frond makes water absorption from blood and blood drying process occur more extensively. In this study, the efficiency of BM production by using oil palm fronds as the absorbent was assessed. The resulted BM was fermented by using BAF and LBP inoculants and their effects on the improvement of nutrient contents ant the use of BM as animal feed were also assessed.

MATERIALS AND METHODS

Site of the study and ethical regulations

The study was conducted at Faculty of Agriculture, Sriwijaya University and supervised by the research committee of the Faculty of Agriculture, Sriwijaya Universisty in compliance with the Government Regulation (PP) Number 95 Year 2012 on Veterinary Public Health and Animal Welfare.

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Blood, oil palm fronds, and bacterial inoculant

Fresh cattle blood was obtained from a slaughterhouse in Palembang City, South Sumatera. Blood was placed in a container containing salt (8 g/liter) to avoid blood coagulation. Blood was immediately stirred and mixed with absorbent (chopped oil palm fronds). Oil palm fronds were obtained from oil palm plantation of Sriwijaya University, Palembang of South Sumatera. Bacterial culture was obtained from Biotechnology Laboratory of Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari, Southeast Sulawesi (BAF FZB42) and Agrotech Laboratory, Yogyakarta (LBP Strain IS-10506).

Experimental design

This study was designed to assess the effects of bacterial inoculant (BAF and LBP) and incubation times (0, 60, and 120 hours) on the quality of BM produced by an absorbance method using oil palm fronds. Six replicates were allocated to each treatment.

Inoculant preparation

Inoculant was prepared by using 100 g rice bran as a medium. Rice bran was sterilized in an autoclave at 121 °C, 1 atm pressure for 15 minutes before it was left cool at room temperature (24°C). A streak of pure BAF culture was diluted in 100 ml distilled water. Ten-millilitre of this mixture was taken and diluted in 90 ml distilled water. This procedure was repeated 6 times until a mixture containing 10^6 cpu of BAF was obtained. No dilution procedure was conducted for LBP culture as it was obtained in the form of diluted one. Diluted culture of both bacteria was each poured into sterilized rice bran medium which was then incubated for 24 hours before it was ready to use in the fermentation process.

Blood meal processing by using absorbent

Blood meal was made by using a method of Makinde and Soniya (2010) with some modifications. Oil palm fronds were chopped into fiber before it was sundried for 24 hours. Chopped oil palm fronds were mixed with fresh blood in 1:1 ratio weight/weight (w/w). This blood-absorbent mixture was dried for 3-4 hours before it was mixed with more blood in 5:4 ratio (w/w). This final mixture was subsequently fermented.

Fermentation process

Aerobic fermentation was conducted in tightly closed plastic containers. Microbial inoculant as much as 3% (w/w) was added to the blood-absorbent mixture. The mixture was further incubated at 40 °C for 0, 60, and 120 hours before they were dried at 60 °C for 24 hours. Dried fermented materials were analysed for their nutrient contents. Fiber fractions were determined by using Van Soest analysis.

In vitro test

In vitro test was conducted to fermented BM-oil palm frond absorbent (BMOPFA) mixtures which were incubated for 120 hours. Three treatments rations including 60% king grass (KG) + 40% BM (P0, control), 60% KG + 40% BMOPFA fermented with BLP (P1), and 60% KG + 40% BMOPFA fermented with BAF (P2) were used.

Digestibility determination

Digestibility was determined by using the in vitro technique of Tilley and Terry (1963). Rumen fluid was obtained from the rumen of a fistulated cow and filtered by using 4-layer cheese clothes. One part of rumen fluid (10 ml) was mixed with 4 parts of media solution (40 ml) consisting of buffer solution, macro and micro mineral solution, resazurin, and reduction solution (Goering and Van Soest, 1970). This mixed solution (50 ml) was added into 1 g of sample placed in a 100-ml incubation tube. CO_2 gas was flown into the tube for 30 seconds before the tube was covered. The filled tubes were incubated for 24, 48, and 72 hours. At the end of each incubation time, 2 drops of $HgCl_2$ were added into each tube. Tubes containing samples and incubation media were centrifuged at 4000 for 10 minutes. Supernatant was taken out from the tubes and analysed for volatile fatty acids and N-NH_3 concentration. Meanwhile, the residue was added with 50 ml pepsin-HCl 0.20% and incubated for 48 hours. When the incubation finished, the solution was filtered by using Whatman No. 41 filter paper and dried at 60 °C for another 48 hours before it was analyzed for its nutrient contents.

Statistical analysis

Data were subjected to an analysis of variance and a Duncan test. Differences were considered to be statistically significant at $P<0.05$.

RESULTS

Changes in fiber fractions of fermented BMOPFA

Types of inoculant and fermentation times gave significant interaction effects ($P<0.05$) on changes in fiber fractions including neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin of fermented BMOPFA (Table 1). Neutral detergent fiber and ADF contents of fermented BMOPFA significantly decreased ($P<0.05$) with

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the interaction of between inoculant type of BAF and BLP and incubation times. Bacterial incubation within 120 hours reduced NDF contents by 1.05 percentage points (BAF)) and 1.60 percentage points (BLP). Significant reduction in ADF content by 2.50 percentage points was found in BMOPFA inoculated with BAF for 120 hours but not in BMOPFA inoculated with BLP. Significant interaction effects of inoculant types and incubation times on hemicellulose, cellulose, and lignin contents of BMOPFA were also revealed. There were fluctuated changes of hemicellulose contents in BMOPFA fermented with both bacteria. Hemicellulose contents were found to increase with the use of BAF and decrease with the use of BLP. However, these changes in hemicellulose contents were not statistically different.

Cellulose contents were found to be significantly reduced ($P<0.05$) as the results of interaction effects of inoculant types and incubation times. Compared to that of unfermented BMOPFA, cellulose content of BMOPFA incubated with bacteria in 120 hour was found to be lower by 3.29 percentage points (BAF) and 5.40 percentage points (BLP). Meanwhile, BMOPFA fermentation within 120 hours by using BAF and BLP was shown to lower lignin content by 0.92 and 1.66, respectively.

Table 1 - Changes in fiber fractions of fermented BMOPFA

Inoculant	Incubation times (hours)	Fiber fractions (%)				
		NDF	ADF	Cellulose	Hemicellulose	Lignin
No inoculant	0	39.11^{c}	27.07^{c}	17.55^{d}	12.04^{ab}	6.69^{c}
B. amyloliquefaciens	60	38.17^{b}	26.87^{bc}	14.32^{b}	11.30^{a}	6.55^{c}
	120	38.16^{b}	26.79^{bc}	14.26^{b}	11.37^{a}	5.77^{b}
L. plantarum	60	38.60^{bc}	26.01^{b}	15.85^{c}	12.59^{b}	5.79^{b}
	120	36.51^{a}	24.57^{a}	12.15^{a}	11.94^{ab}	5.03^{a}

Different superscripts in the same column indicate significant differences ($P< 0.05$), NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Digestibility rates and in-vitro rumen condition characteristics of fermented BMOPFA

It was revealed that inoculant types significantly affected ($P<0.05$) DM and organic matter (OM) digestibility rates of BMOPFA (Figure 1). The highest DM (78.10%) and OM (73.56%) digestibility rates were found in BMOPFA fermented with BLP followed by those in BMOPFA fermented with BAF (66.11 and 60.98%), and those in unfermented BMOPFA (52.59 and 48.54%). Compared to those in unfermented BMOPFA, DM and OM digestibility rates of fermented BMOPFA increased by 48.51 and 51.55% (BLP) and 25.56 and 25.63% (BAF), respectively.

Fermentation also gave significant effects ($P<0.05$) on crude protein (CP) digestibility and rumen N-NH_3 concentration of BMOPFA (Figure 2). BMOPFA fermented with BLP and BAF was found to have increased CP digestibility and rumen N-NH_3 concentration. CP digestibility rates of inoculated BMOPFA were 67.33% (BLP) and 56.08% (BAF). These figures were higher than that (48.72%) of inoculated BMOPFA. These findings indicated that fermentation of BMOPFA with BLP and BAF improved CP digestibility by about 38.42 and 15.11%, respectively. Similar findings were revealed in rumen N-NH_3 concentration, which was higher in inoculated BMOPFA, namely 32.67% (BLP) and 28.67% (BAF), than that in inoculated BMOPFA (25.00%). These were increases of 30.68 and 14.68% of rumen N-NH_3 concentration resulted from BMOPFA fermentation.

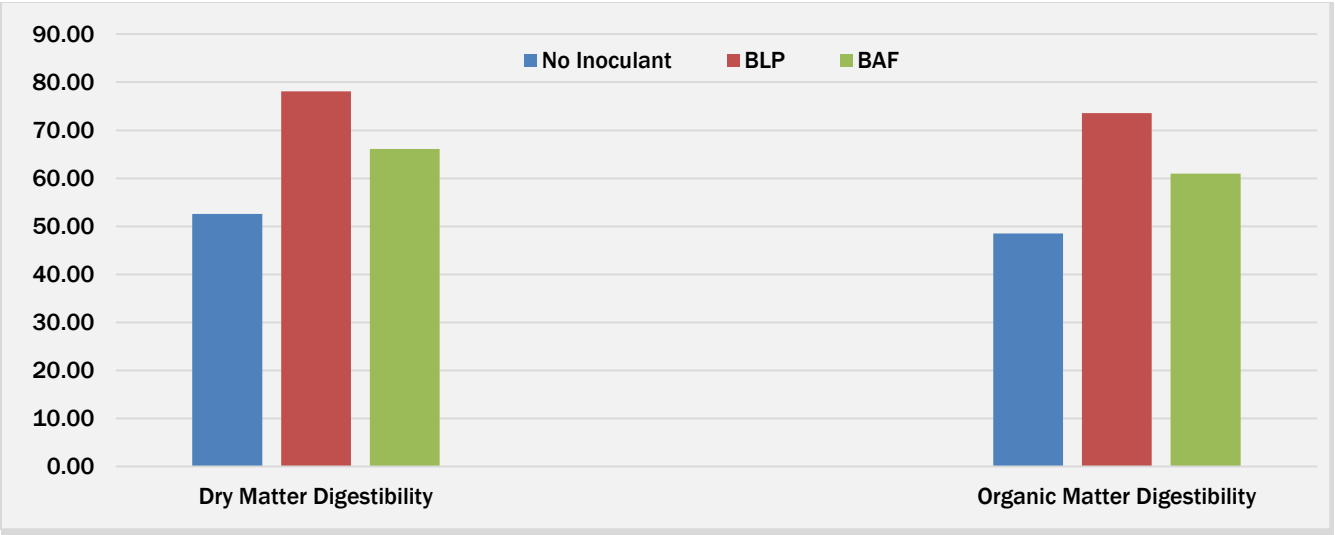

Figure 1 - Dry matter and organic matter digestibility rates of fermented BMOPFA (%). BLP= *Lactobacillus plantarum*; BAF= *Bacillus amyloliquefaciens*

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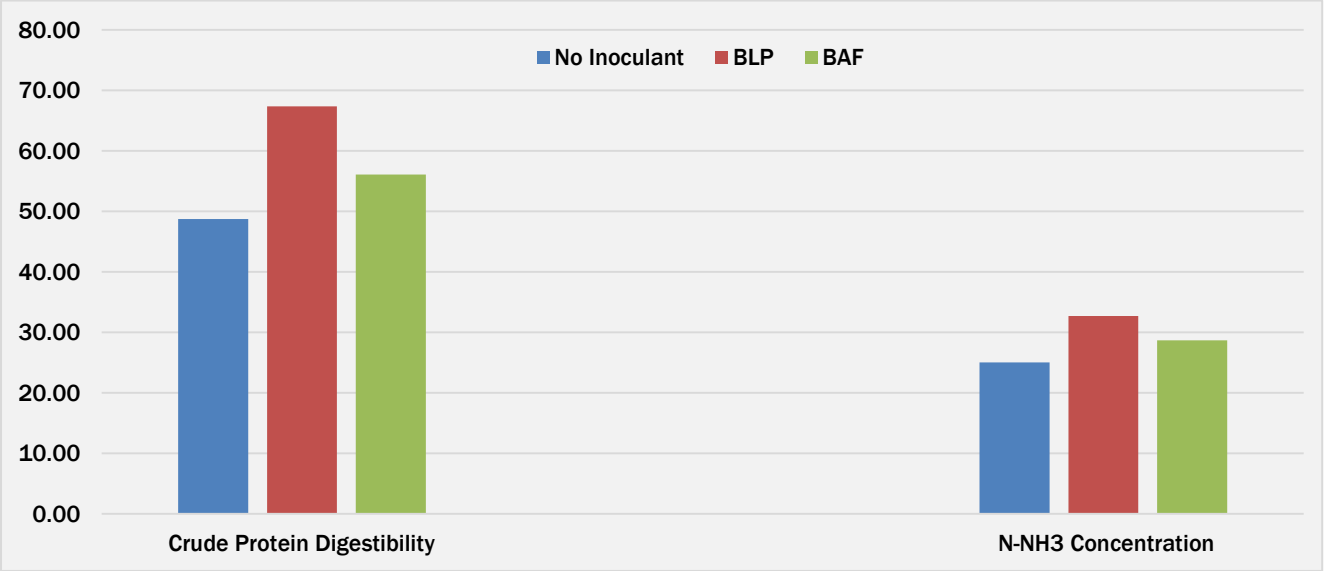

Figure 2 - Crude protein digestibility rates and rumen N-NH_3 concentration of fermented BMOPFA (%). BLP= *Lactobacillus plantarum*; BAF= *Bacillus amyloliquefaciens*

DISCUSSION

In a fermentation process, incubation time and type of inoculant affect nutritive values of fermentation substrate (Wang et al., 2019; Suprayogi et al., 2022). This can be seen from the stages of the fermentation process. In the initial stage, no or very slow growth of inoculant is observed as it is acclimatizing to pH, nutrients, and temperature in a new medium. In an initial (lag) phase, bacteria are adapting to new environment and do not reproduce or undergo cell division (Rolfe et al., 2012). In this lag phase, cell growth but not cell proliferation may take place. As incubation time progresses, inoculants enter exponential phase when and stationary phases. In the exponential phase, bacteria grow very fast while in the stationary phase bacterial growth takes place in the same rate as the bacterial death (Urnemi et al., 2012). Faster growth of inoculant in stationary phase results in production of enzymes to degrade substrates. Changes in dry matter degradation occurs because of the growth of fungi, substrate decomposition, and changes in water content as a result of evaporation, substrate hydrolysis, or metabolic water production (Gervais 2008). Imsya et al. (2013) found that degradation of lignocellulose components in oil palm fronds by fungi increased nutrient availability and improved inoculant growth. Decreased DM and OM contents was caused degradation of OM by fungi which produced more water. This finding was in line with what Dinis et al. (2009) found that cycles of nutrient availability continued to occur during the fermentation process. This led to fluctuations in DM and OM contents as the degradation and utilization of nutrients as an energy source by inoculants progressed. Decreased DM and OM contents of BMOPFA as the substrate used in this study might be attributed to this notion.

Inoculant types also affect nutrient changes in substrate as each inoculant has an ability to produce different kinds of enzymes having different activities. BAFs produce significant amount of amylase making them excellent degraders of simple carbohydrates. Having this characteristic, BAFs are considered as a biocatalyst in starch hydrolysis. Meanwhile, BLPs are cellulase producers (Turker and Ozcan, 2015).

Decreased NDF and ADF contents of BMOPFA in this study might be caused by cell wall degrading activities of enzymes produced by BAF and BLP. Fluctuating decreases in substrate NDF and ADF contents were observed in a fermentation process (Nelson and Suparjo, 2011). Decreases in NDF and ADF contents are the reflections of accumulated decreases in fiber components (cellulose, hemicellulose, and lignin). Cell walls are composed of NDF components including cellulose, hemicellulose, and lignin and ADF components including cellulose and lignin (Van Soest, 2002).

Significant changes in fiber fraction contents, particularly cellulose and lignin of BMOPFA in this study were caused by activities of BAF and BLP inoculants. BAFs and BLPs were found as bacteria which could produce extracellular enzymes including cellulases and hemicellulases (Wizna et al., 2007). In another study by Ramadhan et al. (2016), it was found that the absorption treatments of BM by using coconut pulp and palm kernel cake decreased fiber contents by 6.78-14.72%. Cellulase is a mixture of hydrolytic enzymes which hydrolyse β-1,4-glycocide bonds found in cellulose (Dashtban et al., 2010). Based on their specific activities, these main hydrolytic enzymes include endoglucanase or endo-1-4-β-glucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Rabinovich et al., 2004; MekonnenTeto, 2021). Cellulose degradation is initiated by endoglucanase which hydrolyzes the amorphous part of cellulose to result in end groups of cellulose. These cellulose end groups are further hydrolyzed by cellobiohydrolase to form cellobiose (Rabinovich et al., 2002; Shrotri, 2017). Monomers and dimers are removed from the end of glucan chains by cellobiohydrolase and these dimers are then hydrolyzed by β-glucosidase to produce glucose (Rabinovich et al., 2002).

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Similar kinds of enzymes in greater amount are involved in hemicellulose degradation as hemicellulose is more heterogenous than cellulose (Malherbe and Cloete, 2002). Degradation of hemicellulose results in monomeric sugars and acetic acid. The main carbohydrate contained in hemicellulose is xylan and degradation of it requires collaborative work of various hydrolytic enzymes (MekonnenTeto, 2021). Hemicellulases are divided based on their activities into endo-1,4-β-xylanase (EC 3.2.1.8) producing oligosaccharides from xylan degradation and xylan 1,4-β- xylosidase (EC 3.2.1.37) producing xylose from xylan oligosaccharides. Furthermore, supplementary enzymes including xylan esterases, ferulic and p-coumaric esterases, α-1-arabinofuranosidases, and α-4-O-methyl glucuronosidases which synergically work to hydrolyze xylans and mannans (Perez et al., 2002; Juturu et al., 2013; Houfany et al., 2020). Degradation of O-acetyl-4-O-methylglucuronxylan, the main form of hemicellulose, requires four hemicellulolytic enzymes including endo-1,4-b-xylanase (endoxylanase), acetyl esterase, a-glucuronidase and b-xylosidase. Meanwhile, degradation of O-acetylgalactoglucomannan is initiated with the breakdown of it by endomannases. This is followed by the removal of acetyl groups and galactose residues by acetylglucomannan esterases and a-galactosidases, respectively. In the last stage, endomannases-generated oligomers b-1,4 bonds are broken down by b-mannosidase and b-glycosidase (Perez et al., 2002).

Similarly, lignin degradation also occurs in fermentation processes involving microbial organisms including fungi and bacteria (Janusz et al., 2017). Lignin is the hardest part of cell wall components designed to protect carbohydrates from bacterial degradation. Therefore, in nature, lignin is more effectively degraded by white-rot fungi (Zabel and Morrell, 2020). In ruminant animals, however, rumen bacteria are also found as lignin degraders (Kuhad et al., 2013). There are two important groups of enzymes involved in lignin degradation namely lignin-modifying enzymes (LME) and lignin-degrading auxiliary (LDA) enzymes. Lignin-modifying enzymes including lignin peroxidase, manganese peroxidase, versatile peroxidase, and laccase can work on their own or cooperatively with others. Meanwhile, LDA enzymes cannot work by their own but are required to complete the degradation process (da Silva Coelho-Moreira et al., 2013; Janusz et al., 2017) into simpler components (Nelson and Suparjo, 2011). Changes in lignin degradation in this study were found to be small compared to those of cellulose and hemicellulose. This might be attributed to the fact that the fermentation process in this study were conducted by using bacterial inoculants (BAF and BLP) which were less effective in degrading lignin component than fungi (Zabel and Morrell, 2020).

Increases in BM and OM digestibility of BMOPFA in this study occurred because of changes in nutrient contents resulted from absorbent and fermentation treatments. Fermentation process resulted in decreased fiber fraction contents (Table 1) which, in turn, made it easier for rumen microbes to degrade other nutrients contributing to higher DM and OM digestibility. It is well accepted that DM and OM digestibility is negatively correlated with fiber fraction contents (Cherdthon et al., 2010). Lower fiber content increased DM and OM digestibility and DM digestibility was affected by OM digestibility as OM is part of DM in feeds (Davidson et al., 2003; Griswold et al., 2003; Imsya et al., 2013).

Likewise, increased protein content because of absorbent and fermentation treatments was found to enhance protein digestibility of BMOPFA in this study. Increased activities of proteinase enzymes were observed in BM treated with fermented agricultural wastes (Ramadhan et al., 2016). Fermentation by inoculants degraded protein into simpler peptides and improved protein digestibility. In vitro CP was also found to increase in BM as protein solubility increased (Paz et al., 2013).

Increased CP and OM digestibility was indicated by increased rumen N-NH_3 concentration (Imsya et al., 2013). Rumen N-NH_3 concentration resulted from BMOPFA in this study was adequate to meet the requirement of ruminant animals (10-15.7 mg N/dl) for their optimal growth (Alcaide et al., 2003; Cherdthong et al., 2011).

CONCLUSION

It was concluded that types of inoculants and lengths of incubation time interactively affected changes in concentration and digestibility of fiber fractions of BMOPFA. Fermentation with BLP in 120-hour incubation time produced the best BMOPFA containing 36.51% NDF, 24.57% ADF, 12.15% cellulose, 11.94% hemicellulose, and 5.53% lignin. This BMOPFA had 78.10% DM digestibility rate, 73.56% OM digestibility rate, 67.33% CP digestibility rate, and 32.67 mM rumen N-NH_3 concentration. In-vivo feeding studies on the use of BMOPFA fermented with LBP in 120-hour incubation time and its effects on the performance of ruminant animals are suggested.

DECLARATIONS

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Authors' contribution
All authors contributed equally to this research work. All authors read and approved the final manuscript

Conflict of interests
The authors declare no conflict of interests.

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EFFECTS OF DIFFERENT PROCESSING METHODS ON NUTRIENT CONTENTS AND ACCEPTABILITY OF HOG PLUM (*Spondias mombin* Linn.) LEAF BY WEST AFRICAN DWARF SHEEP

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ABSTRACT: Three experiments were conducted to evaluate the effect of processing method on leaves from *Spondias mombin* tree as fodder for ruminants in the tropics. The leaves were subjected to three different physical processing methods; T1 control (fresh but air drying), T2 (fresh but soaked in ordinary water for 24 h then air drying), and T3 (fresh but soaked in water at 50°C for 20 min then air drying). Nutrient and secondary metabolites content were determined in experiment 1. In experiment II, the Coefficient of preference (CoP) was determined. In vitro gas production was used to predict metabolizable energy (ME), organic matter digestibility (OMD), short-chain fatty acid (SCFA) and methane (CH4) of *S. mombin* leave with different processing methods in experiment 111. Results revealed significant differences in the chemical composition of *S. mombin* leaf subjected to different processing methods. The dry matter value was highest in *S. mombin* leaves soaked in hot water (90.22%), and lowest in *S. mombin* leaves soaked in water at room temperature (85.05%). Crude protein was highest in leaf processed with hot water (11.25 %) and lowest in control (9.59 %). No significant variations were observed for minerals and anti-nutrients investigated. The Vitamin content of leaves of *S. mombin* tree with various processing methods differed significantly except for vitamin E. The preference coefficient value was greater in leaves soaked at 50°C for 20 mins than leaves from the other processing method and control. All leaves of *S. mombin* tree from all processing methods considered in this study were acceptable to the animals, but leaves soaked in 50°C for 20 mins were most preferred. The in vitro gas production parameters and characteristics were not significantly different. In conclusion, *S. mombin* leaf subjected to 50 °C for 20 mins is more advantageous as forage in animal nutrition than unprocessed.

Keywords: *In vitro* gas production, Nutritional value, Processing method; Ruminant, *Spondias mombin* leaf.

INTRODUCTION

The potential of browsing plants as an alternative fodder resource in ruminant nutrition has attracted the attention of researchers worldwide (Mbatha and Bakare, 2018; Medjekal et al., 2020). Several indigenous and exotic browse species have been investigated and evaluated for inclusion in ruminant feeding systems. Unfortunately, farmers' adoption of most of these species faced several challenges, such as pests and disease attacks and the presence of anti-nutritional factors. Therefore, there is the need for continuous screening of browse plants to identify those with good potential as supplements for livestock fodder and which could serve as alternatives to those species that have already been evaluated (Fadiyim et al., 2011). *Spondias mombin*, popularly known as the Caja plant, belongs to the family Anacardiaceae, native to the tropical Americas and have naturalized in many parts of Africa and Southeast Asia (Duarte and Paull, 2015). It is predominant in all Brazilian regions and West Africa (Sofowora, 2013). *Spondias mombin*, a rainforest plant, is commonly found in Nigeria's southwest and coastal region (Fadimu et al., 2018). The leaves are used for animal feed during the drought. Various parts of the plant are being used in traditional medicine (Tabuti et al., 2010).

Similarly, Cordeiro et al. (2021) reported that the leaves of *S. mombin* have antimicrobial and antioxidant substances such as tannins, saponins, resins, sterols, triterpenes, flavonoids, and alkaloids. Njoku and Akumefula (2007) report that the leaves of *S. mombin* are potential feedstuff for ruminants because of their richness in crude protein, vitamins, and minerals. Igwe et al. (2010) reported that the crude protein and fibre content from three different species of Spondias mombin ranged from 11.04 to 14.23% and 8.93 to 10.51%, respectively. This crude protein (CP) content compares well with many other browsing plants. Though, biologically active constituent in plants with potential as animal feeding stuff has been a concern. The treatments to which these plants are subjected could have a significant effect on the availability or unavailability of these constituents to animals (Schmitt et al., 2020; Ntalo et al., 2022).

The use of physical treatment of leaves and seeds in livestock feeds to reduce secondary metabolites has been explored by many researchers. Osum et al. (2013) reported a significant ($P < 0.05$) decrease of micronutrients in the extracts, while it caused a significant ($P<0.05$) increase in the protein, fat, and ash content of the oven-dried leaves of

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Vitex doniana subjected to different processing methods. Sallau et al. (2012) also observed an 88.10%,80.95% and 61.90% reduction of cyanide in the leaf of *Moringa oleifera* after subjection to boiling, simmering, and blanching, respectively. Similarly, following boiling, the trypsin inhibitor in *Arachis hypogaea* L. (groundnut) lowered to 0.09TUI/g from 0.12TUI/g, while the oxalate content also reduced from 3.04 mg/g to 2.62 mg/g. When Asparagus beans (*Vigna Sesquipedis*) were soaked in water, the alkaloids reduced from 0.34 to 0.28%, phytate from 0.18 to 0.09, tannin from 0.23 to 0.09%, trypsin inhibitor from 13.82 to 9.41 TUI/100g: HCN from 8.63 to 5.68% and Saponin from 0.42 to 0.24% (Nwosu, 2010). Most previous studies explored the medicinal benefits of this plant; however, to the best of our knowledge, there are no studies that consider the effect of processing method on the nutritional value and preferences of *S. mombin* leaf for ruminant nutrition.

Therefore, considering this plant's potential to be a browsing plant, this study is designed to evaluate the effect of processing methods on the nutritional value and acceptability of *Spondias mombin* leaves by West Africa Dwarf sheep.

MATERIAL AND METHODS

Ethical approval: Institutional Animal Care and Use Committee Statement (I.A.C.U.C.)

Routine care and experimental protocols used in this study were approved by the Animal Science Unit in Department of Agricultural Science, of Tai Solarin University of Education, Ijagun. Ijebu-Ode, Nigeria, and conformed to published guidelines for ethical conduct and reporting of research in animal science (Jarvis et al., 2005; Kilkenny et al., 2010).

Harvesting of leaves and identification

Spondias mombin leaves were harvested from *Spondias mombin* trees located at the botanical garden of Tai Solarin University. Ijebu Ode, Nigeria and authenticated at the Herbarium unit of the Department of Agriculture at the same University.

Processing techniques

About 15 kg of the leaves were harvested, washed, and processed using different methods for nutrient content evaluation, acceptability, and in vitro gas production. The following processing techniques were used. Fresh and air drying for 48 h (control, T1), Fresh but soaking in water for 24 hours, then air-drying for 48 h (T2), and Fresh but soaking in a water bath of 50 ^{o}C for 20 mins, then air drying for 2-days (T3) as described in the review of Samtiya et al. (2020). Each processing method processed about 5kg of *Spondias mombin* leaves for the nutritional content, acceptability, and in vitro gas production experiments. After this, the leaf samples from all treatments were packed into sealed nylon, labelled accordingly, and kept for experimental use.

Chemical analysis

Dry matter, crude protein, crude fibre, ether extract and total ash of samples were analyzed in triplicates using the standard procedure described in AOAC (2012). The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by the Van Soest (1995) method.

Analysis of minerals

A total of ten minerals were analyzed. All samples were digested with HNO3 / HClO3 mixtures (nitric acid and perchloric acid) (20:5 v/v). The digest was 100 ml in a standard volumetric flask with deionized water. Ca, Na, K, Fe, Cu, Mn, Zn, Mg and Pb in the digest were determined with the atomic absorption spectrophotometer model 420 (Gallenkemp and Co. Ltd). Phosphorus in the digest was estimated with vanadomolybdate solution. The colour that developed was read with a spectrophotometer at 420 nm.

Quantitative determination of Tannin, Saponin, Oxalate and Phytate

Tannin contents were determined as described by Bohm and Kocipai-Abyazan (1994). Peng and Kobayasli (1995) method was used for saponin analysis. While Oxalate and Phytate contents were determined as described by Oke (1969) and Maga (1983).

Determination of Vitamin content

Vitamins A and E were determined as described by AOAC (2000). The water-soluble Vitamin B was determined as follows: B1 as described by Poornima and Ravishankar (2009), B2 as described by Uraku et al. (2014) and B3 as described by Scalar (2000). Vitamin C was determined as described by Hussain et al. (2006).

Acceptability study

Sixteen West African Dwarf sheep of 18±0.5 kg average body weight was individually housed in pen, 1.5×1.5 m in size, using a complete randomized design with four animals per treatment. Each pen has 3 different feeding troughs (150 × 60 cm each) with one water trough. Leaves (about 1 kg) from each processing method were placed in a separate feeding trough and were strategically placed in the pen in the form of cafeteria feeding, with animal having free access to

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each of the three feeding troughs (Mako, 2009). The feed preference study lasted for 10 days, excluding a week for the animals' adaptation to the leaves from different processing methods. The location of feeders was changed every day to prevent the adaptation of the animal to a particular feeding trough. The feeding was allowed from 08:00 to 16:00 hr daily.

The feed consumed was determined by deducting the feed refusal from the quantity offered. Dried samples (about 200 g) of each processing method taken during the 10-day acceptability trial were used to determine the dry matter content. The leaves of processing method preferred were assessed from the Coefficient of preference (CoP) value calculated from the ratio between the intakes of each processing method divided by the average intake of all processing methods (Mako and Babayemi, 2008). Thus, the plant was acceptable, provided the CoP was greater than 1.

***In vitro* gas production**

This was carried out using the method described by Menke and Steingass (1988). A 200 mg milled leaf sample of each processing method was incubated in triplicate with buffered rumen fluid in 120 ml calibrated glass syringes. A total of 12 ewes of West Africa Dwarf sheep were fed concentrate consisting of 40% corn bran, 35% wheat offal, 20% palm kernel cake, 4% oyster shell, 0.5% salt and 0.5% growers' premix for seven days prior to the collection of rumen liquor. Before morning feeding, rumen fluid was collected from the 12 ewes using a suction tube into a pre-heated (39 °C) thermos flask. This was mixed with the buffered mineral solution in the ratio of 1:2 under continuous stirring and flushing with carbon dioxide. Then 30 ml inoculums containing cheesecloth strained rumen liquor and buffer (NaHC03 + Na2HP04 + KCl + NaCl + MgS04. 7H20 + CaCl2. 2H20) were dispensed into a pre-heated sample containing blank syringes and incubated in a water bath maintained at 39 °C. The reading of gas volume was recorded after 3, 6, 9,12,15,18 and 24 h of incubation. The average volume of gas produced from the blanks was deducted from the gas produced per sample. The volume of gas produced at intervals was plotted against the incubation time. From the Graph 1, the gas production characteristics were calculated using the equation, Y= a+b (1-e-ct) described by Orskov and McDonald (1979), where Y= volume of gas produced at the time (t), a = intercept (gas produced from insoluble fraction), c = gas production rate constant for the insoluble fraction (b), and t = incubation time.

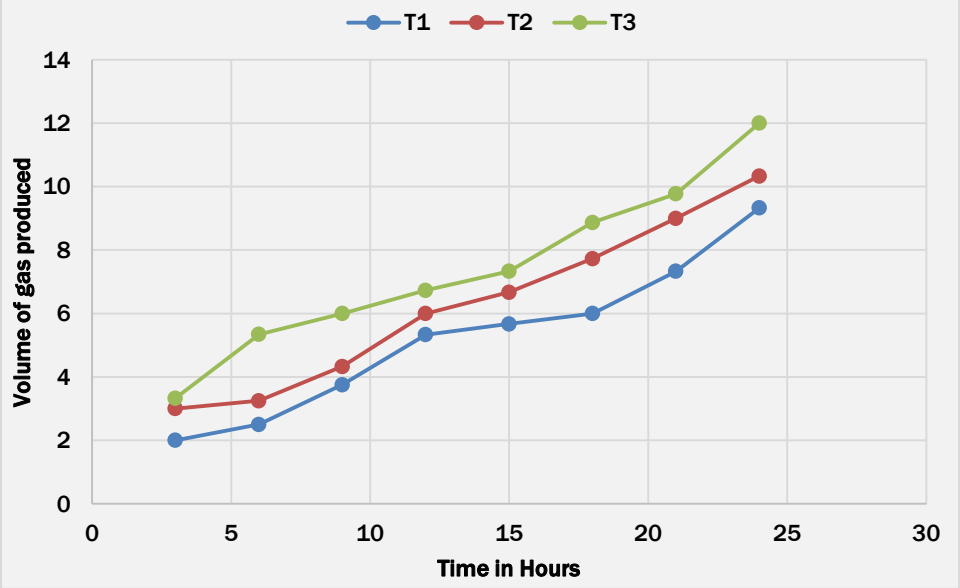

Figure 1 - Gas Production (ml/200mg D.M.) of *Spondias mombin* leaves subjected to different physical processing methods.

The methane gas component was measured at the end of the fermentation period by injecting 4.0 ml of NaOH (10 M) into each syringe containing the incubated samples, following the technique described by Fievez et al. (2005). The measured methane gas volume was related to its respective total gas volume to estimate the methanogenic potential of the digestible OM (Moss et al., 2000). The volume of gas produced after 15 h of incubation was used as an index of energy content and organic matter digestibility (O.M.D.) as described by Menke and Steingass (1988) and short-chain fatty acid (S.C.F.A.) production according to Negesse et al. (2009).

The metabolizable energy (M.E., M.J./Kg D.M.) and organic matter digestibility (O.M.D., %) were estimated as established by Menke and Steingass (1988), and short-chain fatty acids (SCFA, mmol) was calculated as reported (Getachew et al., 1999).

ME = 2.20 + 0.136*GV + 0.057*CP + 0.0029*CF

OMD = 14.88 + 0.889GV + 0.45CP + 0.651XA

SCFA = 0.0239*GV – 0.0601

Where GV, CP, C.F. and X.A. are net gas productions (ml /200 mg D.M.), crude protein, crude fibre, and ash of the incubated samples.

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Statistical analysis

Data obtained were analyzed using SAS's general linear model (GLM) procedure- 2012. Single-factor analysis of variance (ANOVA) was used to assess the effects of the processing method of *Spondias mombin* leaf on nutrient composition, coefficient of preference, in vitro gas production, O.M.D., ME and S.C.F.A. with the following model: Yij = μ + Bi + Eij. Where: Yij is an observation, μ is the overall mean, Bi is the effect of leaf growth stages, and Eij is the experimental error. Significant means were separated by Duncan's (1955) Multiple Range Test. Differences between means were considered statistically significant, P<0.05.

RESULTS

The result of the chemical composition is presented in Table 1. It was observed that all parameters examined varied significantly (P<0.05). The dry matter ranged from 85.05 – 90.22 %, with leaves soaked in water at room temperature for 24 hours having the lowest while leaves soaked in 50 °C for 20 mins the highest value. The crude protein content ranged from 9.59-to 11.25%. The macro and micro mineral content of *S. mombin* leaves subjected to different physical processing methods are presented in Table 2. The result revealed that all leaves from different processing methods contained all minerals investigated. There were no significant variations among the leaves from different processing methods for all the minerals investigated.

Presented in Table 3 is the anti-nutritional content of *Spondias mombin* leaves subjected to different physical processing methods. All the physical processing methods were implicated for all the antinutrients investigated. No significant variation occurred among the different processing methods. The tannin, oxalate, saponin and phytate levels ranged from 0.27-0.88; 0.392-0.432; 0.281-0.397 and 0.6-0.623 %, respectively.

Table 4 presents the vitamin content of leaves subjected to different physical processing methods. All vitamins investigated differed significantly (P<0.05) except vitamin E. In all the vitamins analyzed, the *S. mombin* leaves subjected to hot water (50 °C) for 20 mins had the lowest values while the control had the highest values. Table 5 presents the Coefficient of preference (CoP) of *Spondias mombin* leaves exposed to different physical processing methods by sheep. Table 6 shows the *in vitro* gas production parameters of *Spondias mombin* leaves exposed to different physical processing methods. No significant differences were observed for metabolizable energy (ME), organic matter digestibility (OMD), short-chain fatty acid (SCFA) and methane (CH_4). They ranged from 3.89-4.52 MJ/Kg D.M., 37.13-39.01%, 0.15-0.22 μmol, and 3.50-6.00 ml soaked in hot water for 20 mins, soaked in water at room temperature and the control, respectively.

Figure 1 presents the gas production pattern of the different processing methods *of Spondias mombin* leaves incubated for 24 hr.

Table 1 - Chemical composition (%) *Spondias mombin* leaves subjected to different

Parameters \ Physical processing methods	T1	T2	T3	S.E.M.
Dry matter (DM)	85.05^{c}	89.34^{b}	90.22^{a}	0.42
Crude protein (CP)	9.59^{c}	10.28^{b}	11.25^{a}	0.33
Crude fibre	17.18^{a}	16.64^{b}	15.23^{c}	0.20
Ether extract	8.24^{a}	7.95^{b}	7.50^{c}	0.30
Ash	10.00^{a}	9.79^{b}	8.25^{c}	0.20
Neutral detergent fibre	46.48^{c}	57.94^{b}	55.01^{a}	0.32
Acid detergent fibre	33.63^{c}	35.52^{b}	45.67^{a}	0.40
Acid detergent lignin	10.98^{a}	10.24^{b}	9.56^{c}	0.30

^{abc}= means on the same row with different superscripts differ significantly (*P<0.05); SEM= Standard Error of mean

Table 2 - Macro and micro mineral content of *Spondias mombin* leaves subjected to different physical processing methods

Processing methods	Macrominerals (%)					Micro minerals (mg/kg)				
	Ca	P	K	Na	Mg	Fe	Zn	Cu	Mn	Pb
T1	1.230	0.22	2.156	1.21	0.41	569.0	58.5	13.3	22.4	11.2
T2	1.330	0.17	1.70	1.17	0.38	551.5	54.3	11.0	20.4	9.1
T3	1.341	0. 13	1.65	1.06	0.26	546.1	53.3	10.8	17.6	8.4
SEM	0.29	0.15	0.13	0.30	0.29	10.9	2.5	0.8	7.3	0.9

SEM= Standard Error of the mean (*P<0.05)

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Table 3 - Secondary Metabolites (%) content of *Spondias mombin* leaf subjected to different physical processing methods

Parameters \ Physical processing methods	T1	T2	T3	S.E.M.
Tannin	0.88	0.44	0.27	0.41
Oxalate	0.432	0.408	0.392	0.42
Saponin	0.397	0.368	0.281	0.42
Phytate	0.623	0.617	0.600	0.42

SEM= Standard Error of the mean (*P<0.05)

Table 4 - Vitamin content (mg/100 g) of *Spondias mombin* subjected to different physical processing methods

Vitamins \ Physical processing methods	T1	T2	T3	S.E.M.
A	5.72[a]	5.10[b]	4.38[c]	0.01
B1	0.060[a]	0.040[b]	0.021[c]	0.001
B2	0.31[a]	0.281[b]	0.223[c]	0.001
B3	3.87[a]	3.54[b]	3.21[c]	0.02
C	19.01[a]	17.21[b]	14.43[c]	0.21
E	1.12[a]	1.01[a]	1.10[a]	0.001

[abc]= means on the same row with different superscripts differ significantly (*P<0.05); SEM= Standard Error of mean.

Table 5 - Mean of daily intake of leaves from different parts of *Spondias mombin* and the Coefficient of preference by WAD sheep.

Processing methods	Mean daily (kg/D.M.) consumption by all animals	Coefficient of preference	Ranking
T1	3.26	1.85	3
T2	3.40	1.89	2
T3	4.23	2.05	1

Table 6 - *In vitro* Parameters of leaves of *Spondias mombin* subjected to different physical processing methods

Methods \ *In vitro* parameters	ME (MJ/Kg DM)	SCFA (µmol)	OMD (%)	CH_4 (ml/200mg DM)
T1	3.89	0.15	37.13	6.00
T2	4.12	0.18	38.78	4.15
T3	4.52	0.22	39.01	3.50
SEM	0.24	0.04	1.75	2.00

ME= Metabolizable energy; SCFA= Short chain fatty acid; OMD= Organic matter digestibility; CH_4 - Methane; SEM = standard error of mean. (*P<0.05)

Table 7 - *In vitro* characteristics of *Spondias mombin* leaves subjected to different physical processing methods.

Methods \ *In vitro* characteristics	a (ml/200 mg DM)	b (ml/200 mg DM)	c (ml/h)	a+b (ml/200 mg DM)
T1	1.10	14.45	0.10	16.01
T2	1.30	15.13	0.10	17.23
T3	1.43	18.06	0.11	19.13
SEM	0.1	1.05	0.02	1.52

a= soluble degradable fraction; b= insoluble degradable fraction; a+b= Potential degradability; c= rate of degradation; SEM= standard error of mean (*P<0.05)

DISCUSSION

The proximate analysis results quickly estimate the nutrient content of leaves or feedstuff and show the potential and clues for further research. Though, the proximate analysis of the feed may not be the true reflection of the nutritional value of such feedstuff. It provides the primary guideline in screening feedstuff with the potential of being a browse plant for ruminant animals.

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The dry matter (DM) and the crude protein values obtained in the study are more in the processed leaves than in control. This result agrees with Adebayo et al. (2014) but is lower than the 15.44% CP reported by Omoniyi et al. (2013). The same trend as the dry matter and crude protein was observed for ADF and NDF. These values are lower and at variance with the values reported by Igwe et al. (2010) but in agreement with the values reported by Adebayo et al. (2014). The CP content is more than that proposed as the minimum requirement for growth (113g C.P./Kg D.M.) and lactation (120 CP/Kg D.M.) in ruminants (NRC, 2001). This makes Spondias mombin leaves a good source of protein supplement for low-quality roughage. The values of NDF for physically processed leaves are within the recommended limit of 60.00% guaranteed for forage intake by ruminants (Wanapat et al., 2013). The ash content of the processed leaves was lower than the control but was still high despite the treatment effect. This suggests that many inorganic elements are contained in the leaves (Akinmoladun, 2018; Mishra et al., 2022). The macro and micro mineral content of *S.mombin* leaves subjected to different physical processing methods revealed that all leaves from different processing methods contained all minerals investigated. There were no significant variations among the leaf from different processing methods for all the minerals investigated. However, all the minerals investigated have higher values in control than the processed except for Calcium. This implies that most of the minerals analyzed in this study are vulnerable to water treatment, reducing them when subjected to water treatment. Yet, the values obtained in the processed leaves are within the recommended limit for the proper functioning of the body system and agree with the values reported for Spondias mombin by Ayoola et al. (2010).

Tannins are plant polyphenols that can form complexes with metal ions and macromolecules such as proteins and polysaccharides, thereby protecting them from ruminal degradation (Akubugwo and Ugbogu, 2007; Siemińska-Kuczer et al., 2022). Mlambo et al. (2015) report that 2- 3% of tannin in ruminants' diets is beneficial because it helps in reducing rumen protein degradation by the protein-tannin complex formed. They can reduce feed intake, feed efficiency and weight gain when the value is above 60 to 100g/kg (Ogbe and George, 2012). Saponin suppresses methanogenesis, a significant energy loss to animals (Wang et al., 2009). Agbaire (2012) observed that Saponin higher than 10% in ruminant diets could result in gastroenteritis, diarrhoea, and dysentery. Oxalate can form complexes with most essential trace elements, making them unavailable for enzymatic activities and other metabolic processes. High doses of oxalic acid cause corrosive gastroenteritis, shock, convulsive symptoms, and renal damage (Eneobong, 2001). Phytic acid inhibits the absorption and utilization of some mineral elements (Eneobong, 2001). In this study, the values of all phytochemicals investigated are lower in the processed treatments than in the control. This implies that subjecting S. mombin leaves to water for 24 at room temperature or to hot water (50 ^{o}C) for 20 mins would significantly reduce the secondary metabolites considered in this study. Therefore, S. mombin leaves treated by physical methods in this study lower the antinutrients appreciably without adversely affecting the proximate composition. All phytochemicals investigated were within the safe limit for ruminant consumption, as reported by Teferedgne (2000). This makes the values of these phytochemicals in all the different processing methods in this present study beneficial. Vitamins are organic molecules that are essential micronutrients which an organism needs in small quantities for an efficient functioning of its metabolism system. Vitamins quantity produced via the body is insufficient. Therefore, sources through diet are essential. The values of all the investigated vitamins are lower in the processed leaves compared with the control. This could be because most of these vitamins, except vitamin E, are water-soluble vitamins. However, the values fall within the recommended levels for ruminant animals, as Maduka et al. (2014) reported. The high values of vitamins observed showed that the plant has a high nutritive value which could attenuate physiological oxidative stress due to its high concentration of vitamin C and E (Maduka et al., 2014). Also, feeding this plant to ruminants will boost such animals' immune and reproductive systems because of the high-level vitamin C and E content.

The nutritional content of a feed is crucial; however, an animal's acceptance of such feed is more vital. Hence, this aspect of the study was looked into. The animals' co-efficient of preference (CoP) showed that all leaves subjected to different physical processing methods were acceptable to the animals because the CoP was greater than unity (Mako and Babayemi, 2008). Many factors may influence the acceptability of feed by ruminants. Provenza and Cinocotta (1994) reported that physical plant structure and chemical composition are the most vital factors influencing animals' preference for feed. The crude protein of the leaf subjected to soaking in hot water for 20 mins was the highest. This might be the reason why it is most preferred. Also, this could be because this treatment contains the lowest values of phytochemicals like tannins that can affect the palatability of browse plants compared to others. Similarly, the action of a short time soaking in hot water could make the leaves succulent and soft; hence the most preferred compared with others.

In combination with in vitro digestibility and ME content, chemical composition can be considered valuable indicators for preliminary evaluation of the potential nutritive value of forages (Kafilzadeh and Heidary, 2013). The values of ME, OMD, and SCFA obtained here indicate that animals will obtain energy from the S. mombin leaves subjected to different physical processing methods. Methane production is an energy loss to ruminants. Also, it has environmental implications as a significant contributor to greenhouse gas emissions (USEPA, 2014). Feedstuff that shows a high capacity for gas production is also synonymous with high methane production (Njidda, 2010), hence the reason for high methane production in control compared with others. The result obtained in this present study aligned with the 4.74 MJ/Kg D.M.; 38.03 % and 0.22 µmol reported for Spondias mombin leaf by Omoniyi et al. (2013), but lower and at variance with the report for Spondias mombin leaf by Ogunbosoye and Babayemi (2010). This variance could be due to different processing methods used.

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The same trend as in vitro gas production parameters was observed for a, b, a+b and c. These results are lower and at variance than Ogunbosoye and Babayemi (2010) report for Spondias mombin leaf.

The gas production pattern reflects the effectiveness and extent of forages' degradability because forages with high ruminal degradability of dry matter tend to have high gas production (Njidda and Nasiru, 2010). A high lignin level negatively influences the degradation of fibre and non-fibre (Mizubiti et al., 2011). The lignin content obtained in the control treatment in this study is high, hence the low volume of gas produced. There was a steady increase in the gas production for 24 hours; no significant variations were observed in the gas volume produced at every interval. Many factors may determine the amount of gas produced during fermentation, depending on the nature and level of fibre, the presence of secondary metabolites and the potency of rumen liquor (Babayemi et al., 2004). The values observed in this present study agree with the report for Spondias mombin leaf by Omoniyi et al. (2013) but are lower and at variance than the 43 – 175 ml reported by Ogunbosoye and Babayemi (2010). This could be attributed to the processing methods implored in the research. The processed treatments have lower lignin content compared with unprocessed. The low volume of gas production of the Spondias mombin leaf in control connotes the low digestibility of the leaf, which may be due to the higher values of anti-nutritional factors and fibre compared with the processed.

CONCLUSION

The nutrient, antinutrient, mineral, vitamin content, and *in vitro* gas production fermentation revealed that Hog plum (*Spondias mombin*) leaf has potential forage for ruminants when subjected to physical processing methods such as soaking in hot water (50 °C) for 20 mins. In addition, animals prefer Hog plum leaves soaked in hot water (50 °C) for 20 min. compared with ordinary air-dried leaves before feeding.

DECLARATIONS

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Authors' contribution
Conceptualization, investigation, writing, data collection and methodology were carried out by Ikusika Olusegun and Adejoke Mako. While Thando Mpendulo and Ikusika did the statistical analysis, supervision, and validation of the research work.

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Data Will be available on request.

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EFFECT OF DIFFERENT LEVELS OF FERMENTED WATER HYACINTH LEAF MEAL ON FEED UTILIZATION AND PERFORMANCE OF JUVENILE NILE TILAPIA

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study was conducted to evaluate the effects of different inclusion levels of water hyacinth leaf meal fermented with *Aspergillus niger* on feed utilization efficiency and growth performance of Nile tilapia (*Oreochromis Niloticus* L.). Fermented water hyacinth leaf (FWHL) at 0, 10, 20 and 30% inclusion levels were incorporated into four isonitrogenous (35% CP), and isoenergetic (18 KJ g^{-1} g) test diets. The fishes were stocked in 80 liters aquarium units, in a closed, recirculating indoor system. The diets were fed to triplicate groups of fish fingerlings (1.6 g average body weight) twice a day, at 6% of body weight/day, for three months. The study demonstrated that Nile tilapia fed FWHL at levels 30% had a significant negative impact ($P<0.05$) on weight gain, specific growth rate, feed utilization efficiency, and whole body composition. But, there was no significant change on the performance of Nile tilapia fed diets supplemented with 10% and 20% FWHL when compared with the control group. Therefore, supplementation of fermented water hyacinth leaf meal to diets of Nile tilapia is recommended up to 20% because it is cheaper than fish meal and corn.

Keywords: Body composition, Feed utilization, Growth performance, Nile tilapia, Water hyacinth

INTRODUCTION

Since 1984, aquaculture has experienced the world's fastest rate of animal production growth. Today, more than 61.5% of all fish are produced through aquaculture. With the exception of Sub-Saharan Africa, aquaculture is growing, intensifying, and developing almost everywhere in the world as the demand for aquatic food products is anticipated to rise (FAO, 2012). According to the World Bank's 2013 report, Sub-Saharan Africa's per capita fish consumption will fall by 1% annually to 5.6 kg between 2010 and 30. However, the total demand for fish food would increase significantly (by 30% between 2010 and 2030) as a result of the population's rapid growth, which is projected to average 2.3% annually between 2010 and 2030. Its development can lessen the strain on natural resources and help meet future food needs (FAO, 2006).

The price of conventional feedstuffs has continued to rise due to their scarcity and is no longer affordable for rural livelihoods, despite the significance of aquaculture production (Téguia et al., 2008; Naylor et al., 2021). The price of fish feed makes up between 50 and 60 % of the total operating costs of aquaculture production (Workagegn et al., 2013). The development of low-cost aquaculture systems suitable for small-scale farmers in the developing world has been severely hampered by the scarcity of and high price of conventional pelleted fish feed (Fagbenro and Arowosegbe, 1991; Kaleem and Sabi, 2021; Moyo et al., 2021).

Researchers, particularly those who specialize in fish nutrition are looking for alternative feed components or processes to create environmentally friendly and cost-effective aqua feeds that can lower feed costs and the competition between humans and fish for food. Fish feed ingredients that can be found locally are typically less expensive and more readily available than commercial feeds (Adéyèmi et al., 2020). Finding an alternative protein source for fish feed ingredients is necessary to meet the demand for sustainable aqua-feed production due to the scarcity and limited availability of fish feed ingredients, particularly protein feeds (Makkar et al., 1997). However, a number of factors, including low palatability, poor digestibility, ant nutritional elements, high fiber content, and low protein content, limit the incorporation of plant feed materials in fish feeds (Kokou and Fountoulaki, 2018; Alfred et al., 2020). Plants can be processed and fermented to get around those issues for fish feed uses (Francis et al., 2001; Ansari et al., 2021).

Pontederia crassipes, a free-floating perennial herb of fresh water ecosystems, is native to the Amazon basin and has since naturalized throughout tropical and subtropical South America. It is a member of the Pontederiaceae family (Lu et al., 2007; Adeyemi and Osubor, 2016). It is also widely dispersed in Ethiopia's water bodies, where it causes socioeconomic and environmental issues (Tewabe , 2015; Gezie et al., 2018; Dersseh et al., 2019) that worsen poverty by

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impacting the country's agriculture, fisheries, and biodiversity (Asmare, 2017), all of which are vital to people's ability to support themselves.

It has potential uses despite its negative consequences. It is currently receiving a lot of attention as a substitute plant feed ingredient in fish, poultry, and other animal feed as it is less costly than commonly used ingredients (Aderolu and Akinremi, 2009). However, incorporation is limited because of its relatively high fiber profile and low protein content, which may limit how effectively it is used by fish as a feed ingredient (Konyeme et al., 2006).

Studies have shown that the use of solid state fermentation technologies can address the underutilization of this weed (Singhania et al., 2009; Shamim et al., 2017). Solid-state fermentation is preferred over other fermentation techniques because it is more cost-effective, uses readily available inexpensive substrates, and has a relatively low risk of contamination, as evidenced by the fact that microorganisms can grow on moistened solid substrates without access to free water (Osma et al., 2007). Do Santos et al. (2015) reported that solid state fermentation technology with Rhyzopus sp. and *Aspergillus niger* increased the protein content and decreased the crude fiber content of cactus pears after fermentation. Moreover, the availability of well-established information on the evaluation of *Aspergillus niger* fermented water hyacinth leaf as the alternative plant-based feed source in tilapia diet is scanty.

This study, therefore, evaluates the nutritional potential of fermented water hyacinth leaf meal as an alternative plant feedstuff in Nile tilapia (*Oreochromis niloticus* L.) diets.

MATERIAL AND METHODS

Water hyacinth collection and fermentation

Water hyacinth *(Pontederia crassipes)* was harvested from Qoqa Reservoir and the leaf was separated from the whole plant and washed thoroughly with tap water to remove adhering dirt. Approximately 250 g of fresh leaf was taken from the collected water hyacinth plant, oven-dried at 60 °C for 48 h until a constant weight was obtained for dry matter determination. The remaining sample of leaf was sundried by spreading thinly on plastic sheets until fully dried. Then, the dried water hyacinth leaf was ground and passed through a 2 mm meshed sieve to ensure homogeneity. Finally, water hyacinth leaf powder was fermented by filamentous fungi species, *Aspergillus Niger* following different fermentation procedures. *Aspergillus niger* is nontoxic and environment friendly fungi species that is widely used for nutrient improvement of low quality animal feeds.

Experimental system and animals

The experiment was conducted in aquariums built by Addis Ababa University, Department of Zoological Sciences. The experiment was conducted in 12 aquarium units of 80 L capacity. These are linked to a plumbing system that continuously supplies water. Each aquarium received 2 L m^{-1} of thermo-regulated recirculating water, with a photoperiod of 12 h of light and 12 h of darkness. A compressor that supplied additional aeration through an air tube system and air stones was used. Weekly measurements were made of the dissolved oxygen, pH, nitrite (NO2), nitrate (NO3), and ammonia (NH3) levels in the water. They had the following average values over the course of the study: pH 7.2, 28.7 °C, 0.16 $mg.L^{-1}$ ammonia, 0.20 $mg.L^{-1}$ nitrite, 49 $mg.L^{-1}$ nitrate, and 5.3 $mg.L^{-1}$ dissolved oxygen; and they were within acceptable ranges for tilapia.

The Lake Chamo strain of Nile tilapia brood stock was used for production of fry for this study. An average weight of 1.6 g of Nile tilapia fingerlings were randomly distributed in to 12 aquarium units. The experiment was conducted for about three months.

Animal ethics/rights

An international protocol for animal ethics was followed in this study. As a result, the European Union regulation on the use of animals for research (European Union, 2010) was used in this research.

Diet Formulation and preparation

Generally, all experimental diets were formulated to contain 350 g kg^{-1} protein, 100 g kg^{-1} lipid and 18 kJ g^{-1} energy. These levels were based on requirements for Nile tilapia (NRC, 1993). The grains (Table 1) used in this study were purchased in Addis Ababa from commercial sources. The diets were designed using the as-fed principle. Fish meal and soybean meal are the primary sources of dietary protein used in formulation, while wheat and corn grains are the primary sources of carbohydrates. A binder (high viscosity carboxymethyl cellulose) and a premix of vitamins and minerals for poultry were also added. The lipid in the diets was obtained from soy oil.

Previously fermented water hyacinth leaf meal was used to formulate the diets for Nile tilapia at different inclusion levels. Fermented water hyacinth leaf was incorporated in the diet at inclusion levels of 10% (FWHL10), 20% (FWHL20) and 30% (FWHL30). To create a homogeneous mixture, all ingredients were finely ground and passed through a 500 mm sieve. After adding water (20–30%) slowly while stirring continuously, the resulting homogenate was moistened before being run through an electrical meat mincer. The meat mincer's "expeller-like strands" were dried for 24 h in a convector-fan oven at 35 to 40 °C. They were then reduced to crumbles and put through a sieve with a 1mm mesh size. The resulting pellets were employed in this study, and their proximate composition and energy were also examined.

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Table 1 - Composition of diets (g·kg^{-1} as-fed) fed to Nile tilapia with varying inclusion levels of fermented water hyacinth leaf.

Ingredients	Control	FWHL10 %	FWHL20 %	FWHL30 %
FWHL	-	100	200	300
FM	321	288.9	256.8	222.7
SM	360.2	323.7	287.6	250.3
WG	115	101	89	76.4
CG	103.8	91	79.8	70.9
SB oil	30	25.4	16.8	9.7
VMP[1]	50	50	50	50
CMC[2]	20	20	20	20

[1] contains (mg·kg^{-1}); vitamin A (retinol)=2100, vitamin D3 (chole-calciferol)=50, vitamin E (tocopheryl acetate)=10000 I.U, vitamin k3=2000, Thiamine=1,000, Riboflavin=4,000, Niacin=10,000, Pantothenic acid=5,000, Pyridoxine=750, Folic acid=250, Vitamin B12=8, Vitamin H as Biotin=30, Betain=100,000, Antioxidant=125,000; **Minerals:** Manganese=80,000, Zinc=50,000, Iron=20,000, Copper=5,000, Iodine=1,200, Cobalt=200, Selenium=200; FM=Fish meal; FWHL=Fermented water hyacinth leaf; Soybean meal; WG Wheat grain; CG=Corn grain; SB oil=Soybean oil; VMP=Vitamin mineral premix; [2]Carboxymethyl cellulose (high viscosity).

Chemical composition

In an oven with a 105 °C setting, weight loss was used to measure moisture. A known quantity of the sample was burned at 550°C to a constant weight to produce ash. Crude protein was created by converting the total nitrogen determined using the Kjeldahl method. The 6.25 factor was applied. Using the Soxhlet method, petroleum ether was used to extract the total lipids. The sum of the calories in proteins, lipids, and carbohydrates, which are 5.5, 9.1, and 4.1 kcal • g^{-1}, respectively, was calculated. By difference, carbohydrates were calculated using the following equation:

Carbohydrates are equal to 100 less (moisture, protein, lipid, and ash)

Analysis of experimental data

Multiple biological parameters, including growth performance, food conversion ratio, and diet protein, lipid, and energy utilization, were determined using experimental data gathered during the growth trial and diet analysis results.

Growth efficiency

Fish weight gain and a specific growth rate were the parameters used in this study to evaluate growth performance (SGR). The most typical fish growth expression is SGR. Weight gain (WG) is the variation in fish body weight over time between the initial and final weights: (FBW-IBW)/IBW 100 = WG; Where, FBW (g) stands for final body weight and IBW (g) for initial body weight (g). These are average body weights.

Specific growth rate

The percentage increase in body weight per day over any given time period is used to express the instantaneous change in weight of fish. Natural logarithms of body weight are used in the calculation, and it expresses growth as a percentage. SGR = $\frac{(\text{In FBW} - \text{InIBW})}{\text{D}} \times 100$

Feed conversion Ratio

The amount of dry feed fed per unit of live weight gain is known as FCR. It frequently acts as a gauge of the diet's effectiveness. Less food, or a lower FCR, is needed to produce a unit of weight gain the more growth-friendly the diet (Lucas et al., 2019). It was determined to be: FCR = $\frac{\text{Feed fed (g)}}{\text{Live weight gain (g)}}$

Protein efficiency ratio

According to De Silva and Anderson (1995), the protein efficiency ratio (PER) is the proportion between the weight gain of fish and the protein fed. PER = $\frac{\text{Weight gain (g)}}{\text{Crude protein fed (g)}}$

Productive Protein value

Productive protein value (PPV), also known as efficiency of protein utilization (Gerking, 1971), measures the amount of protein in the diet by comparing it to the amount of protein that is retained in fish tissues. Fish samples taken before and after feeding with the evaluated protein are used to determine PPV, which is typically expressed as a percentage of protein fed. PPV = $\frac{\text{Protein retained in tissue}}{\text{Dietary protein consumed}}$ ×100

In comparison to PER, PPV is a more precise criterion for evaluating dietary protein because it considers the conversion of the dietary protein into body protein rather than the overall increase in body weight (Hepher, 1988).

Nutrient Deposition = $\left[\frac{(\text{IBW}\times\text{FBN}) - (\text{IBW}\times\text{IBN})}{(\text{feed intake} \times \text{feed nutrient}}\right]$×100

Where, FBW= final body weight (g), IBW= initial body weight (g), FBN= final body nutrient and IBN= initial body nutrient.

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Due to practical limitations, it was not possible to guarantee that all food was consumed during experiments with fish or to remove uneaten food from the aquariums. Therefore, the amount of feed fed (instead of feed consumed/intake) was used for the calculation of FCR, PER, and PPV (ANPU- Apparent Net Protein Utilization) without adjustment for any wastage. In actuality, this might cause the ratios to be underestimated and the feed to be overestimated.

Body composition of fish

To determine the body composition of fish, whole body proximate analysis and the hepatosomatic index (HSI) were used. Following the procedures outlined above, the proximate analysis included the analysis and expression of moisture, crude protein, crude lipid, and ash as a percentage of fresh weight. Following the completion of each experiment, 20 randomly chosen fish from each treatment, including the control, were euthanized by clove oil overdose, dissected, and had their livers removed. The livers were then weighed and used to calculate the hepatosomatic index (HSI), which was calculated as follows:

$$HSI=\frac{\text{liver weight}}{\text{body weight}} \times 100$$

Statistical analysis

This study's experimental design was primarily a completely randomized design (CRD), in which various dietary treatments were assigned to the experimental units at random (aquariums). The study's null hypothesis was that there is no discernible difference between the dietary treatment methods. Statistical Packages for Social Sciences were used to conduct the statistical analyses for this study (SPSS ver. 20). One-way analysis of variance (ANOVA) was used to compare the means of the various dietary treatments and to test for differences between the means that might be statistically significant (Duncan, 1955). Differences were considered significant at a p-value of 0.05.

RESULTS

Table 2 lists the approximate composition and energy content of the experimental diets. There were no significant difference between the diets in crude protein (342.5–353.2 g.kg^{-1}), crude lipid (102.9–112.7 g. kg^{-1}) and ash (104.9–139.5 g. kg^{-1}) contents. Whereas, there was a significant difference ($P<0.05$) in nitrogen free extract (256.4-280.2 g. kg^{-1}). Diet 4 (FWHL 30) had significantly higher ($P<0.05$) crude fiber content (356 g.kg^{-1}) than the other treatments. Fermented water hyacinth leaf (FWHL) alone contained the highest crude protein and the lowest crude fiber content than the raw counterparts.

Table 3 indicates the price of each ingredients used in the study. Water hyacinth was the least expensive ingredient (10 birr kg^{-1}) than the other ingredients as its cost is calculated only for harvesting and processing i.e. it was not purchased elsewhere from the market. It was eight and four times less expensive than fish meal and corn grain respectively. Table 4 shows the initial and final mean weights, percentage weight gain (WG), and specific growth rate (SGR) of Nile tilapia fed the experimental diets containing fermented water hyacinth leaf. Inclusion of FWHL at 10 and 20% level resulted comparable growth performance with the control group. Inclusion level of FWHL at 30% resulted significantly lower weight gain ($P<0.05$) than the other treatments (Table 4).

Feed intake of the different diets ranged between 20.06 g and 26.79 g per fish at the end of the experiment. Diets with 30% inclusion of fermented water hyacinth leaf has significantly lower feed intake ($P<0.05$) which could be accompanied by the decrease in feed conversion ratio (FCR). Table 5 displays the fish samples' initial and final whole body proximate compositions. The moisture contents of the fish fed the control diet and diets containing 20% fermented water hyacinth were significantly lower. Except for fish fed 10% and 20% of fermented water hyacinth leaf, which shows relatively comparable whole body contents with the control diet, the inclusion of fermented water hyacinth leaf at 30% significantly affected the CP, CL, and GE contents of fish in the whole body.

Table 2 - Chemical composition of diets fed to Nile tilapia in this study.

Components (g·kg^{-1})	RWHL	FWHL	Control	FWHL10	FWHL20	FWHL30
Dry matter	927.8	913.0	920.2	914.7	910.3	908.6
Crude protein	94.9	195.6	353.2	350.8	348.9	342.5
Crude lipid	47.6	41.1	112.7	102.9	105.6	107.4
Crude fiber	297.8	158.5	259	276	298	356
Ash	191.2	246.3	104.9	119.5	125.9	139.5
NFE	315.6	255.0	256.4	260.8	269.7	280.2
Gross energy (KJ·g^{-1})	16.5	17.3	19.7	18.9	19.4	18.5

NFE = Nitrogen free extract; RWHL=Raw water hyacinth leaf, FWHL = Fermented water hyacinth leaf

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Table 3 - Price of ingredients used in the study (Ethiopian Birr .kg^{-1})

Ingredients	Price
Fermented water hyacinth leaf	10
Fish meal	80
Soya bean meal	65
Wheat grain	50
Corn grain	40

Table 4 - Growth and feed utilization (per fish) of Nile tilapia fed fermented water hyacinth based diets.

Parameter	1	2	3	4
	Control	FWL 10	FWL 20	FWL 30
IBW	1.63±0.12	1.64±0.23	1.66±0.05	1.65±0.20
FBW	10.15±0.19^{a}	10.01±0.02^{a}	9.98±0.12^{a}	7.64b±0.05^{b}
WG	307.4±1.57^{a}	296.14±1.58^{a}	288.48±1.13^{a}	249.28±0.96^{b}
SGR	2.92±0.07^{a}	2.84±0.06^{a}	2.79±0.08^{a}	2.25±0.09^{b}
S	95.3±2.4	95±5	97.23±2.49	98.58±2.19
FCR	1.81±0.01^{a}	1.78±0.02^{a}	1.76±0.03^{a}	1.41±0.02^{b}
FI	26.79±0.10^{a}	25.23±0.14^{a}	24.96±0.07^{a}	20.06±0.04^{b}
PER	1.03±0.08^{a}	0.92±0.02^{a}	0.89±0.03^{a}	0.65±0.0.02^{b}
PPV	17.11±0.18^{a}	16.89±0.04^{a}	16.81±0.12^{a}	12.96±0.07^{b}
HIS	2.56±0.13^{a}	2.51±0.02^{a}	2.47±0.11^{a}	1.9±0.21^{b}

IBW (g) = initial body weight; FBW (g)=final body weight; SGR (% day^{-1})=specific growth rate; FI (g)=feed intake; FCR=feed conversion ratio; PER=protein efficiency ratio; PPV (%)=productive protein value; S(%)=survival; HIS=hepatosomatic index. Values are means±SD of three replicates, and values within the same row with different letters are significantly different (P<0.05).

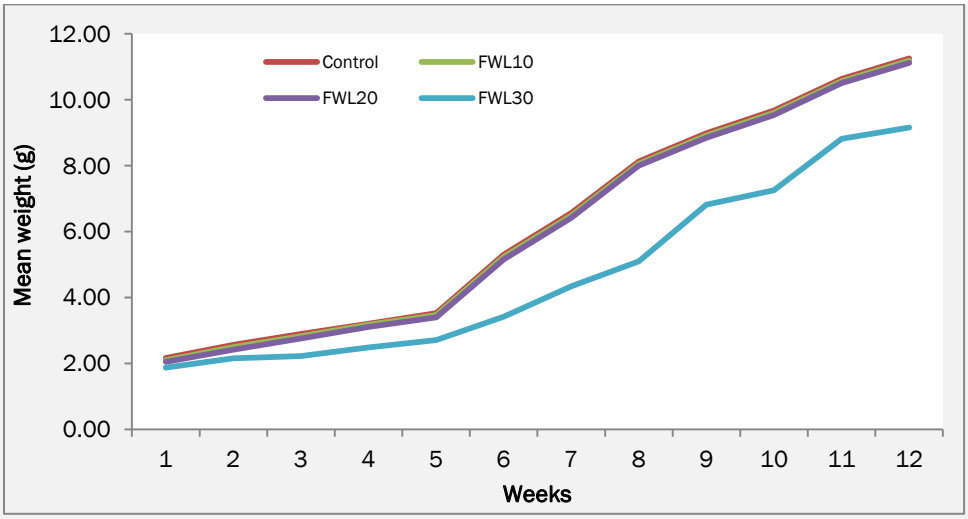

Figure 1 - Shows growth response of Nile tilapia fed fermented water hyacinth based diets for twelve weeks.

Table 5 - Whole body proximate composition (% wet Weight) and energy of Nile tilapia fed fermented water hyacinth based diets.

Components		1	2	3	4
	Initial carcass	Control	FWHL 10	FWHL 20	FWHL 30
MC	78.6	73.27±0.32^{a}	75.03±0.21^{b}	74.91±0.10^{b}	76.30±0.36^{c}
CP	14.9	15.61±0.10^{a}	14.53±0.21^{a}	14.29±0.20^{a}	11.03±0.21^{b}
CL	4.9	6.37±0.15^{a}	6.35±0.21^{a}	6.30±0.10^{a}	3.87±0.15^{b}
Ash	3.9	4.5±0.10^{a}	4.2±0.10^{a}	4.3±0.1^{a}	5.2±0.10^{b}
GE	5.1	5.9±0.10^{a}	5.5±0.10^{a}	4.67±0.15^{a}	3.95±0.10^{b}

MC = Moisture content; CP= crude protein; CL= crude lipid; GE= gross energy; FWHL= fermented water hyacinth leaf with the corresponding number; Values are means SD (n = 3) and values within the same row with different letters are significantly different (p<0.05).

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DISCUSSION

Statistically, inclusion of FWHL at 10 and 20% exhibited equivalent feed utilization and growth performance with the control group. Since inclusion of FWHL at 10 and 20% equivalent in growth and feed utilization efficiency with the control diet, it can be added up to 20% of formulated feeds without negative impact on feed efficiency metrics and growth performance. However, at 30% inclusion level of fermented water hyacinth leaf, growth and feed utilization efficiency was significantly reduced ($P<0.05$). The accuracy of the randomization process between the experimental treatments was demonstrated by statistical analysis, which revealed no significant differences in initial body weight among the various experimental treatments.

In comparison to the other ingredients used in the present study, the cost of water hyacinth was very low (Table 3) as it is not purchased from the market. It is eight times less costly than fish meal and five times less than the cost of wheat grain. Its price is calculated for harvesting and processing.

The fermentation process, which reduces the crude fiber and some of the anti-nutritional contents present in water hyacinth leaf, may be the cause of the relatively similar results in growth performance and feed utilization parameters between 10% and 20% inclusion of fermented water hyacinth leaf with the control group. This is because fermentation is a special process that could increase the nutritional value of feed ingredients while significantly lowering the anti-nutritional factors and fiber content in the plant-based feed ingredients (Bairagi et al., 2002; Uchida and Murata, 2002; Singhania et al., 2009; Shamim et al., 2017). According to Bake et al. (2015), fish fed with ingredients from fermented plant sources showed better growth performance.

According to El-Sayed (2003), the significantly lower weight gains and SGRs in fish fed diets containing water hyacinth meals above 20% inclusion levels may be caused by the plant's high fiber content, which is difficult to reduce through fermentation because of high inclusion level. This claim is in line with research by Jimoh and Aroyehun (2011) and Bake et al. (2015), who found that higher inclusion levels of meals containing the majority of plant protein sources led to poor growth and nutrient utilization. This finding is also consistent with the findings of Nwanna et al. (2008), who found that feeding fish a diet containing crude fiber above 4.7% resulted in poor growth performance. These findings corroborated reports of Nwanna and Ajani's (2005) on the growth and blood parameters of catfish fed diets containing more than 20% water hyacinth meal.

In line with the current study, Sayed-Lafi et al. (2018) found that fermented water hyacinth up to a 20% level was suitable and had no negative effects on the growth and feed efficiency of young grass carp (*Ctenopharyngodon idella*) (Val, 1844). Similar findings to those of the current study were also reported by Konyeme et al. (2006) and Fouzi and Deepani (2018). Cruz et al. (2011) provide additional evidence in support of the idea that feeding *Cachama blanca* a practical diet supplemented with fermented aquatic plants at a 15% level boosts growth rates and feed efficiency equivalent to the control diet.

Other fermented non-conventional feeds, like fermented duckweeds*, Lemna minor* and *Spirodela polyrhiza* and the water fern, *Azolla filiculoides* in *Piaractus brachypomus* and *Oreochromis niloticus* L. feeds have growth performance and feed utilization efficiency that is comparable to the control diet (Bashir and Suleiman, 2018). The ability of Nile tilapia juveniles to utilize the nutrients of hyacinth meal for growth is shown by the improved growth performance obtained from 10 and 20% incorporation of *Pontederia crassipes* leaf meal equivalent to the control diet.

Kraidy et al. (2020) on the other hand found that *Heterobranchus longifilis* fed a diet containing raw water hyacinth at a 20% inclusion level had lower growth performance and feed utilization efficiency than fish fed the Control diet. This discrepancy may be caused by the different fish species, the rearing system, the feed composition, and the use of raw (unfermented) water hyacinth in the prior study, which had high crude fiber and antinutritional contents. Except for ash content, which was significantly higher in diet 4 (30% FWHL), there was a significant decrease in the whole body composition of CP, CL, and GE contents in diets fed 30% inclusion of fermented water Hyacinth.

The fermentation process, which could enhance the nutritional value of feed ingredients and greatly reduce the anti-nutritional factors and fiber content in the plant-based feed ingredients, may be the cause of the significantly higher body composition of CP, CL, and GE in diets containing 10 and 20% fermented water hyacinth leaf equivalent to the control diet. Some authors claimed that feeding fish feed containing fermented plant-based ingredients improved their growth performance and body composition (Bake et al., 2015; Jafer et al., 2018).

CONCLUSION

Nile tilapia fed up to 20% fermented water hyacinth leaf for three months showed acceptable growth and feed utilization efficiency. According to the study, high concentrations (30%) of fermented water hyacinth leaf could harm fish growth and feed utilization. Water hyacinth may be a readily available alternative ingredient for fish meal formulation in underdeveloped nations like Ethiopia. Additionally, incorporating water hyacinth into fish feed may help to slow the spread of this invasive plant in the water bodies. Moreover, the use of fermented water hyacinth leaf in fish feed can reduce the cost as it is less costly than conventional fish feed ingredients.

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DECLARATIONS

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Computing Interest

The authors declare that they have no competing interests

Authors' contribution

All authors are involved in the preparation of this manuscript

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A SYSTEMATIC REVIEW ON THE DEVELOPMENT OF QUAIL OVARY EMBRYOGENESIS (*Coturnix coturnix Japonica*) UNDER DIFFERENT LIGHTING COLORS

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Supporting Information

REVIEW

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ABSTRACT: Quail (*Coturnix coturnix Japonica*) is one of the commercial poultry that is being developed and produced more frequently. Various lighting programs (pre-hatching) on Aves have been carried out to provide an increased biological response, including growth, reproduction, and productivity. The addition of light or the lighting program is also a factor in the growth of Aves which directly plays a role in controlling various physiological processes. The purpose of this study was to review embryogenesis development of the Quail ovary under various lighting conditions. A literature search was carried out systematically through the PubMed, NCBI, and Google Scholar databases using keywords, namely, "embryogenesis development, ovary, quail light color, and lighting". The articles obtained were selected based on these keywords by setting several inclusion criteria. Papers that do not meet the inclusion criteria are eliminated, and articles that meet the criteria will be analyzed to obtain data. Based on the search results in the databases using predetermined keywords, 500 articles were obtained. All articles were selected based on inclusion criteria and exclusion and obtained as many as 35 articles that met the inclusion criteria. From the results of the research, it can be concluded that giving variations in the colour of lighting for 16 hours affects the development of quail ovaries. Because of the significant effect of lighting and its colour on embryo development, pre-hatch lighting programs should be considered in future studies.

Keywords: Egg, Embryogenesis, Incubation, Lighting, Quail.

INTRODUCTION

Quail (*Coturnix coturnix Japonica*) is one of the poultry species that is being developed and increased in commercial production. Apart from meat, quails are egg producers with high productivity, producing between 200 and 300 eggs per head per year (Akarikiya, 2021). The nutritional value of quail eggs is not inferior to other poultry, such as hens (Wilson, 2017). One of the new approaches in this field is providing a lighting program in quail farms. Various lighting programs on Aves have been carried out to increase biological response such as growth, reproduction, and productivity (Yameen et al., 2020; Gharaoghlan et al., 2022).

The age of sexual maturity in female quails is characterised by the first time they lay eggs, while for males it is characterised by the start of crowing with a distinctive sound. Quails first lay eggs between 35-72 days old with an average age of 41. This was also expressed by another research which showed that quails reach sexual maturity on average at the age of six weeks, but it is also found that they are older than that age (El-Sayed et al., 2022; Wiradimadja et al., 2007). This situation caused by health factors, management, and food also affects sexual maturity. Other factors that have an impact are genetics, lighting, and body weight. The growth of Aves, which is directly responsible for controlling various physiological processes, is also influenced by the addition of light or the lighting program (Drozdová et al., 2021; Franco et al., 2022).

Several studies demonstrated that exposing fertile eggs to light can increase the growth of the embryo and decrease the incubation period. Post-hatch artificial lighting is an important management method for the growth and muscle development of meat-type birds. Embryo exposure to light leads to changes in metabolic rate, such as an increase in embryo metabolic rate in the pigeon (*Columba livia domestica*) light compared with darkness. Furthermore, exposure to light increased the heart rate of embryos. Providing light during broiler egg incubation affects production, health, and exhibits the potential to reduce the stress associated with growth and production. Archer G in 2017 reported that providing light for 12 h/day during egg incubation reduced susceptibility to the stress of broilers post-hatch (Abdulateef et al., 2021). The physiological mechanism of light stimulating embryonic growth and development differs before and after

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the formation and maturation of the retinal photoreceptor. The hypothalamic pacemaker and pineal gland are the main parts of the circadian avian system. Embryonic cell proliferation increases with high light intensity. Experimental work in domesticated species of birds showed that light accelerates embryonic development by increasing metabolic activity and increased embryonic development, pineal gland formation, and modifies melatonin synthesis (Yalcin et al., 2022).

Low light intensity (e.g. 10 lx) can entrain embryonic starlings Sturnus valgus to show a light-dark rhythm mediated by high melatonin hormone concentrations in darkness and low levels during light exposure, which is a universal feature of embryonic organisms. The research demonstrated that chicken embryos exposed to light for as little as 1 h decrease melatonin production and affects embryonic development (Kankova et al., 2022).

The light energy that comes from artificial light with a light source will produce light with a single wavelength frequency that is directly related to the color of the light. Each color will have a different effect on behavior, growth, and reproduction (Xie et al., 2008). Another research showed that quail in relation to the color of light showed that blue light causes quails to calm down thereby stimulating growth and reducing the stress response, further, red light can reduce cannibalism, stimulate the growth of wing feathers, and stimulate sexual maturity, furthermore, green light stimulate muscle growth in adolescence and increase antibody production (Abeysinghe, 2019). In addition, the red color also causes quail to be more aggressive in pecking feed. The purpose of this study was to analysis of embryogenesis development of the quail ovary given different lighting color.

METHODS

A literature search was carried out systematically through the PubMed, NCBI, Google Scholar databases using keywords, namely "Development of Embryogenesis, Ovary, Quail (*Coturnix coturnix Japonica*) Light Color, and Lighting". Based on these keywords, the articles obtained were first selected by setting several inclusion criteria including journals are not paid / free articles, research results focus on "Development of Embryogenesis, Ovary, Quail (*Coturnix coturnix Japonica*) Light Color, and Lighting." Articles that do not meet the inclusion criteria are eliminated and articles that meet the criteria will be analyzed to obtain data.

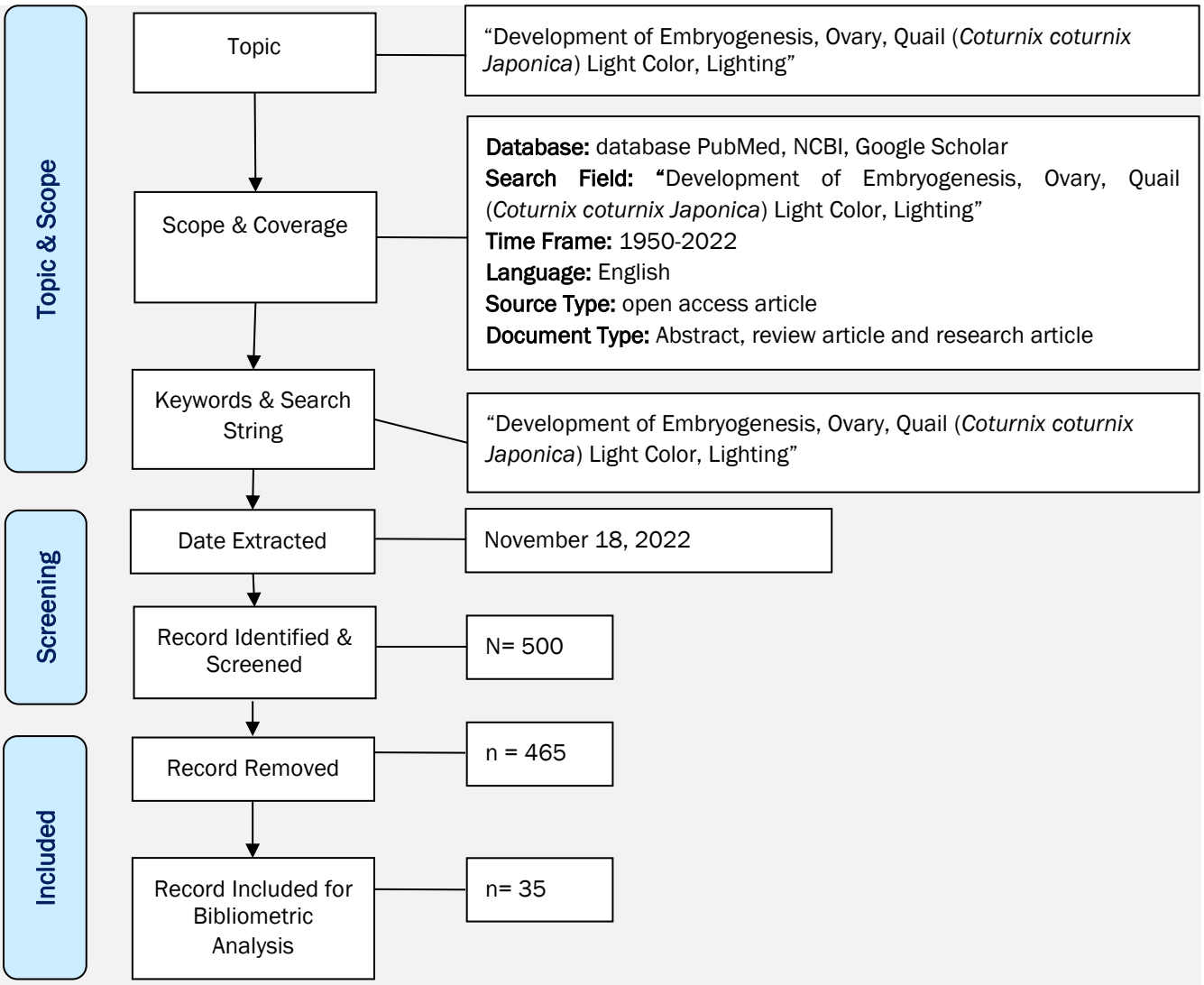

Figure 1 - Flow diagram of search strategy

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RESULTS

Based on the search results in the PubMed, NCBI, Google Scholar databases using predetermined keywords, 500 articles were obtained for the development of embryogenesis, ovary, quail (*Coturnix coturnix Japonica*) light color, lighting. All articles were re-selected based on inclusion criteria and exclusion and obtained as many as 35 articles that meet the inclusion criteria. Figure 1 explains the structure of the ovary based on the treatment group, namely, the control group (K), clear lighting (I), red lighting (II) blue lighting (III), yellow lighting (IV). In the 2nd treatment group (blue lighting) the quail ovarian structure was better than the 3rd treatment group. In Table 1, the ovarian weight of the quail was obtained; the treatment group 2 obtained a better ovarian weight compared to the three treatment groups.

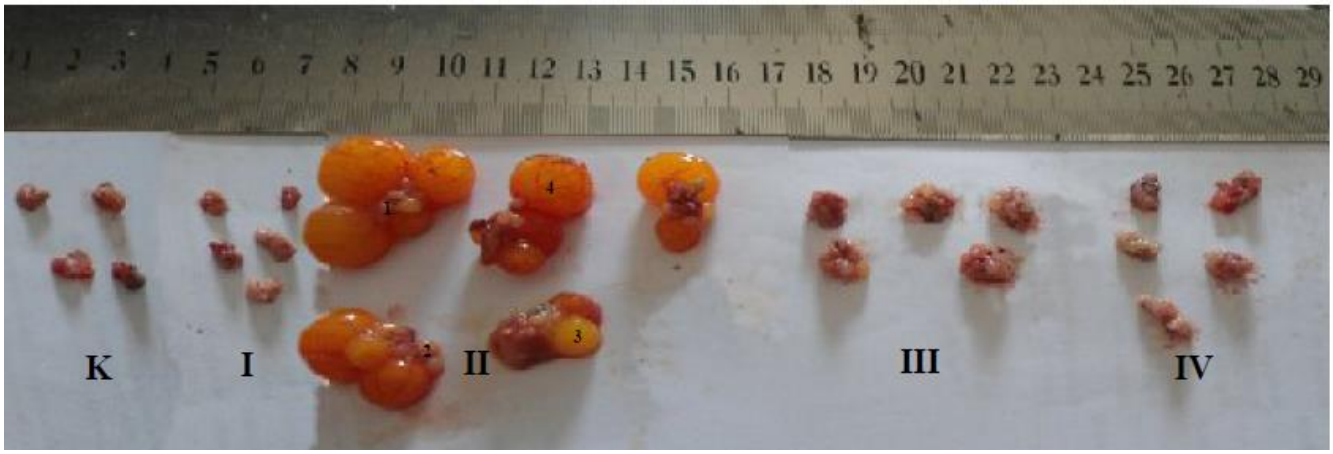

Figure 1 - The structure of the quail ovary in the control group (K). clear lighting (I), red lighting (II) blue lighting (III), yellow lighting (IV). 1 primary follicle, 2 secondary follicles, 3 tertiary follicles, 4 Graff follicles (El-Sayed et al., 2022).

Table 1 - Weight of quail ovaries given 16 hours of light color variations at 6 weeks of age.

Group	Mean ± SEM
Control	0.06 ± 0.02^{a}
I	0.12 ± 0.05^{ab}
II	3.46 ± 2.71^{c}
III	0.26 ± 0.06^{abd}
IV	0.15 ± 0.03^{abde}

Different letters indicate significant differences at P<0.05. I= clear lighting, II=red lighting, III=blue lighting, IV=yellow lighting.

DISCUSSION

The light received by the eye will be brought to extraretinal receptors in the hypothalamus (Mishra et al., 2018). These nerve impulses are transmitted to the hypothalamic suprachiasmatic nucleus via the retinohypothalamic fibres, then proceed to the hypothalamic paraventricular nucleus. Impulses will be forwarded to the spinal cord and pass through the adrenergic preganglionic fibres of the sympathetic nervous system. Through the sympathetic nerves, impulses will reach the pineal gland (the centre of biological clock regulation). This causes the release of norepinephrine (NE) so that through the AMP cycle it will increase the production of tryptophan hydroxylase which plays a role in serotonin synthesis. Serotonin has no direct effect on the reproductive process (Gu et al., 2022).

Serotonin is a building block for melatonin. In dark conditions the activity of Hydroxyindole-O-methyltransferase (HIOMT) increases resulting in the conversion of serotonin to melatonin. However, in bright conditions, melatonin decreases so that it can release the Gonadotropin-releasing hormone (GnRH) from the hypothalamus. Stimulation of the hypothalamus can lead to the development of the reproductive system of quails, which will stimulate the acceleration of sexual maturity marked by the development of follicles. Based on the observation of the macroscopic appearance of quail ovaries, shows that ovarian development in the control group is less than optimal. This can be seen from the weight of the ovaries which has the lowest average compared to the other groups. Lack of light causes stimulation of the release of GnRH from the hypothalamus to experience obstacles. An obstructed hypothalamus will find it difficult to stimulate reproductive hormones, such as Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH). As research said that the lack of light signals causes GnRH to be unable to secrete FSH and LH. Low light causes the HIOMT enzyme to convert serotonin into melatonin. Because low light causes high melatonin, thereby inhibiting the release of the GnRH hormone. This is what causes the development of quail ovaries to be less than optimal (Sharokhyan Rezaee et al., 2022).

One of the researcher reported that if a lighting program of 12 hours of light/a day has no effect on accelerating the maturation of quail ovarian follicles, that would be due to the ovarian follicles not yet developed (Stein, 1974). Then,

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another research added that when quail were given a short period (8 hours of light and 16 hours of darkness) and then maintained normal (12 hours of light and 12 hours of darkness), the concentration of LH in their blood plasma did not change so that giving light for 12 hours was not effective in increasing the concentration of LH in quail (Follett et al., 1977). Quail ovaries in group I that used clear color lighting showed that the ovaries had formed grape-like protrusions.

However, the development of the ovary has not yet reached the Graff follicle, it is still only a hierarchy of follicles. This is almost the same as the control group. However, what distinguishes it is the provision of light, which is for 16 hours. As stated by another researcher which said that providing light for 16 hours/day can provide better productivity and performance than providing light for 12 hours/day (Vanderzwalmen et al., 2003). Based on the observation of the macroscopic appearance of the quail ovaries, it shows that the red color has the most optimal ovarian development compared to the other groups. In addition, red light has the longest wavelength compared to the others, so it has a strong intensity to affect the hypothalamus.

The retina of quails is very sensitive to red lighting. Before receiving red light from the retinal photoreceptors, it must first pass through the ocular media, namely the cornea, aqueous humour, lens, and vitreous humour, so that light is not directly transmitted to the hypothalamus. However, it will go through a series of reactions from light energy to be converted into electrochemical signals (phototransduction). These electrochemical impulses are then sent to the hypothalamus. This statement was reinforced when stated that the red light (700 nm) received by quails will stimulate the hypothalamus to secrete GnRH, which in the next stage the presence of GnRH will stimulate the secretion of reproductive hormones, such as FSH, LH, estrogen, and progesterone which in turn will stimulate egg production and increase fertility (Akyüz and Onbaşilar, 2018). In accordance with what was stated by Onagbesan and Peddie (1988) and Asem et al., (1985) the presence of GnRH will be responded to by the pituitary to secrete FSH and LH (Asem et al., 1985; Onagbesan and Peddie, 1988). The flow of FSH received by the ovary causes the ovary follicles to grow and develop.

Increased development of quail ovaries that get red light requires sufficient energy as a result, quail consume more feed. Lewis et al. (2001) also suggested that the entry of light information into the pineal gland will stimulate the synthesis, release, and metabolism of dopamine (Lewis et al., 2001). The presence of dopamine causes birds to become more active and easily stimulated. Various daily activities require energy both obtained from feed nutrients and from energy reserves stored in the body. Whereas in group III (lighting with blue light), the development of quail ovaries had better development than in the control group, the group I, and group IV. This happens because lighting blue lights makes quail calmer and controlled feed intake. As an argue which stated giving blue to birds can cause birds to become calmer (Akyüz and Onbaşilar, 2018).

In addition, Mardiati stated that blue light which has a short wavelength (450 nm) is able to penetrate directly and be absorbed by the skull bones and cranial tissue which are then received by extraretinal photoreceptors. This causes sexual maturity to turn blue faster than the control group, I and IV. Foster and Soni (1998) and Daghir (2008) stated that extraretinal photoreceptors in the Aves are scattered in the basal part of the brain, lateral septum, hypothalamus (deep brain), intracranial pineal organ, and cerebrospinal fluid connected to neurons. Photoreceptors are nerve cells that are specialized to receive light signals and transduce these light signals into electrochemical signals. Brain tissue is permeable to light and light absorbed by brain tissue will be filtered back by neural tissue, but most of the light with short wavelengths such as blue light will still be able to penetrate the base of the brain. The light signal received by the hypothalamus will stimulate the release of GnRH.

The development of the ovary in blue light occurs through a process proposed by Lewis, and Moris that the Aves hypothalamus is very sensitive to blue light (Akyüz and Onbaşilar, 2018). A blue light will also be received by a quarter of the total number of rod cells in the retina of the avian eye (Lewis and Morris, 2000). The blue light signal received by the rod cells will be directly received by the hypothalamus via the optic fibres. The blue color of the development of the ovaries is not as good as the red color because the red color can be received by the cone cells in Aves, while the blue color cannot be received by the cone cells. As for the yellow light color, in group IV the development of the quail ovarian follicles did not reach the mature follicles. In this group have follicles that begin to develop. This is because the yellow color causes the quail to consume food not as aggressively as the group using red light. This statement is reinforced by the opinion of North and Bell in Madzingira who said that the yellow color can reduce the aggressiveness of quail towards feed, so that the development of the ovaries will decrease (Madzingira, 2018). This statement is reinforced by the research in 1992 which concluded that increasing feed restrictions will result in more pressure on egg production, which is caused by the low energy consumption required for egg production (Olawuni et al., 1992).

The low energy consumption found in group IV makes it difficult for light to stimulate the hypothalamus to secrete GnRH. The difficulty of stimulating the hypothalamus against GnRH causes reproductive hormones, especially FSH and LH, to be unable to be stimulated. This is what causes quail ovaries to not develop properly. As stated by Olanrewaju et al. (2006) states that the speed of cooking quail genitals requires an adequate source of energy from feed intake.

CONCLUSION

From the results of research conducted, it can be concluded that giving variations in the color of lighting for 16 hours affects the development of quail (*Coturnix coturnix Japonica*) ovaries. Pre-hatch lighting program should be considered in future studies.

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DECLARATIONS

We declare that this research work is original and has not been published.

Author's contribution

Maslichah was conducted the supervision, funding acquisition, project administration, writing the draft manuscript, while Akhmad did revision of manuscript. Jonathan analyzed for result and method.

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Conflict of interests

This study has no conflict of interest

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DETECTION OF GENETICALLY MODIFIED SOYBEAN SEED, SOYBEAN MEAL AND RICE IN KARBALA CITY OF IRAQ

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Rice and soybean are two high-demanded grains for human foods and animal feeds. The current study aimed as first time in one of Iraqi region to find genetically modified soybean seed, soybean meal and rice grain samples utilizing the *Cauliflower mosaic virus* (CaMV) 35S promoter and nopaline synthase (NOS) terminator catalyst like *Agrobacterium tumefaciens* NOS (ANOS) terminator, in PCR tests. A total of 55 samples of soybeans seed, soybean meal, and rice cereal were collected from the market in Karbala, Iraq. The samples were collected from markets in Karbala city during January-March 2021, and evaluated in the Food Laboratory, College of Al Safwa University of Karbala, Iraq. DNA was isolated from dry vegetable samples. Two genes, including CaMV-35S and NOS terminator, that are routinely used in genetic engineering were employed to evaluate genetically modified crops. The present study revealed CaMV-35S and NOS genes in soybean meals. In conclusion, the obtained results indicated that all rice samples tested with the same primers were genetically unaltered. Whereas, there is genetically alternations in soybean seeds and soybean meal.

Keywords: Genetically modified seeds, Feedstuff, Soybean meal, Rice, CaMV-35S, NOS terminator.

INTRODUCTION

DNA is the molecule that contains the genetic information of each living organism, and its discovery has revolutionized the field of life sciences (Chawla, 2011). So, due to the universality of genetic codes, scientists have used molecular biology techniques to connect DNA sequences from other animals and insert foreign DNA into plants (Holme et al., 2018). As a result of gene transfer, these genetically modified organisms commonly known as GMOs can synthesize extra proteins that allow them to adapt to their environment, one of the hoped-for benefits of gene transfer (Spök et al., 2007; Kircher et al., 2020).

Any new food items containing genetically modified soy or maize must be labeled under European legislation. Labeling is based on identifying foreign DNA sequences from GMOs (Böschen et al., 2006) using PCR (Broeders et al., 2012). DNA molecules are more thermostable than proteins; hence this approach can be beneficial for their sequence detection (Fraiture et al., 2015). Since any genetic information may be produced in an organism, genetic engineering has been used to address a wide range of issues, including crop protection in agriculture and high value added compound synthesis, such as vitamins or biopolymers (Broeders et al., 2012; Turnbull et al., 2022). A wide range of GMOs is also being investigated and produced, such as genetically modified maize engineered to generate a healthier cooking oil by decreasing the amount of its saturated fat (Uzogara, 2000). In this regard, antifreeze proteins generated from the winter flounder might be used to help strawberries flourish in cold climates and create nutritionally improved strawberries that include greater concentrations of ellagic acid, a natural anti-cancer agent (Mezzetti, 2013).

GMOs detection in different regions of Iraq has been started with using PCR method (Saadedin et al., 2019; Jasur et al., 2020). The study aimed to find genetically modified soybean products and rice yield in Karbala market samples utilizing the CaMV-35S promoter and NOS terminator catalyst in PCR tests.

MATERIALS AND METHODS

The current study included 55 food and feed samples collected randomly from a variety of sources, including the markets in Karbala, as well as other locations in this region, from January to March of 2021. The samples were sent to Kerbala

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University Food Laboratory, College of Alsafwa, Iraq, for analysis. The food samples were pulverized in a ceramic mortar, and liquid nitrogen was used. DNA was isolated from dried vegetable meals, including soybeans, soybean meal, and rice grains. Following that, a spectrophotometer was used to measure the concentration and purity of each DNA sample using a genomic DNA extraction kit (Intron Company, Korea). DNA collected from the samples was studied according to the manufacturer's guidelines. To evaluate the quality of the extracted DNA samples, spectrophotometer readings at two wavelengths (260 and 280 nm) were employed. Absorptance ratios between 1.7 and 2.0 indicate that the extracted DNA was of high purity.

Special primers were employed to identify GMOs in soybeans and their derivatives (soybean feed). The 195-bp primer CaMV35S and 118-bp terminator NOS were employed to identify GM genes in soybeans and their derivatives (Zaulet et al., 2009). Most PCR screening methods rely on identifying the CaMV-35S mosaic virus initiator or the NOS terminator in the examined product. In the current investigation, 123-bp primer CaMV35S and 118-bp terminator NOS were employed to detect genetically modified genes in rice grain genotypes (Safaei et al., 2019). These primers are shown in Table 1.

In addition to utilizing the components of the PCR kit and primer solutions, it was also important to chill the kit by storing it in an ice box, as daily work solutions for PCR interactions. In PCR reaction components, all target genes were examined with primer concentrations of 10 Pmol. The final findings were from 25-ul PCR runs. The gel was prepared in a electrophoresis procedure by dissolving 1 g agarose in 100 ml buffer TBE. Electrical migration commenced (IX) following Sambrook and Russell (2001). Once the agarose was completely dissolved, the mixture was cooled to 50°C and microwaved until the agarose was completely dissolved. Polymerization reaction mixture and DNA marker were each applied to each gel well in 5 microliter portions. Each gel well was filled with 0.5 microliters of ethidium bromide solution per milliliter of gel. At a voltage of 100 volts, the electrodes reached their target, and the models moved electrically for an hour.

Table 1 - The primers used in the present study.

Primer name	Origin sequence	Target sequence	Amplicon (bp)	Sequence
Detection Primer	*Cauliflower mosaic virus*	35S1 Forward	195 bp	F-5'-"GCTCCTACAAATGCCATCA-3"
		35S2 Reverse		R-5'-"GATAGTGGGATTGTGCGTCA-3'"
Detection Primer	*Agrobacterium tumefaciens*	HANos-118 Forward	118 bp	R-5'-GCATGACGTTATTTATGAGATGGG-3'
		HANos-118 Reverse		R-"GACACCGCGCGCGATAATTTATCC-3'"

RESULTS AND DISCUSSION

The GMO screening methods focused on uncovering the regulatory elements and genes responsible for GMOs' unique traits. CaMV-35S promoter (P35S), *Agrobacterium tumefaciens* NOS terminator (ANOS), and others were chosen (TNOS). In many CaMV-transgenic plants, the 35S promoter and/or NOS terminator are present (Querci et al., 2010).

Traditional PCR methods 1 and 2 were employed to detect the CaMV-35S encoder gene and no-terminator in soybean gain, soybean meal, and rice gain. Primers for detecting P-35S promoters for the existence of a package from the inflation process in the fourth, fifth, and sixth wells at 195 base pairs utilizing a soybean meal sample yielded experimental observations (Figures 1 and 2).

Rice is main daily meal in many countries, genetically modified rice strains can be hazardous for consumers (Fu et al., 2019; Hajimohammadi et al., 2022). Consumption of genetically modified foods and feeds are rising and it makes concerns for human and animal health (Nawaz et al., 2019; Turnbull et al., 2021). The discovery of transgenic wheat and soybean meal, which are widely used in livestock feeding, is of great importance in all parts of the world (Matovu, 2021; Sieradzki et al., 2021; Singh et al., 2021).

There are no restrictions on the kinds of GMOs that can be screened using this technology as long as the samples include one of the amplifying genes found by this technique (Nikolić et al., 2008). The advancement of PCR methods has made it possible to swiftly and accurately detect GMOs in food (Chen et al., 2021). There are two important GM features that may be detected using these methods; the CaMV 35S promoter and the Agrobacterium NOS terminator as general recombinant markers, as well as two of the most commonly occurring targets in GMOs so far (Barbau-Piednoir et al., 2012).

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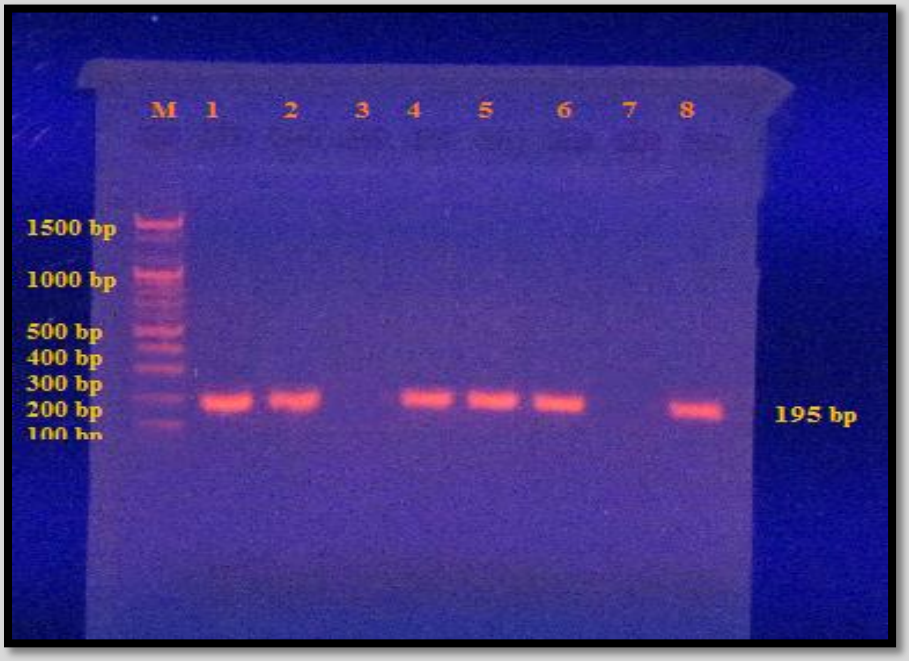

Figure 1 - Uniplex PCR amplification of GMO-specific areas employing primer pairs": P35S (*Cauliflower mosaic virus*), M as DNA ladder 100 base pair, 1,2,4,5,6 and 8 wells GMO positive, 3 and 7 wells without GMO.

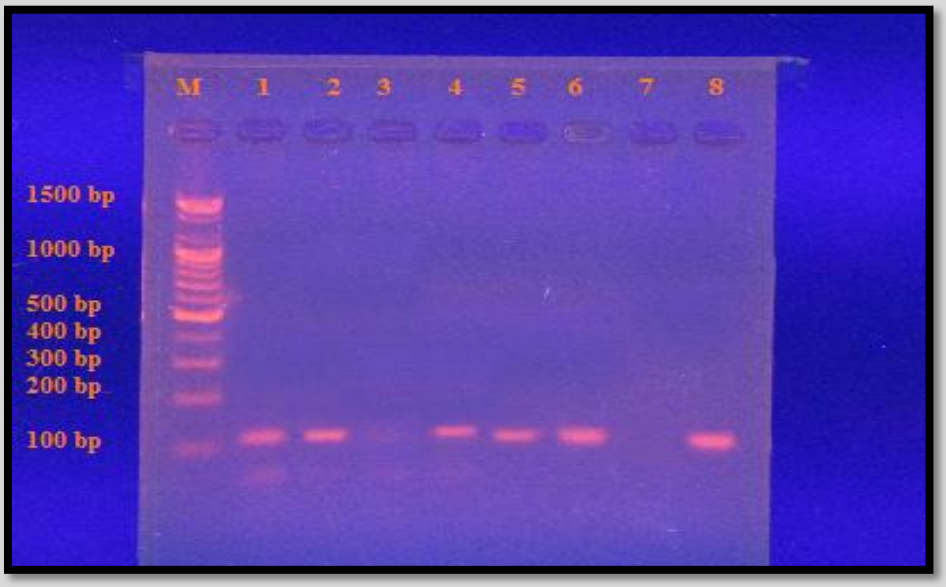

Figure 2 - Uniplex PCR amplification of GMO-specific areas employing primer pairs: HANOS (*Agrobacterium tumefaciens*), M as DNA ladder 100 base pair, 1,2,4,5,6 and 8 wells GMO positive, 3 and 7 wells without GMO.

CONCLUSION

PCR methods developed for the detection of genetically modified soybeans have also been effectively applied to a number of other crops and may be simply adopted to restrict the distribution and usage of genetically modified food. Two genes, including CaMV-35S and NOS terminator, were employed to evaluate genetically engineered crops throughout the current study period. These genes are routinely used in genetic engineering. The present study revealed CaMV-35S and NOS genes in soybean meals. The obtained results indicated that all rice samples tested with the same primers were genetically unaltered.

DECLARATIONS

Novelty statement

Genetically-modified seeds are identified in one of Iraqi region (Karbala city) as first time. Especially, these kinds of grains (rice and soybean) are high-demanded for human foods and animal feeds.

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Author's contribution

Kamal Mathlum Al-khafaji, Ashraf Ayyal Mutar Alrashedi, Wafaa Fawzi Al-Mosawy, and Hayder Ali Muhammed had similar roles and attempts in study, experiments, writing and proof-reading of article.

Conflict of interest

The authors have declared no conflict of interest.

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EFFECT OF GRADED LEVELS OF GUAVA (*Psidium guajava* L.) LEAF MEAL ON PRODUCTIVE PERFORMANCE AND MEAT ORGANOLEPTIC PROPERTIES OF CHICKEN

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RESEARCH ARTICLE

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ABSTRACT: A study was carried out to determine the productive performance and meat organoleptic properties of finisher broiler fed diets supplemented with graded levels of dried guava leaf meal (DGLM) as a phytogenic feed additive. The study was conducted at the livestock experimental unit of National Veterinary Research Institute Vom, Nigeria. Two hundred and forty unsexed 5-weeks-old broilers of similar mean live weight were randomly assigned to 1 of 4 dietary groups with 3 replicates (0, 150g, 300g and 450g of DGLM per 100kg basal diets) over a four weeks finisher period, in completely randomized design. All the diets of iso-nitrogenous and iso-caloric and water were served to the birds *ad libitum*. The results of the finisher phase of the experiment showed that, though the average daily feed intakes of all the treatments were the same, the final body weights of birds fed T_4 diets were significantly ($P<0.05$) higher than those fed other diets. Feed conversion ratio (FCR), protein efficiency ratio (PER) and feed cost/weight gain followed similar trend as in body weight gain. The organoleptic properties (colour, appearance, texture, taste and aroma) of all the treatment groups revealed that DGLM had no adverse effect on broiler meat. The study concluded that the supplementation of DGLM at 300g and 450g/ 100kg enhanced utilization of nutrients in the diets resulting in impressive growth performance, reduced feed cost/weight gain, and high survivability without influencing the organoleptic properties of finisher broiler chickens.

Keywords: Broiler Chickens, Guava Leaf Meal, Herbal additive, Productive Performance, Organoleptic Properties.

INTRODUCTION

Growth promoters are getting popularity as feed additives due to their beneficial effect on gut health and immunity, and growth performance in broiler chicken. Though their mechanism of action varies, positive effect can be expressed through improved feed conversion, better appetite, stimulation of the immune system and increased vitality and regulation of the intestinal microflora (Peric et al., 2009). Antibiotics and other synthetic compounds were hitherto, used globally as feed additives (Lee et al., 2011). Although these substances achieved good performance, their potential side and residual effects both in humans and animals have become a real public health concern globally (Donoghue et al., 2003; Bacanli and Basaran, 2019). This eventually, led to the ban of the products especially in the Western World and specifically in Sweden since 1986 (Bacanli and Basaran, 2019). Some of the banned growth promoting antibiotics as indicated by (Odoemelam et al., 2013) include: avoparcin, tylosin-phosphate, virginiamycin, Zn-bacitracine, spiramycin, olaquindox and carbadox. This scenario has triggered an explosion of interest in the use of herbs and spices and their products as supplements in animal rations (Reyan Mohasesi et al., 2020; Abd El-Hack et al., 2022). Odoemelam et al. (2013) reported that up to one third of all commercial swine and chicken ration producers in Europe now use mixture of herbs and spices as feed additives.

These new class of natural feed additives are currently referred to as "phytogenics" (Singh and Gaikwad, 2020; Bajagai et al., 2022). According to (Odoemelam et al., 2013), some of the phytogenics already in use or undergoing trial are indigenous to Africa and they include: ginger (*Zingiber officinale*), garlic (*Allium sativium*), scent leaf (*Ocimum gratissimum*) bitter leaf (*Vernomnia amygdalia*) and neem leaf (*Azadirachta indica*). Kuralkar and Kuralkar (2021) indicated that the usefulness of these phytogenics lies in some important bioactive chemical constituents like alkaloids, tannins, flavonoids, saponins and phenolic compounds that produce definite physiological actions in the body of animals. Muhammad et al. (2009) posited that these phytogenic substances have been reported to enhance the performance of livestock. Guava (*Psidium guajava*) plants are widely and locally available and they have long history of nutritional and medicinal properties like the earlier mentioned phytogenics already in use. All the body parts of guava plant as well as the by-products have been used effectively and scientifically validated both for nutritional and medicinal purposes (Takeda et al., 2022) except the leaf meal (Okpara, 2006; Joseph, 2011). The determination of possible influence of any new feed additive on meat quality is an important part of the testing of new products for registration by the European Economic Council (EEC) in the annex to the directive on feed additive (Fris-Jensen, 1982). Fris-Jensen (1982) posited that

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organoleptic quality is measured or evaluated both in connection with feeding and other experiments and as a special objective in research work. This study seeks to identify and evaluate the potentialities of another prospective phytogenic plant material; guava (*Psidium guajava*) leaves as feed additive in broiler ration. The objective of this study is to determine the effects of dried guava leaf meal (DGLM) as feed additive on the productive performance and organoleptic properties of broiler chickens.

MATERIALS AND METHODS

The study location

The study was conducted at the experimental unit of Livestock Investigation Division (L.I.D); National Veterinary Research Institute (NVRI) Vom, Plateau State. Vom which is in Jos South Local Government Area of Plateau state is located between latitudes 9^0 $50^{'}$ and 10^0 North and longitudes 8^0 55^{i} and 9^0 East. Vom has a cold climatic condition due to its high altitude measuring over 1290 meters above sea level. The average rainfall is between 1,300 mm to 1500 mm and the rainy season extends from late March to early October, July and August being the wettest months. The average daily maximum temperature is 28.6^{0}C, average minimum temperature is 17^0 C while the mean relative humidity at noon varies between 14 and 17 % (Anon, 2010).

Collection and processing of guava (Psidium guajava) leaves

Fresh and matured green guava leaves used for the experiment were harvested in Vom and its environs in Jos South Local Government Area of Plateau State in the month of October, 2015. Each batch of the collection was washed and air dried. They were considered adequately dried when they became crispy to touch. They were then milled, using a hammer mill with 2 mm sieve to produce dried guava leaf meal (DGLM). The leaf meal was weighed, carefully packed in clean polythene bags, labeled and stored under room temperature until use as prescribed by Okpara (2006).

Nutrient composition of guava (*Psidium guajava* L.) leaf meal

The guava leaf meal sample was analyzed to determine the proximate constituents like moisture, protein, ether extract, ash and crude fiber according to AOAC (2000) at the Science Laboratory Technology Unit, University of Jos, Plateau State.

Experimental birds and general flock management

Two hundred and forty unsexed cobb five weeks old broiler chickens purchased from Zartech farms – Jos, Plateau State were raised in deep litter system following standard management and biosecurity practices specified for broiler chicken production as described by Oluyemi and Robert (2000). This experiment was conducted in the months of November to December and lasted for 6 weeks.

Experimental design

Birds were randomly distributed into 4 dietary treatments comprising of 60 birds per treatment. Each treatment was replicated thrice with 20 birds per replicate using a completely randomized design (CRD). The initial weight of each bird was determined with the aid of electronic weighing scale. Water and feed were made available to the birds *ad libitum* throughout the experimental period.

Experimental diets

Four experimental diets were formulated for finisher phase in accordance with the nutrient requirements of finisher broiler (NRC, 1994). The experimental diets were designed as: Treatment (T_1): 0g DGLM/100 kg basal diet as control; T_2: 150g DGLM/100 kg basal diet; T_3: 300g DGLM/100 kg basal diet; and T_4: 450g DGLM/100 kg basal diet. Ingredients and their proximate compositions are presented in Tables 1 and, 2.

Table 1 - Composition of broiler finisher's diet

Ingredients (kg)	Finisher		Calculated analysis	
Maize	49.02		ME Kcal/kg	2823.97
Wheat offal	7.35		Crude Protein (%)	20.20
Rice offal	4.90		Crude Fiber (%)	4.43
Soybean cake	33.93		Calcium (%)	1.08
Fishmeal	1.50		Phosphorus (%)	0.57
Bone ash	1.50		Feed cost ₦/kg	90.90
Lime stone	1.00			
Common salt	0.25			
Lysine	0.10			
Methionine	0.20			
Premix	0.25			
Total	100			

Bio-mix starter Premix supplied /kg: Vit A: 100000iu,Vit E : 23000mg, Vit.K_3 : 2000mg,Vit B_1: 1800mg,Vit.B_2 : 5500mg, Niacin: 27,500mg, Panthotenic Acid: 7500mg, Vit B_6: 3000mg,Vit B_{12}: 15mg, Folic Acid: 750mg, Biotin H_2: 60mg, Choline Chloride: 300000mg, Cobalt: 200mg, Copper: 3000mg, iodine: 1000mg, Iron: 20000mg, Manganese: 40000mg, Zinc: 300000mg, Selenium: 200mg, Anti-oxidant: 1250mg

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Table 2 - Percentage composition of broiler finisher diets supplemented with graded levels of DGLM

Parameters \ Treatment levels	T_1	T_2	T_3	T_4
Moisture	7.67	7.64	7.62	7.66
Crude protein	20.00	20.04	20.20	20.30
Crude fibre	6.40	6.45	6.57	6.58
Crude Fat	3.85	3.87	4.05	4.06
Ash	6.57	6.45	6.57	6.06
NFE	55.51	55.55	54.99	54.8
ME Kcal/kg	3026	3030	3031	3029

DGLM=dried guava leaf meal. Treatment (T_1): 0g DGLM/100 kg basal diet as control; T_2: 150g DGLM/100 kg basal diet; T_3: 300g DGLM/100 kg basal diet; and T_4: 450g DGLM/100 kg basal diet. NFE: Nitrogen free extract, ME: Metabolizable energy, calculated using the formula 37 × % CP + 81.8 × % EE + 35.5 × NFE (Pauzengua, 1985).

Data collection

Average daily feed intake (ADFI)

The experimental birds were provided feed and water liberally in the course of the experiments. Left over feed was collected and weighed daily. This was then subtracted from the quantity of feed served daily to obtain the daily feed intake. To obtain the average weekly feed intake per bird (AWFI / bird), feed consumed daily was multiplied by 7 (seven) and divided by the number of birds/replicate.

Average daily weight gain (ADWG)

Body weights of birds were taken at the on-set of the study and then weekly until the expiration of the study. The difference between the initial weight and the final weight showed the weight gain/bird. ADWG was obtained by dividing the final weight gain/bird/replicate by the number of days the study lasted.

Feed conversion ratio (FCR)

The records of feed consumed and that of the weight gain by birds in each treatment group were used to compute FCR according to the following formula.

$$FCR = \frac{\text{Feed intake}}{\text{Body weight gain}}$$

Protein efficiency ratio (PER)

This was obtained as

$$PER = \frac{\text{Weight gain}}{\text{Protein intake}}$$

Mortality

This was determined by dividing the total number of dead birds by the total number of birds brought at the beginning of the study and expressed as a percentage.

$$\text{Mortality} = \frac{\text{Number of dead birds} \times 100}{\text{Number of birds housed}}$$

Economics of production

The prevailing market prices of the ingredients at the time of experiment were used to calculate the cost of feed per kilogram (₦), total cost of feed consumed (₦) and cost of feed per kilogram weight gain (₦).

Organoleptic Test

Upon completion of the feeding trial, twelve birds (one per replicate) with the mean weight of the various replicates were slaughtered and processed as described by Aduku and Olukosi (2000). Sensory evaluation was done using breast muscle samples from the processed broiler chickens. The prepared meat was cooked in separate pots to a temperature of 100^{0}C for 20 minutes by braising method without spicing or salting. Hereafter, the cooked meat samples were coded and served at room temperature (27^{0}C) to each member of a 7- man panel comprising of relevant fields; like Food Science, Animal Science, Microbiology and Catering (Zakaria et al., 2010). For the evaluation of the meat quality indices, the Hedonic scale rating of sensory evaluation method was employed as earlier described by FAO (2010); Zakaria et al. (2010). Each of the meat quality parameter was characterized and rated 1-5. While "1" represents 8 points, "5"represents 0 point in that order and without the panelists' fore knowledge. Thereafter, all the points against each parameter by each panelist were subjected to analysis of variance. This was done to determine any differences or similarities in the panelists' opinion and to determine if the test additive had any effect on the natural organoleptic meat quality indices of broiler chickens.

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Statistical analysis

The experimental design was a one-way classification in a Completely Randomized Design (CRD) with the following model: Yij=μ + ai + eij; Where Yij is the observed value of each of the response variables (performance characteristics) arising as a result of μ=the overall population mean; ai=observed effect of the i^{th} dietary treatment; eij=random or residual error due to the experimentation. All data collected were subjected to analysis of variance (Steel and Torrrie, 1980) SPSS 17 Software. Means showing significant differences were separated using the Duncan's Multiple Range Test (Duncan, 1985).

RESULTS

The result of performance indices (Table 3) revealed that mean final body weight and mean daily body weight gain increased with corresponding increase in the level of dietary supplementation with DGLM up to 450 g/100 kg of basal diet. This clearly indicated that DGLM had growth promoting effect on the animals and, that the stated level was well tolerated by the birds. Feed intake did not differ among all the treatment groups (P>0.05) which implies that DGLM was palatable to the birds. This finding was in consonance with the reports of Mahmoud et al. (2013) and Rahman et al. (2013) who reported that feed intake was statistically the same (P >0.05) among treatment groups. For feed conversion ratio (FCR) and protein efficiency ratio (PER), the trend of response observed was that the FCR and PER improved significantly (P<0.05) as the dietary supplementation level of DGLM was increased. This suggests that DGLM enhanced feed and protein conversion efficiency in the basal diets of the experimental birds. According to Anon (2011), FCR is a measure of how well a flock converts feed intake into live weight and any factor which reduces feed intake, growth or health of the broiler will worsen flock FCR. Feed cost per kilogram weight gain improved significantly (P<0.05) following the same trend of FCR and PER whereby a corresponding increase in dietary supplementation of DGLM in broiler diet improved the aforementioned parameters. Low mortality percentages which ranged from 0 to 3.33% were observed in this study. The mortality could not be attributed to any detrimental effect of the test ingredient because the mortality incidence did not follow any definite pattern. This further implies that the broiler chicken tolerated DGLM, up to 450g/100kg basal diet without any deleterious effect. Besides good management practice, DGLM according to Pandey and Shweta (2011) contains anti-inflammatory and antimicrobial properties that could induce positive effects in broiler gut health. Hascik et al. (2015) further posited that positive effects of dietary supplementation of DGLM in broiler diet may boost broiler immunity which could be attributed to the presence of flavonoids which have anti-microbial and anti-oxidative activities. The negligible mortality percentage recorded in this study (0% to 3.33% range) contradicts the reports of Mahmoud et al. (2013) and El-Deek et al. (2009) who recorded significant levels of mortality while testing the effects of DGLM and guava wastes products on broiler chickens. These differences could be attributed to the levels of bio-sanitary and bio-security measures adhered.

Table 3 - Productive performance of finisher broiler chickens fed diets supplemented with DGLM.

Performance indices \ Treatment levels	T_1	T_2	T_3	T_4	SEM	P-value
Initial body weight (g/b)	673.17	676.33	676.83	677.33	2.276	ns
Final body weight (g/b)	3,355.00^{c}	3,381.67^{c}	3,468.33^{b}	3,555.00^{a}	16.915	*
Average daily weight gain (g/b)	63.85^{c}	64.42^{c}	66.46^{b}	68.52^{a}	0.406	*
Average daily feed intake (g/b)	97.47	97.42	97.47	97.45	0.015	ns
Feed conversion ratio	1.53^{a}	1.51^{ab}	1.47^{c}	1.42^{d}	0.013	*
Protein efficiency ratio	3.27^{b}	3.30^{b}	3.41^{a}	3.51^{a}	0.239	*
Cost of feed consumed (₦/b)	372.14	371.93	372.12	372.04	NA	ns
Cost of guava leaf/kg(₦)	0	1.50	3.00	4.50	NA	ns
Feed Cost/Kg (₦)	90.90	90.90	90.90	90.90	NA	ns
Feed Cost ₦/kg weight gain	138.70^{a}	137.30^{a}	133.60^{b}	129.70^{c}	0.66	*
Mortality (%)	3.33	1.67	0.00	1.67	NA	ns
Initial body weight (g/b)	673.17	676.33	676.83	677.33	2.276	ns

DGLM=dried guava leaf meal. Treatment (T_1): 0g DGLM/100 kg basal diet as control; T_2: 150g DGLM/100 kg basal diet; T_3: 300g DGLM/100 kg basal diet; and T_4: 450g DGLM/100 kg basal diet. ^{a,b,c}: Means in the same row with different superscripts are significantly (P<0.05) different; NS: not significant (P > 0.05); *=P value <0.05; SEM: Standard error of mean, g/b: Gram per bird; NA: not analyzed, ₦/b: Naira per bird

Sensory evaluation of organoleptic meat quality indices of broiler chickens fed diets supplemented with dried guava leaf meal

Result of organoleptic indices is shown in Table 4. It was observed that all the organoleptic properties (colour, appearance, texture, taste and flavour) of all the treatment groups evaluated using Hedonic scale rating of sensory method compared favourably (P>0.05) across treatments. This implies that the test additive (DGLM) did not impact any

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negative influence on the broiler meat. Fris-Jensen (1982) had earlier reported that robenidine; an anti-coccidial additive, imparted an off-flavour influence on broiler meat after its administration. Mellen et al. (2014) observed significant differences among experimental groups of cobb 500 chicken with regards to meat quality after administering agolin, agolin tannin, agolin acid and bio-strong additives in their nutrition. Similarly, Wasker et al. (2009) experimented on the effect of phyto-additive methiorep (synthetic methionine) on carcass and cooked meat quality attributes in chicken and reported that the additive had no negative influence on the meat. Pandey and Shweta (2011) reported that bee pollen extract caused a significant increase in the redness value of broiler chicken meat evaluated after use as feed additive in their diet. These findings are indications that additives could influence both the performance of the livestock and the quality of its products. The slightly numerical higher rating (7.4 each) observed in T_3 and T_4 in relation to colour and 6.86 in T_4 in relation to appearance could be attributed to the presence of some oxygenated carotenoids (xanthophils or lutein) in the guava leaf according to Joseph (2011). This may add more market and nutritional value to broiler chicken fed DGLM at the rate of 450g/100kg diet.

Table 4 - Sensory evaluation of organoleptic properties of meat quality of finisher broiler chickens fed diets supplemented with graded levels of dried guava leaf meal.

Treatment levels / Sensory traits	T_1	T_2	T_3	T_4	SEM	P-value
Colour	6.67	6.29	7.14	714	0.680	ns
Appearance	6.29	6.00	6.00	6.86	0.691	ns
Texture	5.71	5.43	4.86	6.57	1.100	ns
Taste	6.00	5.71	5.71	6.29	0.639	ns
Aroma	5.71	6.29	6.29	6.29	0.595	ns

Numbers in the table represent means of 0-8 point ratings by 7 panelists using Hedonic method of sensory evaluation described by El-Deek et al. (2009). ns: not significant (P> 0.05)

CONCLUSION

The study concluded that the supplementation of dried guava (*Psidium guajava* L.) leaf meal at 300g and 450g/ 100kg enhanced utilization of nutrients in the diets resulting in impressive growth performance, reduced feed cost/weight gain (₦/kg), and high survivability without influencing the organoleptic properties of finisher broiler chickens.

DECLARATIONS

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Authors' contribution

F. B. P. Abang performed conceptualization, writing, original draft preparation, review and editing. I. E. Echeonwu performed conceptualization and evaluation of manuscript before submission. M. U. Amu performed conceptualization and evaluation of manuscript before submission.

Ethical approval

The University Committee on Ethical Matters Examined and Approved all the Experiments.

Conflict of interests

The authors declare that there is no conflict of interest.

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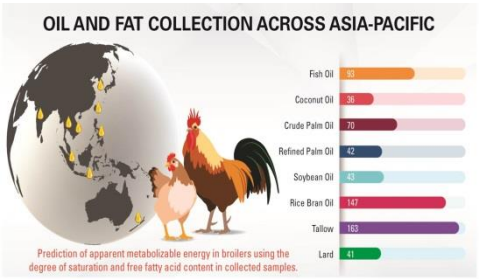

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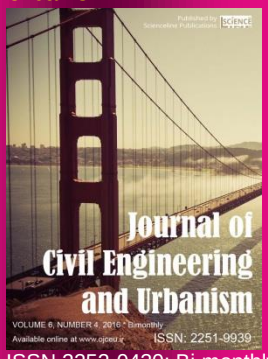

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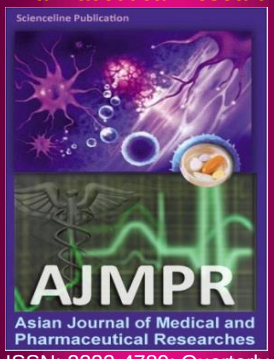

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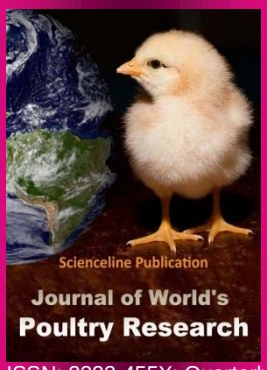

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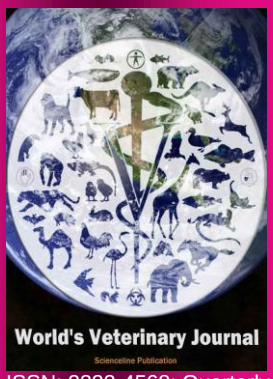

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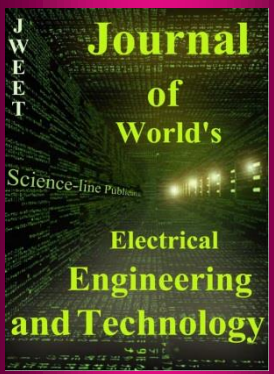

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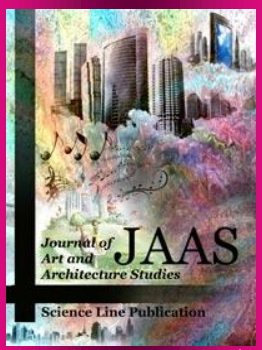

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